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VOLUME 18 ISSUE 2 VERSION 1.0

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Polyphenol Content and in Vitro Antioxidant Activity of Aqueous-Ethanol Extracts of *Pterocarpus soyauxii* and *Pterocarpus santalinoides*

By Emmanuela Nneoma Akaniro-Ejim, Marynn Ifunanya Ibe & Godwill Azeh Engwa
Godfrey Okoye University

Abstract- Recently, medicinal plants are gaining considerable attention for their therapeutic antioxidant activities. Though many studies have investigated the pharmacological and medicinal activities of Pterocarpus soyauxii and Pterocarpus santalinoides, there is limited knowledge of their antioxidant potential. Hence, this study aimed to assess the polyphenol content and investigate the in vitro antioxidant activity of these plants. Aqueous-ethanol extracts of the plants' leaves were obtained by maceration. The total flavonoid content (TFdC) and total flavonol content (TFlC) of the leaf extracts were determined by standard methods, while ferric reducing power and hydrogen peroxide scavenging assays were used to assess their in vitro antioxidant potentials. The mean TFdC of P. santalinoides (1083.33 ± 35.12 mg/g) was higher than that of P. soyauxii (730 ± 40 mg/g), while the mean TFlC was higher in P. soyauxii (390 ± 60.83 mg/g) than in P. santalinoides (260 ± 45.83 mg/g). The reducing potential of extracts of P. santalinoides was significantly higher ($p < 0.05$) than that of P. soyauxii, as well as the standard compound, at all concentrations tested.

Keywords: antioxidants; Pterocarpus soyauxii; Pterocarpus santalinoides; flavonoids; flavonols, ferric reducing potential, hydrogen peroxide scavenging activity.

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Emmanuela Nneoma Akaniro-Ejim [α], Marynn Ifunanya Ibe [σ] & Godwill Azeh Engwa [ρ]

Abstract- Recently, medicinal plants are gaining considerable attention for their therapeutic antioxidant activities. Though many studies have investigated the pharmacological and medicinal activities of *Pterocarpus soyauxii* and *Pterocarpus santalinoides,* there is limited knowledge of their antioxidant potential. Hence, this study aimed to assess the polyphenol content and investigate the in vitro antioxidant activity of these plants. Aqueous-ethanol extracts of the plants' leaves were obtained by maceration. The total flavonoid content (TFdC) and total flavonol content (TFlC) of the leaf extracts were determined by standard methods, while ferric reducing power and hydrogen peroxide scavenging assays were used to assess their *in vitro* antioxidant potentials. The mean TFdC of *P. santalinoides* (1083.33 ± 35.12 mg/g) was higher than that of *P. soyauxii* (730 ± 40 mg/g), while the mean TFlC was higher in *P. soyauxii* (390 ± 60.83 mg/g) than in *P. santalinoides* (260 ± 45.83 mg/g). The reducing potential of extracts of *P. santalinoides* was significantly higher ($p < 0.05$) than that of *P. soyauxii*, as well as the standard compound, at all concentrations tested. The hydrogen peroxide scavenging activity of *P. santalinoides* was superior to that of *P. soyauxii,* as well as ascorbic acid. The results of this study suggest that *P. soyauxii* and *P. santalinoides* are rich in flavonoids and flavonols and exhibit potent hydrogen peroxide scavenging activity and ferric reducing capacity, with the later showing greater activities. These properties may contribute to the therapeutic potential and medicinal applications of these plants and suggests a potential drug candidacy of flavonoid compounds of these species of *Pterocarpus*.

Keywords: antioxidants; Pterocarpus soyauxii; Pterocarpus santalinoides; flavonoids; flavonols, ferric reducing potential, hydrogen peroxide scavenging activity.

I. Introduction

Oxidative stress contributes to many pathological conditions and diseases including cancer, stroke, diabetes, inflammatory diseases such as arthritis, cardiovascular disorders, etc. [1-4]. It results from an overwhelming level of free radicals or reactive oxygen species (ROS) such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, peroxynitrite radical, etc. [5], which contain one or more unpaired electrons thus making them unstable and highly reactive [2]. Due to this high reactivity, these molecules rapidly attack adjacent molecules leading to disruption of membrane fluidity, lipid peroxidation, protein oxidation, alteration of platelet functions, DNA strand breaks, and modulation of gene expression [3, 4].

To circumvent the delirious and detrimental effects of free radicals, antioxidants are naturally present in living organisms and are capable of scavenging these free radicals, converting them to less reactive forms, thereby preventing or inhibiting cellular damage [6]. Antioxidant defense systems in humans include iron transport proteins such as transferrin, albumin, ferritin, and caeruloplasmin; metabolic products such as glutathione, ubiquinol and uric acid; and endogenous enzymes such as superoxide dismutase, catalase, various peroxidises etc. [2, 5, 7]. The antioxidant defense systems under normal physiological conditions are sufficient only to cope with the normal threshold of the physiological rate of free-radical generation. Therefore, any additional burden of free radicals, either from endogenous or exogenous sources on the human physiological system may lead to oxidative stress [2, 7]. Hence, supplementary sources of antioxidants are needed to prevent oxidative stress. Recently, medicinal and dietary plants are gaining considerable concern, as they are rich in micronutrients such as vitamin E (α-tocopherol), vitamin C (ascorbic acid) and β-carotene, as well as plants secondary metabolites such as phenolic compounds, flavonoids, saponins, etc. [4, 5, 8-11] which have been shown to exhibit promising therapeutic antioxidant properties. Though the activity of synthetic phenolic antioxidants is often observed to be higher than that of natural antioxidants, [12] there is evidence of increased predisposition to various fatal diseases following use of synthetic antioxidants [4, 8-10], hence the renewed interest in natural antioxidants.

The genus *Pterocarpus* is a tropical and sub-tropical plant with about 60 species of which 20 of these are found in Africa, particularly in Nigeria, Cameroon,

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Sierra Leone and Equatorial Guinea [13, 14]. *Pterocarpus soyauxii* and *Pterocarpus santalinoides*, locally known as "oha" and "uturukpa" respectively in Igbo, are abundant and widely consumed as vegetables in South-Eastern Nigeria [13, 14]. They are traditionally used in the treatment of headaches, pains, fever, convulsions, skin rashes and respiratory disorders, and as antiabortive, antidiabetic, hepatoprotective and antimicrobial agents [13-15]. Though many studies have investigated the pharmacological and medicinal activities of these species [16-18], little is known about their antioxidant potential. Hence, this study was aimed to determine the polyphenol content of these plants and investigate the in vitro antioxidant activity of *Pterocarpus soyauxii* and *Pterocarpus santalinoides*.

II. Materials and Methods

a) Plant materials

Fresh leaves of *Pterocarpus soyauxii* and *Pterocarpus santalinoides* were purchased from Ahia Abapka in Enugu, Enugu State of Nigeria. The plants were identified taxonomically by Prof C. U. Okeke (Department of Botany, Nnamdi Azikiwe University, Akwa) as *Pterocarpus soyauxii* (*P. soyauxii*) and *Pterocarpus santalinoides* (*P. santalinoides*). The leaves were air-dried at room temperature (28 ± 2°C) in the Biotechnology Laboratory of Godfrey Okoye University Enugu for seven days and thereafter pulverized before further processing.

b) Chemicals and reagents

Ethanol and ascorbic acid were purchased from JHD, Guangdong Guanghua Sci-Tech Co., Ltd., Guangdong, China. Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) for phosphate buffer were purchased from Merck KGaA, Darmstadt, Germany. Potassium ferricyanide and trichloroacetic acid were obtained from Vicker Laboratories Ltd, West Yorkshire, England. Ferrous chloride was obtained from Griffin and George, Wembley, England. Hydrogen peroxide and aluminium chloride were obtained from BDH Laboratory Supplies, Poole, England. Sodium acetate was obtained from Burgoyne Burbidges and Co., Mumbai, India. Rutin was obtained from Sigma-Aldrich, St. Louis, MO, USA. All solvents and reagents used in the study were of analytical grade.

c) Maceration and extraction of plant materials

Extraction was carried out according to the methods of Bothon *et al*. [15] with slight modifications. Hundred gram (g) of the pulverized leaves of *P. soyauxii* and *P. santalinoides* were separately macerated in 500 ml of aqueous-ethanol for 48hours. The aqueous-ethanol extracts were prepared by adding 500 ml of an ethanol-water mixture (70:30) to 100g plant powder and mechanically stirred for 48 hours. The resulting solutions were filtered through Whatman No. 1 filter paper and the extracts obtained were then concentrated and finally dried to a constant weight. The extraction yields of the samples were calculated using the following equation:

$$\text{Total extraction yield, } Y_t\ (\%) = \frac{\text{Mass of extract, } M_t}{\text{Mass of sample, } M_s} \times 100\%$$

Extracts were stored in sterile containers at 4 °C until further use.

d) Estimation of polyphenol compounds

i. Total flavonoids content

Total flavonoids content of the plant extracts was determined based on the formation of an aluminium-flavonoids complex, using the methods described by Ordon Ez *et al*. [19]. A volume of 0.5 ml (2 %) aluminium chloride-ethanol solution was mixed with 0.5 ml of plant extracts (100 mg/l). The mixture was incubated at room temperature for 1 hr and the absorbance measured at 420 nm. All determinations were carried out in triplicates. The same procedure was repeated for the various concentrations (6.25 - 100 mg/l) of a standard solution of rutin, and the rutin calibration curve was constructed. The concentration of flavonoids was expressed as rutin (mg/l) equivalent from the calibration curve of rutin (Figure 1) using the equation: $Y = 0.001X - 0.003$, $R^2 = 0.991$

$$X = \frac{Y + 0.003}{0.001}$$ where, Y was absorbance and X was concentration of rutin (mg/l).

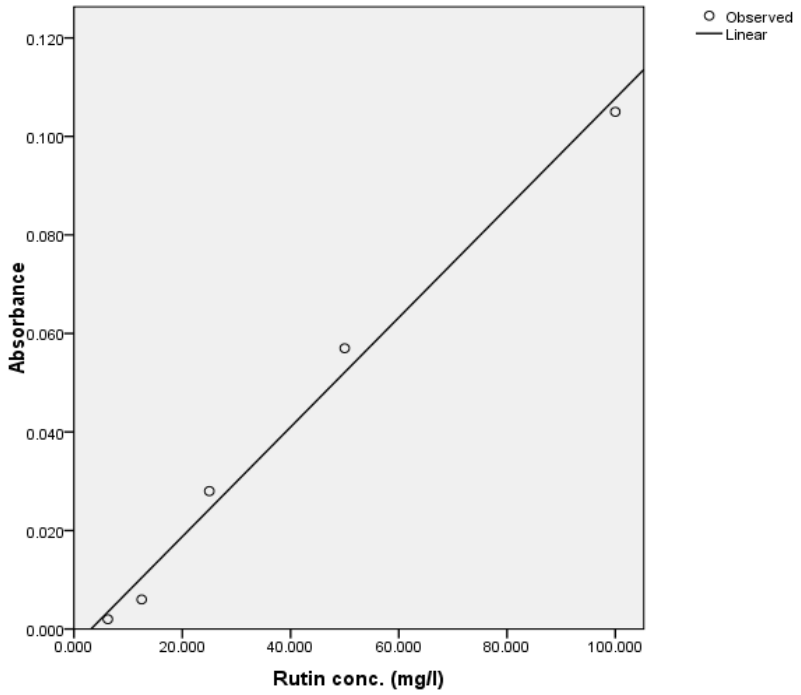

Figure 1: Calibration curve of total flavonoids of rutin

The total flavonoids content (TFdC) of extracts was calculated in terms of rutin equivalent (mg of RU/g of dry weight extract) using the following formula:

$$\text{TFdC (mg RU/g)} = \frac{\text{Concentration of rutin (mg/l)} \times \text{[Total volume of extract solution (ml)} \times 10^{-3}\text{ (l/ml)]}}{\text{Weight of extract (mg)} \times 10^{-3}\text{ (g/mg)}}$$

ii. *Total flavonols content*

The total flavonols content was estimated based on the method of Kumaran and Karunakaran [20], using rutin as a reference compound. Two milliliters of the extracts (100 mg/l) were separately mixed with 2 ml of 2% aluminium chloride-ethanol solution and 3 ml of sodium acetate solution (50 mg/ml). The resulting solution was incubated at room temperature for two and half hours, and the absorbance was read at 440 nm. All determinations were carried out in triplicates. The same procedure was repeated for the various concentrations (6.25 - 100 mg/l) of standard solution of rutin and the rutin calibration curve was constructed. The concentration of flavonols was expressed as rutin (mg/L) equivalent from the calibration curve of rutin (Figure 2) using the equation: $Y = 0.001X + 0.008$, $R^2 = 0.990$

$X = \frac{Y-0.008}{0.001}$ where, Y was absorbance and X was concentration of rutin (mg/l).

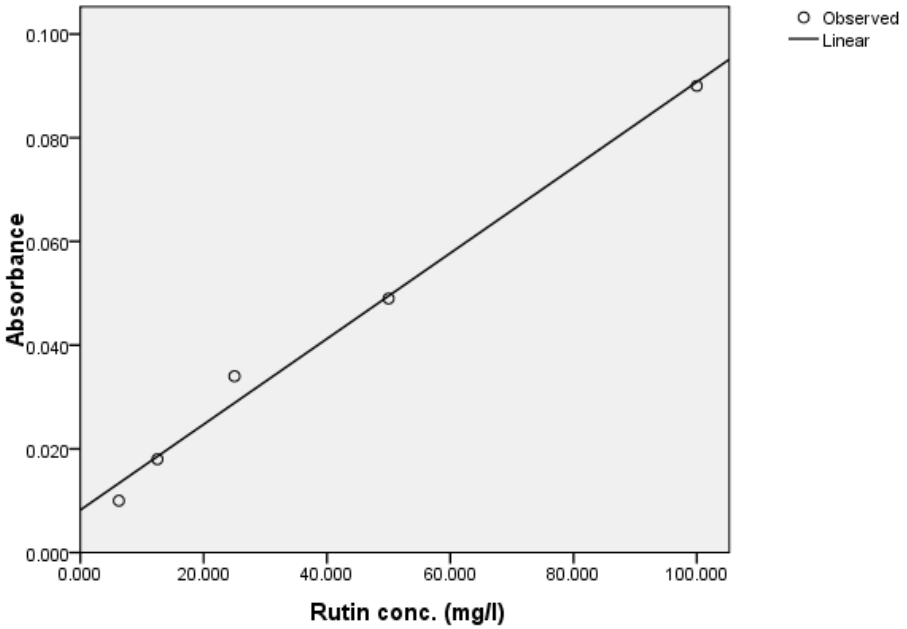

Figure 2: Calibration curve of total flavonols of rutin

The total flavonols content (TFlC) of extracts was calculated in terms of rutin equivalent (mg of RU/g of dry weight extract) using the following formula:

$$\text{TFlC (mg RU/g)} = \frac{\text{Concentration of rutin (mg/l)} \times [\text{Total volume of extract solution (ml)} \times 10^{-3}\ \text{(l/ml)}]}{\text{Weight of extract (mg)} \times 10^{-3}\ \text{(g/mg)}}$$

e) In vitro antioxidant activity

i. *Determination of reducing power*

The reducing power of the extracts was determined according to the methods of Yildirim *et al.* [21], with slight modifications. Extracts (1.25- 10.00 mg) in 1 ml of distilled water were separately mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.0) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe\ (CN)_6$]. The resulting solution was incubated at 50°C for 30 min, followed by addition of 2.5 ml of 10% trichloroacetic acid, and centrifugation of the resulting mixture at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferrous chloride ($FeCl_3$) and the absorbance was measured at 700 nm against a blank sample using a UV-5800(PC) UV/VIS Spectrophotometer. Increased absorbance of the reaction mixture was indicative of high reducing power of the plant extracts. Ascorbic acid was used as standard.

ii. *Hydrogen peroxide scavenging activity*

The ability of the aqueous-ethanol extracts of *P. soyauxii* and *P. santalinoides* to scavenge hydrogen peroxide was determined using the methods of Yen and Chen [22]. A solution of hydrogen peroxide (4mM) was prepared in phosphate buffer (0.1 M, pH 7.0). The hydrogen peroxide solution (0.6 ml) was separately mixed with 4 ml of various concentrations of the extracts (1.25 - 10.00 mg/ml) and incubated at room temperature for 10 min. Absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing plant extracts without hydrogen peroxide. Percent scavenging activity of the plant extracts was determined by following formula:

$$H_2O_2\ \text{scavenging activity (\%)} = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100\%$$

Where, Absorbance of control was the absorbance of hydrogen peroxide radical + solvent; Absorbance of sample was the absorbance of hydrogen peroxide radical + sample extract or standard. Ascorbic acid served as standard.

iii. *Statistical analysis*

Experimental results were reported as mean ± Standard deviation (SD) of three parallel measurements. Unpaired T-test was performed to compare the means of the total flavonoids and flavonols content of the plant extracts. For other analyses, significant differences were established by Two-way ANOVA, followed by Tukey's multiple comparisons test, using GraphPad Prism version 6.05 for Windows. A difference was considered significant at $p < 0.05$.

III. Results

a) Extraction yields

The percentage yield of *Pterocarpus soyauxii* and *Pterocarpus santalinoides* aqueous-ethanol extracts was 6.63% and 5.61% respectively.

b) Estimation of polyphenol compounds

The total flavonoids content (TFdC) and total flavonols content (TFlC) of aqueous-ethanol leaf extracts of *P. soyauxii* and *P. santalinoides* is summarised in Table 1. The mean TFdC level was higher in *P. santalinoides* (1083.33 ± 35.12 mg/g) than *P. soyauxii* (730 ± 40 mg/g), while the TFlC level was higher in *P. soyauxii* (390 ± 60.83 mg/g) *than P. santalinoides* (260 ± 45.83 mg/g).

Table 1: Total flavonoids content (TFdC) and total flavonols content (TFlC)

Compounds	Concentration of rutin (mg/g)	
	P. soyauxii (PSO)	*P. santalinoides* (PSU)
Flavonoids (mg of RU/g of extract)	770	1120
	730	1050
	690	1080
Mean TFdC	730 ± 40	1083.33 ± 35.12
Flavonols (mg of RU/g of extract)	350	220
	360	310
	460	250
Mean TFlC	390 ± 60.83	260 ± 45.83

c) Reducing power activity of P. soyauxii and P. santalinoides leaf extracts

The reducing power of leaf extracts of *Pterocarpus soyauxii* and *Pterocarpus santalinoides* exhibited different degrees of electron donating capabilities, all in a concentration-dependent manner (Figure 3).

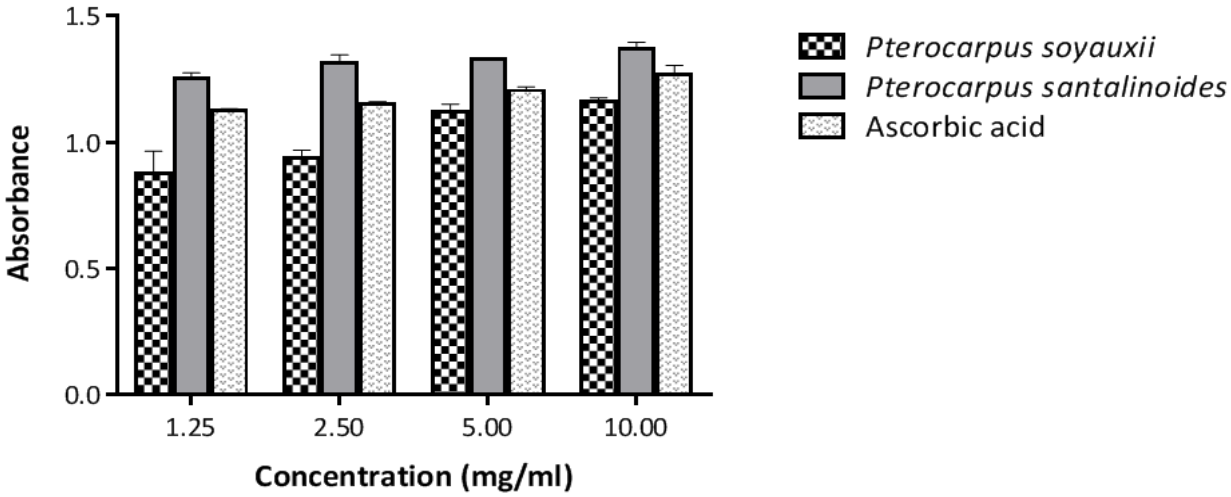

Figure 3: Reducing power of leaf extracts of *P. soyauxii* and *P. santalinoides* compared to ascorbic acid. The results are presented as mean ± SD

The reducing potential of extracts of *P. santalinoides* was significantly higher ($p < 0.05$) than that of *P. soyauxii*, as well as the standard compound (ascorbic acid) at all concentrations tested. The reducing potential of the tested compounds was greatest in *P. santalinoides,* followed by ascorbic acid and least in *P. soyauxii* (*P. santalinoides* > ascorbic acid > *P. soyauxii).*

d) Hydrogen peroxide scavenging activity of P. soyauxii and P. santalinoides leaf extracts

Hydrogen peroxide scavenging activity of aqueous-ethanol leaf extracts of *P. soyauxii* and *P. santalinoides* was observed to be concentration dependent (Figure 4). *P. soyauxii* exhibited the lowest scavenging activity at all concentrations tested, with an exception of the extracts at 10.00 mg/ml concentration, which had a higher percent inhibition of hydrogen peroxide (99.63 %) than the standard antioxidant compound at an equivalent concentration (99.23 %).

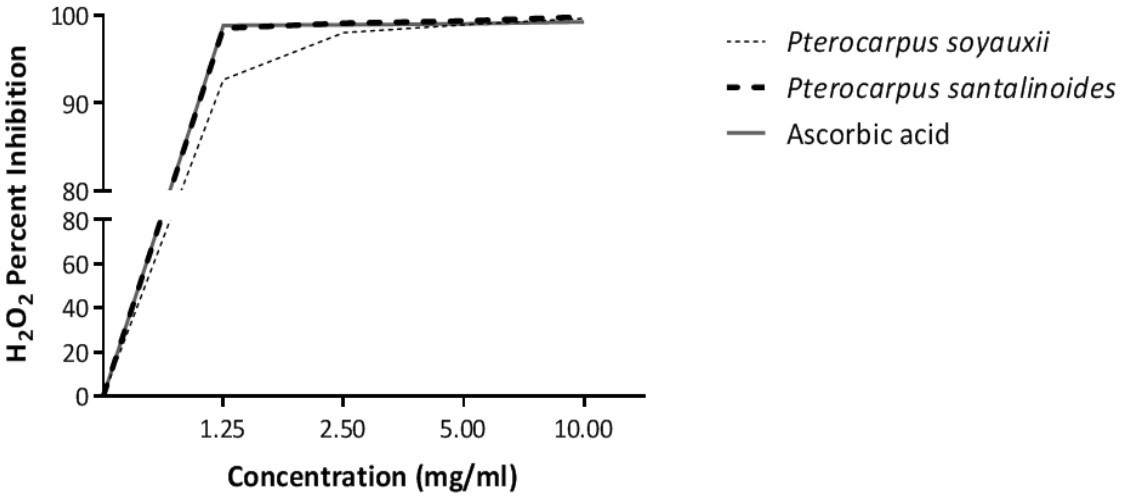

Figure 4: Hydrogen peroxide scavenging activity of *P. soyauxii* and *P. santalinoides* leaf extracts compared to ascorbic acid

Similar to its reducing power activity, the hydrogen peroxide scavenging activity of *P. santalinoides* was superior to that of *P. soyauxii,* as well as ascorbic acid, with percentage inhibitions of 98.50, 99.07, 99.33 and 99.80 at 1.25 mg/ml, 2.50 mg/ml, 5.00 mg/ml and 10.00 mg/ml respectively. The only exception of enhanced hydrogen peroxide scavenging activity of standard compound over *P. santalinoides* was for the starting concentration of 1.25 mg/ml, with a percent inhibition of 98.83 % as against *P. santalinoides* with percent inhibition of 98.50 % at similar concentration.

IV. Discussion

Plant secondary metabolites exert important functions in living plants. Flavonoids for instance, can protect against free radicals generated in plants [23]. High content of phenolics and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in preventing the development of chronic as well as age-related diseases, particularly caused by oxidative stress [6, 10, 24]. Preliminary phytochemical screening of *Pterocarpus soyauxii* and *Pterocarpus santalinoides* has revealed the presence of flavonoids in these plants [13, 14]. Estimation of polyphenols in this study revealed the presence of both flavonoids and flavonols in extracts of both *Pterocarpus* species. The total flavonoid content of aqueous-ethanol leaf extracts of *P. santalinoides* was significantly higher than that of *P. soyauxii* ($p = 0.0003$), while total flavonols concentration was higher in *P. soyauxii* than in *P. santalinoides*.

Flavonoids are well known for their antioxidant activity [8]. They are thought to exert their antioxidant activity by the mechanisms of radical scavenging and metal ion chelation to inhibit lipid peroxidation [4]. Several studies in recent years have shown that flavonoids, like other polyphenols in plants, scavenge reactive oxygen species and effectively prevent oxidative cell damage [1]. The activities of antioxidants have been ascribed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging [5, 21]. The reducing power of a compound may thus serve as an important marker of its possible antioxidant activity [21]. Reducing power of a plant extract correlates with phenolic constituents in the plant [10]. In this assay, the oxidation form of iron (Fe^{+3}) in ferric chloride is converted to ferrous (Fe^{+2}) through electron transfer ability by antioxidant compounds [10, 25]. The aqueous-ethanol extracts of *P. soyauxii* and *P. santalinoides* exhibited good reducing power activity at the different concentrations tested (Figure 3), however extracts of *P. santalinoides* showed a higher ferric reducing power than *P. soyauxii* and ascorbic acid at all concentrations tested. The observed higher ferric reducing activity of *P. santalinoides* over *P. soyauxii* may be attributed to its higher flavonoids content and possibly the presence of other bioactive compounds with antioxidant properties. Bothon *et al.* for instance, has reported the presence of coumarins in extracts of *Pterocarpus santalinoides* [15]. Coumarins are well established antioxidant compounds [26-28], hence their presence in *P. santalinoides* may potentiate the reducing power activity of these plants. The trend in the reducing power of extracts from *P. santalinoides* was similar to those of their hydrogen peroxide scavenging activities and the total flavonoids content, indicating that there is a correlation between the total flavonoids content and the antioxidant activities of plant extracts.

The ability of extracts of *P. soyauxii* and *P. santalinoides* to scavenge free radicals in vitro strongly suggests their antioxidant activity. Percentage inhibition of hydrogen peroxide (H_2O_2) by both extracts was comparable to that exhibited by ascorbic acid, a

standard antioxidant compound. In this study, this relationship was verified by the observation that both the total flavonoids composition and the H_2O_2 scavenging activity of species of *Pterocarpus* tested were in the order of *P. santalinoides* > *P. soyauxii*. Scavenging of H_2O_2 by plant extracts may be attributed to their phenolics and flavonoids which can donate electrons to H_2O_2, thus neutralizing it to water [29]. Although hydrogen peroxide is itself not very reactive, it is converted to highly reactive hydroxyl radicals by Cu^{2+} and Fe^{2+} ions, leading to lipid peroxidation, oxidative stress and cytotoxicity [30-32]. Thus, removing H_2O_2 throughout biological systems, particularly the human body, is very important.

V. Conclusion

Pterocarpus soyauxii and *Pterocarpus santalinoides* are shown to both be rich in flavanoid and flavonols compounds and exhibit potent hydrogen peroxide scavenging activity and ferric reducing capacity. This raises the possibility that phenolic-rich plants such as *Pterocarpus soyauxii* and *Pterocarpus santalinoides* could provide beneficial antioxidant effects in disease states characterized by oxidative stress conditions. Further in vitro and in vivo studies to validate the antioxidant potential of extracts of *Pterocarpus soyauxii* and *Pterocarpus santalinoides* are however suggested, to establish the potential drug candidacy of flavonoid and flavonols compounds from these plants.

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A DFT-Based QSAR and Molecular Docking Studies on Potent Anti-Colon Cancer Activity of Pyrazole Derivatives

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Abstract- Pyrazole derivatives have been described as a group of compounds with various biological activities including anticancer effect. Therefore, a set of twenty Pyrazole based compounds which had been previously shown to be active against human colon cancer cell (HT29) are use in the study. These compounds were optimized using Density Functional Theory (DFT) for the calculations of molecular descriptors that related the bioactivity of these compounds to their structures. The developed quantitative structure activity relation (QSAR) was validated, and it showed the reliability and acceptability of the model. The in silico simulations were carried out on the twenty Pyrazole based compounds with colon cancer cell line, HT29 (PDB ID: 2N8A) using Autodock vina software. The docked complexes were validated and enumerated based on the AutoDock Scoring function to pick out the best inhibitors based on docked Energy. The analysis of the ligand-receptor complexes showed that H-bonds played a prominent role in the binding and posed stability of the ligand in the ligandreceptor complexes. The binding free energy, **Δ**G calculated ranged from - 6.10 kcal/mol – 8.20 Kcal/mol.

Keywords: pyrazole derivatives, DFT-QSAR, molecular docking.

GJMR-B Classification: NLMC Code: QV 4

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I. Introduction

Cancer is not a contagious neither infectious disease, but it has become a second leading cause of death worldwide and travels from one end to the other via bloodstream within the body system [1,2]. It can be caused by both external and internal factors, e.g. tobacco, infectious organisms, chemicals, and radiation are for outside while inherited mutations, hormones, and immune conditions for internal factor. Moreover, all of these factors may work together or in series to start or enhance carcinogenesis[3]. The cure for cancer remain surgery, chemotherapy and radiation therapy and adult, as well as, children can be affected. However, cases of children having cancer are limited[4].

Colon cancer as a worldwide known health problem forays more than a million people every year, which has been the cause of death to over 600,000 people[5]. It is found to be the usual cause of death in comparison to other types of cancer that exist[6,7]. Several features that may cause an increment in colon cancer risks comprise diet, diabetes, aging, obesity, genomic instability, etc. Over the last half-century, the United States Food and Drug Administration (USFDA) has approved more than one hundred drugs for clinical treatment of cancers. Nevertheless the search for new and/or improved chemical compounds as potential anticancer agents continues with the hope that better efficacy and more manageable adverse side effects of pharmaceutical drugs may be achieved. Molecular modeling, screening and mimicking of natural compound derivatives have been among several drug discovery approaches to rationally design and modified structures that may confer a better therapeutic index[8] or that can cure cancer in the human race[9].

Among such compounds are pyrazole and its derivatives, pyrazoles are class of heterocyclic compounds used for the development of drugs, and they have attracted the attention of several researchers due to their extensive biotic actions such as anticancer[10], antifungal[11], antiviral[12], anti-inflammatory[14,15]. More so, pyrazole derivatives such as Pyrazolopyrimidine and pyrazolo[4,3-*d*]pyrimdin-7-one perform some pharmacological activities which can never be put aside in the medical world, for example as antihypertensive[15], antiviral[16,17], tuberculostatic[18], herbicidal agents[19], antileishmanial[20] and treatment of heart diseases[21]. Therefore, the structural features of the Pyrazoles have been recognized as vital parameters due to their bioactivity as therapeutic aids. Several pyrazoles have been commercialized such as omeprazole, Albendazole, mebendazole, candesartan, telmisartan, astemizole[22-24].

Quantitative Structural Activity Relationship (QSAR) as a statistical model embroils the relationship between physicochemical parameters of a chemical compound to its biological activity[25]. It has attracted vast usefulness for linking molecular evidence with biotic activities and many other physicochemical properties as well as its helpfulness for drug design, discovery, and development[26]. QSAR helps in the prediction of toxicity of materials in bulk system, for instance, drug-like compounds and are very useful in case of the classic chemicals[27-30]. The use of molecular descriptors calculated from quantum chemical methods for development of QSAR models has been described to be sufficient for generating comprehensive QSAR. Thus

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the use of quantum chemical descriptors has countless potential[31-34].

Molecular docking studies divulge information on the interaction between the drug-like compound known as a ligand and an enzyme/receptor through recognizing the active positions within the enzyme along with the binding energy calculation[34]. In molecular docking, scoring is a statistical way of predicting the strength of the interactions which are non-covalent in between a ligand and a receptor. Therefore, the calculations of interaction energy can be offered in the form of "dock score"[35].

