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Preliminary Phytochemical Ethnopharmacological Surveys

Moringa Oleifera to Chelate Medicinal Plants uses Discovery

Highlights

Discovering Thoughts, Inventing Future

VOLUME 15 ISSUE 5 VERSION 1.0

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Preliminary Phytochemical Investigations with Quantitative Fractionation of Orange Pulp (*Citrus Aurantium Var.* Dulcis L.): Natural Product Waste as Medicine

By Rufai Y & Fatimah S
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Abstract- Day by day, faith of people on herbal medicine increases due to the side effect of synthetic drugs; this has resulted into people falling back to the traditional knowledge of plant for their health care. Certain local practitioner and traditional healers use the fruits of *Citrus aurantium var.* Dulcis L pulps in various disease management and so, they advise to eat the pulps along with the drinking of the juice. The present study deals with preliminary phytochemical analysis of the fruit of *Citrus aurantium var.* Dulcis L pulp using 95% ethanol for its extraction. The fruits of *Citrus aurantium var.* Dulcis L pulp ethanolic extract revealed the presence of all tested phytochemical compounds except protein and glycoside. These include Alkaloids, Tannins, Phenolic, Quinine, Reducing Sugar, Coumarins, Flavonoids, Saponins, and Steroids. During the analysis, the quantitative fractionation of the ethanolic extract showed a reasonable amount of saturated hexane fraction (40g), unsaturated hexane fraction (2.0g), methanolic fraction (1.3g), acidic fraction (1.2g) and basic fraction (0.3g).

Keywords: citrus aurantium var. dulcis L pulp, preliminary phytochemical analysis, quantitative fractionation.

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Preliminary Phytochemical Investigations with Quantitative Fractionation of Orange Pulp (*Citrus Aurantium Var.* Dulcis L.): Natural Product Waste as Medicine

Rufai Y [α] & Fatimah S [σ]

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Keywords: citrus aurantium var. dulcis L pulp, preliminary phytochemical analysis, quantitative fractionation.

I. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [1]. The most important of these bioactive constituents of plants are Alkaloids, Tannins, Flavonoids, and Phenolic compounds [2]. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [3;4]. This field of natural products research is currently being carried out intensively though it remains far from exhaustion. An attempt to obtain bioactive agents from plants is a worthwhile exercise since only 10% of all plants have been investigated in detail [5]. However, as at the time of this study, a higher percentage of bioactive compounds could have been discovered. The majority of these bioactive compounds are Sesquiterpenes, Diterpenes, Triterpene Saponins, Triterpene Aglycones, and Monoterpenes. It is imperative that ethnobotanical researches and phytochemical tests have led to some patent-able and industrially exploitable compounds for drug development. Plants fulfill the needs of not only human being but also entire animal kingdom.

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Man as a unique creation of God[6] is but a part of the universe that relates domestically with other living creatures, man has been provided with food, water, shelter and herbal medicine around his habitat. However, the orange pulps of the fruits of *Citrus aurantium var.* Dulcis L. which is part of man's food are more beloved to domestic animals like goat and sheep for reason not yet proven scientifically. Domestic animal kept looking at human when eating or drinking such food and most times compete with them self in eating the thrown away part by human.

The popular orange tree (*Citrus aurantium var.* Dulcis L.*)* belongs to the plant family *Rutaceae*. It is a small tree with grayish-brown branches that are widely spread. The petioles of the leaves are winged and the leaves are ova, alternate, and have a deep green colour. The calyx is bell-shaped and bisexual flowers are pure white. The fruit is round and green and yellow when ripe. It is widely used for it juice which is sweet [7].

Table 1 : Showing uses for the various parts of *Citrus aurantium var.* Dulcis L.

Part	Medicinal Uses
Leave	The infusion of the leaves, mixed with a little honey, is used for controlling cough.
Pulp	The pulp should be eaten instead of drinking only the juice as it ease bowel movement.
Fruit	The fruit in general is good for cases of arthritis, asthma, respiratory problems, pneumonia, hysteria, neurasthenia, neuralgia, headache, colds, cough, fevers and influenza. It is highly recommended for scurvy.
Rind	Fresh rind rubbed on the face is a good remedy for acne.
Juice	The consumption of orange juice strengthens the stomach, increases variation, and is refreshing. It purifies the blood, increases thirst in fever, heals inflammation of the mucus membrane, and improves appetite. Orange juice is useful in liver problems. The rind expels gas and is a tonic
Flower	The infusion of the dried flowers is recommended for stress or nervousness.

Source: J.C. Kurian (2010). Healing Wonders of Plant. Vol. 2, Pp 40.ISBN: 978-1-907456-05-3. Zambia Adventist press, P.O. Box 31309, Lusaka, Zambia.

II. Materials and Methods

a) Chemical Used

Ethanol, Methanol, Chloroform, Ethyl Acetate, Hydrochloric Acid, Sodium Hydroxide, Hexane and all others solvents (Analytical grade) from Merck Co. (Darmstadt; Germany), and Distilled Water.

b) Sample Collection

Fresh fruits of *Citrus aurantium var.* Dulcis Lwere collected at Nagazi central market around Federal College of Education Okene, Kogi State, Nigeria. The plant was identified and confirmed at Ahmad Bello University, Zaria, Kaduna; ABU Herbarium (Botany Unit, Department of Biological Science) by Mr. Muhammad Musa, The back of the oranges were pealed, the pulps were collected after juice extraction and washed with pure water, air dried as shown below and pulverized into a fine powder using a commercial blender.

III. Extraction and Fractionation Procedure

Extraction and fractionation of the pulp ethanolic extract was carried out by bioassay guided fractionation protocol [8]. The procedure was carried out using ethanol-water (95:5v/v) and different organic solvent in order of polarity (Hexane, chloroform and Methanol) using separatory funnel to fractionate them into different fractions. One thousand grams of the powdered fruits of *Citrus aurantium var.* Dulcis L pulp materials (20 mesh~1g) were extracted using percolation process in a mixture of 95ml of distilled ethanol and 5 ml of distilled water at ambient temperature overnight. The extractives was filtered and re-extracted three times. The combined extract were filtered through a Whatman No. 1 paper and then concentrated invacuo at 40^0C using a rotary evaporator, model W2-100 SENCO® @ rpm of 100; Shanghai SENCO technology Co, Ltd Japan. The various extractive concentrates were evaporated to dryness using water bath for some days and residues were obtained in gram for basic, acidic, polar and non-polar fraction as 0.3g, 1.2g, 1.3g, and 40g.

IV. Phytochemical Screening of the Ethanolic Extract

Preliminary Phytochemical screening was done using standard procedures to identify constituents, as described [9;10]as follows. It involves testing of different

classes of compounds. The methods used for detection of various phytochemical were followed by qualitative chemical test to give idea regarding the nature of constituents present in the fruit of *Citrus aurantium var.* Dulcis L pulp ethanolic extract.

Tests for carbohydrates Fehling's test: 1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution added to the above mixture. The solution was heated in boiling water bath for 5-10minutes. First a yellow, then brick red precipitate was observed.