Consequently, in this research, twenty pyrazole derivatives with known anti-colon cancer activities[21] as displayed in Figure 1 were optimized using Density Functional Theory (DFT) method so as to obtain molecular descriptors for the compounds. These compounds are 4-amino-3-(4-chlorophenyl)-1H-pyrazolyl-5-yl- (3,5-dimethyl-1Hpyrazol-1 yl) derivatives) methanone (**3a, b**), 2-{[4-amino-3-(4-chlorophenyl)-1H-pyrazol-5-yl]carbonyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one (**4**), 1-{[4-amino-3-(4-chlorophenyl-1H-pyrazol-5-yl]carbonyl} pyrazolidine- 3,5- dione (**5**), 3-(4-chlorophenyl)-5-(1,3,4-oxa/thiadiazol-2yl)-1H-pyrazol-4-amine (**7a, b**), 6-amino-3-(4-chlorophenyl)-5-methyl-1,6-dihydro-7*H*-pyrazolo-[4,3-*d*]pyrimidin-7-one (**9**), 4-amino-3- (4-chlorophenyl)- N'- [arylmethylidene]- 1H-pyrazole-5-carbhydrazide ***(10 a-d)***, 3-(4-chlorophenyl)-5-methyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidine-7-one (**12**), 7- chloropyrazolo [4,3-*d*] pyrimidine (**13**), 3-(4-chlorophenyl)-7-(3,6-dihydro-2H-pyran-4-yl)-5-methyl-1H-pyrazolo[4,3-d]pyrimidine (**14**),7- (4-chlorophenyl)-5-methyl -9H-pyrazolo[3,4-e]tetrazolo[1,5-c]pyrimidine ***(15)***, Ethyl{[3- (4-chlorophenyl)- 5- methyl- 1H- pyrazolo [4,3-d]pyrimidine-7-yl]oxy} acetate (***16)***, 6-amino-3-(4-chlorophenyl)- 5- thioxo- 1,4,5,6- tetrahydro- 7*H*-pyrazolo[4,3-*d*] pyrimidin-7-one (**17**), 2-{[4-amino-3-(4-chlorophenyl)- 1H- pyrazol- 5- yl] carbonyl} hydrazinecarbothioamide (***18),*** 4-amino-3-(4-chlorophenyl)-N'-(4-methyl/ or 4-phenyl-1,3-thiazol-2-yl)-1*H*-pyrazole-5-carbohydrazide (**19a, b**). Thus, the major objectives of this work are: (i) to calculate molecular descriptors with the use of quantum chemical method via Density Functional Theory (DFT), (ii) to develop QSAR model which probe into biological activity of the studied compounds, and (iii) to calculate the free energy of interactions (binding affinity, ΔG) of the ligand with the receptor in the binding site through molecular docking.

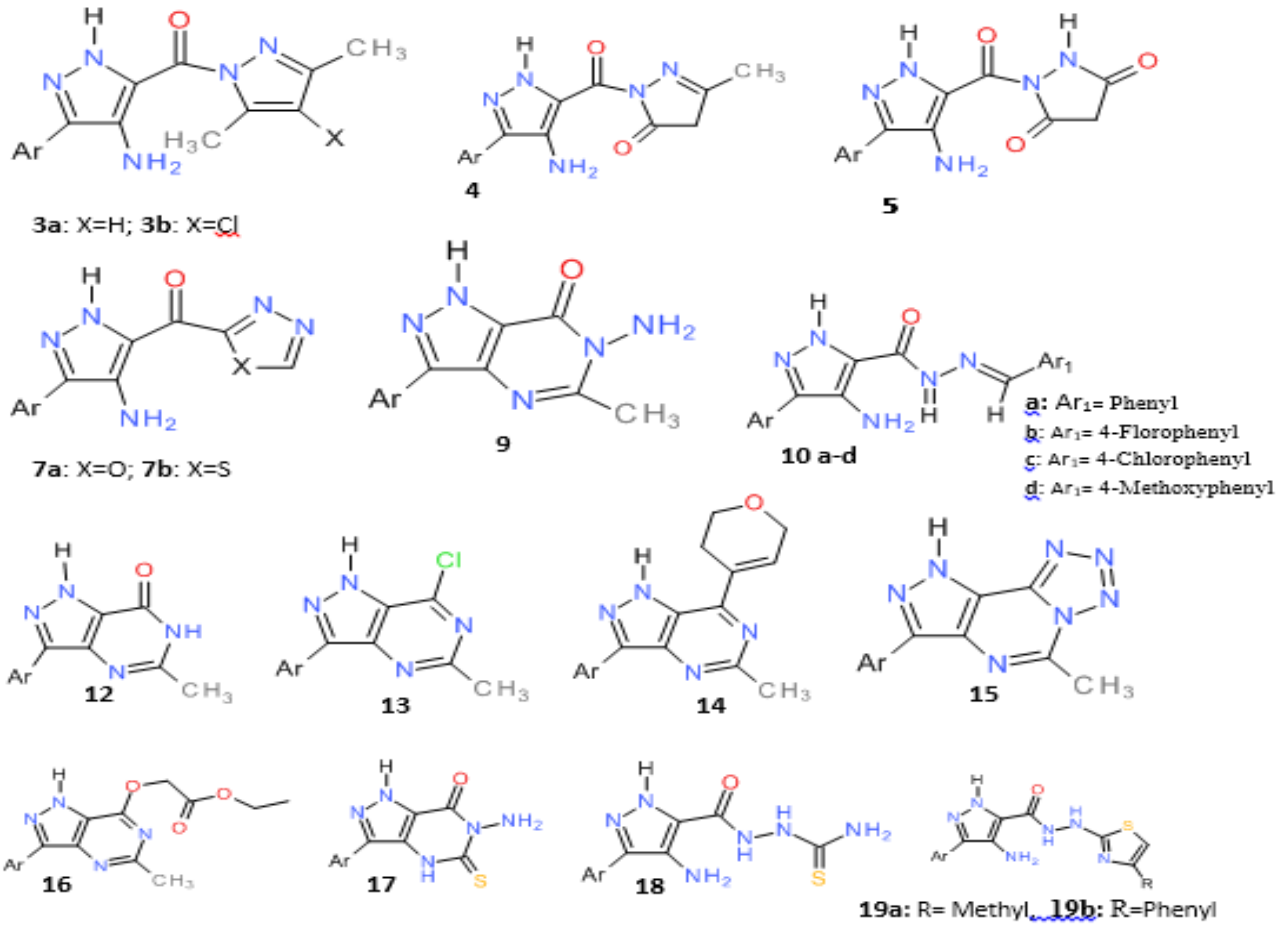

Figure 1: The schematic structures of the pyrazole derivatives, the compounds were numbered as used in [20]

II. Computational Details

a) Ligand optimization and molecular descriptors

The equilibrium geometries for the twenty pyrazole derivatives as reflected in this paper were optimized at Density Functional Theory (DFT). The use of DFT method entails three-parameter density functional, which comprises Becke's gradient exchange correction[37] and the Lee, Yang, Parr correlation functional (i.e., B3LYP)[38]. The accuracy of DFT calculations depends on the particular functional chosen and basis sets. However, 6-31G** basis set has been found to be appropriate for the confirmation search and calculation of drug-like compounds[39]. Therefore 6-31G** basis set was used in this work. Also, the optimized compounds were used to calculate molecular parameters/descriptors that described the bioactivity (IC_{50}) of the compounds. The optimized molecular structures were used for the docking study to estimate the binding affinity of the compounds to the colon cancer cell line, HT29 receptor (PDB ID: 2N8A). The optimization of the compounds was carried out using quantum chemical software Spartan '14 by wavefunctionInc[40].

Table 1: Calculated descriptors used in this study

Descriptors	Symbol	Abbreviation
Quantum chemical descriptors	Molecular dipole moment	DM
	Molecular polarizability	P
	Highest occupied molecular orbital, eV	HOMO
	Lowest unoccupied molecular orbital, eV	LUMO
	Solvation energy (au)	SE
	Natural charge population on nitrogen atoms of pyrazole ring in *e* (Het)	(N+N)/2
	Difference between E_{LUMO} and E_{HOMO}, eV	BD
	Chemical Hardness [$\eta = 1/2 (E_{HOMO} + E_{LUMO})$] eV	η
	Softness ($S = 1/\eta$) eV^{-1}	S
	Electro negativity [$\mu = 1/2 (E_{HOMO} - E_{LUMO})$] eV	μ
	Nucleophilicity ($\omega = \mu^2/2\eta$) eV	ω
Chemical properties	Partition Coefficient	Log P
	Molecular weight	MW
	Volume	V
	Ovality	Ovl
	Polar surface area	PSA
	Bond length between two Nitrogen atoms of the pyrazole ring	NNBL
	Bond length between Nitrogen and the hydrogen atom of pyrazole ring	NHBL
	Natural charge population on Hydrogen atoms of the pyrazole ring	H HET4r
	Hydrogen bond donor	HBD
	Hydrogen bond acceptor	HBA

b) Data processing and QSAR modeling

Furthermore, the chosen calculated parameters were engaged to develop quantitative structure-activity relationship (QSAR) model to link the bioactivity to the calculated molecular descriptors obtained from the studied compounds[41]. This was achieved using multiple linear regression (MLR) method which is a recurrent statistical technique used in developing QSAR model. MLR and correlation analyses were carried out by the statistics software SPSS 13.0 version. Before MLR analysis, the person correlation table was used to examine collinearity among the descriptors ($r > 0.90$). The descriptors with higher correlation with the dependent variable (IC_{50}] were retained, and the others were removed from the descriptor data matrix. The remaining descriptors were used to construct the MLR model, by the stepwise method. Moreover, the QSAR model was validated using some statistical equations such as cross validation (R^2) and adjusted R^2. Cross validation is a mathematical method which oversees the reliability of QSAR model that can be used for a set of facts as shown in equation 1.

$$\mathrm{CV.R^2} = 1 - \frac{\sum(\mathrm{Y_{obs}} - \mathrm{Y_{cal}})^2}{\sum(\mathrm{Y_{obs}} - \bar{\mathrm{Y}}_{\mathrm{obs}})^2} \quad (1)$$

The adjusted R^2 could be calculated using equation (2)

$$\mathrm{R_a^2} = \frac{(\mathrm{N-I})\times\mathrm{R^2-P}}{\mathrm{N-P-1}} \quad (2)$$

where N is number of observations (compounds), *p* is number of descriptors,

Also, for a good model, the standard error of estimate (s) of a set of data should be low, and this is defined as follows:

$$s = \sqrt{\frac{\sum(Y_{obs} - Y_{cal})^2}{N-p-1}} \quad (3)$$

To judge the overall significance of the regression coefficients, the variance ratio (F) which is the ration of regression mean square to deviations mean square can be defined as follows:

$$F = \frac{\frac{\sum(Y_{cal} - \bar{y}_{obs})^2}{p}}{\frac{\sum(Y_{obs} - Y_{cal})^2}{N-p-1}} \quad (4)$$

The F value has two degrees of freedom: p, N − p − 1. The computed F value of a model should be significant at $p < 0.05$; thus for overall significance of the regression coefficients, the F value should be high.

c) Molecular Docking and binding affinity

The downloaded **HT29** receptor (**PDB ID: 2N8A**[42]) from protein data bank was treated i.e., removal of water molecules, ligand, and cofactors from the receptor with the use of discovery studio. Then, both the receptor and ligand were converted to the acceptable format (pdbqt) for AutoDockvina programme. The grid dimension used for all the 2N8A protein was are 50 × 40 ×40 Å (grid size) with point separated by 1.000 Å (grid-point spacing). The docking was done using autodock vina which was inspired by Darwinian evolution theory to be iterative optimization method[43] which involves search Algorithm. At the completion of the docking runs, ligand showing different conformations known as Binding modes were obtained with their respective binding affinity. The stable pattern was assumed to be the one with the lowest binding affinity and was taken for post-docking analysis using Edupymol version 1.7.4.4.

III. Result and Discussion

a) QSAR modeling

The molecular descriptors calculated for the twenty pyrazole compounds served as independent variables, while the observed inhibitory actions ($IC_{50,}\mu M$) against cancer cells line as the dependent variable in the development of QSAR model via multiple linear regression (MLR). These molecular descriptors used for QSAR model were displayed in Tables 2 and 3. In QSAR study, the quality of a model is evaluated by its fitting and prediction abilities; however, for a model to be acceptable, its predictability power is of paramount important. Therefore, Pearson's matrix was used for the selection of suitable descriptors for the QSAR study (Table 4).

Table 2: The calculated molecular descriptors from the compounds

MOL	HOMO	LUMO	BG	DM	SE (au)	(N+N)/2 Het	η	μ	ω
3a	-5.54	-1.66	3.98	5.26	-0.01389	-0.379	3.600	-1.940	0.5227
3b	-5.66	-1.85	3.81	3.33	-0.01208	-0.379	3.755	-1.905	0.4832
4	-5.87	-1.60	4.27	6.35	-0.02134	-0.353	3.735	-2.135	0.6102
5	-6.58	-1.94	4.64	2.54	-0.03482	-0.355	4.260	-2.455	0.7074
7a	-5.74	-1.41	4.33	3.51	-0.02187	-0.398	3.575	-2.165	0.6556
7b	-5.66	-1.80	3.86	3.76	-0.01747	-0.399	3.730	-1.930	0.4993
9	-5.96	-1.36	4.60	4.23	-0.01815	-0.405	3.660	-2.300	0.7227
10a	-5.84	-0.82	5.02	5.19	-0.01575	-0.368	3.330	-2.510	0.9460
10b	-5.84	-1.65	4.33	1.68	-0.01442	-0.368	3.815	-2.165	0.6143
10c	-6.07	-1.80	4.27	1.81	-0.01671	-0.377	3.935	-2.135	0.5792
10d	-5.56	-1.44	4.12	2.91	-0.01841	-0.368	3.500	-3.128	1.3973
12	-5.99	-1.31	4.68	4.11	-0.01977	-0.401	3.650	-2.340	0.7501
13	-6.23	-2.09	4.14	2.26	-0.01402	-0.413	4.160	-2.070	0.5150
14	-5.91	-2.09	3.82	4.67	-0.01784	-0.427	4.000	-1.910	0.4560
15	-5.91	-1.99	4.39	0.76	-0.01789	-0.424	4.185	-2.195	0.5756
16	-5.88	-1.45	4.43	6.68	-0.01861	-0.418	3.665	-2.215	0.6693
17	-6.18	-1.89	4.29	3.02	-0.02548	-0.391	4.035	-2.145	0.5701
18	-5.79	-1.41	4.38	4.84	-0.03355	-0.396	3.600	-2.190	0.6661
19a	-5.73	-1.24	4.49	4.68	-0.02043	-0.372	3.485	-2.245	0.7231
19b	-5.70	-1.30	4.40	4.39	-0.02085	-0.371	3.485	-2.200	0.6914

Table 3: The calculated molecular descriptors from the compounds (Continued)

MOL	MW	Vol	Ova	Log P	PSA	Pol	NNBL	NHBL	H HET4r	HBD	HBA
3a	315.764	297.32	1.48	0.07	63.211	64.57	1.322	1.012	0.294	2	6
3b	350.209	310.90	1.51	-0.06	63.228	65.69	1.322	1.011	0.294	2	6
4	317.736	287.79	1.48	-1.01	85.752	63.71	1.320	1.010	0.296	3	7
5	319.708	275.32	1.46	-1.52	100.075	62.61	1.329	1.010	0.300	3	8
7a	261.672	229.28	1.39	0.26	74.597	58.95	1.333	1.008	0.285	2	6
7b	277.739	238.68	1.40	0.83	64.537	59.82	1.334	1.008	0.282	2	6
9	275.699	246.62	1.42	-0.15	72.744	60.29	1.345	1.008	0.292	2	5
10a	341.802	329.28	1.55	0.60	88.836	66.90	1.329	1.010	0.292	3	6
10b	357.776	327.84	1.45	1.62	79.201	66.94	1.334	1.010	0.294	3	6
10c	374.231	337.13	1.55	2.02	79.118	67.71	1.336	1.010	0.295	3	6
10d	369.812	350.35	1.57	1.34	86.232	68.82	1.333	1.010	0.293	3	7
12	260.684	235.01	1.39	0.10	56.316	59.33	1.345	1.008	0.294	1	4
13	279.130	241.38	1.40	1.55	37.704	59.98	1.354	1.008	0.292	1	4
14	326.787	311.07	1.47	1.67	42.724	65.70	1.352	1.006	0.277	1	5
15	285.698	246.93	1.40	1.83	68.584	60.37	1.352	1.008	0.297	1	6
16	332.747	303.52	1.52	0.76	64.473	64.95	1.351	1.007	0.287	1	6
17	293.738	244.92	1.41	0.15	74.925	60.23	1.339	1.009	0.300	3	7
18	310.679	266.54	1.49	-0.96	106.292	61.96	1.335	1.009	0.287	5	8
19a	348.818	309.47	1.53	0.09	89.093	65.42	1.327	1.009	0.292	3	7
19b	410.889	374.88	1.59	1.49	88.388	70.75	1.327	1.009	0.293	3	7

Table 4: Pearson's correlation matrix

	HT29	HOMC	LUMC	BG	DM	SE	N+N/2HE	CP	CH	GN	MW	VOI	OVA	LOGP	PSA	POL	NNBL	NHBL	HHET4r	HBD	HBA
HT29	1.000																				
HOMO	-0.009	1.000																			
LUMO	0.306	0.476	1.000																		
BG	0.334	-0.349	0.655	1.000																	
DM	0.280	0.520	0.506	0.102	1.000																
SE	-0.370	0.372	-0.028	-0.342	-0.064	1.000															
N+N/2HET	0.274	0.152	0.320	0.219	0.060	-0.173	1.000														
CP	-0.434	0.005	-0.448	-0.472	0.092	0.229	-0.343	1.000													
CH	-0.191	-0.828	-0.888	-0.235	-0.596	-0.177	-0.284	0.283	1.000												
GN	0.423	0.230	0.567	0.397	0.039	-0.111	0.356	-0.970	-0.48	1.000											
MW	0.283	0.285	0.195	-0.044	0.044	0.125	0.533	-0.225	-0.27	0.284	1.000										
VOL	0.295	0.365	0.272	-0.027	0.130	0.233	0.499	-0.272	-0.36	0.355	0.973	1.00									
OVALITY	0.456	0.419	0.414	0.078	0.303	0.061	0.501	-0.349	-0.48	0.442	0.913	0.92	1.00								
LOGP	-0.292	0.049	-0.199	-0.266	-0.375	0.587	-0.434	0.018	0.107	0.018	0.257	0.29	0.15	1.000							
PSA	0.524	0.033	0.483	0.482	0.081	-0.631	0.672	-0.459	-0.32	0.439	0.392	0.31	0.45	-0.488	1.000						
POL	0.288	0.372	0.258	-0.047	0.128	0.240	0.494	-0.262	-0.36	0.347	0.973	1.00	0.91	0.304	0.300	1.000					
NNBL	-0.095	-0.463	-0.331	0.031	-0.245	0.078	-0.861	0.044	0.454	-0.13	-0.422	-0.39	-0.44	0.488	-0.580	-0.395	1.000				
NHBL	0.240	0.221	0.135	-0.013	-0.074	0.098	0.787	-0.137	-0.20	0.174	0.402	0.38	0.38	-0.334	0.435	0.381	-0.802	1.000			
HHET4r	0.088	-0.447	-0.027	0.374	-0.354	-0.135	0.527	-0.289	0.251	0.164	0.143	0.05	0.05	-0.260	0.347	0.050	-0.293	0.606	1.000		
HBD	0.565	0.168	0.346	0.219	0.066	-0.541	0.655	-0.273	-0.30	0.294	0.417	0.32	0.44	-0.432	0.873	0.323	-0.600	0.501	0.234	1.000	
HBA	0.416	0.048	0.131	0.097	0.096	-0.658	0.579	-0.263	-0.10	0.247	0.422	0.30	0.44	-0.451	0.861	0.305	-0.583	0.410	0.267	0.787	1.000

The selected descriptors were used to build a linear QSAR model to understand how multiple linear regression (MLR) equations can explain the structural key points correlating to differential behavior in bioactivity against colon cancer cell (HT29) as shown in equation 4. This model was validated statistically by using the squared fitting factor (R^2), cross validation ($CV.R^2$), adjusted fitting factor ($_{adj}R^2$) and variation ratio (F). The developed model was very robust in predicting satisfactory the experimental values. The high values of F, R^2, $CV.R^2$ and $_{adj}R^2$ as shown in Table 5 indicated that the models are statistically acceptable and also have good external predictability[44,45]. The calculated R^2 is 0.9564; this revealed a reasonable fitness, and it also uncovered the efficiency of the model as displayed in equation 4. The value for $CV.R^2$ was calculated to be 0.9542 which is greater than 0.5 (standard)[46], and this showed the reliability and acceptability of the model as well as the adjusted R^2 with 0.9247 which was greater than 0.6 (Table 5)

$$IC_{50} = -2677.65 - 38.6214(BG) + 0.864947(DM) + 257.863(NNBL) + 2353.57(NHBL) - 190.240(HHET4r) - 39.6798(HOMO) + 37.8067(LUMO) + 0.0699416(PSA) \quad (4)$$

The QSAR model contained eight descriptors in different combinations; each descriptor with either positive or negative coefficient attached to it. However, the magnitudes of the coefficients as well as, the values of descriptors have significant roles in deciding the overall biological activity of the molecule. The descriptors with negative coefficients in the model were very significant because they contributed towards increasing the value of the biological activity of anti-colon cancer agents. Therefore, the descriptors with a negative coefficients were most significant followed by descriptors with low weight positive coefficients and lastly the parameters with high weight positive coefficients. The predicted anti-colon cancer activity of the ligands using the QSAR model as well as deviation from the experimental values was displayed in Table 6 and graphically presented in Figures 2 and 3.

Table 5: Statistical parameters for validation of QSAR model

N	p	R^2	$CV.R^2$	R^2_{adj}	s	F
20	8	0.9564	0.9542	0.9247	0.4141	30.168

Table 6: Stepwise regression result for anti-colon cancer activity

Comp	Observed	Predicted	Residual	Comp	Observed	Predicted	Residual
3A	1.45	1.45	-0.00	10D	3.57	3.05	0.52
3B	1.10	1.57	-0.47	12	0.78	0.54	0.24
4	2.78	2.53	0.25	13	1.52	1.23	0.29
5	2.99	2.82	0.17	14	0.88	0.96	-0.08
7A	0.28	-0.26	0.54	15	0.29	0.70	-0.41
7B	0.20	0.31	-0.11	16	3.88	3.86	0.02
9	1.77	2.19	-0.42	17	1.33	1.24	0.09
10A	4.08	4.16	-0.08	18	5.59	5.65	-0.06
10B	2.10	2.18	-0.08	19A	0.42	1.09	-0.67
10C	2.92	2.83	0.09	19B	0.78	0.62	0.16

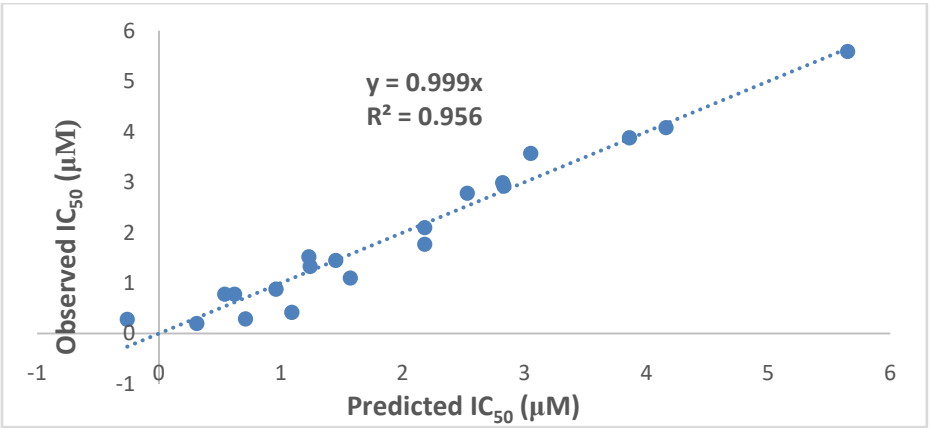

Figure 2: The calculated predicted IC_{50} against the experimental IC_{50}.

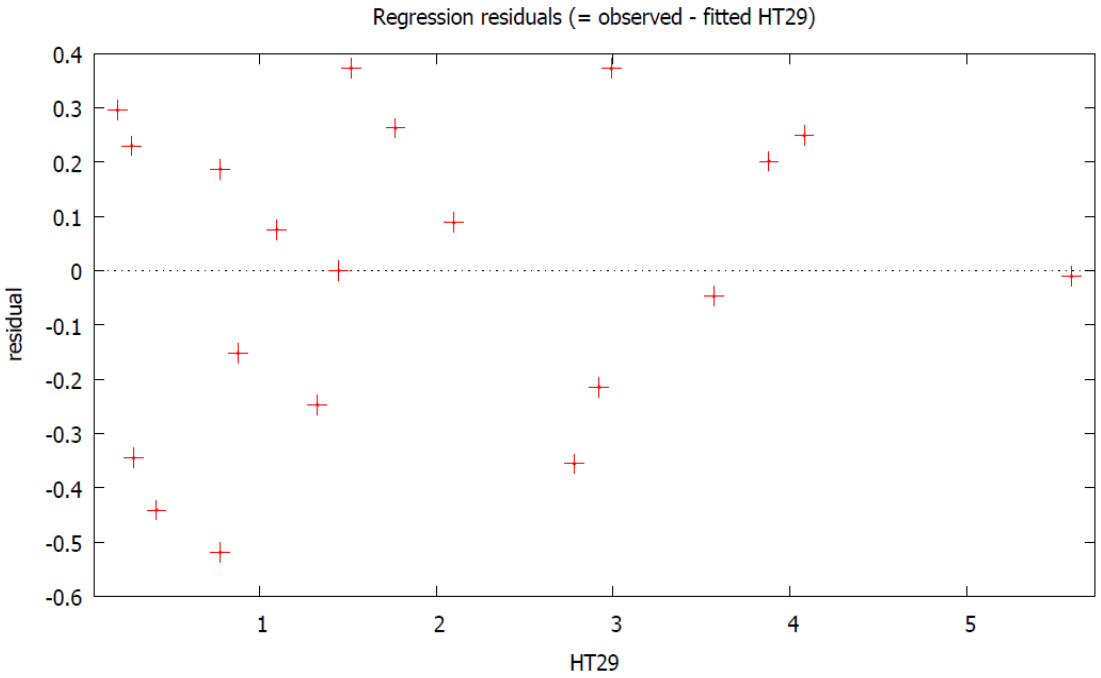

Figure 3: The residuals versus observed IC_{50}

b) Docking and Scoring

The molecular docking studies were performed on the twenty pyrazole derivatives together with colon cancer cells line (PDB ID: 2N8A)[42] obtained from protein data bank. This was achieved with the use of several software such as Discovery studio, Autodock tool, Autodock vina and Pymol as post-dock software. Docking of each compound was carried using autodock vina and conformations obtained varies in number but ranged from 8 – 15 conformations for compounds 3a-19b. The structure with lowest binding energy (i.e., highest negative free energy of binding, ΔG) in each docking simulation was taken to be most stable and analyzed for detailed interactions using Discovery Studio Visualizer4.0 software. Docking simulations can be understood by comparing the values of the free energy of binding (Gibbs energy, ΔG) of the ligands to the protein receptor. ΔG is an indicator to show the stability interaction between ligand and receptor, and it can be used to explain the strength of binding energies of different docking conformation[47,48].

The poses of the lowest conformation of each ligand were examined based on ΔG, and interaction of the ligand with the **2N8A** protein structure in ligand-receptor complex. The free binding energy (ΔG) calculated for the docked twenty pyrazole derivatives ranged from -6.10 kcal/mol (ligand **7a**) to -8.20 kcal/mol (ligand **19b**) as displayed in Table 7. The interaction of ligand with the **2N8A** protein structure was discussed by of H-bonding between the ligand and the receptor molecule as shown in Figure 4. Analysis of the ligand-receptor complex showed that H-bonds played a prominent role in the binding and posed stability of the ligand in the ligand-receptor complex; thus affect the potency/function of biological molecules. The number of H-bonds present in the ligand-protein complex as well as H-bonds distances was shown in Table 7. The ligand **3a** formed one H-bond with **2N8A** receptor involving GLN-40 and LIG: O (carbonyl oxygen) with the bond distance of 2.3 Å; whereas two H-bonds were observed for **3b** with GLN-40 H-bonded to carbonyl oxygen with 2.5 Å bond length and also with hydrogen atoms on N-H of pyrazole ring with 1.8 Å.

Furthermore, ligand **4** two H-bonds, with ALA-64 H-bonded to hydrogen N-H of pyrazole ring of the ligand with 2.5 Å distance apart, and THR-109/LIG:O (pyrazole-carbonyl oxygen) with 1.9 Å bond length. For ligand 5, five H-bonds were observed with ALA-89/LIG:O (pyrazole-carbonyl oxygen) with the distance of 3.0 Å, ILE-64/LIG: O (one of the carbonyl oxygen of pyrazolidine-3,5-dione) with the distance of 3.6 Å, and GLU-107/LIG:O (pyrazole-carbonyl oxygen) with distance of 3.1 Å. Others were GLU-107/LIG:H (hydrogen of N-H of pyrazole ring) with distance of 2.5 Å, GLU-107/LIG:N (amino group of pyrazole ring) with 3.2 Å bond distance, LYS-108/LIG:N (amino group of pyrazole ring) with distance 2.2 Å, and THR-109/LIG:N (amino group of pyrazole ring) with distance 2.8 Å as well as THR-109/LIG:H (H-amino group of pyrazole ring) with distance 2.2 Å.

Table 7: Interactions between ligands and **2N8A** receptor

Comp	Affinity (kcal/mol)	H-Bond Between protein residues in the binding pocket and Drug	Distance
3a	-7.1	GLN-40, LIG:O	2.3
3b	-7.4	(i) GLN-40, LIG:O (ii) GLN-40, LIG:H	(i)2.5 (ii) 1.8
4	-7.1	(i) ALA-106, LIG: H (ii) THR-109, LIG:O	(i) 2.5 (ii) 1.9
5	-6.9	(i) ALA-89, LIG:O (ii) ILE-64, LIG: O (iii) GLU-107, LIG: O (iv) GLU-107, LIG: H (v) GLU-107, LIG:N (vi) LYS-108, LIG:N (vii) THR-109, LIG:N (viii) THR-109, LIG: H	(i) 3.0 (ii) 3.6 (iii) 3.1 (iv) 2.5 (v) 3.2 (vi) 2.2 (vii) 2.8 (viii) 2.2
7a	-6.1	LYS-47, LIG: N	(i) 2.4
7b	-6.4	(i) MET-38, LIG: H (ii) GLN-40, LIG:H	(i) 2.4 (i) 2.3
9	-6.8	(i) THH-109, LIG:N (ii) GLU-107, LIG:H(iii) GLU-107, LIG:H (iv) GLU-107, LIG: O (v) ALA-89, LIG: O (vi) ILE-64 LIG: O (vii) ILE-64, LIG: H	(i) 3.0 (ii) 2.9 (iii) 2.1 (iv) 3.4 (v) 3.2 (vi) 3.6 (vii) 2.5
10a	-7.4	(i) THR-109, LIG: O (ii) GLU-90, LIG:H (iii) GLU-90, LIG: H (iv) ALA-89, LIG: H	(i) 3.3 (ii) 2.1 (iii) 2.7 (iv) 2.0
10b	-7.2	(i) THR-88, LIG: H, (ii) ALA-91, LIG: H	(i) 2.8 (ii) 2.5
10c	-7.2	(i) THR-176, LIG: H (ii) THR-109, LIG: H (iii) GLU-107, LIG:H (iv) GLU-107, LIG: O (v) ARG-65, LIG:O	(i) 2.1 (ii) 2.6 (iii) 2.5 (iv) 2.1 (v) 2.2
10d	-7.5	(i) ARG-65, LIG:O (ii) GLY-111, LIG:O (iii) THR-109, LIG:H (iv) GLU-107, LIG:H (v) GLU-107, LIG: N	(i) 2.3 (ii) 2.2 (iii) 2.1 (iv) 2.4 (v) 2.8
12	-6.4	(i) THR-109, LIG: O (ii) THR-109, LIG:H	(i) 3.3 (ii) 2.2
13	-6.4	MET-38, LIG:H	(i) 2.2
14	-7.0	(i) ASP-6, LIG: H (ii) THR-109, LIG:H	(i) 2.4 (ii) 2.5
15	-7.3	(i) GLU-107, LIG:H (ii) THR-109, LIG: N (iii) THR-109, LIG: N (iv) LYS-108, LIG: N (v) LYS-108, LIG: N	(i) 2.5 (ii) 2.0 (iii) 2.7 (iv) 1.9 (v) 2.1
16	-6.4	LYS-47, LIG: O	2.4
17	-6.8	(i) ALA-89, LIG:H (ii) ALA-89, LIG: O (iii) ILE-64, LIG:O (iv) GLU-107, LIG: O (v) GLU-107, LIG: H (vi) THR-109, LIG: N	(i) 2.2 (ii) 3.2 (iii) 3.5 (iv) 3.4 (v) 2.1 (vi) 3.0
18	-6.8	(i) GLY-93, LIG:N (ii) GLU-90,LIG:H (iii) GLU-90, LIG:H (iv) GLU-107, LIG:H (v) GLU-107, LIG:H (vi) GLU-107, LIG:H (vii) GLU-107, LIG: H (viii) GLU-90, LIG:H (ix) ALA-89, LIG:H (x) GLU-107, LIG:H (xi) GLU-107, LIG: H	(i) 3.5 (ii) 2.5 (iii) 2.2 (iv) 2.1 (v) 2.2 (vi) 2.6 (vii) 2.4 (viii) 2.5 (ix) 2.5 (x) 2.5 (xi) 2.8
19a	-7.2	(i) ALA-106, LIG: N (ii) ALA-106, LIG:H (iii) GLU-107, LIG: H (iv) GLU-107, LIG: H (v) GLU-90, LIG: H (vi) GLY-93, LIG: H	(i) 3.4 (ii) 2.7 (iii) 2.2 (iv) 2.0 (v) 2.2 (vi) 2.3
19b	-8.2	(i) THR-109, LIG: O (ii) THR-109, LIG:O (iii) LYS-108, LIG: O (iv) GLU-107, LIG:H (v) GLU-107, LIG:H (vi) GLU-107 LIG: H (vii) ALA-106, LIG: H (viii) GLU-90, LIG: H	(i) 2.3 (ii) 2.8 (iii) 2.0 (iv) 2.9 (v) 2.4 (vi) 2.5 (vii) 2.5 (viii) 2.0

However, for ligand **7a**, one H-bond was observed between LYR-47 and LIG:N (amino group of pyrazole ring); whereas two H-bonds for **7b** with MET-38/LIG:H (hydrogen of N-H of pyrazole ring) with distance of 2.9 Å, and GLN-40/LIG:H (H-amino group of pyrazole ring) with distance 2.3 Å. Seven H-bonds were observed for ligand **9;** THR-109/LIG:N (amino-group of pyrazole ring), GLU-107/LIG:H (H-amino group of pyrazole ring), GLU-107/LIG:H (hydrogen of N-H of pyrazole ring), GLU-107/LIG: O (Carbonyl oxygen of pyrimidinone). Others were ALA-89/LIG: O (Carbonyl oxygen of pyrimidinone), ILE-64/LIG:O (Carbonyl oxygen of pyrimidinone) and ILE-64/LIG: H (hydrogen N-H of pyrazole ring) with distance of 2.5 Å. Also, four H-bonds were observed for ligand **10a;** THR-109/LIG: O(pyrazole-carbonyl oxygen), GLU-90/LIG:H (hydrogen of N-H of carboxamide), GLU-90/LIG:H (H-amino group of pyrazole ring) and ALA-89/LIG:H (hydrogen of N-H of pyrazole ring). Two H-bonds were observed in docked complex of ligand **10b** and receptor**;** THR-88/LI G: H (H-amino group of pyrazole ring) and ALA-91/LIG:H (hydrogen of N-H of pyrazole ring). For ligand **10c**, five H-bonds were observed via THR-176/LIG:H (hydrogen of N-H of carboxamide), THR-109/LIG:H (H-amino group of pyrazole ring), GLU-107/LIG:H (hydrogen of N-H of pyrazole ring), GLU-107/LIG:O (pyrazole-carbonyl oxygen) and ARG-65/LIG:O (pyrazole-carbonyl oxygen). Also, five H-bonds were recorded for ligand **10d**; ARG-65/LIG:O (pyrazole-carbonyl oxygen), GLY-111/LIG:O (pyrazole-carbonyl oxygen), THR-109/LIG:H (H-amino group of pyrazole ring), GLU-107/LIG:H (hydrogen of N-H of pyrazole ring) and GLU-107/LIG: N (amino group of pyrazole ring).

Likewise, ligand **12**receptor complex presented two H-bonds; THR-109/LIG:O (Carbonyl oxygen of pyrimidin-one) and THR-109/LIG:H (hydrogen of N-H of pyrazole ring); whereas MET-38 was H-bonded with hydrogen of N-H of pyrazole ring of the ligand **13** with bond distance of 2.2 Å. For ligand **14,** two H-bonds were observed in the ligand-receptor complex; ASP-6/LIG:H (hydrogen of N-H of pyrazole ring) with the distance of 2.4 Å and THR-109/LIG:H (hydrogen of N-H of pyrazole ring) with of distance 2.5 Å. Also, for ligand **15**, five H-bonds were identified through GLU-107/LIG:H (hydrogen of N-H of pyrazole ring), THR-109/LIG: N (triazolyl ring), THR-109/LIG: N (N of N-H of pyrazole ring), LYS-108/LIG: N (one of N of triazolyl ring) and LYS-108/LIG: N (one of N of triazolyl ring). Moreover, one H-bond was observed for ligand **16**–receptor complex viaLYS-47/LIG:O(carboxylic-group) with H-bond distance of 2.4 Å; whereas for ligand **17**-receptor complex, six H-bonds were detected. The H-bonds were ALA-89/ LIG:H (hydrogen of N-H of pyrazole ring), ALA-89/LIG:O (First ketonic-group of pyrimidine-dione ring), ILE-64/LIG:O (Second Ketonic-group of pyrimidine-dione ring), GLU-107/LIG:O (First ketonic-group of pyrimidine-dione ring), GLU-107/LIG:H (hydrogen of N-H of pyrazole ring) and THR-109/LIG:N (N of N-H of pyrazole ring).

Also, for ligand **18**, eleven H-bonds were detected between the ligand and receptor residues vis-à-vis GLY-93/LIG:N, GLU-90/LIG:H, GLU-90/IG:H, GLU-107/LIG:H, GLU-107/LIG:H, GLU-107/LIG:H, GLU-107/ LIG: H, GLU-90/LIG:H, ALA-89/LIG:H, GLU-107/LIG:H and GLU-107LIG:H. Similarly, for ligand **19a** formed six H-bonds with **2N8A;** ALA-106/LIG: N, ALA-106/LIG:H, GLU-107/LIG: H, GLU-107/LIG:H, GLU-90/LIG:H, GLY-93/LIG:H. However, for ligand **19b,** eight H-bonds were observed between the ligand and **2N8A** residues. These were THR-109/LIG:O, THR-109/LIG:O, LYS-108/LIG:O, GLU-107/LIG:H, GLU-107/LIG:H, GLU-107/LIG:H, ALA-106/LIG:H and GLU-90/LIG: H. Some selected ligand – receptor (2N8A) complexes showing stable conformation, as well as Van Waal interactions, were displayed in Figure 4.

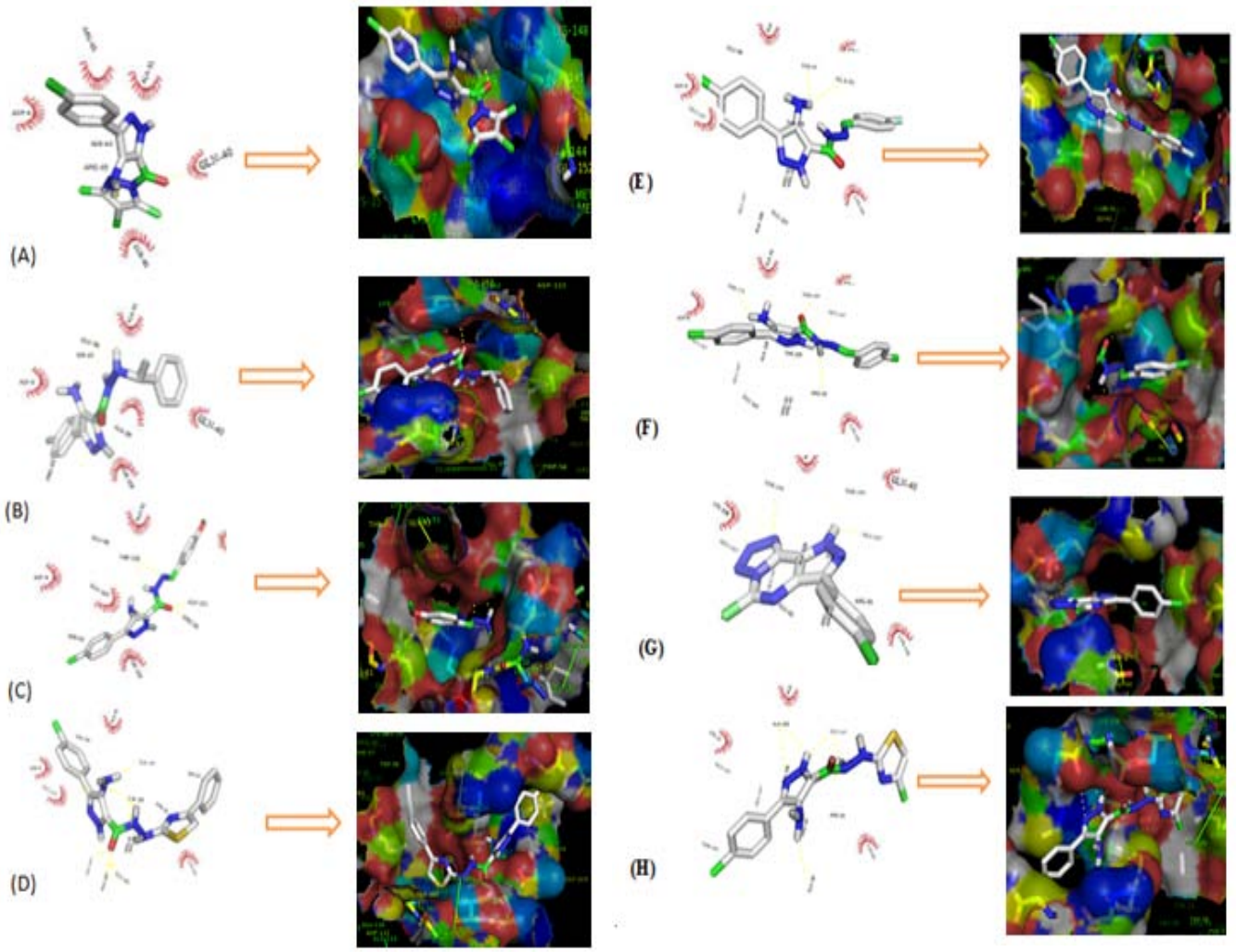

Figure 4: Binding interactions: (A) for 3b, (B) for 10a (C) for 10d and (D) for 19b (E) for 10b (F) for 10c (G) for 15 (H) for 19a with 2N8A

IV. Conclusion

In this study, the quantum chemical method via density functional theory (DFT) method was used for calculation of molecular descriptors relating to the anticancer activity of pyrazole derivatives. The QSAR analysis revealed the efficiency of the model developed using multiple linear regression (MLR), and that the QSAR model replicated the observed bioactivities of the studied compounds against colon cancer cells line (ID: 2N8A). Furthermore, the simulated molecular docking predicted stable conformations of the drug-like molecules (Pyrazolederiatives) in the active gouge of the receptor. Also, the binding energy as well as, nature of electrostatic interactions of the ligands in the ligand-receptor complexes were obtained for the twenty compounds.

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The Medicinal Flora in Gurunanak School of Pharmacy, GNITC, Ibrahimpatnam, Hyderabad, Telangana, India: A Research Based Review

By M. V. N. L. Chaitanya & P. Suresh
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Abstract- Telangana is a part of Deccan Plateau, located in the central part of the Indian Peninsula. The Hyderabad is now a state capital of Telangana after separation from Andhra Pradesh. It is situated in central Telangana and is spread over an area of 260 km2 and a heart of Eastern Ghats and is rich in different varieties of medicinal flora and many floras yet to be explore and there is less scientific literature focusing on these flora. The Guru Nanak School of Pharmacy is a part of GURU NANAK INSTITUTIONS TECHNICAL CAMPUS (AUTONOMOUS) located in Ibrahimpatnam, R.R.District, Nagarjuna Sagar Road, Hyderabad-501506, Telangana. The Ibrahimpatnam is a heart core of Eastern part of Hyderabad where most of the educational institutions are present and also it is rich in medicinal flora. However, awareness on these medicinal plants to the public in this area is not up to the mark. As a part of the Pharmacognosy curriculum and also to create awareness, the Guru Nanak School of Pharmacy is maintaining a medicinal plant garden in an approximate area of 1000 sqft with around 50 varieties of medicinal plants.

Keywords: *medicinal flora, guru nanak school of pharmacy, eastern ghats, euraca sativa, folklore uses.*

GJMR-B Classification: *NLMC Code: QV 752*

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The Medicinal Flora in Gurunanak School of Pharmacy, GNITC, Ibrahimpatnam, Hyderabad, Telangana, India: A Research Based Review

M. V. N. L. Chaitanya [α] & P. Suresh [σ]

Abstract- Telangana is a part of Deccan Plateau, located in the central part of the Indian Peninsula. The Hyderabad is now a state capital of Telangana after separation from Andhra Pradesh. It is situated in central Telangana and is spread over an area of 260 km2 and a heart of Eastern Ghats and is rich in different varieties of medicinal flora and many floras yet to be explore and there is less scientific literature focusing on these flora. The Guru Nanak School of Pharmacy is a part of GURU NANAK INSTITUTIONS TECHNICAL CAMPUS (AUTONOMOUS) located in Ibrahimpatnam, R.R.District, Nagarjuna Sagar Road, Hyderabad-501506, Telangana. The Ibrahimpatnam is a heart core of Eastern part of Hyderabad where most of the educational institutions are present and also it is rich in medicinal flora. However, awareness on these medicinal plants to the public in this area is not up to the mark. As a part of the Pharmacognosy curriculum and also to create awareness, the Guru Nanak School of Pharmacy is maintaining a medicinal plant garden in an approximate area of 1000 sqft with around 50 varieties of medicinal plants. The current review is discussing on important 21 medicinal plants (For example Erythrina variegata (Fabaceae), Euraca sativa (Brassicaceae), Psidium guajava (Myrtaceae), Tylophora Indica (Asclepiadaceae), (etc.) that are maintaining at our garden focusing on the botanical description, Vernacular names, habitat, chemistry, folklore uses, medicinal values and Nutritional values to create an awareness to public through the literature.

Keywords: medicinal flora, guru nanak school of pharmacy, eastern ghats, euraca sativa, folklore uses.

I. Introduction

a) Geographical Distribution of Hyderabad

Hyderabad is located in central Telangana and is situate over an area of 260 km2. It lies in the Deccan Plateau with an average height of 536 m above the sea level. The latitude is 17.3660 N and longitude is 78.4760 E [1]. The Average temperature is 130c to 390 c, June to September is the south- west monsoon season, and Humidity is with an average of 25 to 30% [2].

b) Medicinal Plant flora of Hyderabad district

As per the recent surveys there, are more than 583 reported genus and 1335 species belonging to 160 different families and predominant to 120 families [3].

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c) Medicinal Flora in Guru Nanak School of Pharmacy

The Guru Nanak School of Pharmacy is a part of GURU NANAK INSTITUTIONS TECHNICAL CAMPUS (AUTONOMOUS), situated in the eastern core of Hyderabad called Ibrahimpatnam. The school is maintaining a medicinal plant garden with around 50 varieties in an area of around 1000 sqft as a part of its curriculum and also to create awareness on how these plants are being use by common man as a medicine or as a nutritional supplement. The current review is focusing on the importance of 20 important flora of Hyderabad.

i. *Erythrina variegata*

a) Botanical description: Scientific Name: Erythrina variegata L. Family: Fabaceae (legume family)
b) Habitat: A deciduous tree with 15-18 m tall and leaves are 6 inches long having spiny branches [4].
c) Vernacular Names: English: Indian Coral Tree, Lenten tree, Tiger claw, Hindi: Pangara Manipuri: Kurao, Tamil: Kalyana murungai, Telugu: mulla moduga, tella-varjam., Tangkhul: Thikchowon kahunga [5].
d) Phytochemical Constituents: Alkaloids, flavonoids, pterocarpans, triterpenes, steroids, alkyl trans-ferulates, proteins, and lecithin [6].
e) Isolated Phytoceuticals: Erythratidine [7], N, N-dimethyl tryptophan [8], erystagallin A [9].
f) Nutritional Values: The amino acid composition of seed protein is as follows: alanine (7.2), arginine (3.4), aspartic acid (12.9), glutamic acid (13.4), glycine (7.6), histidine (3.9), isoleucine (3.6), leucine (7.1), lysine (5.1), methionine (0.5), phenylalanine (3.3), proline (4.7), serine (7.1), threonine (5.7), tyrosine (2.2), and valine (4.8) g/100g [10].
g) Pharmacological uses: Antioxidant [11], Analgesic and anti-inflammatory [12], Antisclerotic effect [13].
h) Traditional claims: used especially for menstrual disorders and fissures at penis tip [14].
i) Folklore uses: The tribes of Hingoli, Maharashtra, India use this bark powder as Antirheumatic and internally as a decoction to treat tetanus [15].

ii. *Eruca sativa*

a) Botanical description: Scientific Name: Eruca sativa, Family name: Brassicaceae.

b) Habitat: An Annual, erect herb which grows up to 25-100 cm high. Roots are slender, taproot, glaucous. The stem is simple or branched, stiff, glabrous above, hispid below. Flowers are racemes; Fruits are erect grows up to 3 cm long and glabrous. Seeds are subglobose, flattened, 2-seriate and brownish [16]
c) Vernacular Names: English name: Rocket Salad, Hindi: Tara Mira, Malayalam: Rucola Bengali: Suffed shorshi, Sanskrit: Sarishapa [17]
d) Phytochemical constituents: Alkaloids, Cardiac glycosides, Flavonoids, Phenolics, Ascorbic acid, Saponins and Tannins [18].
e) Isolated Phytoceuticals: kaempferol, Rhamnocitrin [19]
f) Nutritional values: The values mentioned are per 100 g
1. Calories: 25 K.calories, 2. Total Fat: 1 g, 3. Sodium: 27 mg, 4. Potassium: 369 mg, Carbohydrates: 4 g, Dietary fibre: 2 g, Sugars: 2 g, Protein: 2 g, Vitamin A: 47 %, Vitamin C: 25%, Calcium: 16%, Iron: 8% [20].
g) Pharmacological uses: Anticancer [19], Antimicrobial [21].
h) Traditional claims: The Israelites uses this plant in the treatment of aphrodisiac, for eye infections, and for digestive and kidney problems [22].
i) Folklore uses: The local people of Mihalgazi district (Turkey) uses the leaves in treatment of Diabetes, ulcer, kidney diseases, asthma, high cholesterol [23].

iii. *Euphorbia tirucalli*

a) Botanical description: Scientific Name: Euphorbia tirucalli, Family name: Euphorbiaceae
b) Habitat: E. tirucalli is a many-branched succulent plant. Trees or shrubs, producing abundant milky latex when injured, 2-6 m tall, dioecious, having a trunk 10-25 cm, with rugose, gray or light bark. Stems are green, succulent, finely, longitudinally striate. Leaves are alternate, present only on new growth; stipules very small, caducous; base attenuate, margin entire, apex obtuse. Male flowers many, exserted from involucre. Female flower: ovary glabrous, exserted from involucres [24].
c) Vernacular Names: English names: Pencil Tree, Firestick Plants, Indian Tree Spurge, Naked Lady, Pencil Tree, Pencil Cactus, Sticks on Fire, Hindi: anglithor, Kannada: bonta-kalli, Malayalam: guda, Marathi: sher-kandvel, Sanskrit: saptala, Tamil: cakkalavi, Telugu: cemudu, [25].
d) Phytochemical constituents: Alkaloids, Terpenoids, Flavanoids, Tannins, Saponins [26].
e) Isolated Phytoceuticals: β-amyrin acetate, lupenone, daucosterol [27].
f) Nutritional values: NA
g) Pharmacological uses: analgesic, anthelmintics, antiarthritic, antibacterial/ antifungal/ antimicrobial, anti-HIV, anti-inflammatory, antioxidant, antiviral, biodiesel production, CNS depressant/neuropathic pain, cytotoxicity/ anticancer, genotoxic/ mutagenic, hepatoprotective, insect repellants, immune-modulatory, larvicidal, molluscicidal/ ovicidal/ piscicidal, myelopoiesis, proteolytic/ chitinolytic [27].
h) Traditional claims: In India, it is useful in treatment of biliousness, leprosy and leucorrhea. In Brazil, it is used against cancroids, cancer, sarcomas, tumors, etc [27].
i) Folklore uses: In Malabar of India and Moluccas, the latex is use as an emetic and an anti-syphilitic [28].

iv. *Alstonia scholaris*

a) Botanical description: Scientific Name: Alstonia scholaris, Family name: Apocynaceae
b) Habitat: Large trees; height to 30 m; bark 10-15 mm thick, surface grey-brown, irregularly cracked and shallowly fissured, subverrucose, lenticellate; blaze creamy yellow, outer layer thin, corky, inner layer brittle; latex milky white; branchlets whorled. Leaves simple, whorled, estipulate; petiole 5-12 mm long, stout, glabrous; lamina 5-20 x 3-7 cm, obovate, oblanceolate or obovate-oblong; base cuneate or attenuate; apex obtuse or emarginate; margin entire, glabrous, subcoriaceous; lateral nerves many, slender, prominent, glabrous, parallel, looped near the margin forming intramarginal nerves; intercostae reticulate, obscure [29].
c) Vernacular Names: English: Devil tree, Dita bark tree, Shaitan wood Tamil: Elilaipalai, Malayalam: Ezhilampala, Kannada: Doddapala, Telugu: Edakula Ponna [30].
d) Phytochemical constituents: hydrocarbons, triterpenes and phytosterols [31].
e) Isolated Phytoceuticals: sarpagine [32].
f) Nutritional values: Fats: 1.4%, Proteins: 2.8%, Dietary fibre: 12.4 %, [33].
g) Pharmacological uses: anti- malarial [33], anti-cancer [34].
h) Traditional claims: The bark of the Alstonia scholaris is used in Ayurvedic medicine to treat fever, malaria, troubles in digestion, tumors, ulcers, asthma, and so forth. The leaves and the latex are applied externally to treat tumors. The bark and roots are boiled with rice and eaten by girls daily for several weeks to treat excessive vaginal discharge. The dried leaves of the Alstonia scholaris are used as an expectorant [35].
i) Folklore uses: In Bay islands, the tribes used it as an antimicrobial [36].

v. *Aloe vera*

a) Botanical Description: Scientific Name: Aloe vera, Family: Liliaceae.

b) Habitat: Aloe vera having a less stem or very short-stemmed plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. Like other Aloe species, Aloe vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil [35].
c) Vernacular Names: English name: Aloe vera, Medicinal aloe, Burn plant, Hindi: Gheekumari Marathi: Khorpad, Tamil: athalai, Malayalam: Chotthu kathalai, Nepali: Ghyu Kumaari, Telugu: Kalabanda [36].
d) Phytochemical constituents: Tannin, Saponin, Flavonoids and Terpenoids [37].
e) Isolated Phytoceuticals: p-coumaric acid [38], aloe-emodin & chrysophanol [39].
f) Nutritional values: stated below are ingredients per 100 g of juice Sodium: 3%, Total carbohydrate: 1%, vitamin C: 17%, Dietary fiber: 1%, Calcium: 1%, Iron: 2% [41].
g) Pharmacological uses: Wound healing, Anti-aging and Anticancer [42].
h) Traditional claims: Purgative, In treatment of arthritis, Sinusitis, conjunctivitis, Ophthalmia, treatment of wounds, anti-hypertension, anti-stress, Energy booster and liver cleanser [41].
i) Folklore uses: During the 16th century, the Indian tribes uses this as healing plant, it is one of the 16 holy plants having divine status, the Indian tribes uses this plant as mosquito repellent, it also used on wood as insect repellent [43].

vi. *Catharanthus roseus*

a) Botanical Description: Scientific Name: Catharanthus roseus, Family: Apocynaceae
b) Habitat: A tender, perennial subshrub with standing erect (30 cm to 1 m in height). The sap is a milky latex [44].
c) Vernacular names: English name: Periwinkle, Madagascar periwinkle, Rosy periwinkle, Vinca Hindi: Sadabahar, Malayalam: Shavam Naari, Marathi: Sadaphuli • Bengali: Nayantara, Telugu: billaganneru [45].
d) Phytochemical constituents: organic acids, reducing sugars, phenols and tannins, depsides and depsidones, steroids and triterpenoids, alkaloids and saponins [46].
e) Isolated phytoceuticals: Rhazimol [47], Vindoline [48].
f) Nutritional values (per 100 g): carbohydrate: 46.02±0.01%, lipid: 19.68±0.01%, crude protein: 7.05 ± 0.01%, crude fibre: 1.04 ± 0.02% and had Caloric value of 369.37±0.02 kcal. The mineral element analyzed were Calcium (Ca) 232.90 ± 0.01mg/ kg, Iron (Fe) 154.39 ± 0.02mg/kg [49].
g) Pharmacological uses: Antineoplastic, Antidiabetic, Antioxidant [50].
h) Traditional claims: Relieving muscle pain, depression of the central nervous system, also used to heal wounds [50].
i) Folklore uses: The Bhilla tribe of Maharashtra, India uses this leaf decoction in treatment of leucorrhoea/ menstrual complaint [51].

vii. *Bougainvillea glabra*

a) Botanical description: Scientific name: Bougainvillea glabra, Family: Nyctaginaceae
b) Habitat: Bougainvillea glabra is a climbing shrub with thorny stems, usually grows 3-4m (10–12feet) tall, The flowers are white and tiny appears in cluster surrounded with papery bracts, hence the name known as paper flower [52].
c) Vernacular Names: English Name: Paper Rose, Hindi: Booganbel, Manipuri: Cherei, Bengali: Baganbilas, Marathi: Booganvel, Konkani: Bouganvila, Telugu: Kagithala Puvvu [53].
d) Phytochemical constituents: Alkaloids, flavonoides, phlobatannins and terpenoids. Steroids, phenol, tannins, cardinolides [54].
e) Isolated Phytoceuticals: Betacyanins [55], Oleananoic acid acetate [56], Pinitol, Quercetin [57], Bougainvinones A-H [58].
f) Nutritional Values: Unknown
g) Pharmacological uses: Antioxidant, Antimicrobial [59], Anticancer [58].
h) Traditional claims: diarrhea, excess acidity, cough and sore throat, in treatment of low blood pressure Leucorrhea, Hepatitis [60].
i) Folklore uses: The leaf decoction is used in treatment of cough and sore throat by Mexican folks/tribes [61].

viii. *Psidium guajava*

a) Botanical description: Scientific Name: Psidium guajava, Family: Myrtaceae
b) Habitat: Psidium guajava is a large dicotyledonous shrub, or small evergreen tree, generally 3-10 m high, many branches; stems crooked, bark light to reddish brown, thin, smooth, continuously flaking; root system generally superficial and very extensive, frequently extending well beyond the canopy, there are some deep roots but no distinct taproot [62].
c) Vernacular Names: English: common guava, Hindi: Amrud, Malayalam: Pela, Marathi: Jamba, Sanskrit: amrutha phalam, Tamil: Koyya phalam, Telugu: Jaama pandu [63].
d) Phytochemical constituents: Tannins, flavonoids, saponins, glycosides and phenols [64].

e) Isolated Phytoceuticals: Lyxopyranoside [65], lanost-7-en-3β-ol-26-oic acid [66].
f) Nutritional values (per 100 g): Total fat: 1 g, Sodium: 4 mg, Potassium: 150 mg, Carbohydrates: 9 g, Dietary fiber: 5 g, Sugar: 3 g, Protein: 1 g, vitamin A: 72%, calcium: 10%, vitamin C: 243% (Percent Daily Values are based on a 2000 calorie diet.).
g) Pharmacological uses: Antimicrobial [65], Antidiabetic [66].
h) Traditional claims: Dysentry, Diarrhea [67].
i) Folklore uses: The local tribes of Saint Lucia uses a tea made up of new leaves of Psidium guajava, guava, with sugar used as a de-worming agent and in treatment of stomach ache [68].

ix. *Phyllanthus niruri*

a) Botanical description: Scientific Name: Phyllanthus niruri, Family: Euphorbiaceae
b) Habitat: P. niruri is an erect, slender, branched, annual herb from 10 to 50cm, of a light green to whitish. The leaves of the main stem fall very early, so that the side, horizontal and rather brief twigs, look like compound leaf. It has numerous small leaves which are simple, elliptic.
Flowers are of small size and greenish. On half lower of the twigs, flowers are solitary and wedge shape [69].
c) Vernacular Names: English: gale of the wind, Telugu: Nela usiri, Kannada: Nela nelli, Tamil: Keezha nelli, Keezhar Nelli in malyalam [70].
d) Phytochemical constituents: Alkaloids, Saponins, glycosides, Flavonoids and Carbohydrates [71].
e) Isolated Phytoceuticals: Neonirtetralin [72], 4,4,8-trimethoxy chroman [73], Corilagin, ethyl brevifolin-carboxylate [74].
f) Nutritional values (%w/w): Crude fibre 6.95± 0.03, Crude protein 10.50 ± 0.15, Lipids 6.07 ± 0.03, Carbohydrate 65.28 ± 0.04, Caloric value (kcal/100g) 357.75 ± 0.03, Iron: 172 ppm, Magnesium: 327 ppm [71].
g) Pharmacological uses: hepatoprotective, antiviral, antibacterial, hypolipidaemic, hypoglycaemic, analgesic, anti-inflammatory, cardioprotective, anti-urolithiatic and antihyperuricaemic properties [75].
h) Traditional claims: antimicrobial, antioxidant, anticancer, antiinflammatory, antiplasmodial, antiviral, diuretic and hepatoprotective [76].
i) Folklore uses: The local person of India, china and Africa uses this plant paste to treat hepatic disorders [77].

x. *Cymbopogon citratus*

a) Botanical description: Scientific Name: Cymbopogon citratus, Family: Poaceae
b) Habitat: Lemon grass is a tufted perennial grass growing to a height of 1 meter with numerous stiff leafy stems arising from short rhizomatous roots. It has an economic lifespan for about 5 year [78].
c) Vernacular Names: Common name: English name: Lemon Grass, Hindi: Gandhatrina, Manipuri: Haona, Marathi: Olecha, Tamil: Karppurappul, Malayalam: Vasana Pullu, Telugu: Nimmagaddi, Kannada: Majjigehullu, Bengali: Gandhabena, Konkani: Olicha, Gujarati: Lilicha • Sanskrit: Bhustrina [79].
d) Phytochemical constituents: hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes [80].
e) Isolated Phytoceuticals: myrcene, citronellal, citronellol and geraniol [78].
f) Nutritional values (Per 100 g): Total fat: 0.5 g, Sodium: 6 mg, Potassium 723 mg, Total Carbohydrate 25 g, Protein 1.8 g, vitamin C: 4% w/w, Iron: 45% w/w, vitamin B6: 5% w/w, Magnesium: 15% w/w.
g) Pharmacological uses: antimicrobial [81], antimutagenic [82], antidiarrhoeal [83], antimalarial [84].
h) Traditional claims: In Ayurveda, this ancient herb is useful to treat weak digestion, poor concentration, poor circulation, varicose veins, fever, intestinal infections, and certain skin conditions [85].
i) Folklore uses: Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Studies have shown that the lemon grass has antibacterial and antifungal properties. Mixed with pepper, it's a home therapy for menstrual troubles and nausea [78].

xi. *Emblica officinalis*

a) Botanical description: Scientific Name: Phyllanthus emblica , Family: Phyllanthaceae
b) Habitat: The tree is small to medium in size, reaching 1–8 m (3 ft 3 in–26 ft 3 in) in height. The branchlets are not glabrous or finely pubescent, 10–20 cm (3.9–7.9 in) long, usually deciduous; the leaves are simple, subsessile and closely set along branchlets, light green, resembling pinnate leaves [86].
c) Vernacular Names: English name: Amla, Indian gooseberry, Hindi: Aonla, Manipuri: Amla • Marathi: Amla, Tamil: Nelli, Malayalam: Nelli, Nellikka, Telugu: Usiri, Usirikaya, Kannada: Betta nelli, Amalaka, Oriya: Aonla, Gujarati: ambala, Sanskrit: Dhatri, amalaka [87].
d) Phytochemical constituents: Tannins, Alkaloids, Phenolics [88].
e) Isolated Phytoceuticals: β-glucogallin [89], Pectin [90], Phyllemblin [91], Emblicanin A and B [92].
f) Nutritional Values (% w/w per 100g): Protein: 0.5, Fat: 0.1, Mineral matter: 0.7, Fibre: 3.4, carbohydrate: 14.1, Calcium: 0.05, Phosphorous: 0.02, Iron: 1.2 mg, vitamin C: 600 mg, nicotinic acid: 0.2 mg [93].

g) Pharmacological uses: Antioxidant [93], Anticancer [93], Enhance food absorption, regulates elimination, nourishes the brain and mental functioning, act as an antioxidant and chelating agent [94].
h) Traditional claims: In India, it is a belief that this tree is a holy, uses in many prayers and homams in belief that its medicinal air will heal [95], Snake venom neutralizer [96]. As an ayurvedic immuno-booster and digestive aid [95].
i) Folklore uses: In Unani folklore, The plant can use both as a medicine and as a tonic to build up lost vitality and vigor [96].