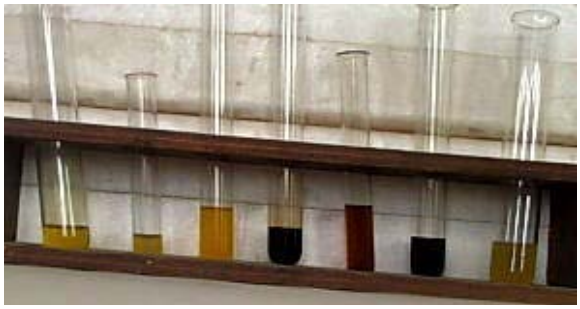

Figure (a)

a) *Benedict's Test*

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solutions appeared green showing the presence of reducing sugar.

b) *Molisch's Test*

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

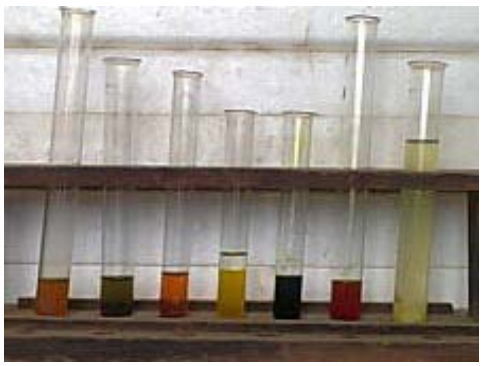

Figure (b)

c) *Test for Proteins (Biurret Test)*

To the small quantity of extract 1-2 drops of Biurret reagent was added. Formation of violet colourprecipitate showed presence of proteins.

d) *Million's Test*

To the small quantity of extract 1-2 drops of Million's reagent was added. Formation of white colour precipitate showed presence of proteins.

e) *Test for Anthraquinone glycosides*

f) *Borntrager's Test*

To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

g) *Test for Cardiac glycosides (Keller-Killiani Test)*

To the 5ml of extract, 1ml of conc. H_2SO_4, 2ml of Glacial acetic acid and 1 drop of $FeCl_3$solutions was added. Appearance of Brown ring shows the presence of cardiac glycosides.

h) *Test for Coumarins*

To the 2ml of extract 10% NaOH was added and shake well for 5mm shows the yellow colour.

i) *Tests for Quinone*

To the 2ml of extract conc. H_2SO_4 added and shake well for 5 mm shows the Red colour.

j) *Test for steroids (Salkowski Test)*

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Figure (c)

k) *Test for alkaloids(Hager's Test)*

To the 2-3 ml of filtrate, 1ml of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

l) *Mayer's Test*

To the 2-3 ml of filtrate, 1 ml of dil. HCl and Mayer's reagent was added and shake well. Formation of yellow precipitate showed the presence of alkaloids.

m) *Dragendroff's Test*

To the 2-3ml of filtrate, 1ml of dil. HCl and Dragendroff's reagent was added and shake well. Formation or orange-brown precipitate showed the presence of alkaloids.

n) Wagner's reagent test

To the 2-3ml of filtrate, 1ml of dil. HCl and Wagner's reagent was added and shake well. Formation of reddish-brown precipitate showed the presence of alkaloids.

o) Test for Flavonoids (With Lead Acetate)

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

p) Test for Tannins and Phenolic compounds ($FeCl_3$ Solution Test)

On addition of 5% $FeCl_3$ solution to the extract, deep blue black colour appeared.

q) Lead Acetate Test

On addition of lead acetate solution to the extract white precipitate appeared.

r) Test for Saponins (Foam Test)

To 1mol extract 20ml distilled water was added and shakes well in measuring cylinder for 15min. Then 1cm layer of loam was formed. Above phytochemical analysis will be carried out using standard procedure [11;12].

Figure (d)

V. Result and Discussion

The Phytochemical analysis for ethanolic extract was determined. It revealed the presence of all tested phytochemical compounds except protein and glycoside. Fig (a), (b), (c), & (d) show the phytochemical analysis bench work photograph. The (a), (b), (c) are the qualitative test for the majority of the secondary metabolites while the (d) shows the test for Saponins in particular. The sharp colour changes during the analysis showed the level of the quantity of such phytochemical compounds present. However, isolation of each component is in progress for further analysis with their quantitative test.

Table 2 : Preliminary screening of *Citrus aurantium var.* Dulcis L. pulp

S/NO	Constituent	Chemical	Observation
1	Alkaloids	Hager's Reagent	+
		Dragendroff's Reagent	+
		Mayer's Reagent	+
		Wagner's Regent	+
2	Carbohydrate & reducing sugar	Fehling's Regent	+
		Benedict's Regent	+
		Molisch's Regent	+
3	Steriods	Salkowski Regent	+
4	Saponins	Foam	+
5	Phenolics& Tannin	$FeCl_3$ Sol.	+
		Lead Acetate	+
6	Fixed oil & fats	Spot test	+
7	Proteins	Biurret Reagent	-
		Million's Regent	-
8	Anthraquinone glycosides	Borntrager's Reagent	-
9	Cardiac glycosides	Keller-Killiani Reagent	-

10	Flavonoids	Lead Acetate	+
		Extract + NH_3	+
11	Quinone	Extract + Conc. H_2SO_4	+
12	Coumarins	Extract + 10% NaOH	+

Key: + = Present - = Absent

These include Alkaloids, Saponins, Steroid, Carbohydrate, Tannins, Quinone, Coumarins, Phenolics, Terpenoids Fixed Oil, Fat and Flavonoids as shown in Table 2. As it is expected for ethanolic solvent used being an active component extractor [13]. Therefore, the presence of these secondary compounds validates the use of oranges pulps as herbal drugs anywhere they are found. On carrying out phytochemical analysis, crude extracts were fractionated into acidic, basic, polar and nonpolar fractions as shown in Table 3. The highest quantity of phytochemical was found to be oil from hexane fraction thereby indicating steroidal properties responsible in the hormonal production and enhancement. Most Alkaloid fraction is known to be poisonous. Thus, it was the least fraction obtained from the fruitof *Citrus aurantium var.* Dulcis L. pulp showing their friendly and less harmful as to be used in medicine.

Each fraction obtained through the bioassay fractionation protocol showed fluorescence under the UV observation. Thus, wavelength between 254–365nm has indicated the presence of secondary metabolites in the fractions. The ultraviolet region extends from about 10 to 380nm, but the most useful region in analysis is from 200 to 380nm, called the near-ultraviolet or quartz UV region. This is as a result of chromophores acting as chromatogram and conjugation (where multiple e.g., double and triple bonds are separated by just one single bond each) between the double bonds from oxygen atoms with the single bonds present in the structure. The different colours of the fluorescence rings are due to different atoms present in the compound having different wavelengths. When atoms are excited to a higher energy level, they may fall back to their original position using the same or a different wavelength resulting to emission of different colours [14]. At still higher energies (visible and ultraviolet wavelengths) different levels of electronic transition take place, and rotational and vibrational transitions are superimposed. Thus, indicating that important medicinal compound could be present in the fruit of *Citrus aurantium var.* Dulcis L. pulp fractions.

Table 3 : Physical parameters of *Citrus aurantium var.* Dulcis L. pulp fractions

S/No	Extractives	Weight	Colour	Texture
1	Methanolic	1.3g	Yellow	Viscous
2	Basic	0.3g	Light brown	Solid
3	Hexane(unsaturated)	2.0g	Orange	Oily
4	Acidic	1.2g	Brick red	Solid
5	Hexane(saturated)	40.0g	Red oxide	Oily

Phytochemicals are known to possess antimicrobial properties as reported [13]. This showed that the orange pulps were rich in chemical constituents. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antiturmour, and anthehelmintic activity [16]. Many alkaloids are known to have effect on the central nervous system and some act as antiparasitic (such as morphine, a pain killer). Quinine was widely used against *Plasmodium falciparum*. In this respect, it is found from the phyto-chemical screening that most plants traditionally used to treat malaria contain alkaloids among other things. Analgesia is another property of many alkaloids containing plants used in traditional medicine. Degenerative disorders, such as gouts and rheumatism, have also been traditionally treated with alkaloid-containing plants. Cochicine compounds are well known in treating gouts [14]. Alkaloids which have anti-inflammatory activity were present in the orange pulp and Saponins which have anti-inflammatory and considered as hemotoxic. Coumarins were present which is precursor for several anticoagulants. Tannins were present which have astringent and detergent properties were also present and can be used against diarrhea [15]. The presence of these compounds in *Citrus aurantium var.* Dulcis L pulpwill be useful in the treatment of diseases associated with the heart, anti-inflammatory action, anticoagulant, diarrhea and dysentery. Steroidal compounds are known to behave like hormones [16] have reported oils, alkaloids and associated with plants to have medicinal value. Others are Tritepenoids, which include: Cardiac Glycosides, Sterols, Saponins and Tritepenes. Mode of action of compounds present in the extracts indicates that the extracts from these pulps have the potential of solving the problem of multi-drug resistance.

VI. Conclusion

The study is useful for the utilization of natural product waste fruit such as the fruit of *Citrus aurantium var.* Dulcis L. pulpas therapeutic agents especially those that are thrown away been considered not very necessary. These may be more needed for the body wellbeing as it contains very important phytochemicals. Thus, it provides an ethnobotanical data of the medicinal fruits as used by the local practitioners, traditional healers to cure different diseases, and promote a practical use validation and to bring back the extinct knowledge for medicine. Further detailed exploration and collection of ethnobotanical information, chemical studies and screening for medicinal properties which are ignored will also provide less cost effective and reliable source of medicine for the welfare of humanity. However, the observations from the present study need to be further validated with isolations of compounds and pharmaco-chemical studies, in order to confirm their efficacy of such components present in the phytochemicals as a future drug.

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Elecrochemical Study of the Capacity of *Moringa Oleifera* to Chelate p-Aminophenol

By A. Zaroual, S. El Qouatli, A. Bellochou, M. Boulghallat, A. Gamouh, R. Najih & A. Chtaini

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Abstract- We report a sensitive electrochemical voltammetric method for analyzing p-Aminophenol, using a Natural phosphate (NP) modified carbon paste electrode NP-CPE, in Na_2SO_4, solution. Operational parameters have been optimized, and the stripping voltammetric performance has been studied using square wave voltammetry. The use of Moringa oleifera as natural chelating agent gave a significant improvement in the depollution of contaminated water sample.

Keywords: *electroanalysis; modified electrodes; paminophenol; moringa oleifera.*

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A. Zaroual [α], S. El Qouatli [σ], A. Bellochou [ρ], M. Boulghallat [ω], A. Gamouh [¥], R. Najih [§] & A. Chtaini [χ]

Abstract- **We report a sensitive electrochemical voltammetric method for analyzing p-Aminophenol, using a Natural phosphate (NP) modified carbon paste electrode NP-CPE, in Na_2SO_4, solution. Operational parameters have been optimized, and the stripping voltammetric performance has been studied using square wave voltammetry. The use of *Moringa oleifera* as natural chelating agent gave a significant improvement in the depollution of contaminated water sample.**

Keywords: electroanalysis; modified electrodes; p-aminophenol; moringa oleifera.

I. Introduction

Aminophenols are interesting electrochemical materials since, unlike aniline and other substituted anilines [1-2], they have two groups (- NH_2 and OH), which could be oxidized. Therefore, they could show electrochemical behavior resembling anilines and/or phenols [3-5]. An important factor would be the relative position of the amino and hydroxyl group in the aromatic ring. Accordingly, the reported electrochemical properties of the three positional isomers (ortho, meta and para) are strongly different. P-Aminophenol (p- AP) is a well-known compound which, in its simple form, or derivative [6], has been used as redox agent in photography. In neutral media, it is oxidized to complex oligomeric deys that could be used in enzymatic assays [7]. konopelnik et al. [8] have studied the oxidation of P-aminophenol (P-AP) in aqueous solution on SnO electrodes. According with these authors, only the amino group of m-aminophenol undergoes oxidation while the hydroxyl group remains unchanged. Common laboratory-based analytical methods for determining para-aminophenol compounds such as primarily gas and liquid chromatography (HP LC) [9-13], UV-vis spectrophotometry [14-15] and spectrofluorimetry [16] have been reported. The use of enzyme- link ed immunosorbent assay (ELISA) has been studied [17]. However, some sample pretreatment involving separation, extraction and/or adsorption is generally necessary, and this can also be time-consuming and complex. Electrochemical methods, such as differential pulse polarography (DP P), anodic stripping voltammetry (ASV) and differential pulse voltammetry (DPV), have been widely applied for the determination of pharmaceuticals, dyes, insecticides and pesticides [18-20]. In recent years, chemically modified electrodes (CMEs) were used for the voltammetric quantification of various organic and inorganic species after their open circuit accumulation [21-22] Much of the work in this field was directed to exploit the chemical reactivity of the modifier towards a target analyte for electroanalytical purpose. Multitudes of modifying agents were used either as coatings on solid electrode surfaces or dispersed within a conductive matrix. It is noteworthy that this last approach is well suited when using electronically insulating modifiers requiring a direct contact to an electronically conducting substrate as used in connection with electrochemistry. The application of silicates and related mineral materials in electrochemistry is rather recent and was directed to combine their intrinsic properties to selected electrochemical reactions in order to improve the response of the electrode. Modified electrodes are being used frequently in the voltammetric determination of organic compounds because of their efficiency, the selectivity that can be obtained by varying the modifier and the sensitivity which is equivalent to that reached in anodic and cathodic stripping. In doing so, zeolite and silica-modified electrodes were prepared, characterized and applied (sometimes tentatively) in various fields including for example electroanalysis and sensors, electrocatalysis, photochemistry, thin-film technology, fuel cells, molecular recognition. Kauffmann [23] has reported that a carbon paste electrode (CPE) modified with lipids and proteins (enzymes) have potential application in environmental analysis. Recent works, reported in the literature, have shown several applications and electroanalytical methodologies employing glassy carbon electrode as working electrodes [24-25]. Luz and al. [25] constructed a glassy carbon electrode impregnated with a lithium tetracyanoethylenide (LiTCNE) for the determination of para-aminophenol. The oxidation and reduction of this compound has been carried out on a modified glassy carbon electrode using cyclic and DPV [26-28]. This study proposed a new modified carbon paste electrode which has been prepared by the Natural Phosphate (NP) for para-aminophenol detection. It has shown a selective preconcentration and quantization of para-aminophenol by cyclic voltammetry (CV). This study has

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led to the development of a new modified electrode for the determination of para-aminophenol with improved qualities such as simplicity of electrode preparation, wider linear range, and low detection limit (DL), high selectivity and very good stability of modifier. The procedure is based on the oxidation and reduction of para-aminophenol after it was preconcentrated on a carbon paste electrode modified with the clay, under open circuit conditions.

In this work we prepared and characterized the phosphate Natual modified carbon paste electrode, which successfully exploits the favorable mechanical and electrochemical properties of carbon paste electrodes. Also, this study therefore focused on the determination of the effectiveness of the Moringa oleifera in the purification of water contaminated by 4-Aminophenol.

II. Experimental

a) Reagents

p-Aminophenol and sodium sulfate were of analytical grade and from Aldrich. A natural phosphate (NP) used in this work was obtained in the Khouribga region (Morocco). Stock solutions of p-Aminophenol were prepared by dissolving p-Aminophenol in deionized water. All preparations and dilution of solutions were made deionized water. Provisions were made for oxygen removal by bubbling the solution with azotes gas for about 5 min then the solution was blanketed with azotes gas while the experiment was in progress. For reproducible results, a fresh solution was made for each experiment. Carbon paste was supplied from (Carbon, Lorraine, ref. 9900, French).

b) Electrodes preparation

Firstly, the carbon-paste electrode was prepared according the following procedure [29]. The carbon-paste electrode was prepared by mixing the graphite powder with paraffin oil used as a binder.