xii. *Vetivera zizanioides*

a) Botanical description: Vetivera zizanioides, Family: Poaceae
b) Habitat: It is a perennial; cespitose, not stoloniferous. It is not branched and not woody. Sheaths glabrous, keeled; Ligules 0.3-1.5 mm, of hairs; blades 23-140 cm long, 2.5-13 mm wide, flat or folded, mostly glabrous but the adaxial surfaces usually pilose basally [97].
c) Vernacular names: Hindi, Bengali: Khas, Gujarati: Valo, Marathi: Vala, Telugu: Kuruveeru, Vettiveellu, Tamil: Vattiver, Kannad: Vattiveeru, Malyalam: Ramaccham, Ayurvedic name Ushira [98].
d) Phytochemical constituents: carbohydrates, proteins, steroids, alkaloids, flavanoids, phenols and tannins [99].
e) Isolated Phytoceuticals: vetiverol, vetivenene [100].
f) Nutritional values (ppm): Iron: 415.3, Copper: 61.27, Manganese: 45.72, Zinc: 64.82, Selenium: 0.02, Cobalt: 0.12 [101].
g) Pharmacological uses: Antiinflammatory, Aphrodisiac, tonic, Cicatrisant, healing, calming and sedative [102].
h) Traditional claims: In Indonesia, it is used for treatment of rheumatism, In Pakistan, it is used in treatment of cholera, In senegal, it is used as an aphrodisiacs, In Traditional Medicine: In Mauritius, vetiver is used as an abortifacient [103].
i) Folklore uses: The Santhal tribe of Bihar and West Bengal use the paste of fresh roots for burns, snakebite and scorpion stings; decoction of the roots has been used as tonic for weakness.
The Lodhas of West Bengal region use the root paste for headache, rheumatism and sprain; also in treatment of urinary infections [103].

xiii. *Tylophora indica*

a) Botanical description: Tylophora indica, Family: Apocynaceae
b) Habitat: Indian Ipecac is a small, slender climbing herb with yellowish sap and distribute in the sub-himalayan tract from Uttarakhand to Meghalaya and in the central and peninsular India [104].
c) Vernacular names: English name: Indian Ipecac, Bengali: antomula, Gujarati: dum vel Hindi: antamul, Kannada: adumuttadagida, Konkani: pitvel, Malayalam: allippala, Marathi: antamul, Oriya: mehendi, Sanskrit: antrapachaka, Tamil: kalutai-p-palai, Telugu: kakapala [104]
d) Phytochemical constituents: alkaloids, flavonoids, terpenoids, glycosides, saponins [105].
e) Isolated Phytoceuticals: Tylophorine, kaempferol, α-amyrin [106].
f) Nutritional values: No details available.
g) Pharmacological uses: asthma, bronchitis, bronchial asthma, hay fever and rheumatism [107].
h) Traditional claims: In Ayurveda, the plant has been used in treatment of asthma [108].
i) Folklore uses: anti-Allergy, Antirheumatic and dermatitis [109].

xiv. *Jatropha curcas*

a) Botanical description: Jatropha curcas, Family: Euphorbiaceae
b) Habitat: Physic nut is a perennial poisonous shrub, up to 5 m high. It is an uncultivated non-food wild-species. The plant, originating in Central America, whereas it has been spread to other tropical and subtropical countries as well and is mainly grown in Asia and in Africa. It is used as a living fence to protect gardens and fields from animals. The plant sports large green to pale-green leaves, 8-15 cm, broadly ovate, cordate, having 3 lobed petioles around 5-15 cm [110].
c) Vernacular names: English name: Physic Nut, Jatropha, Barbados nut, Hindi: Jamal ghota, Marathi: Mogli, Tamil: Kattukkotai • Malayalam: Kattamank, Telugu: Nepalam, Kannada: Kananeranda, Bengali: Bagbherenda, Oriya: Jahazigabai [110].
d) Phytochemical constituents: diterpenes, triterpenes, lignanes and coumarins, flavonoids, alkaloids, phytosterols, [111].
e) Isolated Phytoceuticals: Curcacycline-A [112].
f) Nutritional values (per 100 g): Proteins -38%, 55-58% lipids [113].
g) Pharmacological uses: wound healing, antimalarial and antimicrobial [114].
h) Traditional uses: In India, the leaf paste is used in treatment of hemorrhoids and jaundice [115].
i) Folklore uses: In Nigeria, the decoction of boiled leaves is used to treat diabetes, in cameroon, the leaves are used to treat arthritis [115].

xv. *Cinnamomum zeylanicum*

a) Botanical description: Cinnamomum verum, Family: Lauracaeae
b) Habitat: The C. verum tree grows to around 10 m (30 ft), and has leathery leaves with lanceolate shape of 11 to 16 cm long with pointed tips. Flowers are yellow, tubular, fruits are berries [116].

c) Vernacular names: English Cinnamomum, Assamese: Dalchini, Hindi: Dalchini
Kannada: Dalchini, Malayalam: Edana, Sanskrit: Darusita; Tamil: annalavangam, Telugu: Dasini Chekka [117].
d) Phytochemical constituents: Terpenoids [118].
e) Isolated Phytoceuticals: Cinnamaldehyde and Eugenol [118]
f) Nutritional values (per 100g): Total fat: 1.2 g, Sodium: 10 mg, Potassium: 431 mg, Protein: 4 g, vitamin-A: 5%, calcium: 100% [119].
g) Pharmacological uses: antioxidant, anti-inflammatory, anti-bacterial, antipyretic and analgesic [118].
h) Traditional claims: Local antiseptic and for treatment of cold and sore throat, gastrointestinal complaints [120, 118]
i) Folklore uses: In treatment of impotence, rheumatism, vaginitis and diabetes [118].

xvi. *Cascabela thevetia*

a) Botanical description: Cascabela thevetia, Family: Apocynaceae
b) Habitat: It is a small ornamental tree which grows to about 1.5 - 2.3 m high. The leaves are spirally arranged, linear and about 13-15 cm in length. Flowers are bright yellow and funnel-shaped with 5 petals spirally twisted. The fruits are somewhat globular, slightly fleshy and have a diameter of 4-5 cm. The fruits, which are green in colour, become black on ripening. Each fruit contains a nut which is longitudinally and transversely divided. All parts of the plant contain the milky juice [121].
c) Vernacular Names: English name: Mexican oleander, Lucky Nut, Hindi: Peeli kaner, Manipuri: Utonglei, Bengali: Kolkaphul, Telugu: Bila ganeru [122].
d) Phytochemical constituents: Alkaloids, glycosides, tannins, phenolic compounds, proteins, essential oils, gums, mucilage and fixed oils [123].
e) Isolated Phytoceuticals: thevetin A and B, acetylthevetin A and B [124].
f) Nutrition values (per 100g): Fats (60%), Proteins (40%) [125].
g) Pharmacological uses: Anticancer [126].
h) Traditional claims: In ayurveda, it is a cardiotonics [127].
i) Folklore uses: In treatment of amenorrhoea, jaundice, antiulcer, febrifuge [128].

xvii. *Simarouba glauca*

a) Botanical description: Simarouba glauca, Family: Simaroubaceae
b) Habitat: It is an evergreen, small or medium-sized tree with a narrow crown; it usually grows up to 15 metres tall, but specimens up to 27 metres. The straight, cylindrical bole can be free of branches for up to 9 metres, 30cm or more in diameter, sometimes to 60cm [129].
c) Vernacular names: English: Paradise Tree, Tamil: Sorgamaram, Malaiyalam: Lakshmitaru, Hindi: Hartho [130].
d) Phytochemical constituents: alkaloids, flavonoids, carbohydrates, glycosides, phenolic compound, tannins, triterpenoids, cardinolides, saponins, fixed oils [130].
e) Isolated Phytoceuticals: glaucarubine [131]
f) Nutrition values (per 100 g): Proteins (47.7 g), Calcium (143 mg), Sodium (79 mg) [132].
g) Pharmacological uses: antiamoebic, antifungal, antibacterial, anticancer, antiulcer [133].
h) Traditional claims: antidiahrreal and antimalarial [134].
i) Folklore uses: Indigenous tribes throughout the South American rainforest uses bark decoction in treatment of fevers, malaria, and dysentery, as a hemostatic agent [135].

xviii. *Putranjiva roxburghii*

a) Botanical description: Putranjiva roxburghii Family: Putranjivaceae
b) Habitat: Putranjiva is a famous, moderate-sized, evergreen tree, growing up to 12 m in height. It has pendant branches and dark grey bark having horizontal lenticels. Leaves are simple, alternately arranged, dark green, shiny, elliptic-oblong, distantly serrated. Male flowers, with short stalks, in rounded axillary clusters, female flowers 1-3 in leaf axil. Fruits ellipsoid or rounded drupes, white velvety; seed normally one, stone pointed, rugose, very hard [136].
c) Vernacular names: English name: Putranjiva, Lucky Bean Tree, Hindi: Putijia, Marathi: Jivanputra, Patravanti, Tamil: Irukolli, Karupala, Malayalam: Pongalam, Telugu: Kuduru, Putrajivika, Kannada: Amani Putrajiva, Bengali: Putranjiva, Jioysuta, Oriya: Poilundia, Konkani: Saman, Urdu: Paishandia, Gujarati: Putranjiva, Sanskrit: Putrajivah [136].
d) Phytochemical constituents: glycosides, saponins, triterpenes and flavonoid [137].
e) Isolated Phytoceuticals: glucoputranjivin, putranjivoside, β-sitosterol [137].
f) Nutrition values (per 100 g): No information available over literature.
g) Pharmacological uses: anti- hyperglycemic, analgesic, antipyretic and anti-inflammatory and cytotoxic [137].
h) Traditional claims: In Ayurveda it is used for the treatment of eye disorders, burning sensation, elephantiasis, difficulty in micturition, azoospermia and habitual abortions [138].
i) Folklore uses: used in the treatment phlegm and rheumatism [139].

xix. *Mirabilis jalpa*

a) Botanical description: Mirabilis jalapa, Family: Nyctaginaceae
b) Habitat: Four o'clock flowers having trumpet shape and grows about two inches, opens in evening, shurblike appearance, erect and grows up to 2-3 feet tall. The flowers are fragrant and forms in clusters [140].
c) Vernacular names: English name: Four O'clock, Beauty-of-the-night, Marvel of Peru, Hindi: Gul abbas, Manipuri: Mukak lei, Marathi: Gulabas, Tamil: Pattarashu, Malayalam: Anthimalari, Telugu: Chandramalli, Kannada: Gulamaji Bengali: Sandhya malati, Oriya: Rangini, Sanskrit: Krishnakeli [140].
d) Phytochemical constituents: alkaloids, flavonoids, phenols, steroids, triterpenes, glycosides, tannins, saponins and lignins [141].
e) Isolated Phytoceuuticals: Miraxanthins, Indicaxanthin [141].
f) Nutritional values (per 100g): Potassium: 500 mg, Sodium: 38 mg, Dietary fibre: 1.85 g, proteins: 2.29 g [142]
g) Pharmacological uses: Antistress [141], HIV1 reverse transcriptase inhibitor [143].
h) Traditional claims: Purgative, Emetic [144].
i) Folklore uses: The native people of Mexico use M. jalapa uses in gastrointestinal disorders [145].

xx. *Saraca asoca*

a) Botanical description: Saraca asoca, Family: Fabaceae
b) Habitat: Small trees, up to 5 m tall, Trunk often tubercled; bark lenticellate, dark, shallowly fissured; blaze purplish, Branchlets terete, glabrous, Leaves compound, paripinnate, alternate, distichous; stipules caducous; rachis pulvinate, 7-30 cm long; petiolule 0.1-0.6 cm long; leaflets opposite, 4-6 (-12) pairs, lamina 6-31 x 1.5-9 cm, narrow elliptic-oblong or lanceolate, apex acute to acuminate, base acute to rounded or subcordate, subcoriaceous, glabrous; midrib raised above; secondary nerves ca. 11 pairs, looped; tertiary nerves reticulate[146].
c) Vernacular names: English name: Sita Ashok, Sorrowless tree, Hindi: Sita Ashok, Gujarati: Ashopalava, Kannada: Achenge, Malayalam: Hemapushpam, Marathi: Jasundi, Tamil: Asogam, Telugu: Asokamu, Nepali: Ashok [147].
d) Phytochemical constituents: phenol, tannins and steroid [148].
e) Isolated Phytoceuticals: schizandriside, epicatechin [149]
f) Nutritional values: NA
g) Pharmacological uses: Antidysentric and Antidiabetic [150]
h) Traditional claims: bleeding problems, irregular menses, fibroids, dysmenorrhea [151].
i) Folklore uses: Dried flowers: The womenfolk of Chhattisgarh uses the bark decoction, prepared from cow milk and sugar to prevent gynecological disorders [152].

The above described medicinal flora of school of pharmacy, GNITC, Hyderabad also represented in table 1 below for easy understanding to the researchers, the pictures of the various medicinal flora were given below in the figure 1 and the mol format of Phytoceuticals isolated from various medicinal flora were represented in the below pages as figure 2.

II. Current Challenges

The maintenance of medicinal park / garden is not an easy task, however the school of pharmacy, GNITC, Hyderabad, India is striving hard to maintain these flora, the main challenges we are facing are

a) Weed

Occurrence of unnecessary flora in desired environment are called weeds, this is one of the major challenges we are facing. Removal of these weeds is again a big challenging task as it requires a separate manpower to maintain the flora and protect from these weeds. As our motto is to maintain healthy organic fields, we are not using any chemicals and use of natural herbicides is again a challenging task so that many of these natural herbicides are failure in eradicating the weeds.

b) Water

Now days, as water is one of the sacred element in the nature especially in summer season and most of these flora need more water at their development stages, this became a ultimate challenge to get quality water and the underground water in this area is absent. However, the Management, School of Pharmacy, GNITC, Hyderabad, outsourcing quality water to safe guard and maintain this medicinal flora.

c) Sudden climatic changes

Even sudden changes in the climatic conditions also became a challenge to get uniformity in the growth and quality of the medicinal flora.

d) Genetic information

Getting a genetic barcode of this flora became a challenging task even though we maintaining a authentic flora in the garden, still there genetic identification is missing.

e) Variation in the phytochemical uniformity

Getting an uniform phytochemical fingerprint from these medicinal flora is a big challenge now a days we are facing due to variation in exogenous and endogenous factors.

f) Loss of fertility

The cultivation fields are losing their fertility due to various environment factors.

III. Future Directions to Develop this Medicinal Flora in Campus

The School of pharmacy, GNITC, Hyderabad, India is striving hard to get funds from various organizations to develop a medicinal park/garden with a theme heal through breath under medicinal floral air. Genetic marking and monographic labeling of this flora and to start research and development on these medicinal flora focusing major diseases like cancer, tuberculosis, STD etc towards a preventive measure. The School of Pharmacy, GNITC, Hyderabad also striving hard to conduct awareness programs, workshops, empowerment programs and seminars focusing on the theme of Medicinal and Nutritional values of the flora of the Telangana, India, so that even a common man knows about the nutritional and medicinal values of the flora that grows near to his environment.

IV. Conclusion

The main focus of this article is to create awareness to the public/ researchers about the medicinal importance of the common flora that distributed widely inside the Hyderabad and many of us don't know the importance of these flora and deforesting this flora and many of these flora due to ignorance will be under endangered list in future, if utmost care is not taken place. This can happen only through organizing workshops or seminars on the significance of this medicinal flora and how to gain money from this flora through some homemade elixirs prepared from these plants. The government bodies must encourage these types of organizations where there ambition is to promote medicinal flora for healthy and wealthy India.

Author Contribution

This work was carried out in collaboration between both authors. Author MVNLC (Dr. M.V.N.L. Chaitanya) managed the Literature searches and drafted the manuscript, Author PS (Dr. P. Suresh) guided, corrected the manuscript and arranged it in a scientific manner. Both authors read and approving the final manuscript.

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Table 1: Folklore uses of Medicinal Flora of School of Pharmacy, GNITC, Ibrahimpatnam, Hyderabad, Telangana, India

S. No.	Name of the Flora & Family	Institutional Reference Number	Phytoceuticals identified from Flora	Folklore uses
1.	*Erythrina variegata* (Fabaceae)	GNITCSP001	Erythratidine [7], N, N-dimethyltryptophan [8], erystagallin A [9].	The tribes of Hingoli, Maharstra, India use this bark powder as Antirheumatic and internally as a decoction to treat tetanus [15]
2.	*Eruca sativa* (Brassicaceae)	GNITCSP002	Kaempferol, Rhamnocitrin [19]	The local people of Mihalgazi district (Turkey) uses the leaves in treatment of Diabetes, ulcer, kidney diseases, asthma, high cholesterol [23].
3.	*Euphorbia tirucalli* (Euphorbiaceae)	GNITCSP003	β-amyrin acetate, lupenone , daucosterol [27].	In Malabar of India and Moluccas, the latex is used as an emetic and an anti-syphilitic [28].
4.	*Alstonia scholaris* (Apocynaceae)	GNITCSP004	Sarpagine [32].	In Bay islands, the tribes used it as an antimicrobial [36].
5.	*Aloe vera* (Liliaceae)	GNITCSP005	P-coumaric acid [38] , aloe-emodin & chrysophanol [39].	During the 16th century, the Indian tribes uses this as healing plant, it is one of the 16 holy plants having divine status, the Indian tribes uses this plant as mosquito repellent, it also used on wood as insect repllent [43].
6.	*Catharanthus roseus* (Apocyanceae)	GNITCSP006	Rhazimol [47], Vindoline [48].	The Bhilla tribe of Maharashtra, India uses this leaf decoction in treatment of leucorrhoea/menstrual complaint [51].
7.	*Bougainvillea glabra* (Nyctaginaceae)	GNITCSP007	Betacyanins [55], Oleananoic acid acetate [56], Pinitol, Quercetin [57], Bougainvinones A-H [58].	The leaf decoction is used in treatment of cough and sore throat by Mexican folks/tribes [61].
8.	*Psidium guajava* (Myrtaceae)	GNITCSP008	Lyxopyranoside [65] , lanost-7-en-3β-ol-26-oic acid [66].	In Saint Lucia, the local tribes uses a tea of the new leaves of Psidium guajava, guava, with sugar is given for

				worms and bellyache but the most common medicinal use is to stop diarrhea. [68].
9.	*Phyllanthus niruri* (*Euphorbiaceae*)	GNITCSP009	Neonirtetralin [72], 4,4,8-trimethoxy chroman [73], Corilagin, ethyl brevifolincarboxylate [74].	The local person of India, china and Africa uses this plant paste to treat hepatic disorders [77].
10.	*Cymbopogon citratus* (Poaceae)	GNITCSP010	myrcene, citronellal, citronellol and geraniol [78].	Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Studies have shown that the lemon grass has antibacterial and antifungal properties. Mixed with pepper, it's a home therapy for menstrual troubles and nausea [78].
11.	*Emblica officinalis* (Phyllanthaceae)	GNITCSP011	β-glucogallin [89], Pectin [90], Phyllemblin [91], Emblicanin A and B[92].	In Unani folklore, The plant is used both as a medicine and as a tonic to build up lost vitality and vigor [96].
12.	*Vetivera zizanioides* (Poaceae)	GNITCSP012	vetiverol, vetivenene [100].	The Santhal tribe of Bihar and West Bengal use the paste of fresh roots for burns, snakebite and scorpion stings; decoction of the roots has been used as tonic for weakness. The Lodhas of West Bengal region use the root paste for headache, rheumatism and sprain; also in treatment of urinary infections [103].
13.	*Tylophora indica* (Apocynaceae)	GNITCSP013	Tylophorine, kaempferol, α-amyrin [106].	anti-Allergy, Antirheumatic and dermatitis [109].
14.	*Jatropha curcas* (Euphorbiaceae)	GNITCSP014	Curcacycline-A [112].	In Nigeria, the decoction of boiled leaves is used to treat diabetes, in cameroon , the leaves are used to treat arthritis [115].
15.	*Cinnamomum zeylanicum* (Lauracaeae)	GNITCSP015	Cinnamaldehyde and Eugenol [118]	In treatment of impotence, rheumatism, vaginitis and diabetes [118].
16.	*Cascabela thevetia* (Apocynaceae)	GNITCSP016	thevetin A and B, acetylthevetin A and B [124].	In treatment of amenorrhoea, jaundice, antiulcer, febrifuge [128].
17.	*Simarouba glauca* (Simaroubaceae)	GNITCSP017	glaucarubine [131]	Indigenous tribes throughout the South American rainforest uses bark decoction in treatment of fevers, malaria, and dysentery, as a hemostatic agent [135].
18.	*Putranjiva roxburghii* (Putranjivaceae)	GNITCSP018	glucoputranjivin, putranjivoside, β-sitosterol [137].	used in the treatment phlegm and rheumatism [139].
19.	*Mirabilis jalpa* (Nyctaginaceae)	GNITCSP019	Miraxanthins, Indicaxanthin [141].	The native people of Mexico use M. jalapa uses in gastrointestinal disorders [145].
20.	*Saraca asoca* (Fabaceae)	GNITCSP020	schizandriside, epicatechin [149]	The womenfolk of Chhattisgarh uses the bark decoction , prepared from cow milk and sugar to prevent gynecological disorders [152].

1. *Erythrina variegata* (Fabaceae)

2. *Eruca sativa* (Brassicaceae)

3. *Euphorbia tirucalli* (Euphorbiaceae)

4. *Alstonia scholaris* (Apocynaceae)

5. *Aloe vera* (Liliaceae)

6. *Catharanthus roseus* (Apocyanceae)

7. *Bougainvillea glabra* (Nyctaginaceae)

8. *Psidium guajava* (Myrtaceae)

9. *Phyllanthus niruri* (Euphorbiaceae)

10. *Cymbopogon citratus* (Poaceae)

11. *Emblica officinalis* (Phyllanthaceae)

12. *Vetivera zizanioides* (Poaceae)

13. *Tylophora indica* (Apocynaceae)

14. *Jatropha curcas* (Euphorbiaceae)

15. *Cinnamomum zeylanicum* (Lauracaeae)

16. *Cascabela thevetia* (Apocynaceae)

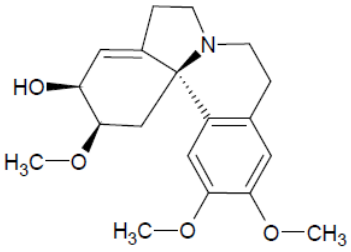
17. *Simarouba glauca* (Simaroubaceae)

18. *Putranjiva roxburghii (Putranjivaceae)*

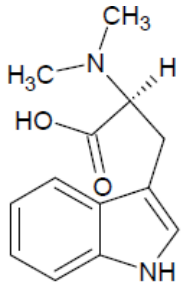
19. *Mirabilis jalpa* (Nyctaginaceae)

20. *Saraca asoca* (Fabaceae)

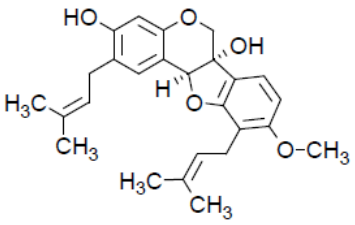
Figure 1: Medicinal flora of School of Pharmacy, GNITC, Hyderabad, India



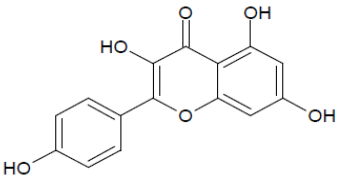
1. Erythratidine



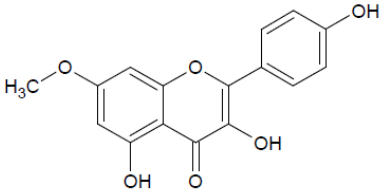
2. N, N-dimethyltryptophan



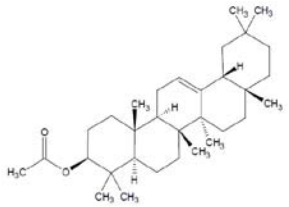
3. Erystagallin A



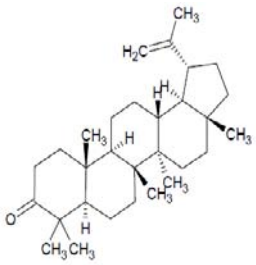
4. kaempferol



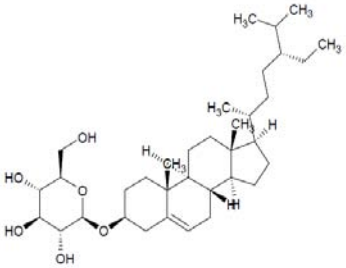
5. Rhamnocitrin



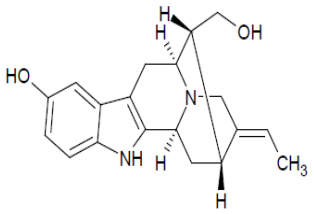
6. β-Amyrin acetate



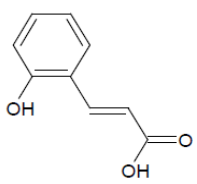
7. Lupenone



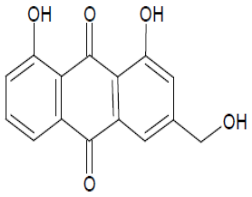
8. Daucosterol



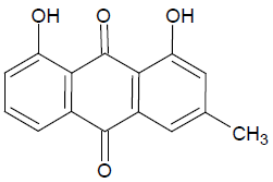
9. Sarpagine



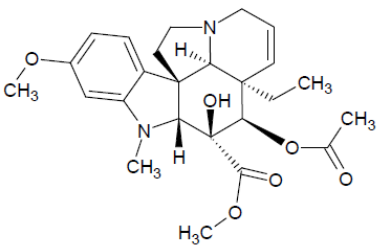
10. P-coumaric acid



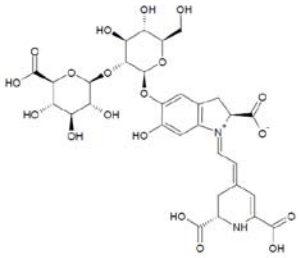
11. Aloe-emodin



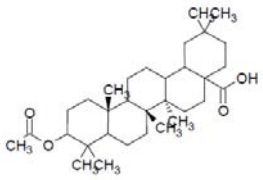
12. Chrysophanol



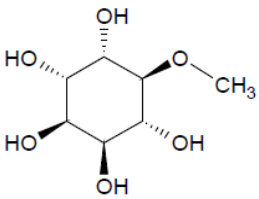
13. Vindoline



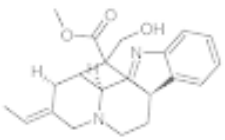
14. Beta-cyanin



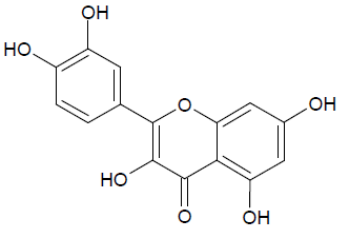
15. Olenalic acide acetate



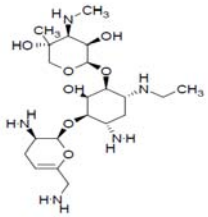
16. Pinitol



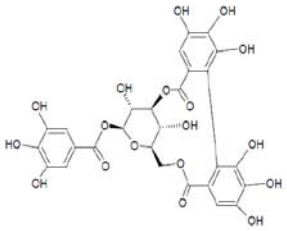
17. Curcacyclin



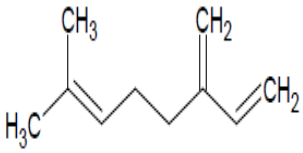
18. Quercetin



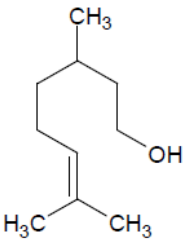
19. Lyxopyranoside



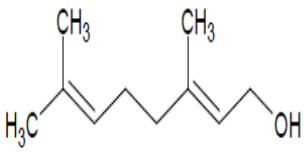
20. Corilagin



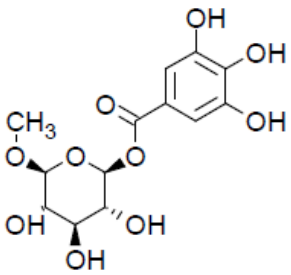
21. Myrcene



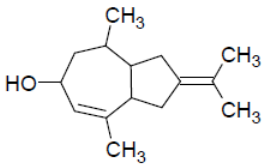
22. Citronellol



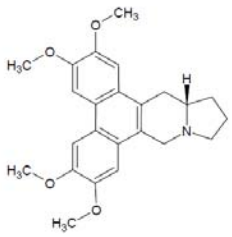
23. Geraniol



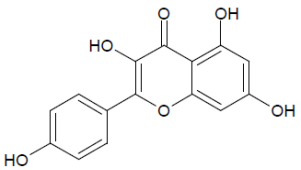
24. Glucogallin



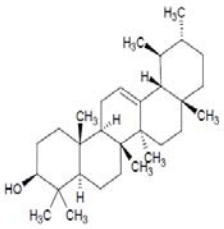
25. Vetiverol



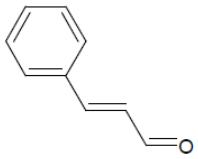
26. Tylophorine



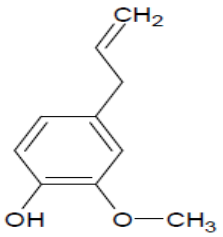
27. Kaempferol



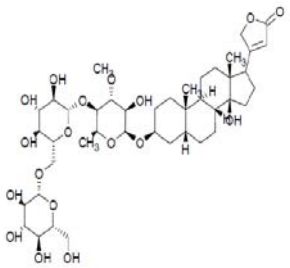
28. Beta Amyrin



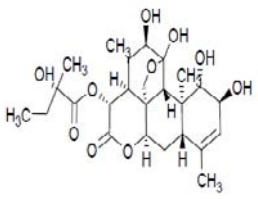
29. Cinnamaldehyde



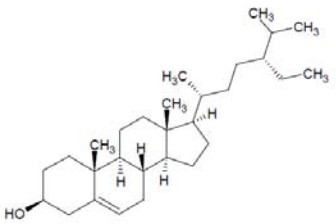
30. Eugenol



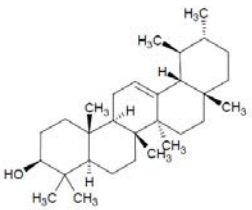
31. Thevetin



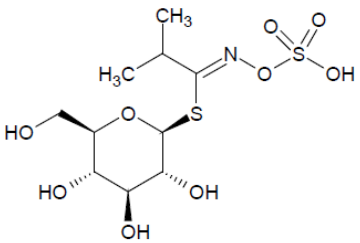
32. Glaucarubine



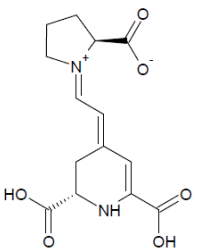
33. Sitasterol



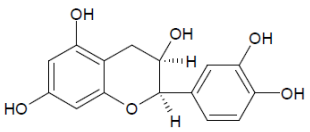
34. Beta-amyrin



35. Glucoputaranjvin



36. Indicaxanthin



37. Epicatechin

Figure 2: Phytoceuticals Reported from the Medicinal Flora, School of Pharmacy, GNITC, Hyderabad

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By Riddhi Trivedi & Shrenik Shah
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Methods: Microspheres were prepared by emulsion crosslinking method, with mucoadhesive polymers (Sodium alginate-carbopol-934P), for potential nasal delivery bypassing first-pass metabolism. Relevant physicochemical properties, in vitro release and Mucoadhesion, drug content and Particle size analysis were investigated. Microspheres were spherical in shape, distinct, with smooth properties favorable for intranasal absorption.

Results: Kinetic modeling result suggested that the prepared formulation followed the korsemeyer-Peppas. Pronounced sustained drug release over 8 hours was exhibited upon incorporation of Carbopol 934P and Sodium alginate. An invitro mucoadhesive test showed that Hydralazine Hydrochloride mucoadhesive microspheres adhered more strongly to the mucous layer and retained in the gastrointestinal tract for an extended period of time.

GJMR-B Classification: *NLMC Code: QV 704*

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Conclusion: The prolonged gastrointestinal residence time and slow release of Hydralazine Hydrochloride resulting from the mucoadhesive microspheres, could contribute to the provision of a sustained anti-hypertensive effect.