The mixture was grinding in a mortar agate and then a portion of the resulting composite material was housed in PTFE cylinder. The geometric surface area of the working electrode was 0.1256 cm2. A bare of carbon vitreous inserted into carbon paste provided the electrical contact, and then the NP film is electrodeposited onto carbon paste electrode. The deposit of Phosphate natural on carbon paste electrode surfaces was processed at 20 V. The current was maintained by a galvanostat with a function generator.

c) Prepared electrode characterization

All the electrochemical experiments were performed in a standard one–compartment three-electrode cell. The reference electrode was SCE and the counter electrode was platinum. All electrode potentials were referred to this reference electrode. The working electrode was NP modified carbon paste electrode (NP-CPE).

d) Apparatus

Electrochemical experiments were performed using a voltalab potentiostat (model PGSTAT 100, Eco Chemie B.V., Ultrecht, The Netherlands) driven by the general purpose electrochemical systems data processing software (voltalab master 4 software).

III. Results and Discussion

a) Characterization of prepared electrodes surfaces

The surface structure of natural phosphate modified carbon paste surface was observed using scanning electron microscopy (Fig. 1). The film layer of NP was formed on the surface of carbon paste electrode; it was not disintegrated or detached from the surface when immersed in the electrolytic solution (0.1M Na2SO4). The morphology of the phosphate rock surface was observed by scanning electron microscopy (Figure 1). The treatment described previously gives compact particle fractions between 100 and 400 μm rich in phosphate. Rock phosphate treaty has the following chemical composition: CaO (54.12%), P_2O_5 (34.24%), F (3.37%), SiO_2 (2.42%), SO3 (2.21%), CO_2 (1.13%), Na_2O (0.92 %), MgO (0.68%), Al_2O_3 (0.46%), Fe_2O_3 (0.36%), K_2O (0.04%) and order of several ppm metals.

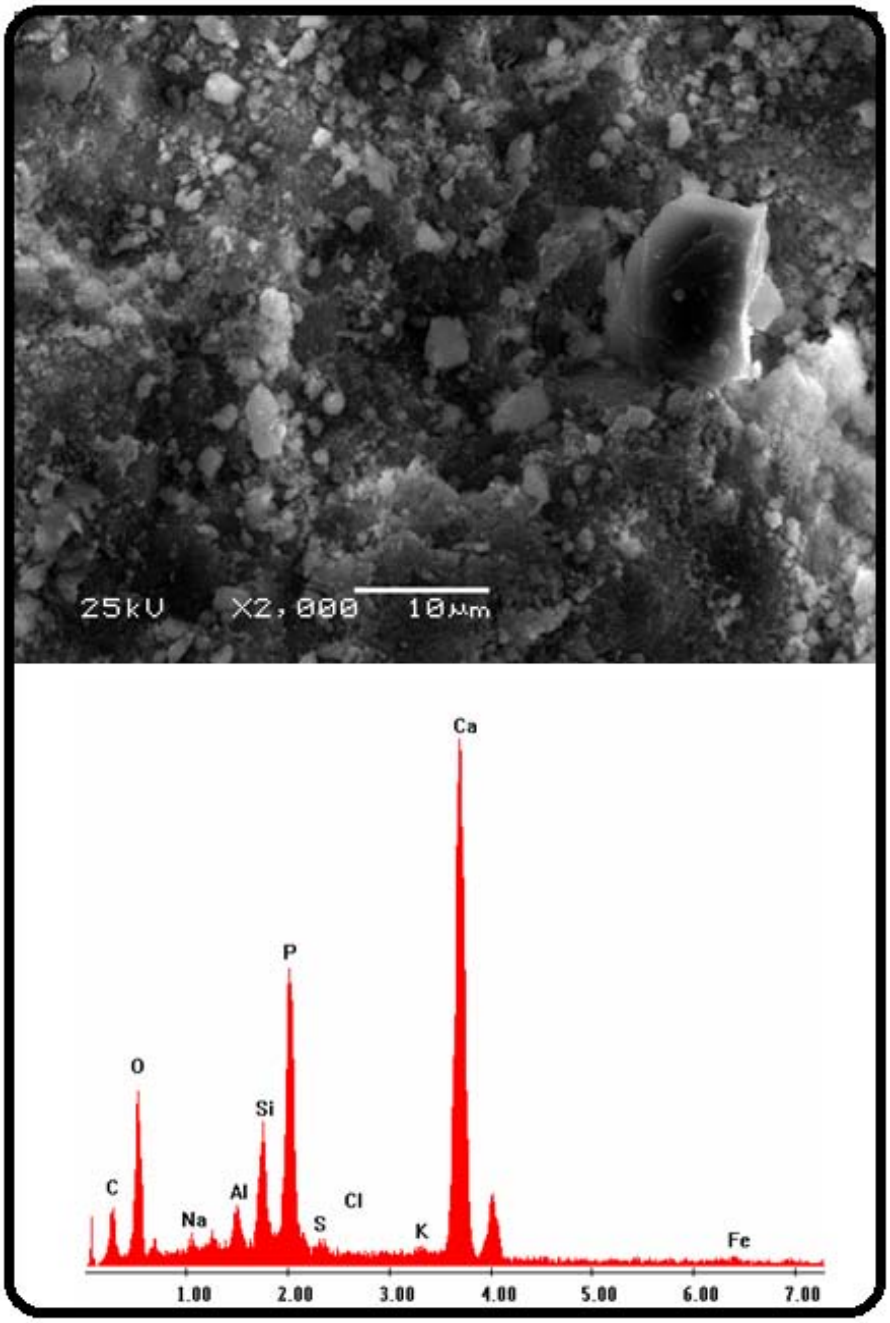


Figure 1: Scanning electron micrograph of NP-CPE

The cyclic voltammograms (CVs) of the NP modified carbon paste electrode and carbon paste electrode (CPE) were recorded in the supporting electrolyte (0.1 M Na_2SO_4) (Fig. 2).

We can see that the shape of the cyclic voltammogram was modified in the presence of NP at CPE surface, suggesting that the carbon paste electrode was effectively modified by natural phosphate.

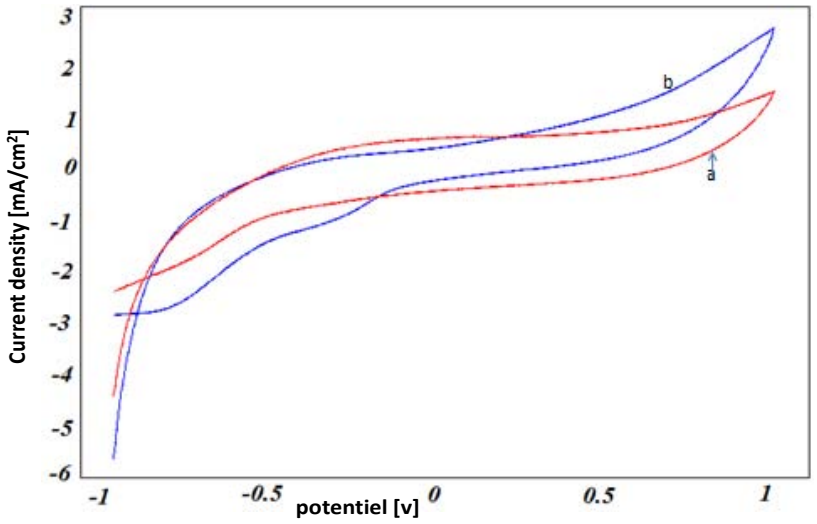


Figure 2 : Cyclic voltammograms recorded in electrolytic solution, at 100 mV/s, at a- carbon paste electrode, b- NP modified carbon paste electrode

b) Electrochemical detection of studied metals

The experimental conditions have been optimized and the response characteristics determined in a previous work [29]. The results obtained are:

- pH 7
- Preconcentration time = 13 min.

In order to avoid the strong residual of reduction, the starting potential was fixed at -1V versus SCE. Fig. 3 shows a cyclic voltammograms performed between-1 V and 1 V for NP-CPE, in 0.1M Na2SO4 solution (curve a), and in 0.1M Na_2SO_4, after exposure NP-CPE to 0.510-3 mmol/L P-Aminophenol for 13 min, in a stirred solution (curve b). The reversible system could be observed at NP-CPE, with cathodic potential value, of -0.3 V and anodic potential value of -0.1 V.

The square wave voltammetry (SWV) corresponding to the determination of P-Aminophenol, was recorded in the supporting electrolyte (curve a) and after, 13 min of accumulation in a solution containing P-Aminophenol (curve b).

The Square wave voltammograms are showed in Figures 4. A well-defined and enhanced peak is observed at NP-CPE, imprinted in P-Aminophenol solution. This peak is attributed to P-Aminophenol oxidation. The scheme 1 shows the proposed mechanism of this oxidation.

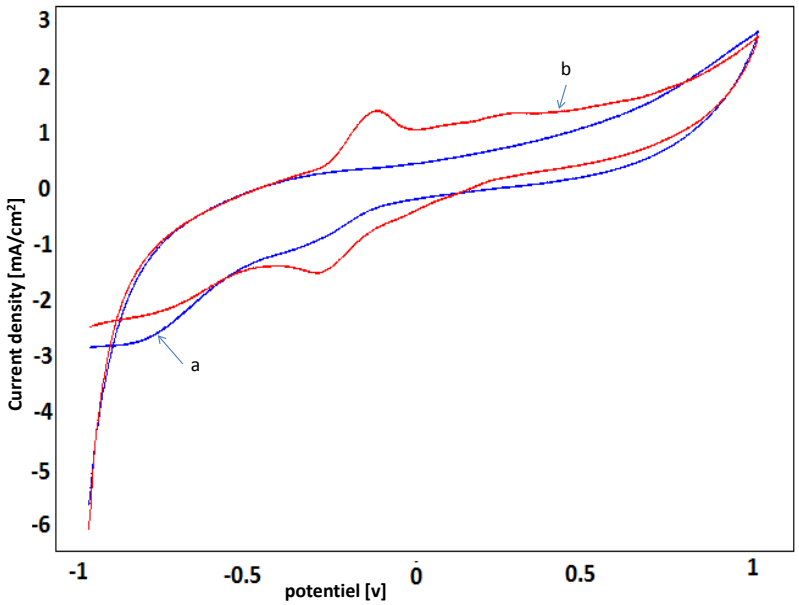


Figure 3 : Cyclic voltammograms recorded in electrolytic solution, at 100 mV/s, at NP modified carbon paste electrode, in 0.1 M Na_2SO_4 solution (a), and after exposing to P-Aminophenol solution

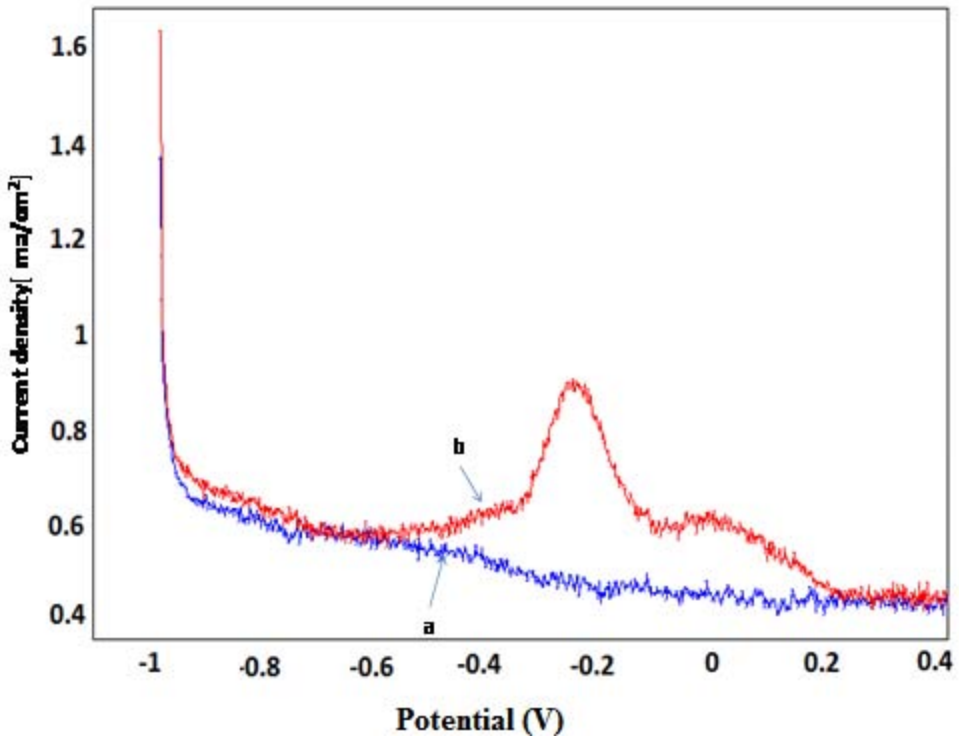
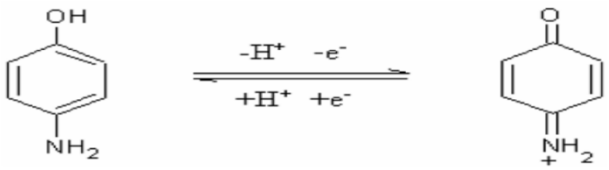


Figure 4 : Square wave voltammograms obtained for PN-pectivelly, in supporting solution (curve a), and after exposing electrode to P-Amnophenol solution (curve b)



Scheme 1: mechanism of electrochemical redox reaction of p-aminophenol at NP-CPE

c) Chelating effect of Moringa oleifera

Moringa oleifera is the best known species of the Moringaceae family. Moringaceae is a family of plants belonging to the order Brassicales. It is represented by fourteen species and a single genus (Moringa), being considered an angiosperm plant. It is a shrub or small tree which is fast growing, reaching 12 meters in height. It has an open crown and usually a single trunk. It grows mainly in the semi-arid tropics and subtropics. Since its preferred habitat is dry sandy soil, it tolerates poor soils, such as those in coastal areas [31].

Firstly, the Moringa oleifera was prepared according the following procedure [30]. Moringa oleifera seeds collected for the analysis were shelled off and sun dried to maintain constant weight. The sun-dried seeds were grinded into powdered form using machine. The powdered was added to the solutions containing heavy metals. After 15 min of contact with moringa oleifera, the solutions were purified and analysis in electrochemical sensor.

The SQWV's recorded at prepared electrode, in supporting solution containing p-aminophenol (curve a) and after addition of the moringa oleifera (curve b), are shown in Figure 7. The peak current decreased considerably after moringa treatment. This current density reduction is due to a sharp decline in p-aminophenol concentrations, which suggests that moringa has a strong complexing power of p-aminophenol (Figs. 7 and 8).We not that the solution pH was varied after moringa treatment. It was decreased from 7.2 to 6.5.