I. Introduction

Microsphere systems which are made from the naturally stirring biodegradable polymers have attracted considerable attention for past few years in sustained release drug delivery. Recently, dosage forms that can accurately control the release rates and target drugs to a specific body site have made the massive impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems (1-3). They have varied applications and are prepared using various polymers (4). However, the success of these microspheres is limited owing to their short residence time at the site of absorption. Hence the efforts have been made for providing an intimate contact of the drug delivery system with the absorbing membranes (5-8). This can be achieved by blend of mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site (9-12). Carbopol-934P (acrylic acid homopolymer) is an anionic polymer that has been used in mucoadhesive systems by several researchers (13-17). Carbopol-934P has been selected as a polymer in the preparation of mucoadhesive microspheres because of its good mucoadhesive properties and is not absorbed by body tissues and being totally safe for human oral consumption. Whereas Sodium alginate Used for the aqueous microencapsulation of drugs in contrast with the more conventional microencapsulation techniques used in combination with an H2 receptors antagonist in the management of gastro esophageal reflex.[18] Hydralazine Hydrochloride is Antihypertensive, Cardiovascular agent, having biological Half life 3-7 hrs necessitates the need for its administration 50 mg at Every 6 hrs. Thus, the development of controlled-release dosage forms would clearly be advantageous.

In context of the above principles, a strong need was felt to develop a dosage form that delivered Hydralazine Hydrochloride as mucoadhesive nasal microspheres and would increase the efficiency of the drug, providing a sustained action. Thus, an attempt was made in the present investigation to use Carbopol-934P as a mucoadhesive polymer and sodium alginate as carrier polymer, in order to prepare mucoadhesive nasal Hydralazine Hydrochloride microspheres. The microspheres were characterized by in-vitro and iv-vivo tests and factorial design was used to optimize the variables. [19]

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II. Experimental

a) Materials

Hydralazine hydrochloride (powder) was obtained as a gift sample from Zydus Cadila (Ahmedabad, India). Carbopol-934P (CP, molecular weight of 3×10^6 Da,) was obtained as a gift sample from Noveon®(Mumbai, India). Sodium alginate and petroleum ether 80:20 were procured from Willson Lab (Mumbai, India) and S. D. Fine Chemicals Ltd. (Mumbai, India), respectively. Liquid paraffin and span 80 were purchased from Loba Chemie Pvt Ltd. (Mumbai, India). All other ingredients were of analytical grade.

b) Animals

Six-months-old mixed sex, specific pathogen-free healthy Indian white rabbits (lupas) (Body weight 2.3 to 2.65 kg), were gifted from Zydus Cadila (Ahmedabad, India) and maintained under standard laboratory conditions (room temperature, 23 ± 2°C; relative humidity, 55 ± 5%; 12/12 h light/dark cycle) with free access to a commercial rodent diet and tap water.

c) Preparation of mucoadhesive hydralazine hydrochloride microspheres

Microsphere were prepared by emulsification method. Sodium alginate and the mucoadhesive carbopol 934 polymer were dispersed in purified water to form a homogeneous polymer mixture. The Hydralazine *hydrochloride* was added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. Polymer solution were dispersed in by a syringe with a needle in 30 ml of DCM containing 2 %w/w span 80 using a using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. Stirring was continued for 3 h. The prepared emulsion was added into a syringe and allow to fall as a droplet into a calcium chloride (10 %w/v) containing 1% tween 20 and allow to react with the polymer globules for the 30 min to induce cross linking and solidify the microspheres.

The amount of emulsifying agent and time for stirring were varied in preliminary trial batches from 1-3 % v/v and 1-3 h, respectively. In factorial design batches F1- F9, 2.0 % v/v Span 80 was used as an emulsifying agent and time for stirring was kept to 3 h. The drug-to-polymer-to-polymer ratio and stirring speed were varied in batches F_1- F_9, as shown in Table 1. All other variables were similar to the preliminary trial batches. Microspheres thus obtained were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room temperature (25 °C and 60 % RH) for 24 h. The effect of formulation variables on characteristics of the microspheres of factorial design batches has been summarized in Table 1.

Various batches of Hydralazine hydrochloride mucoadhesive microspheres, prepared using the 3^2 full factorial design layout.

Table 1: Hydralazine hydrochloride mucoadhesive microspheres, using the 3^2 full factorial design layout

	Variable levels					
Batch code	X_1	X_2	*In-vitro* wash- off test (% mucoadhesion after 1 h)	Drug entrapment efficiency (%)	Particle size (µm)	t_{80} (min)
F_1	-1	-1	58	25	100	589
F_2	-1	0	56	20	95	640
F_3	-1	1	42	29	88	720
F_4	0	-1	82	54	110	496
F_5	0	0	78	51	103	537
F_6	0	1	70	40	96	579
F_7	1	-1	92	45	115	294
F_8	1	0	80	40	111	306
F_9	1	1	74	33	102	333

Translation of coded levels in actual unit

Variable levels:	Low (-1)	Medium (0)	High (+1)
Drug-to-polymer-to polymer ratio (X_1):	1:3:0.5	1:3:1	1:3:1.5
Stirring speed (X_2) rpm:	800	1000	1200

All the batches were prepared using 2 % v/v Span 80 and a stirring time of 3 h.

d) Optimization of microspheres formulation using 3^2 full factorial design

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

Where *Y* is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-

linearity. On the basis of the preliminary trials, a 32 full factorial design was employed to study the effect of independent variables, i.e. drug-to-polymer-to-polymer (X_1) and the stirring speed (X_2) on dependent variables which were the percentage of mucoadhesion, drug entrapment efficiency, particle size and the time required for 80 % drug dissolution (t_{80}).

e) Determination of Hydralazine hydrochloride

The amount of Hydralazine hydrochloride was estimated, using a UV/Vis spectrophotometric method (Shimadzu UV-1700 UV/Vis double beam spectrophotometer, Kyoto, Japan). Aqueous solutions of Hydralazine hydrochloride were prepared in 7.2 phosphate buffer and absorbance was measured on a Shimadzu UV/Vis spectrophotometer at 272 nm nm. The method was validated for linearity, accuracy and precision. The method obeyed from the Beer's Law in the concentration range of 5-40 µg/ml. When a standard drug solution was analyzed repeatedly (n = 5), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.86% and 1.1%, respectively.

f) Drug entrapment efficiency

Two hundred milligrams of accurately weighed microspheres were crushed in a glass mortar and the powdered microspheres were suspended in 10 mL of 0.1 N hydrochloric acid (pH=1.2). After 24 h, the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula:

Practical drug content/Theoretical drug content × 100.

The drug entrapment efficiency for batches F1-F9 has been reported in Table 1.

g) Particle size of microspheres

The particle size of the microspheres was determined, using an optical microscopy method (20). Approximately 300 microspheres were counted for particle size, using a calibrated optical microscope (Labomed CX RIII, Ambala, India). The particle size of microspheres of batches F_1-F_9 has been reported in Table 1.

h) In-vitro wash-off test for microspheres

The mucoadhesive properties of the microspheres were evaluated, using an *in-vitro* wash-off test, as reported by Lehr et al (21). A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (3 inch-by-1 inch), using thread. Microspheres were spread (approximately 50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegration test apparatus, with continuous oxygen supply. The disintegration test apparatus was operated, giving the tissue specimen was given regular up and down movements within the beaker of the disintegration apparatus, which contained the simulated gastric fluid (pH=1.2). At the end of 30 min, 1 h and at hourly intervals up to 12 h, the number of microspheres still adhering onto the tissue was counted. The results of *in-vitro* wash-off test of batches F_1-F_9 have been shown in Table 1.

i) Scanning electron microscopy

Scanning electron photomicrograph of drug-loaded Carbopol-934P mucoadhesive microspheres were taken. A small amount of microspheres was spread on a glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. A scanning electron photomicrograph was then taken at an acceleration voltage of 20 KV, and a chamber pressure of 0.6 mm Hg, at different magnifications. The photomicrograph of batch F_4 has been depicted in Figure 1.

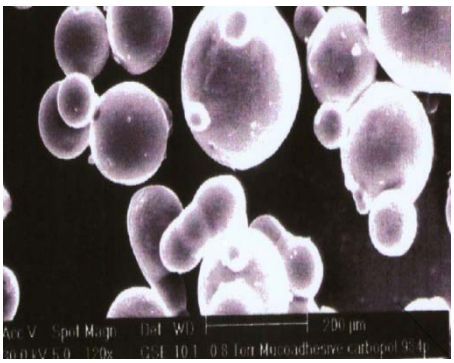

Figure 1: SEM of F4 batch

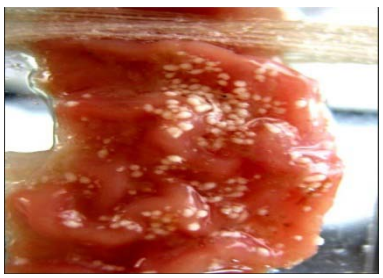

Figure 2: In-vitro wash-off test after 1 h of F4 batch

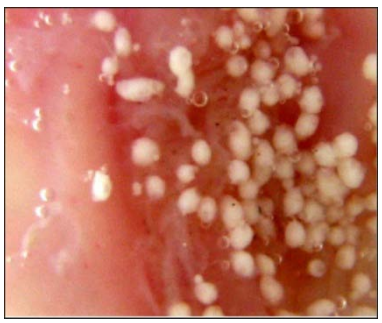

Figure 3: In-vitro wash-off test carried out on Hydralazine hydrochloride loaded Carbopol-934P mucoadhesive microspheres (batch F_4), using rat Mucosa after 8hrs

j) Drug release study

The drug release studies were carried out using a USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C ± 0.5°C and at 100 rpm, within 900 mL of 0.1 N hydrochloric acid (pH=1.2) as the dissolution medium, as per USP XXVI dissolution test described for Hydralazine hydrochloride tablets. Microspheres equivalent to 40 mg of Hydralazine hydrochloride were used for this purpose. Five milliliters of the sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analysed spectrophotometrically at 289nm. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage of drug dissolved at different time intervals was calculated, using the Beer-Lambert equation. The t_{80} was calculated, using the Weibull equation (22). The average values of t80 for batches F_1-F_9 have been shown in Table 1. The percentage of drug released from batch F_4 in pH 1.2 has been shown in Figure 4.

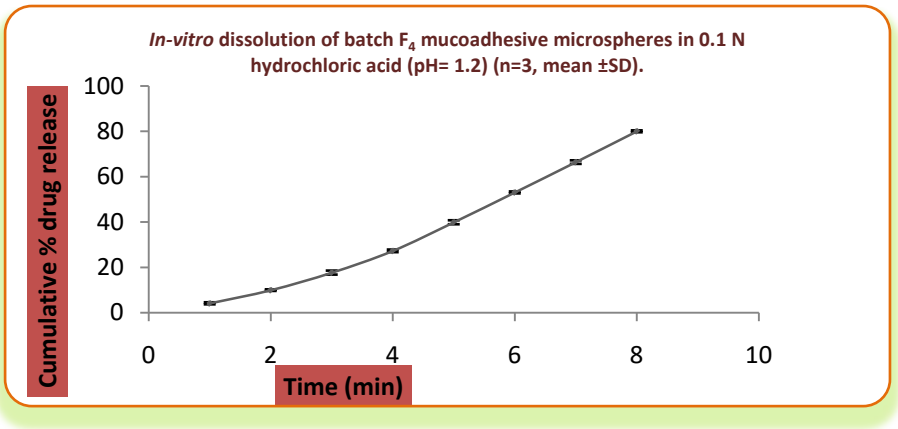

Figure 4: In-vitro dissolution of Hydralazine hydrochloride loaded Carbopol-934P mucoadhesive microspheres (batch F_4), up to 8hrs

k) Data fitting

The curve fitting, simulation and plotting were performed, using the Excel software (Microsoft Software Inc., USA) and Sigma plot® version 10.0 (Sigma plot software, Jangel Scientific Software, San Rafael, CA). The effects of independent variables on the response parameters were visualized from the contour plots. Numerical optimization, using the desirability approach, was employed to locate the optimal settings of the formulation variables so as to obtain the desired response (23). An optimized formulation was developed by setting constraints on the dependent and independent variables. The formulation developed was evaluated for the responses, and the experimental values obtained were compared with those predicted by the mathematical models generated. Counter plots showing the effect of drug-to-polymer-to-polymer (X_1) and stirring speed (X_2) on the percentage of mucoadhesion, drug entrapment efficiency, particle size and t_{80} have been shown in Figure 5.

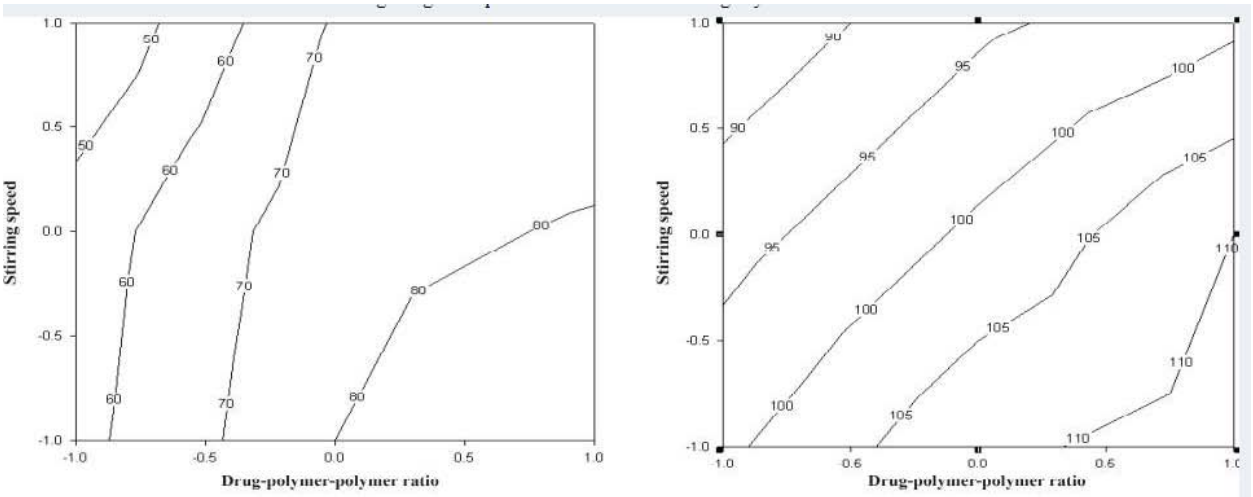

Figure 5: Results of the mathematical models fitted on batch F_4

Table 2: Hydralazine hydrochloride mucoadhesive microspheres F4 batch using release models

Batch	Zero Order	First Order	Higuchi Model	Hixson Crowell Model	Korsemeyer Peppas Model
Slope	10.316	0.2623	38.444	-3.437	2.121
Intercept	-15.479	-0.043	-46.688	38.49	-2.045
Correlation Coefficient	0.9803	0.9619	0.9492	-0.9803	0.999

l) In-vivo study

In-vivo studies on Hydralazine hydrochloride mucoadhesive microspheres were performed on normal healthy Indian white rabbits (lupas) of mixed sex, weighing 2.3 to 2.65 kg each. The approval of the Institutional Animal Ethics Committee was obtained, before starting the study. The study was conducted in accordance with the standard intuitional guidelines. Two groups of normal healthy rabbits (three in each group) that were fasted (with water) at least 12 h before studies, were used for this investigation. Before oral drug administration of respective dosage forms, normal heart rate was recorded for 15 min. After recording the normal heart rate, i.v. isoprenaline (0.25 µg/kg) was administrated for induction of heart rate at a fixed interval. A dose of 2.5 mg/kg of Hydralazine hydrochloride-containing mucoadhesive microspheres and Hydralazine hydrochloride powder were administrated orally using long food needles. After oral administration of dosage forms, heart rate was continuously recorded for 12 h, using pulse transducer (MI) through a Powerlab-multichannel computerized data acquisition system (AD Instruments, Australia) from each rabbit.

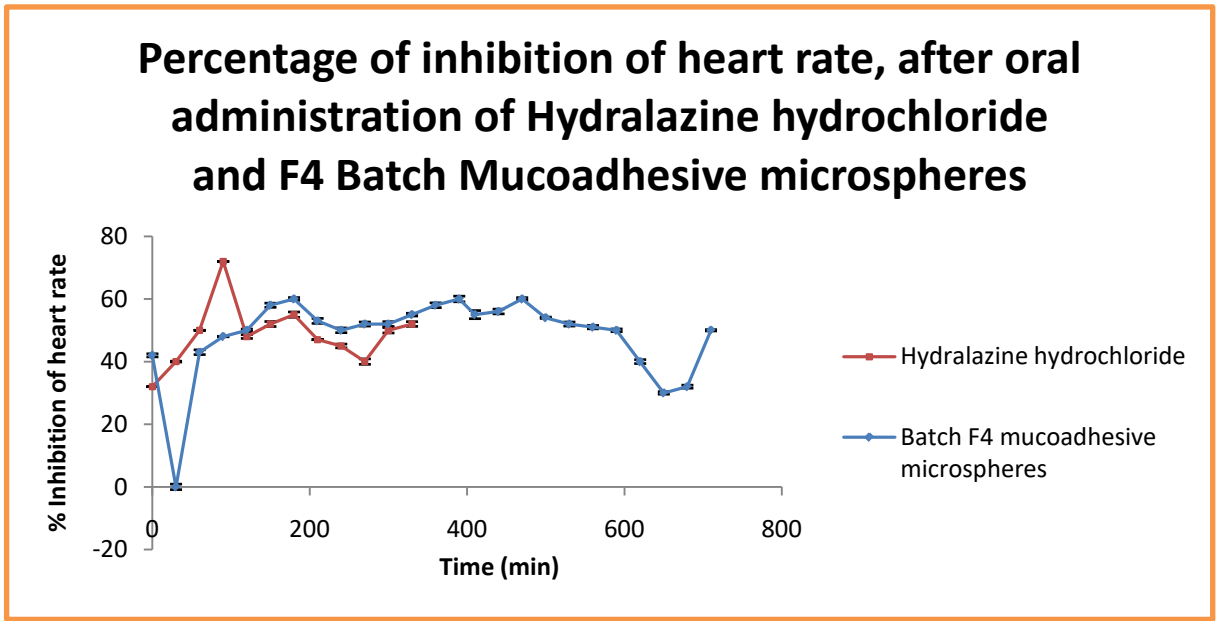

Figure 5: The percentage of inhibition of heart rate after oral administration of pure drug and mucoadhesive microspheres (F4)

III. Results and Discussion

a) Preliminary trials

The mucoadhesive Hydralazine hydrochloride microspheres prepared from Carbopol-934P and sodium alginate were made using the emulsion cross-linking technique. Carbopol-934P choosen for the preparation of mucoadhesive microspheres, owing to its good mucoadhesive properties. Sodium alginate was used as a carrier polymer. Different concentrations of span 80, from 1-3% v/v, were used as the emulsifying agent. Span 80 was found to have a significant influence on the percentage of mucoadhesion observed (i.e. percentage of microspheres adhered and remained on the mucous layer), particles size and drug entrapment efficiency. Results showed that increase in the concentration of span 80, increased the particle size of microspheres, as well as the percentage of mucoadhesion. However, the drug entrapment efficiency was decreased. At a 1% v/v span80 concentration, percentage of mucoadhesion, particle size and drug entrapment efficiency of microspheres were 62%, 100 µm and 56 % respectively. However, formation of irregularly shaped microspheres was observed. On the other hand, at 3% v/v span 80 concentration, the percentage of mucoadhesion, particles size and drug entrapment efficiency of microspheres were 78%, 200 µm and 49 % respectively. The shapes of microspheres were found to be spherical, particles were coalesced. However, a 2 % v/v of concentration of span 80 was used for further studies.

One of the important factors related to microspheres, as reported by Lee et al (24), is the viscosity of the polymer solution. Polymer concentrations of 0.5%, 1%, and 2% w/v were selected for preliminary trials. Flake formation was observed when Sodium alginate and Carbopol-934P concentration was used at a level of 0.5% w/v, whereas maximum Sperical particles were observed at the 1% w/v level. Non-spherical microspheres were formed, when 2% w/v using polymer concentrations. Therefore, a 1% w/v concentration of Sodium Alginate and Carbopol-934P, each in ethanol, was found to be the optimum concentration for the polymer solution. A 1:1 mixture of heavy and light liquid paraffin was found to be suitable as the dispersion medium.

Preliminary trial batches were prepared, in order to investigate the effect of stirring time and speed on the percentage of mucoadhesion, drug entrapment efficiency, and characteristics of the resulting microspheres. An increase in the stirring time 1 h to 3 h, showed an increase in the percentage of mucoadhesion, but a decrease in drug entrapment efficiency and particles size of microspheres. Thus, a stirring time 3h was selected for further studies. Since, the stirring speed had a significant effect on the percentage of mucoadhesion, drug entrapment efficiency and particles size of microspheres, it was selected as an important factor for further studies.

On the basis of the preliminary trials, a 3^2 full factorial design was employed to study the effect of independent variables (i.e. drug-to-polymer-to-polymer ratio [X1] and the stirring speed [X2]) on dependent variables, which were the percentage of mucoadhesion, drug entrapment efficiency, particle size and t80. The results depicted in Table 1 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables, as they show a wide variation among the nine batches (F1-F9). The fitted equations (full models), relating the responses (i.e. percentage of mucoadhesion, drug entrapment efficiency, particle size and t80) to the transformed factor are shown in Table 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). The high values of correlation coefficient (Table 3) for the dependent variables indicate a good fit. The equations may be used to obtain estimates of the response, since a small error of variance was noticed in the replicates.

Table 3: Hydralazine hydrochloride mucoadhesive microspheres F4 batch using release models

Coefficient	b 0	b 1	b 2	b 11	b 22	b 12	R^2
% Mucoadhesion	77.42	14.47	-8.19	-2.81	-9.60	-0.50	0.9809
Drug entrapment efficiency	48.14	7.71	-3.28	-4.57	-16.71	0.28	0.9165
Particle size	103.77	7.30	-6.60	-1.10	-1.01	-1.25	0.9955
t_{80}	533.41	167.50	43.88	-46.19	3.18	-23.49	0.9997

b) Factorial equation for the percentage of mucoadhesion

The *in-vitro* mucoadhesiveness test represented the percentage of mucoadhesive microspheres remaining on the mucosal layer (Table 1). The mucoadhesive microspheres of all the factorial design batches were spherical (Figure 1, batch F_4) and free-flowing.

The linear model generated for the percentage of mucoadhesion was found to be significant, with an *F*-value of 20.64 ($p < 0.0001$) and R^2 value of 0.9809:

$$\% \text{ mucoadhesion} = 77.42 + 14.47X_1 - 8.19X_2 - 2.81X_1X_2 - 9.6X_2^2 \quad (5)$$

The counter plot shows that the in-vitro wash-off test carried out for determining the percentage of mucoadhesion of microspheres, increased from 42 to 58 and 74 to 92, at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as the stirring speed decreased. Results obtained, indicated that the effect of X_1 (drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, stirring speed had a negative effect on the percentage of mucoadhesion (i.e. as the stirring speed increased, the percentage of mucoadhesion decreased). This finding may be attributed to a change in particle size, which can consequently affect mucoadhesion. As the drug-to-polymer-to-polymer ratio increases; the percentage of mucoadhesion also increases; since the greater amount of polymer results in a higher amount of free –COOH (carboxyl) groups (17), which are responsible for binding to the sialic acid groups within the mucus network. Hence, it results in an increase in the mucoadhesive properties of microspheres. *In-vitro* mucoadhesive tests showed that hydralazine hydrochloride mucoadhesive microspheres adhered more strongly to the gastric mucosa and could be retained in the gastrointestinal tract for an extended period of time (Figures 2 and and3). Figure 3 also showed that even after 8 h some of the microspheres were remained on the gastric mucous layer.

c) Factorial equation for particle size

The linear model generated for particle size of microspheres was found to be significant, with an *F*-value of 88.76 ($p < 0.0001$) and R^2 value of 0. 9955:

$$\text{Particle size} = 103.77 + 7.3X_1 - 6.6X_2 - 1.0X_1X_2 - 1.0\,X_2^2 \quad (6)$$

The counter plot showed that the particle size of microspheres increased from 88 to 100 μm and 102 to 115 μm, at lower and higher levels of drug-to-polymer-to-polymer ratio, respectively, as the stirring speed decreased. The results obtained indicate that the effect of X_1 (drug -to-polymer-to-polymer) is more significant than X_2 (stirring speed). This means that, as the stirring speed increases, the particle size decreases, and as a result the percentage of mucoadhesion could be directly affected.

Thus, it can be concluded that the amount of polymer (Carbopol-934P) and the stirring speed directly affect the percentage of mucoadhesion, as well as the particles size of microspheres.

d) Factorial equation for drug entrapment efficiency

The drug entrapment efficiency and t_{80} are important variables for assessing the drug loading capacity of microspheres and their drug release profile. These parameters are dependent on the process of preparation, physicochemical properties of drug, and formulation variables.

The linear model generated for drug entrapment efficiency was found to be significant, with an *F*-value of 4.39 ($p < 0.0001$) and R^2 value of 0.9165:

$$\text{Drug entrapment efficiency} = 48.14 + 7.71X_1 - 3.28X_2 - 4.57X_1X_2 - 16.71X_1^2 + 0.28\,X_2^2 \quad (7)$$

The results obtained, indicate that the effect of X_1(drug-to-polymer-to-polymer) is more significant than X_2 (stirring speed). Moreover, the stirring speed had a negative effect on the percentage of drug entrapment efficiency (i.e. as the stirring speed increased, the particle size decreased and consequently the drug entrapment efficiency also decreased).

e) Factorial equation for t_{80}

The linear model generated for t_{80} was found to be significant, with an *F*-value of 115.91 ($p < 0.0001$) and R^2 value of 0.9997:

$$t_{80} = 533.47 - 167.16X_1 + 43.88X_2 - 25.57X_1X_2 - 58.71X_1^2 + 5.57\,X_2^2 \quad (8)$$

The results depicted in Table 3 indicate that the percentage of drug released *in-vitro* is highly dependent on the drug-to-polymer-to-polymer ratio and the stirring speed. The drug-to-polymer-to-polymer ratio has a negative effect on t_{80}, while stirring speed has a positive effect on t_{80}. Consequently, as the particle size decreases, the drug release also decreases.

A numerical optimization technique, using the desirability approach, was employed to develop a new formulation with the desired responses. Constraints like maximizing the percentage of mucoadhesion, drug entrapment efficiency, particle size and the amount of drug released after 12 h, in addition to minimizing the t_{80}, were set as goals to locate the optimum settings of independent variables in the new formulation.

The optimized microsphere formulation was developed, using a 1:3:1.25 drug-to-polymer-to-polymer ratio and a stirring speed of 950 rpm. The optimized formulation was evaluated for the percentage of mucoadhesion, drug entrapment efficiency, particle size and the amount of drug released. The results of experimentally observed responses and those predicted by mathematical models, along with the percentage prediction errors were compared. The prediction error, for the response parameters ranged between 0.52 and 2.18%, with an absolute error value of 1.26 ± 0.72%. The low values of error indicate the high prognostic ability of factorial equation methodology. The amount of drug released from the optimized formulation was found to be low and it had a t_{80} value of 405 min. Thus, batch F_4 was selected for further studies, since it exhibited a high t_{80} value of 496 min and seems to be a promising candidate for achieving drug release up to 12 h. The drug release profile of batch F_4 is shown in Figure 4. This graph revealed that drug release rate slowed down after 2 h.

The results of curve fitting of the best batch into different mathematical models are given in Table 2.

The mechanism of drug release from the microspheres was found to be diffusion controlled, since the plots of the cumulative percentage of drug release versus the square root of time were found to be linear with the regression coefficient (R^2) values, ranging from 0.9784 to 0.9879 for the best batch. The release profile fitted to the Korsmeyer-Peppas equation, as the value of correlation coefficient was found to be 0.999. The values of slope and intercept were found to be 2.121and -2.045, respectively.

f) In-vivo studies

A rapid reduction in heart rate was observed with pure hydralazine hydrochloride and the heart rate also recovered rapidly to the normal level within 5 h (Figure 6). In the case of hydralazine hydrochloride mucoadhesive microspheres, the reduction in heart rate was slow and reached a maximum reduction of 47 percent within 5 h after oral administration. This reduction in heart rate was sustained over a longer period of time (10 h). The 40 percent reduction in heart rate could be considered as a significant anti-hypertensive effect. In pure drug, the significant anti-

hypertensive effect (40 percent) was maintained during the periods from 0.5 to 5 h following oral administration of hydralazine hydrochloride. Whereas, in case of mucoadhesive microspheres, the effect was maintained for a period of 0.5 to 10 h. The sustained anti-hypertensive effect observed over a longer period of time in case of mucoadhesive microspheres was due to a slow release rate of drug, as well as the mucoadhesive properties of microspheres.

IV. Conclusion

lit could be said that the Hydralazine hydrochloride mucoadhesive microspheres developed, using a 3^2 full factorial design, showed a high percentage of mucoadhesion and drug entrapment efficiency. They also exhibited a sustained drug release property for peroral use in the form of capsule. Drug-to-polymer-to-polymer (Hydralazine hydrochloride-Sodium Alginate-Carbopol-934P) ratio, as well as the stirring speed had a significant influence on the percentage of mucoadhesion, drug entrapment efficiency, particle size and t_{80}. The optimized Hydralazine hydrochloride mucoadhesive microsphere formulation, developed using the desirability approach, showed a greater effect over a period of 8 h, compared to the Hydralazine hydrochloride powder. This would indicate the potential of mucoadhesive Hydralazine hydrochloride microspheres for use in the provision of a sustained therapeutic effect.

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Impact of Simulated Nitric and Sulphuric Acid Rain on the Medicinal Potential of *Telfairia occidentalis* (Hooker Fil.)

By A. J. Mofunanya
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Abstract- This study evaluated the impact of simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR) on the nutrient status of Telfairia occidentalis. Results of phytochemical screening of T. occidentalis revealed the presence of alkaloids, saponins, flavonoids, reducing compounds, polyphenols in all parts with the absence of phlobatanins, anthraquinones and hydroxymethyl anthraquinones in aqueous and ethanol extracts of SNAR and SSAR as well as the control. Tannins, steroids, terpenoids, and glycosides were present in some plant parts and absent in others. Impact of simulated HNO3 and H2SO4 acid rain on qualitative phytochemicals resulted in significant (P=0.05) increase and adecrease in phytochemicals. Ash, protein, fat, fiber and carbohydrate showed decrease owing to SNAR and SSAR impact with an increase in root fat content. Protein was significantly reduced at all levels of acidity with leaf percentage reduction of 54.3%, 37.7%, 20.3% and 62.0%, 42.6%, 10.4% for SNAR and SSAR at pH 2.0, 3.0, and 4.0 respectively. Impact of SNAR and SSAR causedadecrease inhistidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, cysteine, glycine, serine, tyrosine with anincrease in glutamic acid, aspartic acid, proline, and alanine. K, Na, Ca, Mg, Mn, Cu, Zn, Fe, and P were also reduced.

Keywords: *nutrient status, telfairia occidentalis, plant parts, simulated nitric acid rain, sulphuric acid rain.*

GJMR-B Classification: *NLMC Code: QV 704*

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Abstract- This study evaluated the impact of simulated nitric acid rain (SNAR) and sulphuric acid rain (SSAR) on the nutrient status of *Telfairia occidentalis*. Results of phytochemical screening of *T. occidentalis* revealed the presence of alkaloids, saponins, flavonoids, reducing compounds, polyphenols in all parts with the absence of phlobatanins, anthraquinones and hydroxymethyl anthraquinones in aqueous and ethanol extracts of SNAR and SSAR as well as the control. Tannins, steroids, terpenoids, and glycosides were present in some plant parts and absent in others. Impact of simulated HNO_3 and H_2SO_4 acid rain on qualitative phytochemicals resulted in significant (P=0.05) increase and adecrease in phytochemicals. Ash, protein, fat, fiber and carbohydrate showed decrease owing to SNAR and SSAR impact with an increase in root fat content. Protein was significantly reduced at all levels of acidity with leaf percentage reduction of 54.3%, 37.7%, 20.3% and 62.0%, 42.6%, 10.4% for SNAR and SSAR at pH 2.0, 3.0, and 4.0 respectively. Impact of SNAR and SSAR causedadecrease inhistidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, arginine, cysteine, glycine, serine, tyrosine with anincrease in glutamic acid, aspartic acid, proline, and alanine. K, Na, Ca, Mg, Mn, Cu, Zn, Fe, and P were also reduced.

Keywords: nutrient status, telfairia occidentalis, plant parts, simulated nitric acid rain, sulphuric acid rain.

I. Introduction

The plant *Telfairia occidentalis* (Hooker F.) is a member of the family Cucurbitaceae, tribe; Jolifeae and sub-family Cucurbitoidea. It is a crop of commercial importance grown across the lowland humid tropics in West Africa; Nigeria, Ghana and Sierra Leone being the major producers. *Telfairia occidentalis* is one of the most important vegetables grown in southern Nigeria mainly for its leaves which constitute a significant component in the diet of the people. It is not uncommon to find large quantities of the vegetable being hauled around on the streets and markets in the southern belt of Nigeria (Mofunanya et al., 2008). The nutritional interest in *Telfairia occidentalis* stems from its high contents of essential amino acids, vitamins and mineral nutrients (Fasuyi and Aletor, 2005; Mofunanya et al., 2009) and as a result, hectares of land under cultivation is on the increase to meet the nutritional needs of the ever-increasing population. Locally it is called 'ikong' by the Efiks and Ibibios, 'ugu' by the Igbos, 'egusi iroko' by the Yorubas, 'uwmenkhen' by the Benins in Nigeria. The leaves which are harvested at all stages of growth are used in the preparation of ''edikang ikong'' a popular delicious soup in Cross River and Akwa Ibom States and ''ofe ugu'' in the Igbo-speaking States.