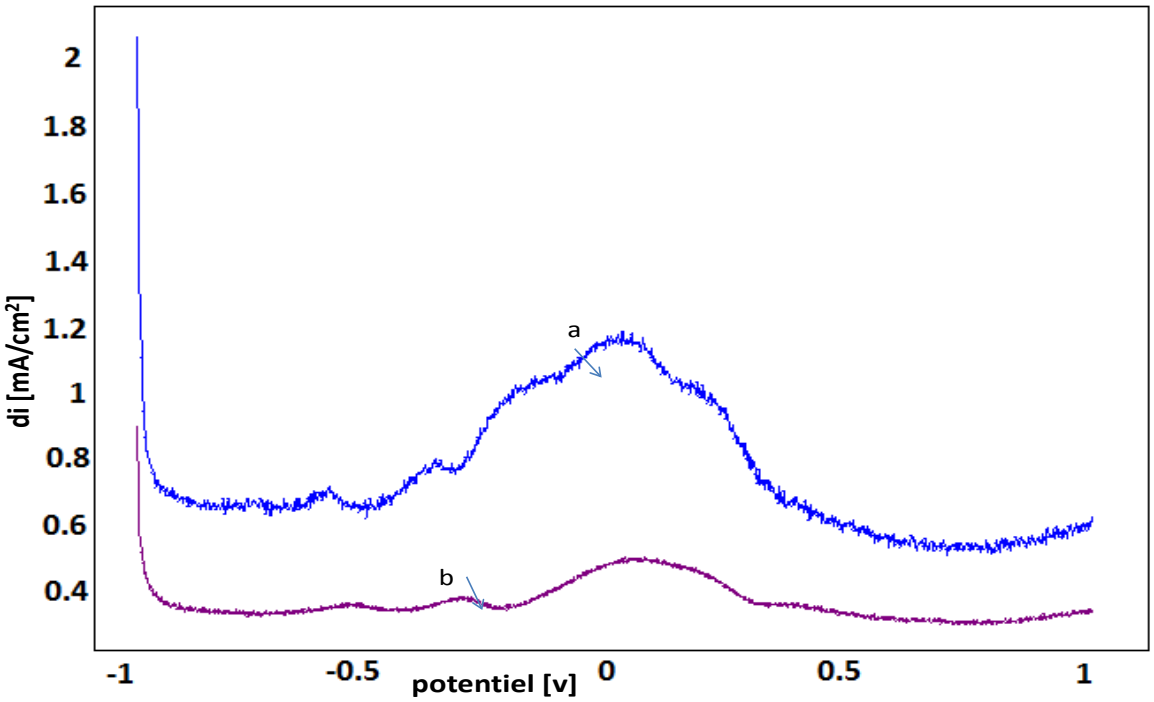


Figure 7 : Square wave voltammograms obtained for NP-respectively, in supporting solution, after exposing electrode to p-aminophenol contaminated solution. (a) - before moringa treatment, b-after moringa treatment

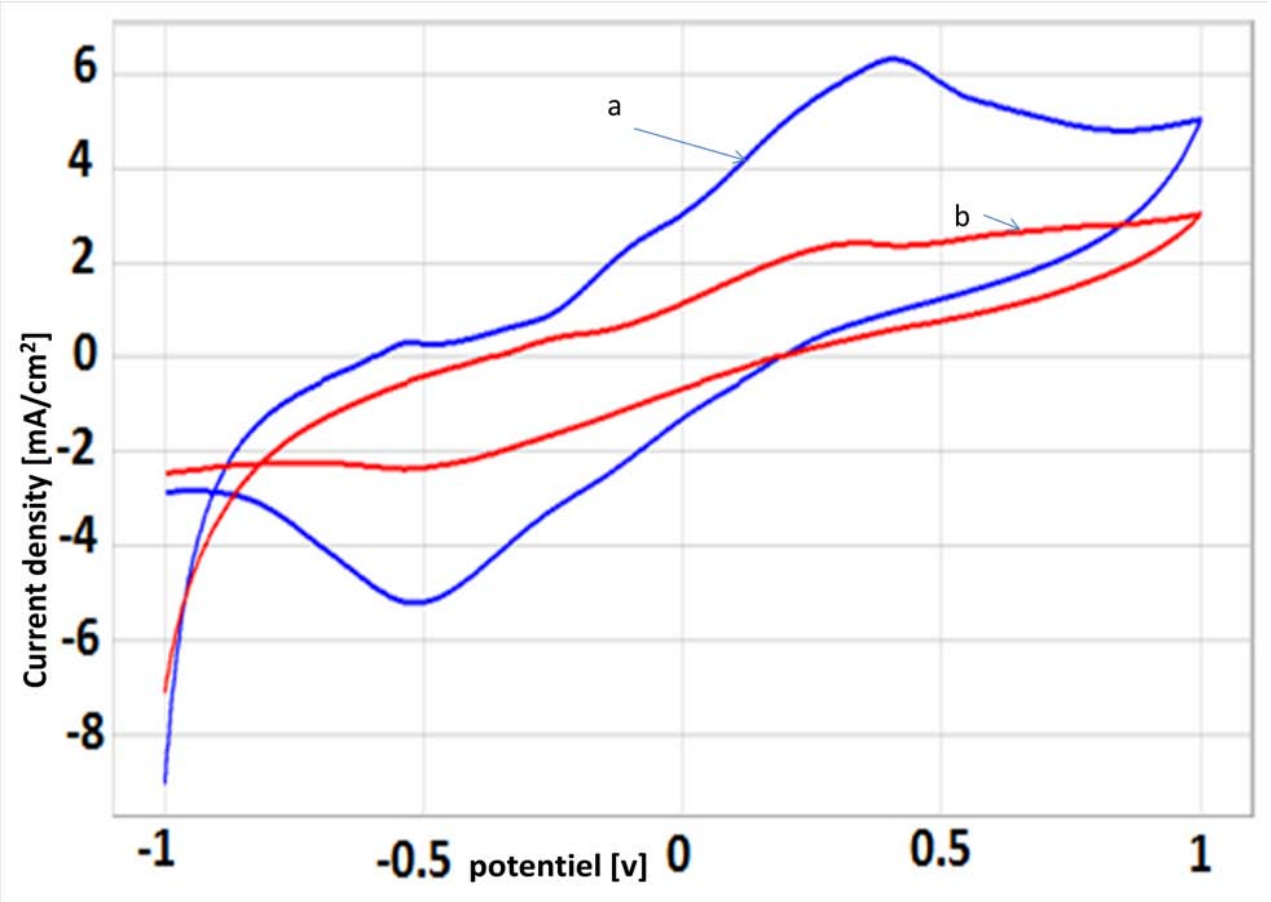


Figure 8 : Cyclic voltammograms obtained for NP-respectively, in supporting solution, after exposing electrode to p-aminophenol contaminated solution. (a) - before moringa treatment, b - after moringa treatment

d) Optimization of experimental conditions

i. The Moringa oleifera concentration effect on p-aminophenol

In order to obtain an analytical curve for the developed sensor, we carried out cyclic voltammograms and Square wave voltammograms to show the Moringa oleifera effect on p-aminophenol at different concentrations, in 0.1M Na_2 SO_4 (pH 7), the sweep rate was fixed at 100 mVs-1.

Figure 7 and 8 shows the CV and SQW curves recorded, for different concentrations of moringa oleifera (MO), at NP-CPE was increased from 2 ml/200 ml (electrolytical solutions) to 13 ml/200 ml (electrolytical solution). Both the anodic and cathodic peak current increases linearly with the concentration of moringa oleifera and the plot of current versus concentration obeys Randles-Sevic equation, which implies that the electrode process is adsorption controlled reaction. It was also observed that the cathodic peak potential shift towards negative values and anodic peak potential shift towards positive side. This kind of shift in Ep in the cathodic and anodic.