Telfairia occidentalis is vegetable with high medicinal values. The leaves are rich in protein (25%), fat (18%), ash (14%) fiber (13%) and minerals and vitamins (20%) (Akanbi et al.,2007).The leaves of this vegetable have a high amount of vitamin A adequate to sustain the vitamin A requirement of consumers. It also possesses the hypolipidemic effect and may be used in hypercholesterolemia therapy (Adaramoye et al., 2007). It is also rich in Fe, Mg, K, carotene and vitamin C is remarkable in making the leaves potentially useful food supplements. The essential amino acids contents compared favorably to those of legumes. It is observed that the vegetable provides little dietary energy, making it valuable in energy-limited diets (Aletor and Adeogun, 1995). In traditional medicine, fluted pumpkin is used in reproduction and fertility; it also has the potential to regenerate testicular damage and to increase spermatogensis (Nwangwa et al., 2007).

Fluted pumpkin has a high amount of antioxidant, free radical scavenging potential and phytochemicals which are of health benefits. Aqueous and ethanol extracts of *T. occidentalis* leaves have the potential to suppress or prevent the production of free radical and to scavenge already produced ones, lower lipid peroxidation status and elevates catalase and superoxide dismutase (antioxidant enzymes) both in vivo and in vitro. The vegetable has been found to protect and ameliorate oxidative brain and liver damage induced by malnutrition in rats (Kayode, 2010). Hepatoprotective property of polyphenol extracts of leaves on acetaminophen-induced liver damage has been reported (Nwanna and Oboh, 2007). Ethanol and aqueous extracts of fluted pumpkin also protect the liver cells against garlic-induced oxidative damage with aqueous extract being more effective than ethanol extract. The utilization of *T. occidentalis* leaves in folk medicine in the treatment of certain diseases in Nigeria in which reactive oxygen species involvement could be

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attributed to antioxidant and free radical scavenging ability.

Telfairia occidentalis has the potential to boost blood level and improves diabetes. In recent time, this vegetable had gained medicinal recognition and subsistence. Fresh leaf is a high-valued health tonic for the treatment of acute anemia. In many tropical countries, anemia constitutes a serious health problem because of the prevalence of malaria and other parasitic infections. In anemic condition, there is a decrease in the level of circulating haemoglobin, less than 13 g dL-1 in males and 12 g dL-1 in females (Okochi et al., 2003). Where, malaria is endemic in the tropics, between 10 to 20% of the populace presents less than 10 g dL-1hemoglobin (Diallo, 2008). The more vulnerable are the children. *Telfairia occidentalis* leaves are rich in iron which plays a role in the cure of anemia. It has been shown to be blood purifiers (Aletor et al., 2002) and could, therefore, be useful in the maintenance of good health most especially among the poor rural community in developing countries. Vegetables are used to fend off illnesses, help nursing mothers build up their milk and assist rural communities to survive long periods of drought. Furthermore, the fiber content has been reported to have beneficial effects on blood cholesterol and aids in the prevention of bowel diseases, while in diabetic subjects (patients). *T. occidentalis* improves glucose tolerance (Hart, 2005). Diabetes Type 2 associates with the increase in oxidative stress, which probably results either from excess generation of reactive oxygen species or a decrease in antioxidant defenses. But recently, it has come to the knowledge that the most significant factor to increase the free radicals production in diabetes is the hyperglycemic status, which induces damage through overproduction of superoxide radical in the mitochondria (Brownlee, 2001). Superoxide converted to hydroxyls, diffuses through membranes and initiates lipoperoxidation. The oxidation of unsaturated lipids has implications not only for atherosclerosis but also for stability and integrity of the red cell membranes.

The root of *Telfairia occidentalis* possesses antiplasmodial and antimicrobial properties. The blood schizontocidal activity of the root extracts is comparable to that of chloroquine (Okokon et al., 2007). Ethanol and aqueous extracts of *T. occidentalis* root exhibit =inhibitory effect on the growth of some Enterobacteriaceae commonly encountered in Nigeria; *Escherichia coli*, *Salmonella typhii*, *Pseudomonas aeroginosa,* and *Proteus sp.* Both extracts did not inhibit the growth of some tested fungi; *Aspergillus fumigatus, A. flavus, Penicillium italium* and *Geotrichum albidun* (Oboh et al., 2006). Root extracts of *T. occidentalis* also possess antibacterial activity against *Staphylococcus aureus, Streptococcus pyrogenes, Kliebsiella pneumonia* and *Shigella dysenterae* (Kayode and Kayode. 2011). Locally, the roots are known as potent human poison and there are reports of their use as fish and human poison. The root extract, though has not been found to be of any practical use in pharmacy, could be used as rodenticide because of high saponin content and cucurbitacin-β which has been reported to cause pulmonary edema (Ajibesin et al., 2002). The vegetable also possesses anti-inflammatory activity.

The environment is the immanent part of human life, the quality of which plays a critical role in human health. Thus, human health is linked to the quality of the environment. The air quality is of great importance for all living things. The health of plant, animal and human depends on a clean atmosphere. Human activities have continually released into the air elements that have the potential to cause pollution such as sulfur dioxide (SO_2), oxides of nitrogen (NO_x), carbon dioxide (CO_2) and hydrogen fluoride (HF) producing acid deposition (acid rain) as a result of complex physical and chemical reactions. These reactions are accelerated by sunlight. The transportation of compounds, which convey acid rains through the prevailing wind for thousands of miles raises the pollution to very high rates. Sulphuric acid and nitric acid are the components of acid rain derived largely from fossil fuels combustion (Mofunanya and Soonen, 2017). In Nigeria, increase in population has led to high demand in automobiles, biomass combustion, burning of refuse and traffic emissions have released large quantities of substances into the atmosphere, acidic rain occurs in Nigeria with resultant effect on crop plants. Information on the impact of simulated acid rain on the plant medicinal quality is scares. In-view of the importance of *Telfairia occidentalis* in the diets of the Nigerian people and the antagonizing effect of acid rain, the present study was carried out to evaluate the impact of simulated nitric and sulphuric acid rain on the medicinal potential of *Telfairia occidentalis* (Hooker Fil.).

II. Materials and Methods

a) Seeds collection and planting

Seeds of T. occidentalis were provided by a farmer in Akparabong, Ikom Local Government Area of Cross River State, Nigeria. Polyethylene bags of 16 mm in diameter were bought from the Ministry of Agriculture, Calabar. Nitric and sulphuric acids were purchased from a Scientific Shop all in Calabar, Nigeria. The seeds were sorted for uniformity of size. They were sun-dried for two days to enhance germination and planted in polyethylene bags filled with loamy soil. Two seeds were planted in each bag. On germination, the seedlings were watered with distilled water for a period of two weeks. Simulated nitric and sulphuric acid rain application began after two weeks of germination. The experiment was conducted in the Department of Plant and Ecological Studies greenhouse, University of Calabar, Calabar, Nigeria (latitude 4.952'N and

longitude 8.341'E) at 25±3°C to check pest infestation and uniformity of sunlight and water supply.

b) Preparation and application of simulated acid rain

Simulated nitric and sulphuric acid rain (SNAR and SSAR) concentrations of pH 2.0, 3.0, 4.0 were separately prepared and a controlled pH of 6.0. Each acid (HNO_3 or H_2SO_4) pH concentration was prepared using different quantity of acid. For pH 2.0 concentration, 30 ml of each acid was used, 20.1 ml for pH concentration 3.0 and 10.2 ml for pH 4.0 using a pH meter and distilled water. The distilled water of pH 6.0 was used as control (Mofunanya and Egah, 2017). A total of thirty-five poly bags were used; fifteen for simulated nitric acid rain, fifteen for simulated sulphuric acid rain and five for the control that is, five for each pH concentration replicated three times. Before SNAR and SSAR application, the poly bags were arranged in a completely randomized block design. Application began with 50 ml of simulated acid rain at the initial growth period. The amount varied with the increase in plant growth. Simulated acid rain of various concentrations was applied using a domestic hand-spraying unit on the plants as well as soil. Application was carried out at an interval of two days for thirteen weeks.

c) Sample preparation

After thirteen weeks of post application of simulated acid rain, the whole plants of *T. occidentalis* grown at various pH concentrations were harvested, and the plant parts (Leaf, Stem and Root) separated. The roots were washed in tape water to remove soil before sun-drying along with other plant parts for one week, and milled separately into powder in an electric mill (National Food Grinder, Model MK 308, Japan). The powdered samples were used to evaluate the impact of simulated nitric and sulphuric acid rain on the medicinal quality (qualitative and quantitative phytochemicals, proximate, amino acids and minerals) contents of *T. occidentalis.*

d) Sample Analysis

The presence of phytochemicals in *T. occidentalis* leaf, stem and root were analyzed using standard methods. Alkaloids and glycosides were identified in samples by the method of Sofowora (1993). The presence of tannins, flavonoids, reducing compounds, polyphenols, phlobatanins, anthraquinones and hydroxymethyl anthraquinones were tested for by the method of Trease and Evans (1989). Quantitative determination of flavonoids, alkaloids, saponins in SNAR and SSAR treated samples of the vegetable was carried out by the method of Harbone (1993). Reducing compounds and polyphenols were determined by method described by AOAC (2006). Phytonutrients were analyzed by standard methods. Crude protein was analyzed using the Kjeldahl method. Fat content was determined by the method of AOAC (1995). Ash, fiber, carbohydrate contents of *T. occidentalis* were analyzed by the method of AOAC (2006), minerals (AOAC, 2006) and amino acids (Speckman, 1956; AOAC, 2006).

e) Data analysis

Data obtained in this study were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science, (SPSS), Version 15.0 (SPSS, 2003). Results were also expressed as percentage difference and differences between mean values were determined at 5% probability.

III. Results

Table 1: Simulated nitric and sulphuric acid rain impacts on qualitative phytochemicals of *Telfairia occidentalis* leaf, stem, and root

Phytochemicals	Plant part	HNO_3 Concs.						H_2SO_4 Concs.						Control	
		pH 2.0		3.0		4.0		pH 2.0		3.0		4.0		pH 6.0	
		Aq.	Eth.	Aq.	Eth.	Aq.	Eth.	Aq.	Eth.	Aq.	Eth.	Aq.	Eth.	Aq.	Eth.
Alkaloids	Leaf	++	+	++	+	+	+	++	+	++	+	++	+	+	+
	Stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Root	++	+	++	+	+	+	+	+	++	+	++	+	+	++
Glycosides	Leaf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stem	+	+	+	+	+	++	+	+	+	+	+	+	+	+
	Root	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Leaf	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Root	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Leaf	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Root	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	Leaf	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++
	Stem	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++
	Root	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++
Reducing compounds	Leaf	++	++	++	++	++	+++	+	+	+	+	+	+	+	++
	Stem	+	+	+	+	++	+++	+	+	+	+	+	+	+	++
	Root	+	+	+	+	+	+	+	+	+	+	+	+	+	++
Polyphenols	Leaf	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++
	Stem	++	++	++	++	++	++	++	++	++	++	++	++	++	+++
	Root	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Phlobatanins	Leaf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	Leaf	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxymethyl anthraquinones	Leaf		-	-	-	-	-	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steriods	Leaf	-	+	-	+	-	+	-	+	-	+	-	+	-	++
	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	+	+	+	+	+	+	+	+	+	+	+	+	+	++
Terpenoids	Leaf	+	+	+	+	+	+	+	+	+	+	+	+	+	++
	Stem	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	+	+	+	+	+	+	+	+	+	+	+	+	+	++

+ = Present, +++ = Present in large amounts, - = Absent, Aq. = Aqueous, Eth. = Ethanol

a) *Simulated nitric and sulphuric acid rain impacts on qualitative phytochemicals of Telfairia occidentalis leaf, stem, and root*

Telfairia occidentalis leaf, stem, and roots (plant parts) were screened for the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds, polyphenols, anthraquinones, hydroxymethyl anthraquinones, steroids and terpenoids (Table 1). Results revealed the presence of alkaloids, saponins, flavonoids, reducing compounds, polyphenols in aqueous and ethanol extracts of all plant parts of simulated acid rain treated (HNO_3 and H_2SO_4) and control plant. Tannins were absent in root samples of both extracts. Steroids and terpenoids were present in the leaf and root of *T. occidentalis* but absent in stem samples. Glycosides were present in the stem and root samples but absent in the leaf. Phlobatanins, anthraquinones, and hydroxymethyl anthraquinones were absent in both aqueous and ethanol extracts of leaf, stem and root extracts of acid treated and control samples. Steriods were absent in aqueous extract of leaf treated with both simulated acid rain and in the control.

Table 2: Simulated nitric and sulphuric acid rain impacts on quantitative phytochemicals of *Telfairia occidentalis* leaf, stem, and root

mg/100 g dry matter								
Phytochemicals	Plant part	HNO_3 concs.			H_2SO_4 Concs.			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Alkaloids	Leaf	1.50 ± 0.1	2.04 ± 0.01	2.35 ± 0.01	1.60± 0.01	2.16± 0.01	2.35 ± 0.01	2.05± 0.01
	Stem	1.60 ± 0.1	1.80 ± 0.1	1.83 ± 0.01	2.31 ± 0.1	2.40 ± 0.1	2.70 ± 0.1	1.50 ± 0.1
	Root	2.50 ± 0.1	2.53 ± 0.01	2.59 ± 0.02	2.04 ± 0.1	2.16 ± 0.1	2.18 ± 0.01	3.10 ± 0.1
Glycosides	Leaf	ND	ND	ND	ND	ND	ND	ND
	Stem	2.21 ± 0.1	2.45 ± 0.1	2.53 ± 0.01	1.68 ± 0.1	2.30 ± 0.1	2.38 ± 0.1	3.16 ± 0.1
	Root	1.45 ± 0.01	1.54 ± 0.01	1.61 ± 0.01	2.45± 0.01	2.78 ± 0.01	3.06 ± 0.01	1.30 ± 0.1
Saponins	Leaf	2.60 ± 0.1	1.95 ± 0.02	1.55 ± 0.1	2.70 ± 0.1	2.01 ± 0.01	1.70 ± 0.1	1.40 ± 0.1
	Stem	2.05 ± 0.2	1.77 ± 0.01	1.41 ± 0.1	1.40 ± 0.1	1.42 ± 0.01	1.65 ± 0.01	1.35 ± 0.1
	Root	1.35 ± 0.02	1.38 ± 0.02	1.40 ± 0.1	2.33± 0.01	2.11 ± 0.01	2.05 ± 0.02	1.80 ± 0.01
Tannins	Leaf	0.12 ± 0.1	0.14 ± 0.01	0.16 ± 0.01	0.13± 0.01	0.14 ± 0.01	0.17 ± 0.01	0.26 ± 0.01
	Stem	0.16 ± 0.01	0.17 ± 0.1	0.19 ± 0.01	0.09 ± 0.1	0.10 ± 0.1	0.11 ± 0.01	0.12 ± 0.01
	Root	ND	ND	ND	ND	ND	ND	ND
Flavonoids	Leaf	11.40 ± 0.1	13.13± 0.01	14.14 ±0.01	8.8 0± 0.1	10.20 ± 0.1	10.57±0.01	18.30 ± 0.1
	Stem	13.10 ± 0.1	11.30 ± 0.1	10.75 ± 0.01	14.10± 0.1	12.31 ±0.01	11.36± 0.1	10.60 ± 0.1
	Root	8.23 ± 0.02	9.44 ± 0.01	10.60 ± 0.1	8.29± 0.01	10.20 ± 0.1	11.30 ± 0.1	13.71± 0.01
Polyphenols	Leaf	12.40 ± 0.1	13.71± 0.02	14.68 ±0.01	10.60± 0.1	12.40 ± 0.1	18.14±0.01	27.31 ±0.01
	Stem	11.81 ± 0.01	12.70 ± 0.1	13.43 ± 0.1	17.65±0.02	20.10 ± 0.1	21.30± 0.1	23.19± 0.01
	Root	10.94 ± 0.2	11.84± 0.01	12.35± 0.02	13.71±0.01	14.68±0.01	21.19 ±0.02	17.46± 0.01
Reducing compounds (Sugars)	Leaf	7.68 ± 0.01	8.26 ± 0.01	9.14 ± 0.01	8.29± 0.01	9.32 ± 0.02	10.81 ± 0.1	12.73± 0.01
	Stem	5.45 ± 0.01	6.59 ± 0.01	8.26 ± 0.01	6.32± 0.01	7.62 ± 0.1	8.71± 0.01	9.80 ± 0.1
	Root	5.17 ± 0.01	6.18 ± 0.01	7.30 ± 0.1	5.42± 0.02	6.51 ± 0.01	7.59 ± 0.01	8.73 ± 0.02
Steriods	Leaf	1.51 ± 0.1	1.76 ± 0.1	2.22 ±0.1	1.06 ± 0.2	1.45 ± 0.2	2.07 ± 0.1	2.93 ± 0.2
	Stem	ND	ND	ND	ND	ND	ND	ND
	Root	0.73 ± 0.2	0.77 ± 0.2	0.94 ± 0.2	0.58 ± 01	0.69 ± 0.2	0.85 ± 0.1	1.51 ± 0.2
Terpenoids	Leaf	0.65 ± 0.1	0.75 ± 0.1	1.23 ± 0.2	0.64 ± 0.1	0.74 ± 0.2	1.20 ± 0.2	1.71 ± 0.2
	Stem	ND	ND	ND	ND	ND	ND	ND
	Root	0.54 ± 0.1	0.49 ± 0.1	0.82 ± 0.2	0.54 ± 0.2	0.50 ± 0.2	0.82 ± 0.1	1.03 ± 0.1

- Results are mean of three replicates on a dry weight basis ± standard deviation; P=0.05
- Simulated nitric acid rain (SNAR), Simulated sulphuric acid rain (SSAR)

b) Simulated nitric and sulphuric acid rain impacts on quantitative phytochemicals of Telfairia occidentalis leaf, stem, and root

Simulated acid rain (SNAR and SSAR) impacts resulted in significant (P=0.05) decrease and increase in phytochemical contents of *T. occidentalis* with pH 2.0 depicting the highest decrease and pH 4.0 the lowest decrease. The quality of phytochemicals varied according to concentrations of simulated acid rain and plant parts. The leaf had the highest amount of phytochemicals, followed by the stem and root. Alkaloids and saponins were higher in the root than in the leaf and stem. Impacts of SNAR and SSAR caused asignificant increase on glycosides content of root, leaf saponins, root saponins in SSAR treated and adecrease in root saponins content of SNAR treated plant part. SSAR impact was more on reducing compounds, steroids, and terpenoids with higher reductions in these phytochemicals than with SNAR. Mean value decrease induced on leaf and root flavonoids at pH 2.0 and 4.0 for SNAR impact were 11.40 ± 0.1, 14.14 ±0.01 and 8.23 ± 0.02, 10.60 ± 0.1 mg/100 g. Impact of SSAR had decrease in values of 8.80± 0.1, 10.57±0.01 and 8.29 ± 0.01, 11.30 ± 0.1 mg/100 g as against control values of 18.30 ± 0.1 and 13.71± 0.01 mg/100 g respectively. However, impact of SNAR led to increase in stem flavonoids contents with values of 13.10 ± 0.1, 10.75 ± 0.01 for SNAR and 14.10 ± 0.1, 11.36 ± 0.1 mg/100 g for SSAR compared to control pH 6.0 value of 10.60 ± 0.1 mg/100 g. Results showed reductions in reducing

compounds of *T. occidentalis* at all levels of simulated acids rain compared to the control. Mean reduction values for leaf at pH 2.0, 3.0 and 4.0 were 7.68 ± 0.01, 8.26 ± 0.01, 9.14 ± 0.01 and 8.29 ± 0.01, 9.32 ± 0.02, 10.81 ± 0.1 for SNAR and SSAR as against control pH 6.0 value of 12.73± 0.01 mg/100 g respectively. The stem had mean values of 5.45 ± 0.01, 6.59 ± 0.01, 8.26 ± 0.01 and 6.32 ± 0.01, 7.62 ± 0.1, 8.71± 0.01 compared to control value of 9.80 ± 0.1 mg/100 g respectively. Corresponding values for reducing compounds of root were 5.17 ± 0.01, 6.18 ± 0.01, 7.30 ± 0.1 for SNAR and 5.42 ± 0.02, 6.51 ± 0.01, 7.59 ± 0.01 for SSAR when compared to value of 8.73 ± 0.02 mg/100 g for the control. Simulated nitric acid rain caused more reductions in reducing compounds than simulated sulphuric acid rain. At pH 2.0 flavonoids content of stem sample was lower than the control pH 6.0 for SNAR but higher than the control at pH 3.0 and 4.0. The impact SSAR on stem flavonoids was higher in content than the control. A similar trend of increase and decrease in phytochemicals due to SNAR and SSAR impacts on alkaloids, glycosides, tannins, polyphenols, reducing sugars, steroids, and terpenoids were obtained. Glycosides was not detected in the leaf, tannins was not detected in the root samples of simulated acid rain treated and control plant parts. Steroids and terpenoids were absent in the stem samples of *T. occidentalis* (Table 2).

Table 3: Simulated nitric and sulphuric acid rain impacts on proximate nutrients of *Telfairia occidentalis* leaf, stem, and root

mg/100 g dry matter								
Proximate Nutrients	Plant part	HNO_3 Concs.			H_2SO_4 Concs.			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Ash	Leaf	3.20± 0.01(50.8)	3.72± 0.02(42.8)	3.81 ± 0.01(41.4)	3.60 ± 0.01(44.6)	4.20± 0.02(35.4)	5.22± 0.01(19.7)	6.50± 0.01
	Stem	4.20 ± 0.1(20.9)	4.35 ± 0.2(18.1)	4.46 ± 0.1(16.0)	2.84 ± 0.1(46.5)	2.96 ± 0.2(44.3)	3.00 ± 0.2(43.5)	5.31± 0.1
	Root	2.81± 0.01(39.8)	2.90 ± 0.1(37.9)	3.10 ± 0.1(33.6)	3.20 ± 0.1(31.5)	3.40 ± 0.1(27.2)	3.45 ± 0.01(26.1)	4.67 ± 0.01
Protein	Leaf	3.38 ± 0.2(54.3)	4.61 ± 0.1(37.7)	5.90± 0.1(20.3)	2.81 ± 0.02(62.0)	4.25± 0.01(42.6)	6.63 ± 0.1(10.4)	7.40 ± 0.01
	Stem	2.08 ± 0.2(65.9)	3.00 ± 0.2(50.8)	4.61± 0.1(24.4)	2.37± 0.1(61.1)	4.51 ± 0.2(26.1)	4.67 ± 0.1(23.4)	6.10 ± 0.1
	Root	3.02 ± 0.1(20.5)	3.08 ± 0.01(18.9)	3.19 ± 0.1(16.1)	2.81 ± 0.01(26.1)	3.25 ± 0.01(14.5)	3.70 ± 0.1(2.6)	3.80 ± 0.1
Fat	Leaf	4.43 ± 0.1(29.0)	4.97 ± 0.02(20.4)	5.37 ± 0.01(13.9)	3.75± 0.1(39.9)	4.43± 0.1(29.0)	4.90 ± 0.01(21.5)	6.24 ± 0.1
	Stem	3.10 ± 0.1(39.2)	4.50 ± 0.1(11.8)	4.72 ± 0.01(7.5)	3.10 ± 0.2(39.2)	4.77± 0.1(6.5)	4.82 ± 0.1(5.5)	5.10 ± 0.1
	Root	5.41 ± 0.01(12.7)	4.97 ± 0.1(3.5)	4.90 ± 0.1(2.1)	4.10 ± 0.1(16.7)	4.43 ± 0.01(7,7)	4.70 ± 0.1(2.1)	4.80 ± 0.1
Fibre	Leaf	12.24 ± 0.01(12.9)	13.20 ± 0.01(6.1)	13.24± 0.02(5.8)	13.50 ± 0.2(4.0)	13.60 ± 0.1(3.3)	14.00 ± 0.1(0.4)	14.06 ± 0.1
	Stem	14.92 ± 0.1(11.2)	15.21± 0.1(9.5)	15.72 ± 0.2(6.4)	13.85 ± 0.1(17.6)	14.21 ± 0.1(15.4)	14.38 ± 0.2(14.4)	16.80 ± 0.01
	Root	12.40 ± 0.1(13.0)	12.85 ± 0.1(9.8)	13.20 ± 0.1(7.4)	13.45±0.01(5.6)	13.60 ± 0.1(4.6)	13.01 ± 0.1(8.7)	14.25 ± 0.1
Carbohydrate	Leaf	85.65 ± 0.02(5.4)	86.10 ± 0.01(4.9)	88.24 ± 0.2(2.6)	84.30±0.02(6.9)	86.10 ± 0.1(4.9)	89.70 ± 0.2(0.9)	90.56 ± 0.1
	Stem	84.60 ± 0.1(6.5)	85.78 ± 0.2(5.2)	86.39±0.01(4.6)	84.24 ± 0.1(6.9)	85.53± 0.01(5.5)	88.01 ± 0.1(2.8)	90.52 ± 0.1
	Root	83.24 ± 0.1(3.3)	84.64 ± 0.2(1.7)	85.01 ± 0.1(1.3)	83.01 ± 0.1(3.6)	85.02± 0.02(1.3)	85.29± 0.01(0.9)	86.10±0.02

- Results are mean of three replicates on a dry weight basis ± standard deviation; P= 0.05
- Simulated nitric acid rain (SNAR), Simulated sulphuric acid rain (SSAR).

c) *Simulated nitric and sulphuric acid rain impacts on proximate nutrients of Telfairia occidentalis leaf, stem, and root*

Impacts of simulated nitric (SNAR) and sulphuric SSAR) rain acid on the proximate nutrients revealed asignificant (P=0.05) decrease in ash, protein, fiber, and carbohydrate content of *T. occidentalis* plant parts. The amounts of these nutrients varied in plant parts. Proximate nutrients at control (pH 6.0) revealed that ash, protein and fat contents were more in leaf sample than in the stem and root. Fiber content was more in stem sample than in leaf and root. Fiber content of leaf and root did not differ significantly. While carbohydrate content in leaf, stem and root did not differ statistically. The decrease in proximate nutrients varied according to levels of acidity; pH 2.0 depicted highest reductions in proximate nutrients followed by pH 3.0 and lowest reductions at pH 4.0. Reductions obtained at pH 4.0 for all proximate nutrients were not statistically significant (P=0.05) when compared to the control pH

6.0. The root sample had the least amount of all the proximate nutrients investigated. Reductions induced by SNAR impact on the protein content of leaf at pH 2.0 was 3.38 ± 0.2, lower than reduction at pH 3.0 (4.61 ± 0.1) and pH 3.0 had lower value than pH 4.0 value of 5.90 ± 0.1 g/100 g. The lowest mean value (pH 2.0) indicate the highest reduction and the highest mean value (pH 4.0) indicate the lowest reduction when compared to value for the control. Corresponding mean value of protein at pH 2.0 for SSAR was 2.81 ± 0.02, this value was lower than the value of 4.25 ± 0.01 at pH 3.0, and this lower than the value of 6.63 ± 0.1 g/100 g at pH 4.0 compared to control pH 6.0 value of 7.40 ± 0.01 g/100 g. Mean value reduction of 2.08 ± 0.2 obtained for protein content of stem at pH 2.0 was lower than value of 3.00 ± 0.2 at pH 3.0, and this lower than value of 4.61 ± 0.1 g/100 g at pH 4.0 for SNAR impact. Reduction impacted by SSAR on protein content of stem at pH 2.0 had lower value of 2.37± 0.1 than value at pH 3.0 of 4.51 ± 0.2; pH 3.0 had lower value than pH 4.0 value of 4.67 ± 0.1 g/100 g compared to control pH value of 6.10 ± 0.1 g/100 g. The root mean reduction values were 3.02 ± 0.1, 3.08 ± 0.01 and 3.19 ± 0.1 g/100 g respectively for SNAR. While SSAR had mean reduction values of 2.81 ± 0.01, 3.25 ± 0.01 and 3.70 ± 0.1 g/100 g for pH 2.0, 3.0 and 4.0 respectively compared pH 6.0 value of 3.80 ± 0.1 g/100 g. A similar trend of lowest decrease at pH 2.0, lower decrease at pH 3.0 and low decrease at pH 6.0 were obtained for ash, fat, fiber and carbohydrate. Simulated nitric acid rain caused more impact on ash and fiber content of *T. occidentalis* while impact of SSAR was more on protein and fat (Table 3). All control plant parts had higher proximate nutrient contents than simulated acid train treated plant parts.