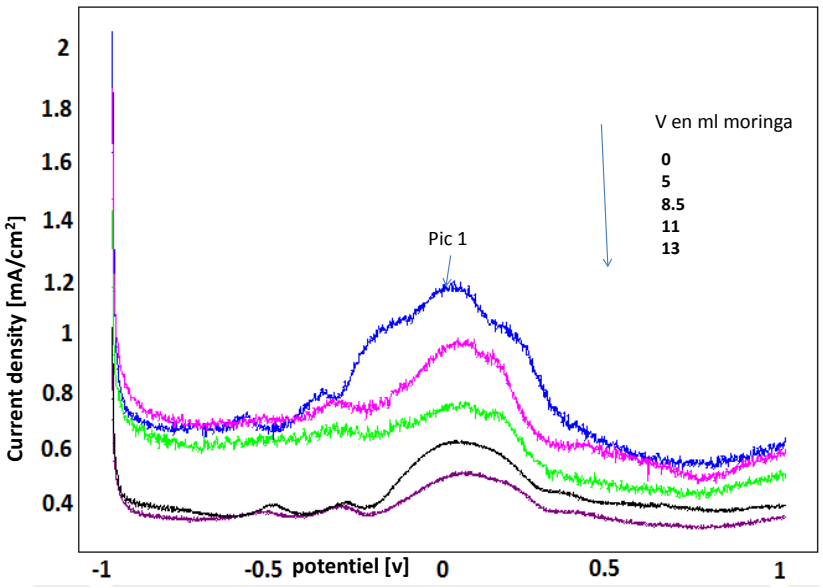


Figure 7 : Cyclic Voltammogram of different concentration of MO (0 ml to 13ml) in (0.5 mM) aminophénol at NP-CPE in 0.1 M Na_2SO_4, Scan rate 100 mV/s

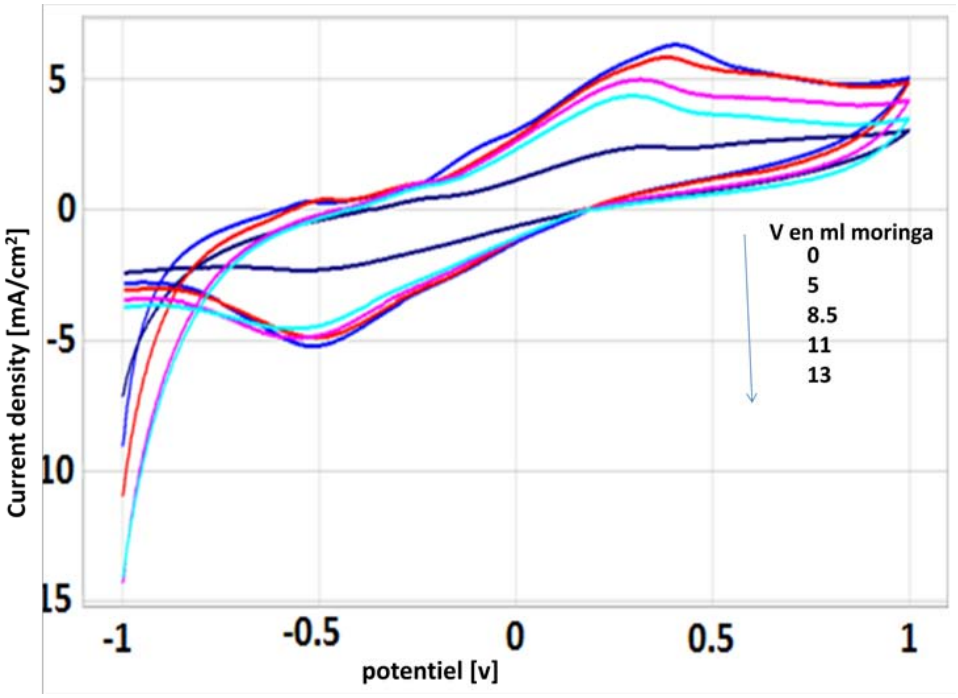


Figure 8 : Square wave voltammograms of different concentration of MO (0 ml to 13ml) in (0.5 mM) aminophenol at NP-CPE in 0.1 M Na_2SO_4, Scan rate 100 mV/s

The effect of the MO concentration of MO is investigated (Figure 9), this significantly affects the oxidation peak current of p- aminophenol. The peak current decrease greatly with MO concentration.

Similarly, the effectiveness of the Moringaenol to chelate p- aminophenol increaszs considerably with the MO concentration. (Fig. 10)

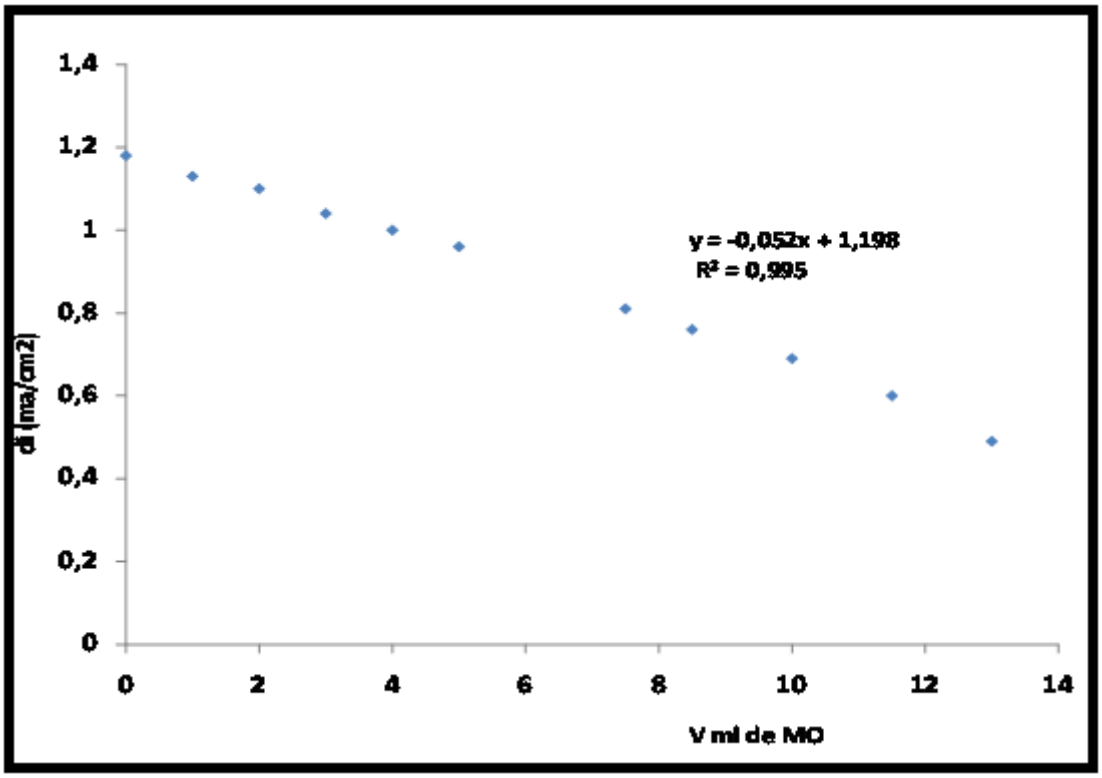


Figure 9 : Peak area of land depending on the concentration of added MO on Peak 1