Table 4: Simulated nitric and sulphuric acid rain impacts on amino acids of *Telfairia occidentalis* leaf, stem, and root

g/16 N								
Amino acids	Plant part	HNO_3 Concs.			H_2SO_4 Concs.			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Histidine	Leaf	1.88±0.1 (25.1)	1.91±0.01 (23.9)	2.01±0.01 (19.9)	1.89±0.1 (24.7)	1.97±0.1 (21.5)	2.20±0.1(12.4)	2.51±0.06
	Stem	1.88±0.02 (29.9)	1.92±0.01 (28.4)	2.09±0.01(22.0)	1.88±0.02(29.9)	1.99±0.2 (25.7)	2.30±0.01 (14.2)	2.68±0.03
	Root	1.52±0.03 (22.4)	1.60±0.03 (18.4)	1.70±0.01(13.3)	1.54±0.03(21.4)	1.61±0.03(17.9)	1.85±0.03 (5.6)	1.96±0.03
Lysine	Leaf	3.16±0.1 (35.6)	3.35±0.06 (31.8)	3.75±0.03(23.6)	3.18±0.02(22.4)	3.51±0.01(28.5)	3.80±0.02(22.6)	4.91±0.1
	Stem	3.10±0.02(43.7)	3.30±0.03 (40.1)	3.74±0.03 (32.1)	3.21±0.33 (41.7)	3.59±0.06 (34.8)	3.94±0.03 (28.5)	5.51±0.03
	Root	2.32±0.03 (25.6)	2.46±0.06 (21.2)	2.88±0.06 (7.7)	2.33±0.06 (25.3)	2.37±0.01 (24.0)	3.00±0.01 (3.8)	3.12±0.06
Arginine	Leaf	3.42±0.1 (39.1)	3.66±0.01 (34.9)	4.20±0.01 (25.3)	3.83±0.01(31.9)	4.11±0.01(26.9)	4.52±0.01 (19.6)	5.62±0.03
	Stem	3.18±0.1 (41.0)	3.28±0.01(39.1)	4.02±0.01(25.4)	3.28±0.01(39.1)	4.02±0.01(25.4)	4.25±0.01(21.2)	5.39±0.03
	Root	1.45±0.01(60.9)	1.59±0.1(57.1)	1.85±0.01(50.1)	1.41±0.01(62.0)	1.63±0.01(56.1)	2.86±0.01(22.9)	3.71±0.03
Aspartic acid	Leaf	14.53±0.02(51.2)	11.55±0.02(20.2)	9.61±0.02(0.0)	14.78±0.06(53.0)	11.70±0.01(21.7)	9.61±0.01(0.0)	9.61±0.06
	Stem	12.82±0.01(30.0)	10.64±0.01(7.7)	9.90±0.01(0.2)	12.91±0.01(30.7)	11.46±0.06(16.0)	9.90±0.01(0.2)	9.88±0.06
	Root	8.26±0.01(34.7)	7.00±0.01(14.2)	6.14±0.01(0.2)	8.32± 0.01(35.7)	7.20±0.01(17.5)	6.15±0.01(0.3)	6.13±0.03
Threonine	Leaf	2.21±0.01(45.6)	2.40±0.01(40.9)	3.51±0.01(13.5)	2.10±0.02(48.3)	2.20±0.01(45.8)	3.01±0.01(6.4)	4.06±0.1
	Stem	2.11±0.02(43.7)	2.29±0.01(38.9)	2.76±0.01(35.9)	2.02±0.01(46.1)	2.11±0.1(43.7)	2.57±0.01(31.5)	3.75±0.01
	Root	1.41±0.06(51.4)	1.76±0.01(39.3)	2.11±0.02(27.2)	1.32±0.01(54.5)	1.53±0.02(47.2)	2.00±0.1(31.0)	2.90±0.01
Serine	Leaf	2.89±0.01(9.9)	2.79±0.01(5.7)	2.70±0.01(2.7)	2.81±0.01(6.8)	2.73±0.1(3.8)	2.66±0.1(1.1)	2.63±0.0
	Stem	2.70±0.1(12.5)	2.50±0.01(4.2)	1.95±0.01(18.8)	2.72±0.2(13.3)	2.54±0.1(5.8)	1.89±0.01(21.3)	2.40±0.01
	Root	1.65±0.01(13.8)	1.53±0.01(5.5)	1.15±0.1(20.7)	1.61±0.01(11.0)	1.49±0.02(2.8)	1.20±0.1(17.2)	1.45±0.01
Glutamic acid	Leaf	13.68±0.01(12.0)	12.52±0.03(2.5)	12.10±0.02(0.9)	13.70±0.01(12.2)	12.50±0.01(2.4)	12.13±0.02(0.7)	12.21±0.2
	Stem	12.59±0.02(11.4)	11.31±0.02(0.1)	11.15±0.03(1.3)	11.52±0.02(1.9)	11.28±0.01(0.2)	11.17±0.02(1.2)	11.30±0.1
	Root	8.26±0.02(23.8)	7.43±0.03(11.4)	6.42± 0.2(3.7)	8.20±0.03(22.9)	7.33±0.01(9.9)	6.28±0.01(5.8)	6.67±0.2
Proline	Leaf	4.53±0.1(15.9)	4.02±0.1 (2.8)	3.60±0.1(7.9)	4.49±0.1(14.8)	4.06±0.1(3.8)	3.48±0.2(11.0)	3.91±0.02
	Stem	4.47±0.1(14.9)	4.30±0.01(10.5)	3.25±0.1(16.5)	4.47±0.1(14.9)	4.40±0.1(13.1)	3.38±0.1(13.1)	3.89±0.01
	Root	2.55±0.1(107.3)	1.81±0.1(47.2)	1.07±0.1(13.0)	2.45±0.1(99.2)	1.75±0.1(42.3)	1.19±0.01(3.3)	1.23±0.01

Glysine	Leaf	5.01±0.01(11.3)	4.61±0.01(2.4)	4.13±0.01(8.2)	5.04±0.01(12.0)	5.63±0.01(25.1)	4.10±0.01(8.9)	4.50±0.03
	Stem	2.69±0.01(18.5)	2.34±0.01 (3.0)	1.07±0.01(5.3)	2.75 ± 0.01(21.1)	2.40±0.01(5.7)	1.02±0.01(55.1)	2.27±0.03
	Root	1.77±0.01(38.3)	1.45±0.01(13.3)	0.90±0.01(29.7)	1.80±0.01(40.6)	1.22±0.1(4.7)	0.80±0.1(37.5)	1.28±0.03
Alanine	Leaf	2.66±0.02(32.8)	3.81±0.1 (3.8)	4.19±0.01 (5.8)	2.60±0.01(34.3)	3.71±0.01(6.3)	4.13±0.01(4.3)	3.96±0.1
	Stem	2.39±0.02(26.7)	3.67±0.1(12.6)	3.84±0.02(17.8)	2.32±0.01(31.6)	3.50±0.01(7.4)	3.71±0.01(13.8)	3.26±0.1
	Root	0.79±0.02(22.5)	0.82±0.02(19.6)	1.67±0.02(63.7)	0.81±0.02(20.6)	0.85±0.02(16.7)	1.55±0.2(52.0)	1.02±0.1
Cysteine	Leaf	0.67±0.1(25.6)	0.78±0.1 (13.3)	0.89±0.1 (1.1)	0.60±0.1(33.3)	0.74±0.1 (17.8)	0.85 ± 0.1(5.6)	0.90±0.06
	Stem	0.59±0.1(27.2)	0.77±0.1(4.9)	0.77±0.1(4.9)	0.55±0.1(32.1)	0.70±0.1(13.6)	0.71 ± 0.1(12.3)	0.81±0.03
	Root	0.33±0.1 (40.0)	0.40±0.1(27.3)	0.50±0.1(9.1)	0.30±0.1(45.5)	0.37±0.1(32.7)	0.47±0.1(14.5)	0.55±0.1
Valine	Leaf	3.32±0.06(47.2)	3.41±0.03(24.4)	3.99±0.2(11.5)	3.29±0.1(27.1)	3.37±0.1(25.3)	3.81±0.1(15.5)	4.51±0.1
	Stem	3.32±0.06(47.2)	3.41±0.1(24.4)	3.97±0.2(12.0)	3.26±0.1(27.7)	3.37±0.1(25.3)	3.80±0.1(15.7)	4.51±0.2
	Root	1.58±0.2 (27.5)	1.65±0.2(24.3)	1.88±0.1(13.8)	1.50±0.1(31.2)	1.62±0.1(25.7)	1.73±0.1(20.6)	2.18±0.2
Methionine	Leaf	0.81±0.03(35.2)	0.85±0.03(32.0)	1.05±0.03(16.0)	0.78±0.2(37.6)	0.82±0.2(34.4)	0.99±0.03(20.8)	1.25±0.06
	Stem	0.80±0.03(34.4)	0.85±0.03(30.3)	1.04±0.03(14.8)	0.76±0.2(37.7)	0.80±0.2(34.4)	0.96±0.2(21.3)	1.22±0.03
	Root	0.77±0.01(22.2)	0.78±0.01(21.2)	0.82±0.01(17.2)	0.70±0.1(29.3)	0.70±0.2(29.3)	0.75±0.2(24.2)	0.99±0.03
Isoleucine	Leaf	2.10±0.1 (47.9)	2.75±0.3 (31.8)	3.01±0.01(25.3)	2.55±0.01(36.7)	3.00±0.03(25.6)	3.81±0.02(5.5)	4.03±0.2
	Stem	2.66±0.1 (44.0)	3.05±0.2 (35.8)	3.99±0.2(16.0)	2.69±0.1(43.4)	3.23±0.03(32.0)	3.97±0.01(16.4)	4.75±0.1
	Root	1.50±0.1(34.5)	2.25±0.2 (18.7)	2.61±0.2(20.6)	2.12±0.2(32.5)	2.34±0.03(25.5)	3.00±0.1(4.4)	3.14±0.1
Leucine	Leaf	5.11±0.06(36.3)	6.52±0.01(18.7)	7.71±0.2(3.9)	5.83±0.1(27)	6.81±0.02(15.1)	7.99±0.1(0.4)	8.02±0.1
	Stem	4.66±0.03(51.7)	6.41±0.1 (33.5)	7.95±0.1(17.5)	5.01±0.2(48.0)	7.23±0.1(25.0)	8.13±0.1(15.7)	9.64±0.12
	Root	4.92±0.03(4.8)	5.04±0.01(2.51)	5.46±0.1(5.6)	4.96±0.1(4.1)	5.44±0.2(5.2)	5.61±0.2(8.5)	5.17±0.02
Tyrosine	Leaf	2.61±0.03(24.3)	2.85±0.2 (17.4)	3.00±0.03(13.0)	2.79±0.3(19.1)	2.96±0.01(14.2)	3.14±0.01(9.0)	3.45±0.13
	Stem	2.53±0.02(29.7)	2.53±0.03(27.4)	2.97±0.03(17.5)	2.70±0.2(25.0)	2.90±0.1(19.4)	3.11±0.2(19.4)	3.60±0.02
	Root	1.91±0.01(23.6)	2.02±0.01(19.2)	2.20±0.01(13.6)	1.99±0.01(20.4)	2.19±0.01(12.4)	2.37±0.02(5.2)	2.50±0.02
Phenylalanine	Leaf	2.37±0.2 (40.2)	2.68±0.2(32.3)	3.01±0.2(24.0)	2.56±0.2(35.4)	2.77±0.01(30.1)	3.10±0.02(21.7)	3.96±0.3
	Stem	2.59±0.1(33.6)	2.70±0.1 (30.8)	3.00±0.2(23.1)	2.58±0.01(33.8)	2.70±0.2(30.8)	2.98±0.02(23.6)	3.90±0.02
	Root	2.15±0.2(59.4)	2.61±0.2 (31.9)	2.89±0.1(24.5)	2.43±0.01(36.6)	2.60±0.2(32.1)	2.96±0.01(22.7)	3.83±0.1

- Results are mean of three replicates on a dry weight basis ± standard deviation; P=0.05
- Simulated nitric acid rain (SNAR), Simulated sulphuric acid rain (SSAR)

d) Simulated nitric and sulphuric acid rain impacts on amino acids of Telfairia occidentalis leaf, stem, and root

Analysis of variance revealed that all amino acids of *Telfairia occidentalis* were severely affected by simulated acid rain. Significant (P=0.05) reductions were obtained for both essential (Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and nonessential (alanine, aspartic acid, arginine, cysteine, glycine, proline, serine, and tyrosine) amino acids (Table 4). Amino acids reductions varied according to acidity levels with the highest reductions occurring at pH 2.0, followed by pH 3.0 and the lowest reductions occurring at pH 4.0 for both simulated nitric and sulphuric acid rain. The quality of amino acids in the various plant parts also varied with the leaf having the highest amount, followed by the stem and root having the lowest amount. Amino acids; lysine, threonine, glysine and leucine in leaf, stem and root in control pH 6.0 varied significantly. Histidine, arginine, aspartic acid, serine, glutamic acid, proline, alanine, cysteine, valine, methionine, isoleucine, and tyrosine did not differ in leaf and stem but varied in root samples. Phenylalanine did not differ in leaf, stem, and root. Impacts of simulated acid rain resulted in significant (P=0.05) increase in aspartic acid, glutamic acid, proline, and alanine. Lowest values at pH 2.0 indicate highest reductions and highest values indicate lowest reductions. Highest values at pH 2.0 indicate highest increases and lowest values at pH 4.0 indicates lowest increases in amino acids compared to the control. Arginine content in simulated acid rain treated plants

was significantly (P=0.05) impacted with reductions at all pH concentrations compared to the control. Highest (pH 2.0) and lowest (pH 4.0) percentage reductions in leaf arginine content were 25.1%, 19.9% and 24.7%, 12.4% for stem arginine were41.0%, 25.4% and 39.1, 21.2%, and values of 60.9%, 50.1 and 62.0%, 22.9% for root arginine respectively for SNAR and SSAR. Highest and lowest impacts on aspartic acid by SNAR revealed values of 51.2%, 0.0% (leaf) 30.0%, 0.2% (stem) and 34.7%, 0.2% (root). SSAR impact on aspartic acid had percentage increase in values of53.0%, 0.0% (leaf), 30.7%, 0.2% (stem) and 35.7%, 0.3% (root). Phenylalanine revealed percentage reduction values at pH 2.0, 2.0 and 4.0 of 40.2%, 32.3%, 24.0% for leaf, 33.6%, 30.8%, 23.1% (stem), 59.4%, 31.9%, 24.5% for SNAR respectively. Reductions impacted on phenylalanine by SSAR had reduction values of 35.4%, 30.1%, 21.7% (leaf), 33.8%, 30.8%, 23.6% (stem) and 36.6%, 32.1%, 22.7% respectively. Isoleucine depicted percentage reduction of 47.9%, 31.8%, 25.3% (leaf), 44.0%, 35.8%, 16.0% (stem) and 34.5%, 18.7%, 20.6% (root) due to SNAR impact while SSAR had reduction values of 36.7%, 25.6%, 5.5% for leaf, 43.4%, 32.0%, 16.4% (stem) and 32.5%, 25.5%, 4.4% for root samples respectively.

Table 5: Simulated nitric and sulphuric acid rain effect on elemental nutrients of *Telfairia occidentalis* leaf, stem, and root

mg/100 g dry matter								
Elements	Plant part	HNO_3 Concs.			H_2SO_4 Concs.			Control
		pH 2.0	3.0	4.0	pH 2.0	3.0	4.0	pH 6.0
Potassium (k)	Leaf	601.30±0.2(24.3)	620.05±0.2(21.9)	694.19±0.2(12.6)	601.15±0.2(24.3)	615.23±0.1(22.5)	653.13±0.1(17.8)	794.11±0.2
	Stem	600.32±0.2(24.9)	620.17±0.2(22.4)	695.45±0.2(13.0)	587.29±0.1 (26.5)	601.32±0.1(24.8)	669.75±0.2(16.2)	799.16±0.1
	Root	615.08±0.1(25.8)	639.21±0.2(22.8)	699.86±0.2(15.5)	600.36±0.2(27.5)	613.54±0.2(25.9)	642.91±0.2(22.4)	828.41±0.2
Sodium (Na)	Leaf	6.03±0.1(44.0)	7.01±0.1(33.7)	8.00±0.1(24.3)	6.00±0.2 (43.2)	6.98±0.2(34.0)	7.54±0.1(28.7)	10.57±0.1
	Stem	4.02±0.1 (54.7)	5.55±0.2(37.5)	6.71±0.1(24.4)	4.01±0.2(54.8)	5.51±0.3(38.0)	6.53±0.1(26.5)	8.88±0.1
	Root	9.99±0.2 (28.8)	10.20±0.2(27.3)	12.01±0.2(14.4)	9.94±0.2(29.2)	10.00±0.2(28.7)	11.66±0.2(16.9)	14.03±0.1
Calcium (Ca)	Leaf	26. 21±0.2(20.9)	27. 61±0.2(16.7)	30.96±0.2(6.6)	25.12±02(24.2)	27.66±0.1(16.5)	29.59±0.2(10.7)	33.14±0.20
	Stem	35.46±0.1(12.5)	37.47±0.2(7.5)	38.89±0.2(4.0)	34.81±0.1(14.6)	35.82±0.2(11.1)	36.03±0.2(11.1)	40.51±0.2
	Root	30.50±0.1 (21.8)	33.55±0.2(14.0)	37.79±0.2(3.1)	30.06±0.2(22.9)	31.58±0.2(19.0)	34.09±0.2(12.6)	39.01±0.2
Magnesium (Mg)	Leaf	60.04±0.1(24.0)	64.87±0.1(17.9)	69.97±0.1(11.5)	58.73±0.1(25.7)	61.22±0.1(22.5)	65.15±0.1(17.6)	79.02±0.1
	Stem	60.00±0.1(23.9)	63.03±0.1(20.0)	69.25±0.1(12.1)	58.70±01(25.5)	60.67±0.1(23.0)	66.01±0.1(16.2)	78.81±0.1
	Root	59.10±0.1(16.0)	62.81±0.1(10.7)	66.33±0.1(5.7)	57.02±0.1(18.9)	59.26±0.1(15.8)	64.20±0.1(8.7)	70.34±0.1
Iron (Fe)	Leaf	19.18±0.1(30.6)	20.03±0.1(27.6)	23.15±0.1(16.3)	18.80±0.1(32.0)	19.12±0.1(30.8)	20.13±0.2(27.2)	27.65±0.2
	Stem	16.80±0.2(16.4)	17.78±0.1(11.5)	18.00±0.1(10.4)	16.10±0.2(19.9)	17.44±0.1(13.2)	17.91±0.1(10.9)	20.10±0.1
	Root	10.64±0.2(24.4)	10.88±0.2(22.7)	11.99±0.2(14.8)	10.05±0.2(28.6)	10.32±0.2(26.7)	10.68±0.2(24.1)	14.07±0.1
Copper (Cu)	Leaf	0.31±0.1(66.3)	0.46±0.2 (50.0)	0.50±0.1(45.7)	0.34±0.1(63.0)	0.61±0.1(33.7)	0.74±0.2(19.6)	0.92±0.1
	Stem	0.34±0.02(2.9)	0.40±0.02(14.3)	0.41±0.02(17.2)	0.30±0.2(14.3)	0.34±0.1(2.9)	0.35±0.1(0.00)	0.35±0.01
	Root	0.24±0.1(54.7)	0.32±0.1(39.6)	0.46± 0.2(13.2)	0.20±0.1(62.3)	0.31±0.1(41.5)	0.38±0.2(28.3)	0.53±0.1
Zinc(Zn)	Leaf	5.12±0.2(48.0)	5.13±0.2(47.9)	8.90±0.1(9.6)	5.24±0.2(46.8)	6.79±0.2(31.1)	9.44±0.2(4.2)	9.85±0.1
	Stem	5.00±0.1(33.8)	5.05±0.1(33.1)	6.88±0.1(8.9)	5.12±0.2(32.2)	6.01±0.2(20.4)	7.13±0.1(5.6)	7.55±0.1
	Root	5.00±0.1(32.7)	5.02±0.1(32.4)	6.81±0.1(7.4)	5.11±0.2(31.2)	5.99±0.2(19.4)	7.01±0.2(5.7)	7.43±0.2
Manganese (Mn)	Leaf	11.25±0.1(30.3)	13.78±0.1(14.6)	15.98±0.1(0.9)	11.03±0.2(31.6)	12.63±0.2(21.7)	14.70±0.2(8.9)	16.13±0.1
	Stem	12.63±0.1(26.0)	14.95±0.1(12.4)	16.32±0.1(4.3)	12.00±02(29.7)	13.91±0.2(18.5)	15.04±0.2(11.8)	17.06±0.1

	Root	10.42±0.1(31.8)	12.13±0.1(20.6)	15.02±0.1(1.7)	10.10±0.2(33.9)	11.65±0.2(23.8)	14.06±0.2(8.0)	15.28±0.2
Phosphorus (P)	Leaf	8.22± 0.1(41)	8.34 ± 0.1(40.5)	10.41±0.01(25.7)	8.00±0.1(42.9)	8.17±0.1(41.7)	9.19±0.1(34.5)	14.02±0.1
	Stem	8.08 ± 0.1(32.8)	8.16 ± 0.1(32.2)	10.01±0.1(16.8)	7.86±0.1(34.7)	8.00±0.2(33.5)	9.01±0.2(25.1)	12.03±0.1
	Root	4.23 ± 0.1(45.7)	5.30 ± 0.1(33.0)	6.33±0.1(18.7)	5.02±0.2(35.6)	5.11±0.1(34.4)	6.06±0.2(22.2)	7.79±0.1

- Results are mean of three replicates on a dry weight basis ± standard deviation; P=0.05,
- Simulated nitric acid rain (SNAR), Simulated sulphuric acid rain (SSAR).
- Percentage difference values were obtained by expressing the difference between the value for the control and simulated acid rain treated sample as a percentage of the control.

e) Simulated nitric and sulphuric acid rain impacts on elemental nutrients of Telfairia occidentalis leaf, stem, and root

The results in Table 4 highlight impacts of simulated nitric and sulphuric acid rain on the mineral nutrient contents of *T. occidentalis*. Analysis of variance revealed significant (P=0.05) reduction in all mineral nutrients posed by simulated acid rain impact compared to the control. Mineral nutrients reductions varied according to acidity levels and plant parts. The leaf had higher mineral nutrients than the stem, and the stem had higher mineral nutrients than the roots with the exceptions of K and Na which were more in the root than in the leaf and stem. However, Ca and Mn were higher in the stem than in the leaf and root of simulated acid rain treated plants and the control. Highest nutrient reductions occurred at pH 2.0, followed by pH 3.0, pH 4.0 had the lowest reductions in all mineral nutrients. However, reduction on Ca, Zn, and P induced by SNAR did not differ statistically at pH 2.0 and pH 3.0. Simulated SNAR impact was higher on K, Zn, Mn, and Cu with a higher reduction in contents than with SSAR, SSAR impact was higher on Na, Ca, Mg, and Fe content of the vegetable with significant reductions obtained than with SNAR. Reductions were highest at pH 2.0 and lowest at pH 4.0. Na, Ca, Fe, Mn, and P varied significantly according to plant parts, while K and Mg were not statistically different in leaf and stem parts. Reductions posed on Ca in leaf by SNAR at pH 2.0, 3.0 and 4.0 were 26.21 ± 0.2, 27.61 ± 0.2 and 30.96 ± 0.2 mg/100 g, SSAR impact caused reductions of 25.12 ± 02, 27.66 ± 0.1 and 29.59 ± 0.2 mg/100 g. In the stem, SNAR had mean Ca reduction values of 35.46 ± 0.1, 37.47 ± 0.2 and 38.89 ± 0.2 mg/100 g, SSAR had values of 34.81 ± 0.1, 35.82 ± 0.2 and 36.03 ± 0.2 mg/100 g respectively compared to value of 40.51 ± 0.2 mg/100 g for the control. Reductions posed by SNAR on root Ca had values of 30.50 ± 0.1, 33.55 ± 0.2 and 37.79 ± 0.2 mg/100 g, SSAR had values of 30.06 ± 0.2, 31.58 ± 0.2 and 34.09 ± 0.2 mg/100 g compared to control pH 6.0 value of 39.01 ± 0.2 mg/100 g. Reduction in Iron content in leaf, stem and root caused by SNAR had mean values of 19.18 ± 0.1, 16.80 ± 0.2 and 10.64 ± 0.2 mg/100 g at pH 2.0, 20.03 ± 0.1, 17.78 ± 0.1 and 10.88 ± 0.2 mg/100 g at pH 3.0, 23.15 ± 0.1, 18.00 ± 0.1 and 11.99 ± 0.2 mg/100 g at pH 4.0. Values obtained for SSAR were 18.80 ± 0.1, 16.10 ± 0.2 and 10.05 ± 0.2 mg/100 g at pH 2.0, 19.12 ± 0.1, 17.44 ± 0.1 and 10.32 ± 0.2 mg/100 g at pH 3.0, 20.13 ± 0.2, 17.91± 0.1 and10.68± 0.2 mg/100 g respectively as against control pH value of 14.07 ± 0.1 mg/100 g. A similar trend of reduction in mineral nutrient contents according to pH levels and variations in plant parts was also obtained for K, Na, Mg, Cu, Mn, and P (Table 5).

IV. Discussion

a) Impact of SNAR and SSAR on phytochemicals

Quantitative determination of phytochemicals in plant parts of *T. occidentalis* treated with SNAR and SSAR and the untreated control revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing sugars, steroids, and terpenoids in both aqueous and ethanol extracts while phlobatanins, anthraquinones, and hydroxymethyl anthraquinones were absent in both extracts. The impact of simulated nitric and sulphuric acid rain on *T. occidentalis* provoked significant (P=0.05) reductions in some of the investigated phytochemicals; alkaloids, glycosides, saponins, tannins, flavonoids, polyphenols, reducing sugars, steroids, and terpenoids. Phytochemicals are of great importance to the health needs of the people. The reduction in these phytochemicals orchestrated by SNAR and SSAR impact has positive and negative effects. *Telfairia occidentalis* is a rich source of phytochemicals like other vegetables. These plant chemicals have both therapeutic and protective potentials essential in disease prevention and maintenance of the state of well being, by stimulating catalysts (enzymes) in the liver that neutralize some carcinogens and helping the body stimulate others.

Phytochemicals present in this vegetable are responsible for these health benefits. Tannins are used in the treatment of ulcerated or inflamed tissues and cancer prevention. Thus, tannins contained in this vegetable may serve as a potential source of bioactive compound in the prevention and treatment of cancer. Plants rich in tannins have been used in Ayurvedic medicine for the treatment of diarrhea, leucorrhoea, and rhinorrhoea (Douglas et al., 2009). Tannins are polyphenols that are astringent, making them useful in drawing tissues together thus, limiting blood flow. They help to maintain healthy circulation and strengthens capillary (Ejike and Ajileye, 2007). Flavonoids are polyphenolic compounds widely distributed in many

plant varieties. Flavonoids are antiviral, anti-inflammatory, antitumor and anti-platelets agents. They are potent antioxidants that are soluble in water, they are free radical scavengers, which prevent oxidative cell damage and possess anticancer activity. Hydroxyl flavonoids are responsible for the free radical scavenging effects of most plants (Usunobun and Egharebva, 2014). Flavonoids aid in the alleviation of cholesterol levels in patients with cardiovascular complication reduces high blood pressure. They also reduce the chances of heart disease. Isoflavones help to reduce osteoporosis and menstrual pains in women. The phytochemical; proanthocyanidins possess the ability to improve dental health and also to reduce urinary tract diseases. They fight atherosclerosis. Phytochemicals help to boost the immune system, reduce chronic inflammation which is of immense benefit to obese individuals with inflammatory markers (http://benefits-of-phytochemicals/). Saponins; triterpenoid saponins are useful for skin care that found in a herb licorice root (Glycyrrhiza glabra). This saponin promotes nutrients absorption. The major ingredient in many medicinal plants is saponins. Triterpene, sponins, and their aglycones are used as analgesic, antioedema and antimicrobial, anti-inflammatory, antipyretic, antiulcerogenic, and fibrinolytic agents (Mofunanya and Nta, 2016).

b) Impact of SNAR and SSAR on proximate nutrients

Proximate nutrients of acid rain treated and control plant parts (leaf, stem and root) varied in amount of nutrients. SNAR and SSAR plant parts had lower proximate nutrients than the control. The leaf samples of *T. occidentalis* had the highest amount of nutrient, followed by the stem and then the root. However, fiber content was higher in stem than in the leaf and root. Acid rain impact was evident in the reduction of ash, protein, fat, fiber and carbohydrate when compared to the control. Reductions in these proximate nutrients due to acid rain stress are in line with similar reductions in these nutrients in Amaranthus hybridus leaf, stem, and root treated with SNAR and SSAR (Mofunanya and Egah, 2017). Reduction in N which is a component of protein in apple leaves at low pH has been documented (Proctor, 1983). Kong et al. (2000) in their research reported that acid rain caused an increase in free oxygen radicals and a decrease in protein in various organs. The reduction posed by acid rain impact on these nutrients is disturbing because of their essentiality to health and wellbeing. Proteins are compounds made up of smaller units of amino acids. When they are broken down during digestion amino acids are released, which are the building blocks of all protein. Once present in the human body, these amino acids are used in the synthesis of new proteins including enzymes which are proteins and hormones such as adrenalin (''fight and flight' hormone). Proteins are also energy source. Proteins are vital in the maintenance of muscle mass and helpful after strenuous exercise. The needful role of dietary protein in the body is to supply amino acids for the construction of human proteins. All amino acids are necessary for the synthesis of protein, although cells in the human body have the potential to synthesize eleven (11) amino acids from raw materials; the remaining nine (9) cannot be synthesized by the body. These nine amino acids are called essential amino acids and must be obtained from plant food (diet). These essential amino acids cannot be stored by the body. Insufficient amount of these essential amino acids prevents the synthesis of necessary proteins resulting in protein deficiency diseases (Levetin and McMahon, 1999). Deficiency of protein causes wasting and shrinkage of muscle, anemia resulting from the inability to deliver enough oxygen to the cells, caused by lack of dietary iron, edema; a build-up of fluids in the feet and ankles. It causes slow growth in children. Fibers in diet play a role in disease prevention and treatment of colorectal cancer, diabetes, weight loss, high cholesterol, obesity, heart disease and gastrointestinal disorders such as constipation, diarrhea. It promotes Ca absorption. Ash in plant food is very important in that it contains all the mineral nutrient; micro and macronutrients. Carbohydrates are indispensable energy source. Fat in food increases the palatability of food by absorbing and retaining flavors (Antia et al., 2006).

c) Impact of SNAR and SSAR on amino acids

Amino acids profile of T. occidentalis leaf, stem and root revealed the presence of histidine, lysine, arginine, aspartic acid, threonine, cysteine, glycine, glutamic acid, serine, valine, proline, methionine, leucine, isoleucine, tyrosine, and phenylalanine. These amino acids were present in both SNAR and SSAR and in control plant parts. The present of these amino acids have been reported in T. occidentalis (Tindall, 1992; fasuyi, 2006) and in other cases of biotic stress (Mofunanya et al., 2009; Mofunanya, 2016).

Amino acids are the core of orthomolecular medicine. The field of medicine that describes the practice of optimizing bodily functions, in the prevention and treatment of diseases by providing the body with optimal amounts of natural nutrients such as vitamins, dietary minerals, proteins, antioxidants, amino acids and fatty acids. Findings of this research revealed significant reductions in histidine, lysine, arginine, threonine, cysteine, glycine, serine, methionine, leucine, isoleucine, tyrosine, and phenylalanine in plant parts of *T. occidentalis* due to SNAR and SSAR impact. SNAR and SSAR impact however, caused asignificant increase in glutamic acid, aspartic acid, proline, and valine. Accumulation or increase in proline and other amino acids is a usual response of higher plants to biotic and abiotic stress. Many plants accumulate high quantity of

proline in their tissues (Mazid et al., 2011; Mofunanya et al., 2009; Mofunanya et al., 2015). The reduction in amino acidsis of concern as they keep the body healthy and regulate virtually almost all of the metabolic processes in the human body. The human body uses amino acids in three ways; in the synthesis of protein, acting as precursors of other compounds example, the brain chemical serotonin (neurotransmitters) and as anenergy source. The human cannot store amino acid, so they must be obtained from plant food daily. Some of these amino acids, for example methionine, is an essential amino acid required for the synthesis of another amino acid cysteine. Like carbohydrates and fats, amino acids are also sources of energy but differ from carbohydrates and fat because they contain nitrogen. They are precursors of enzymes and neurotransmitters. Amino acids have the capability of forming tissues, organs, muscles, skin, and hair. Singly or in combination, amino acids are vital for body functions. Methionine and arginine help to fight arthritis. Amino acids glutamine and cysteine and arginine strengthen the immune system. Leucine aids in muscle and strength improvement. Carnitine and leucine, isoleucine and valine support weight loss. Arginine, methionine, and cysteine are responsible for better hair, skin and nails. Lysine, glycine and proline help in boosting the natural skin and nails beauty. Arginine and carnitine in combination treatment with zinc, magnesium, Chromium, and omega-3 regulate better blood sugar, therefore used in blood management. Arginine, lysine, zinc, and vitamin C improve digestion and protects from rectal diseases. Arginine and Ginkgo biloba help to improve blood circulation, increase oxygen, and nutrient availability with the ear thus, their use tinnitus treatment. Arginine, carnitine, and taurine provide cholesterol-lowering effects. Tryptophane is used in sleep and mood management. Arginine aid in the production of keratin, to minimize disease-related hair loss by enhancing immune function and protects from damaging of hair color and bleaching. Cysteine is a component of keratin, it reduces symptoms of androgenic alopecia and as the precursor to glutathione indirectly protects the hair follicles from oxidative stress. Methionine is a component of keratin, a vital synthesis of the precursor of collagen called pro-collagen, it also protect the hair follicles from oxidative damage and slows the graying of hair and hair thinning. Lysine simulates collagen to repair hair. Hair follicles also require lysine to function. Hair loss is reduced with lysine. Glycine is not only important for the digestive and central nervous system but also help in collagen production. Proline a non-essential amino acid helps in the production of collagen and cartilage, as well as maintaining the muscle tissues. SNAR and SSAR impact caused a significant increase in amino acids aspartic acid, glutamic acid, proline, and alanine. Plant disturbances with other stressors have been reported to cause increase in these amino acids in leaves of *Telfairia occidentalis*, Amaranthus hybridus and seeds of Sphenostlylis stenocarpa (Mofunanya et al., 2009; Mofunanya et al., 2015; Mofunanya, 2016).

d) Impact of SNAR and SSAR on mineral nutrients

Mineral nutrients reduction induced by simulated acid rain impact is threatening as these are essential to human health. Results of mineral nutrient of leaf, stem, and root revealed a significant decrease in K, Na, Ca, Mg, Mn, Cu, Zn, Fe and P due to SNAR and SSAR impacts when compared to the control. Results of this investigation are in agreement with previous findings of the minerals in A. hybridus due to acid rain effect (Mofunanya and Egah, 2017). Acid rain of low pH caused a decrease in nitrogen concentration in apple leaves with no effect in levels of K+, Ca2+ and Mg2+. Reductions in these mineral nutrients present problems, when in excess are harmful to the body and when available in desired levels, they contribute immensely to wellbeing. Minerals exercise a role in nearly every human body function ranging from building healthy bones and teeth to energy production, support to the immune system. They are so crucial to health that even slight imbalances of some minerals can produce harmful effects; ranging from low energy levels to severe gastrointestinal problems (http://younevity.com/index.cfm/my-profile). Trace minerals are used to neutralize the raw, infected cells in the throat, help the skin to heal faster and reduce scarring, and also for wound cloterization. They heal from sickness. Trace minerals contain Fe which help in the formation of hemoglobin in the blood, and in turn transports oxygen to the cells (http:// www. oohoi. com/ healthy- living/ vitamin- info. benefits-of-iron.htm). They also contain Mg and are used in painful menstrual cycles to proffer fast and better pain relief in women than placebo. The Fe present in trace minerals replaces the Fe lost due to high menstrual flow. Low Fe levels can lead to fatigue (http:// www. elliotthealthcare. com/ iron- decficiency. htm). Trace minerals improve sight. Zinc is essential for the transport of vitamin A from the liver to the eye, also needed to quench free radicals. Trace minerals help to curb cravings.

V. Conclusion

Fluted pumpkin leaf, stem, and root showed a wide array of nutritional distinctiveness. This study has revealed that individually simulated nitric acid rain and simulated sulphuric acid rain impacted the medicinal status of *Telfairia occidentalis*. Fluctuation in nutrient contents occasioned by simulated acid rain does not allow the consumers of this vegetable to know the exact amounts of nutrients taken in at any given time. The increase in content of these nutrients creates excess amounts and decrease, insufficient amounts altering their efficacy with attendant health problems.

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Conflicts of interest

I declare that no conflicts of interest exist.

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Cosmetics and its Health Risks

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Abstract- The use of cosmetic products is increasing around the world and a variety of chemical compounds used in the manufacture of these products grows at the same time. In this way, the risk of intoxication, allergic processes, prolonged chemical exposure, side effects and indiscriminate use are also increased. The present work aims to highlight the biological risks that cosmetics can pose to human health against the toxic substances used in its formulation. This work is an integrative literature review structured according to the methodology described by Whittemore, R. and Knafl, K., 2005. This work relates the main toxic chemical substances present in cosmetic products to the possible health complications reported in the scientific literature. Currently, the cosmetic industries have increased the use of compounds with preservative action, surfactant, fragrances, stains, etc. in the formulation of cosmetic products. Such substances potentiate the quality, property and shelf life of cosmetics, but on the other hand, many of these substances are toxic to the human body, presenting health risks ranging from a simple mild hypersensitivity reaction to an anaphylactic process or even a lethal intoxication. Thus, the indiscriminate use of cosmetics may present itself as an emerging issue of public health. In view of the above, this work seeks to encourage improvements in the search for new methodologies for quality control in the production and consumption of cosmetic products around the world.

Keywords: *cosmetics, biological risks, toxicity, quality control, adverse effects.*

GJMR-B Classification: *NLMC Code: QT 275*

COSMETICSANDITSHEALTHRISKS

Strictly as per the compliance and regulations of:

Cosmetics and its Health Risks

Jonathas Xavier Pereira [α] & Thaís Canuto Pereira [σ]

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I. Introduction

Historically, cosmetics began to be used 6.000 years ago and its use has spread throughout the world. Such products had the purpose of adorning and perfuming the body, so as not to alter the structure and function of the skin. In ancient Egypt, records point to the use of cosmetics containing lead-rich black pigments (Kohl) applied to the eye region. Lead in turn, when in contact with the skin, releases gaseous nitric oxide capable of activating the immune system through vasodilation and activation of macrophages with cytotoxic characteristics [1].

Cosmetics and skin care products are currently consumed worldwide, with frequent use, increasing the exposure of the human body to the various chemical compounds that make up its formulas. Stipulating the incidence of cosmetic side effects is quite difficult because users with weak side effects often do not seek medical advice [1,2].

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The side effects derived from the use of cosmetics pose health risks mainly due to exposure to numerous chemical substances. Its consequences can range from a simple mild hypersensitivity reaction to an anaphylactic process or even a lethal intoxication [1–3].

a) The beginning of complications due to the use of cosmetics

The use of pigmented lead-based cosmetics used by Egyptians is characterized as the earliest evidence of the use of cosmetics and their complications. Subsequently, rouges and lipsticks appeared whose reddish coloration was attributed to mercury sulfide. Such a compound, when ingested by pregnant women, caused miscarriage spontaneously. Another toxic compound capable of causing damage to the organism was the arsenic used by Greeks and Romans in chemical depilatory solutions [1].

With the advancement of knowledge about the physiology of skin and its components, the pharmaceutical industries started to invest in new active principles and vehicles for the production of cosmetics. Thus, new quality control tests in the manufacture of such cosmetics must be also updated in order to ensure safety in the use of such compounds [4].

Several regulatory agencies around the world are dedicated to the control and regulation of commercial activities, safety and quality control of cosmetics. Although there are rules and quality control tests to be followed for the manufacture of a cosmetic, these regulatory mechanisms are not fully effective, as the adverse effects still persist in the cosmetic consumer population. [2].

b) The cosmetic risk and the public health

In front of the arising use of cosmetic products and the larger exposure to the formulas compounds for a large time and frequency, the side effects of these products become more frequent in the population around the world. Women and men all over the world use large amount of cosmetic products in pursuit of everlasting youth, ignoring the probable health risks [5].

Cosmetic ingredients are emerging pollutants too. Their environmental monitoring is at a very early stage. However, it is known that they reach the environment in multiple ways, often through water, posing health risks to marine and freshwater ecosystems and to humans [5].

Thus, in public health science, the term “cosmetovigilance” began to represent a kind of health surveillance where the aim is the safety of the cosmetic

product for commercial purposes. This surveillance is very important to control potentially hazardous ingredients and can thus set our minds at ease on the products placed on the market [6].

The restrictions on the use of some cosmetic ingredients are stipulated by several health surveillance agencies around the world mean that any ingredient not on the restriction's list be allowed. Thus, as the industry is quite creative and is always looking to improve its products, it is constantly using new ingredients not listed in the restriction's list. Such ingredients are new potential allergens. Unlike medicines, there is no a specific agency to assess the safety of cosmetic products, no marketing authorization with specific requirements, no evaluation of the risk-benefit ratio and no guarantee of constancy from one batch to another [6].

The health risks associated with the use of cosmetic products become currently an emerging public health problem, where about 12% of users in the general population had experienced undesirable effects with one or several cosmetic products in the last nine years [5,6].

II. Methodology

This study is an integrative literature review based on the model proposed by Whittemore and Knafl, where it aims to relate clinical and toxicological aspects in the use of cosmetic products [7]. This review has selected a total of 32 studies published between 1998 and 2015, derived from the PubMed database, as shown in Figure 1.

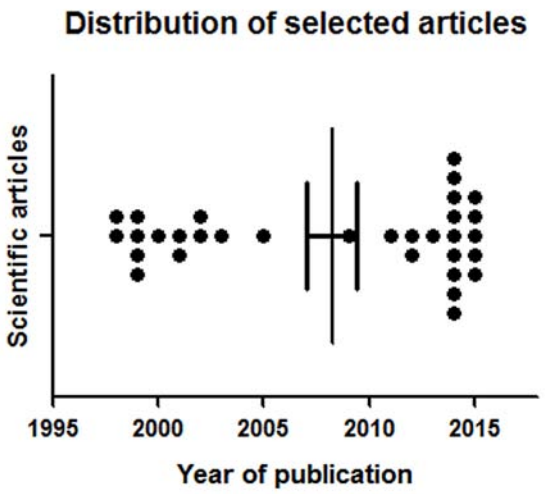

Fig. 1: Distribution of selected articles by year of publication. The graph shows the average year of publication and the standard deviation of the mean

The scientific articles followed the inclusion criteria: articles that contain the descriptors "cosmetics toxicity"; "cosmetic intoxication"; "cosmetic risk"; "cosmetic danger"; "cosmetic side effects".

The scientific articles followed the exclusion criteria: articles related to cosmetic surgery or that do not relate to cosmetic products were excluded.

The selection of scientific articles included reviews and original articles with a toxicological and clinical approach. This methodology aims to relate the main toxic chemical substances present in cosmetic products to the possible health complications reported in the scientific literature. Through this integrative study, the clinical-toxicological correlation becomes a valuable instrument to clarify and understand the side effects in using cosmetics, drawing attention to the neglected use of these products and highlighting the associated health risks.

III. Substances with Toxic Potential in the Formulation of Cosmetics

In face of technological innovations in the cosmetics industry, many of these products have been added with chemical additives to increase their performance, effectiveness and viability [8]. Some examples of these chemical additives are Diazolidinyl Urea, Dioxane, Formaldehyde and Paraformaldehyde, Imidazolidinyl urea, heavy metals, Methylchloroiso-thiazolinone-methylisothiazolinone (MCI-MI), Methyld-ibromoglutaronitrile-phenoxyethanol (MDBGN-PE), Parabens, Phthalate, Quaternium-15, Thimerosal and others.

a) Diazolidinyl Urea

It is an additive used since 1982 in the manufacture of personal care products such as child care products, eye and face make-up, skin care products, hair and nails [9]. Figure 2 shows its chemical formula.

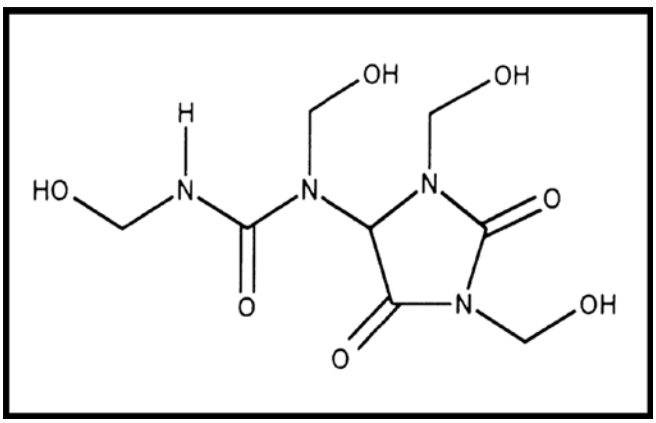

Fig. 2: Chemical formula of Diazolidinyl Urea

Exposure to this compound is capable of causing allergic contact dermatitis, as well as being characterized as a mutagenic and carcinogenic agent since it is capable of releasing formaldehyde, a fixative and preservative which will be discussed later [9].

b) Dioxane

1,4-dioxane (Figure 3) is an ether with an emulsifying, detergent and solvent function commonly found in products such as shampoo, toothpaste and mouthwash [8].

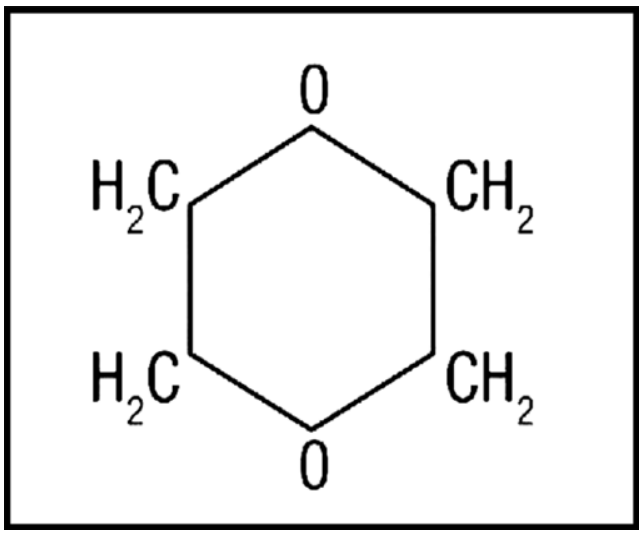

Fig. 3: Chemical formula of 1,4-Dioxane

Although this compound is not listed as a cosmetic component, this substance is a contaminant in the manufacturing ethoxylation step, creating other ingredients such as polyethylene glycol, polyethylene and polyoxyethylene. Thus, high levels of this contaminant can be observed in cosmetic products, being such chemical substance a potent carcinogen, capable of triggering cancer of breast, skin and liver. [8].

c) Formaldehyde and Paraformaldehyde

Formaldehyde and paraformaldehyde are toxic preservatives, the paraformaldehyde being a polymer derived from formaldehyde (Figure 4) [10].

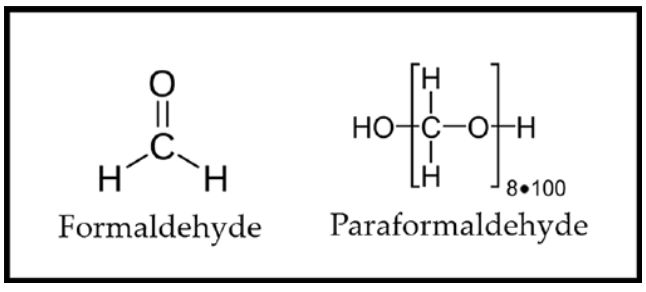

Fig. 4: Chemical formula of Formaldehyde and Paraformaldehyde

Formaldehyde has molecular characteristics that result in an agent of great potential risk to cancer [9]. Clinical studies show that 13% of a sample of 957 participants had allergic contact dermatitis caused by formaldehyde, which is the second largest cause of contact dermatitis from cosmetic products [11].

In a study conducted by Agner, et al., 1999, 57 patients were evaluated for their exposure to formaldehyde. For this analysis, the participants were asked to bring the main cosmetics that were used by them on a daily basis. In total, 409 products were cataloged, and among them, 103 had formaldehyde in their compositions [12].

d) Imidazolidinyl Urea

Imidazolidinyl urea (Figure 5) is a preservative used in cosmetic products which also has the property of releasing formaldehyde as a consequence of its degradation, like Diazolidinyl Urea [3,13].

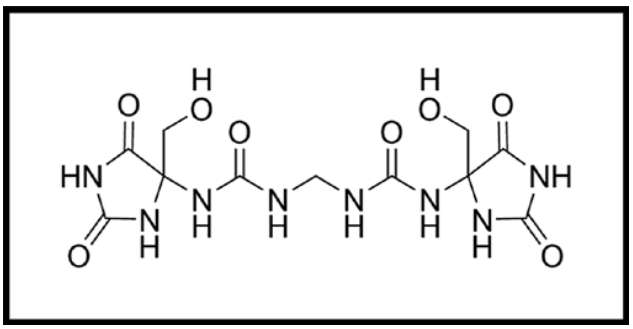

Fig. 5: Chemical formula of Imidazolidinyl Urea

Concentrations of 0.01% to 1% of Imidazolidinyl urea at 24 hours in contact with a culture of cells from human peripheral blood were considered moderately cytotoxic doses. At concentrations of 0.1% to 0.5%, the same effect was observed in only 3 hours [14].

Clinical trials point to Imidazolidinyl urea as a causative agent of contact dermatitis allergies [3,11,15].

e) Heavy metals

A group of hazardous substances in the manufacture of cosmetics is toxic heavy metals, such as lead (Pb), cadmium (Cd), nickel (Ni), arsenic (As) and mercury (Hg). Some cosmetics may contain aluminum (Al), classified as a light metal. As there is no single and effective regulation around the world, some cosmetics like colorful cosmetics, face and body care products, hair cosmetics and herbal cosmetics may contain in their formulation relatively high amounts of these heavy metals. These elements can accumulate on the skin and internal organs, causing toxic effects that can be classified into topical (mainly contact dermatitis), and systemic (systemic allergic dermatitis) [16].

Some metals serve as pigment substances, for example, chromium (Cr) used mainly eyeshadows and blushes. Pigmented cosmetics of a reddish color, for example, are commonly contaminated with arsenic (As), Lead (Pb) e mercury (Hg) [17].

Antimony (Sb) can cause pneumoconiosis, alterations in pulmonary function, bronchitis, emphysema, abdominal pain, vomiting, diarrhea and ulcers. This metal is mainly found in lipsticks, eye pencils and face powder. Arsenic (As) can cause skin disorders, circulatory and peripheral nervous disorders, an increased risk of lung cancer, and a possible

increase in the risk of the gastrointestinal tract and the urinary system cancers. This metal is mainly found in make-up powder and skin cream. Cadmium (Cd) may accumulate in the kidneys, with possible damage. Chronic exposure to low levels of cadmium can also cause bone fragility and consequent bone fractures. Cadmium is commonly found in hair creams, lipsticks and skin cream. The Chromium (Cr) in its oxidized state can cause contact allergies. Its presence in cosmetics is mainly associated with eyeliner, eye pencil, eyeshadow, lipstick and make-up powder. Cobalt (Co) and nickel (Ni) can cause allergies such as contact dermatitis, and these metals are commonly present in cosmetics such as eye shadow, face paint, hair cream and lipstick. The lead (Pb), when ingested in large quantities, may interfere with the synthesis of hemoglobin and calcium channels, whose functions are important for nerve conduction. Lead is found in dyes for hair (such as lead acetate whose chemical formula is shown in Figure 6) and lipsticks, eyeliner, eye pencil, hair cream in their inorganic form, and can be minimally absorbed by the skin [8,17].

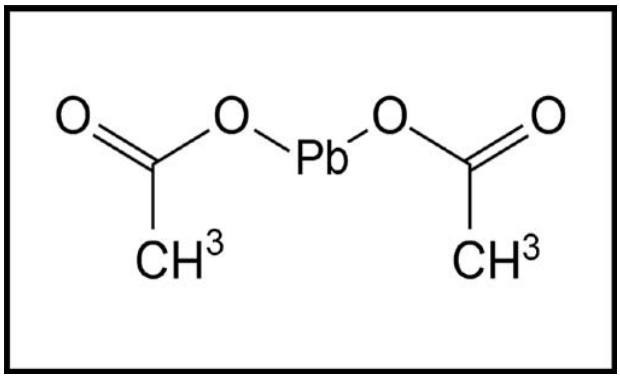

Fig. 6: Chemical formula of Lead acetate

f) Methylchloroisothiazolinone-methylisothiazolinone (MCI-MI)

MCI-MI (Figure 7) is a preservative widely used in the manufacture of personal hygiene products, which has a high degree of cytotoxicity and is dose-dependent when tested in vitro, in cultured cells [14,18]. In cosmetic products, this compound represents an important allergen and may cause hypersensitivity when there is constant exposure to this substance. [19].

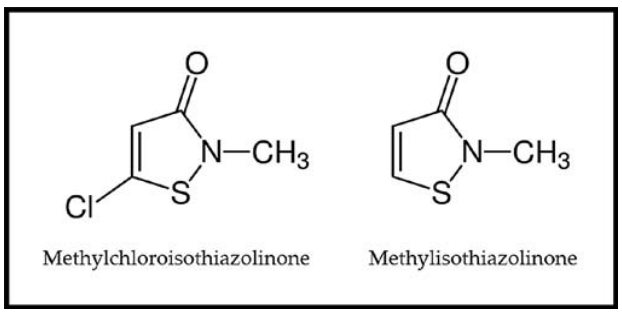

Fig. 7: Chemical formulas of Methylchloro-isothiazolinone-methylisothiazolinone (MCI-MI)

g) Methyldibromoglutaronitrile-phenoxyethanol (MDBGN-PE)

MDBGN-PE (Figure 8) is also a preservative used in the manufacture of cosmetics such as, for example, moisturizing creams. This compound is the preservative with the highest potential to cause contact dermatitis, presenting an average of 14.5% incidence on the population [19,20].

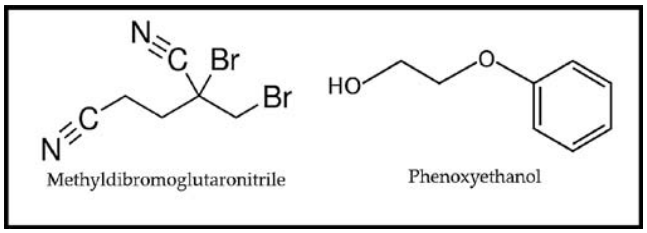

Fig. 8: Chemical formulas of Methyldibro-moglutaronitrile-phenoxyethanol (MDBGN-PE)

h) Parabens

Parabens are esters of p-hydroxybenzoic acid, with alkyl substituents ranging from methyl to butyl or benzyl groups. Parabens are also a group of substances for the purpose of preservation. Some examples of these chemical additives are methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben (Figure 9). Its use is also associated with antimicrobial action and is widely used in the cosmetics industry because it has a low cost for its implementation in the production. Methylparaben and propylparaben are the most commonly used and often present in the cosmetic products together [21,22].

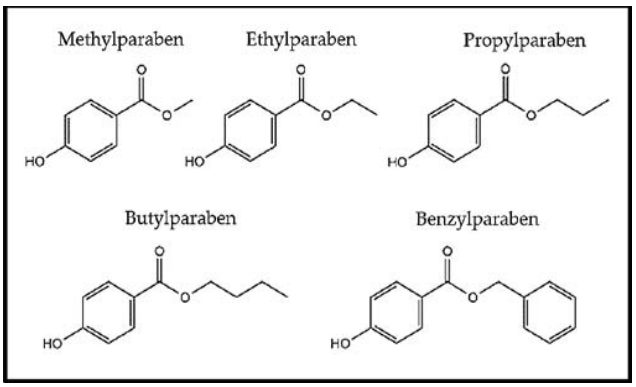

Fig. 9: Chemical formulas of Parabens

In the past, the use of parabens in higher concentration triggered allergic reactions in the population. Nowadays, in conjunction with the change of safety resolutions for cosmetics and hygiene and beauty products, the use of parabens has become very low, being used in low concentrations, which reflects in a reduction in the positive cases related to allergic reactions or contact dermatitis [23].

i) Phthalate

Phthalate esters are derivatives of phthalic acid and often found in products as plasticizers, solvents,

and alcohol denaturants. Phthalate compounds may be found in a diverse number of cosmetics (nail polish, lotions, hair care products, perfumes). The most commonly encountered forms are dimethyl phthalate and diethyl phthalate, and their chemical formulas are shown in Figure 10 [8].

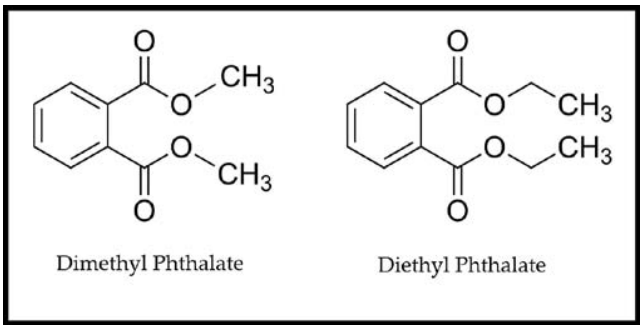

Fig. 10: Chemical formulas of Dimethyl phthalate and Diethyl phthalate

Exposure to these components can trigger developmental disorders and breast cancer. Some experimental studies have shown that high levels of phthalate could change hormone levels and cause genital-related birth defects in rodents [8].

j) Quaternium-15

Quaternium-15 (Figure 11) is a common contact allergen included in the European baseline series. The prevalence of contact sensitization is as low as 1.2–1.6%, but could represent the first event leading to systemic anaphylactic reaction through a switch from type IV to type I allergy [24].

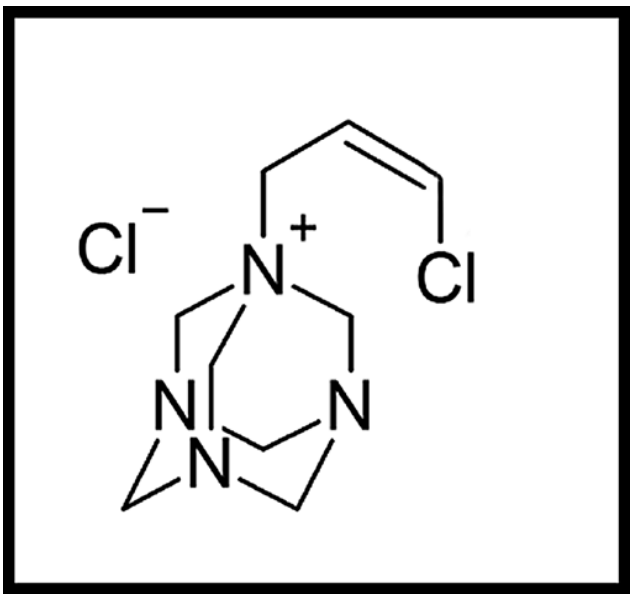

Fig. 11: Chemical formula of Quaternium-15

k) Thimerosal

Thimerosal (Figure 12), a mercuric derivative of the thiosalicylic acid, is a preservative used in several types of consumer products, including cosmetics, ophthalmic and otolaryngologic medications, and vaccines [26].

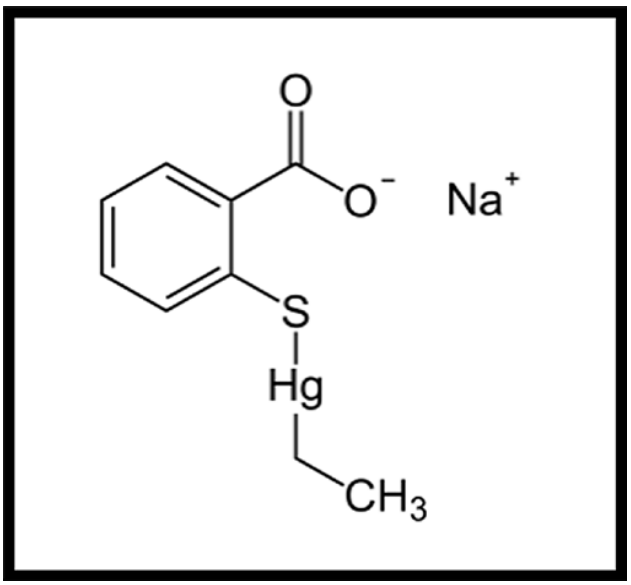

Fig. 12: Chemical formula of Thimerosal

Due to a large number of allergic reactions and environmental problems, its use has been diminished in the last two decades [26]. Thimerosal is used primarily for the conservation of eye shadows, make-up removers, masks, and soap-free cleansers [27].

l) Others substances

Although the preservatives mentioned above are the substances responsible for most adverse effects from cosmetics, other substances have allergenic potential, for example:

- Fragrances (Myroxylon Pereirae);
- Chemical contamination to obtain surfactants (Cocamidopropyl betaine) used in the production of shampoo, liquid soap, skin cleansers, shower gels, and deodorants;
- Oxidizing agents (Paraphenylenediamine) very common in hair dye composition;
- Glyceryl monothioglycolate, used in permanent hair solutions (to be curled or waved hair);
- Toluenesulfonamide-formaldehyde resin, used in the manufacture of nail polishes and lacquers;
- Propyl gallate, octyl gallate, and dodecyl gallate are all antioxidants used to prevent the deterioration of unsaturated fatty acids that can cause discoloration and odor, present in the composition of cosmetic creams and lotions [25,28].

IV. Possible Health Complications Associated with the use of Cosmetics

Due to the presence of numerous components in the formulation of cosmetic products, such products

have the potential to cause side effects and their consequences can range from a simple mild hypersensitivity reaction to an anaphylactic process or even a lethal intoxication [1–3].

There are many types of adverse reactions caused by cosmetics. Most adverse reactions are irritant, however, type IV hypersensitivity, contact urticaria, photosensitization, pigmentary disorders, damage of hair and nails, paronychia, acneiform eruptions, folliculitis, and exacerbation of an established dermatosis may also occur. Side effects of cosmetic products do occur. It is to be expected that the improvements in safety, tolerance, and skin compatibility will not prevent side effects to cosmetic products from increasing in the future because of the continuing goals to intensify their biological activity and therapeutic efficacy. [25,29].

The areas of the body most affected by adverse reactions attributed to the use of cosmetics are head and neck, and irritant dermatitis is the most common type of complication [25,29]. The health complications associated with the use of cosmetic products can be:

a) Allergic reactions to cosmetics

Allergic reactions to cosmetics constitute a small but significant portion of the complications associated with the use of cosmetics. Allergic contact dermatitis represents true delayed-type (type IV) hypersensitivity that presents eczematous dermatitis and comprises approximately 10% to 20% of all cases of contact dermatitis. Type IV is hypersensitivity reaction that is T-cell mediated, wherein circulating or resident sensitized T cells are activated by the offending allergen to release pro-inflammatory cytokines. Sensitization depends on several factors including product composition, a concentration of potential allergenic components, amount of product applied, site application, skin barrier integrity, and frequency and duration of application [2,19,25,29].

This clinical scenario can range from mild erythema and scale with a minimal itch to vesicular, bullous, indurated plaques that are intensely pruritic. Initial sensitization is required for the subsequent expansion of a reaction when exposure occurs again [19].

Allergic contact urticaria is immediate-type hypersensitivity that represents a true allergic reaction. As the name implies, the reactions occur within minutes to hours and might be limited to the site of exposure on the skin or, in severe cases, reactions can be generalized. Contact urticaria is a rarer reaction to cosmetics and skin care products that may be an immunologic or nonimmunologic reaction. It is characterized by the development of a wheal-and-flare response to a topically applied chemical. The spectrum of clinical presentation ranges from itching and burning to generalized urticaria to anaphylaxis. In highly allergic individuals, mucosal exposures, or large exposures, the symptoms of immediate-type hypersensitivity can generalize and include conjunctivitis, cough, bronchoconstriction, hypotension, anaphylaxis, and, occasionally, death. [2,3,19].

b) Irritant contact dermatitis

This is the most commonly encountered type of complication due to the use of cosmetics, especially those containing methylchloroisothiazolinone-methylisothiazolinone (MCI-MI) in its formulation. There are currently over 57.000 described irritants worldwide, ranging from weak or marginal irritants to strongly corrosive acids and bases. Then, the majority of facial problems that arise with skin care products and cosmetics are of the irritant contact dermatitis type manifesting as erythematous, burning, pruritic skin that may develop microvesiculation and later desquamation. The dermatitis is characterized by stratum corneum damage without immunologic reaction [3,19].

Facial irritant dermatitis, which results mostly from cosmetics, presents as papules and plaques. Another common presentation is a “seborrheic-like dermatitis” with pink scaly plaques on the cheeks and chin. Less commonly, patients may develop urticarial or infiltrated plaques [28].

c) Photoallergic dermatitis

This type of allergic reaction occurs after contact with cosmetic products and subsequent exposure to light. Usually, such a reaction presents itself as sunburn that may be followed by hyperpigmentation and desquamation. This reaction is formed by chemical substances capable of absorbing radiation, especially ultraviolet A, in addition to having no definite immune mechanism. Its clinical manifestations vary from erythema, edema to vesiculation. The incidence of photoallergic dermatitis is low, and is mainly caused by fragrances methylcoumarin and musk ambrette, antibacterial agents and the para-aminobenzoic acid esters as sunscreening agents [3].

Photoallergy is an uncommon acquired altered reactivity dependent on an immediate antibody or a delayed cell-mediated reaction [30].

d) Facial stinging

There is a group of patients who note stinging or burning within several minutes after applying a cosmetic that intensifies over 5 to 10 minutes and then resolves after 15 minutes. This effect occurs without the patient exhibiting allergic contact dermatitis or irritant contact dermatitis with the applied substance. Tests should be done on the skin of the patient before using such components. Usually, substances such as benzene, phenol, salicylic acid, resorcinol and phosphoric acid are the main cause of facial stinging [3].

e) Redness

The redness of the skin caused by cosmetic products, especially soaps, is associated with the unbalance in cutaneous pH. Modern soap is a blend of tallow and nut oil, or the fatty acids derived from these products, in a ratio of 4:1. This fact allows the pH of these soaps to be commonly alkalized (pH 9-10), which can generate redness in the skin, which normally has a pH of 5.2-5.4. Ideally, such compounds should have neutral or slightly acidified pH [3].

Another reason redness can occur is the use of moisturizers with a greater oily proportion, allowing skin warming throughout the day [3].

V. Results

Today, innovation, research and development of new cosmetic products have increased the use of compounds with preservative action, surfactant, fragrances, stains, etc. Such substances enhance the quality, property and shelf life of cosmetic formulations, but on the other hand, many of these substances can be harmful to human health because of frequent, prolonged and indiscriminate exposure.

There are several agencies around the world to regulate the quality control, safety and production of cosmetic products, which are responsible for adjusting the standards and guidelines for the safe and healthy use of such products by the population, minimizing health risks. However, there is no specific agency that regulates the cost-benefit, guarantee of safety in the use of substances with toxic potential when applied to cosmetic products.

This work reviewed several substances with potential toxic to the human body, which can be found in the formulation of various cosmetic products around the world. We also reviewed the possible health complications reported by the scientific literature that are associated with the use of cosmetics and attributed to such toxic substances.

The scientific literature reveals that high amounts of chemical preservatives, perfumes and emulsifiers used in the manufacture of cosmetic products increase side efects and health risks through chemical and physical principles [31].

Chemically, preservative compounds generally have chemical structures associated with aromatic rings which generally have toxic potential, and ability to bind to metal elements that promote bioaccumulation in the body [32]. Although not all compounds that present toxic potential have their toxicity mechanism clarified in the literature, clinical evidence obtained by the side effects after the use of these substances point to the health risk associated with the use of cosmetics. The health risk associated with the use of cosmetics can ranging from a simple mild hypersensitivity reaction to an anaphylactic process or even a lethal intoxication. Cancer is also a complication associated with the use of cosmetics in the face of clinical evidence reported in the literature [33].

Faced with the occurrence of side effects, the imminence of complications associated with the use of cosmetics contribute to an emerging public health problem, it is concluded that the process of quality control in the manufacture of cosmetic products is not completely effective in preventing health risks associated with the use of cosmetic products.

VI. Conclusion

The cosmetic products may present health risks and recurrent adverse effects are attributed to the toxic substances commonly found in their formulations. Although the various structures for the regulation and quality control of cosmetics around the world are quite complex and comprehensive, they should be more rigorous in the inclusion of new substances with toxic potential in the formulation of cosmetics to avoid damages to human health.

To encourage improvements in the manufacture, marketing and use of cosmetic products by the population, it is necessary to apply a unified cosmetovigilance around the world. This public health strategy are a genuine means of obtaining information on the safety of cosmetic products and their ingredients, preventing the risks associated with using cosmetics become a serious public health problem.

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Conflict of Interest

The authors state that there is no conflict of interest.

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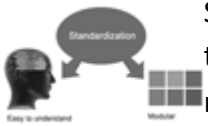

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Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

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Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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