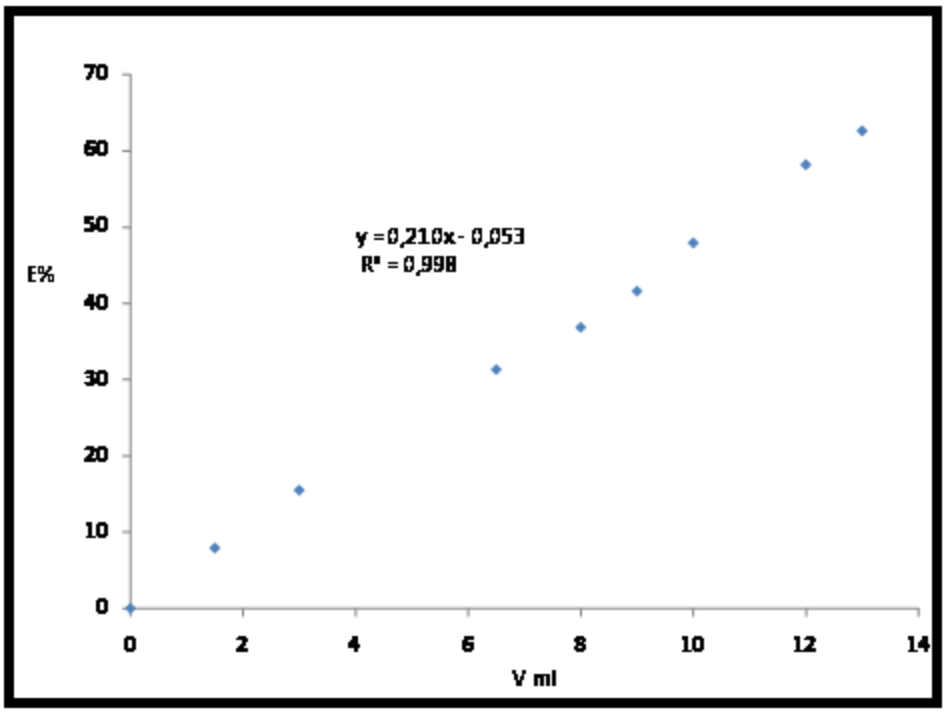
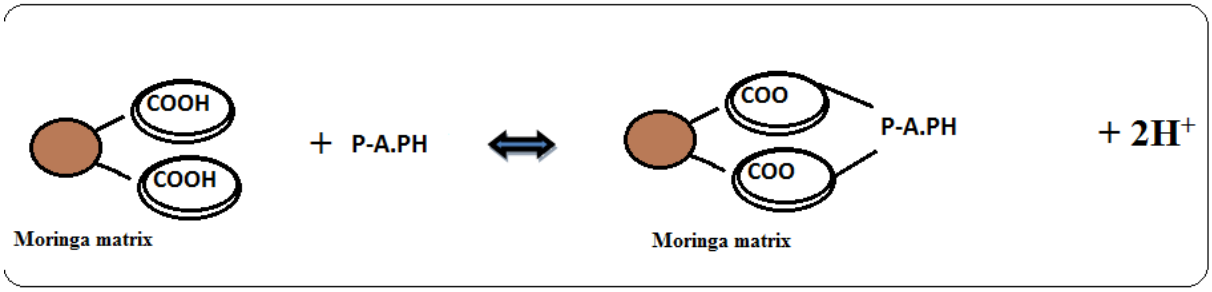


Figure 10 : The MO efficiency on 0.5 mM[p-aph] vs MO concentration

Based on the pH variation of the solution after treatment with MO, we proposed the following mechanism:



P-A.PH (p-aminophenol)

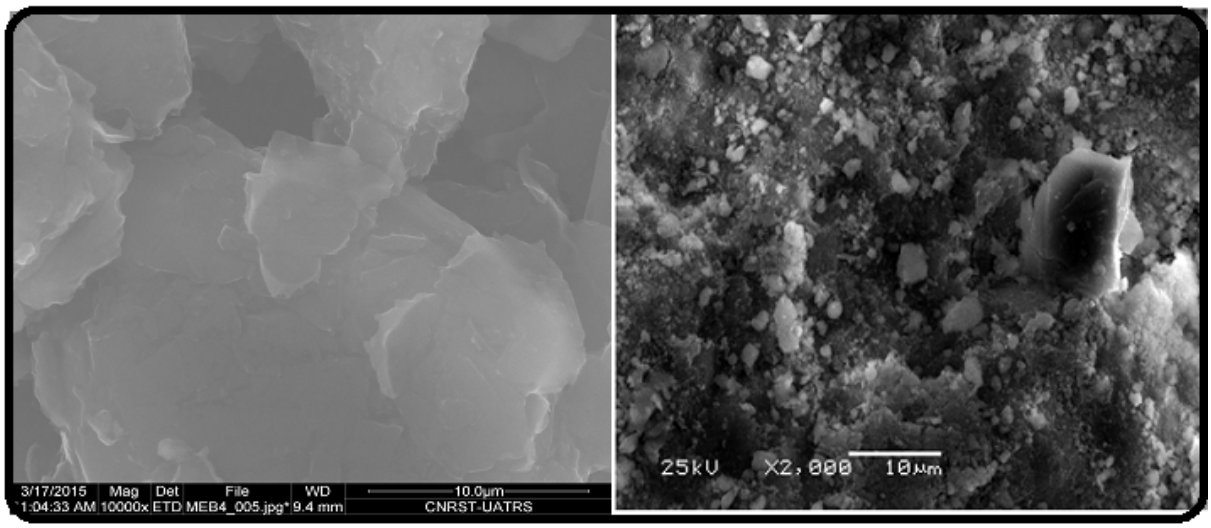


Figure 11: MEB images of NP modified carbon paste electrode, a-before and b- after MO treatment

In Figure 11, we present the SEM images, taken to the surface of the modified electrode; we can see that after MO treatment, the surface morphology changed dramatically with the advent of large compact crystals, leaving suggest that MO-p-aminophenol complex is adsorbed on the electrode by forming a continuous film.

IV. Conclusion

In conclusion, it was possible demonstrating the potentiality of the proposed electrode (NP–CPE) for determining P-aminophenol. Such a sensor is characterized by a higher sensitivity and reproducibility.

The Moringa oleifera seeds have the ability to retain p-aminophenol. The metal is sequestered by chemical sites naturally present in the moringa matrix. The chelating process is rapid and takes place under normal temperature and pressure. Moringa oleifera is an environmentally-friendly natural complexing most suitable for the treatment of water containing undesirable p-aminophenol concentrations. The removal efficiencies were 60 % for P-aminophenol. It is an eco-friendly technology that is economically more advantageous than other treatment alternatives.

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Ethnopharmacological Surveys' Methodologies for Medicinal Plants uses Discovery and Environmental Threatens on Recorded Plants from Indigenous Knowledge in Cameroon

By Nole Tsabang, Lionel Wilfried Djeufack Tsambang, Armel Tedjou Nouboudem, Denis Sonwa, Alain Bertrand Dongmo, Gabriel Agbor Agbor & Bernard Aloys Nkongmeneck

University of Douala, Cameroon

Abstract- Nowadays, more than 80% of the world population is depending on medicinal plants based medicines to satisfy their healthcare needs. Medicinal plants have served as valuable starting materials for drug development. The general objective of the present work was to exploit the strong experience, developed since the antiquity on medicinal plants uses in Cameroon, for their future valorization by the scientific community. The accustomed medicinal plants are species known by traditional healers for the treatment of given diseases. The species which successfully treats or relieved patients are known to be convinced medicinal plants. Those known as suspected medicinal plants are plants used to treat diseases indirectly from their signs, their symptoms or their complications.

Keywords: usual, potential, convinced, suspected and threaten medicinal plants.

GJMR-B Classification : NLMC Code: QV 37

Strictly as per the compliance and regulations of:

Ethnopharmacological Surveys' Methodologies for Medicinal Plants uses Discovery and Environmental Threatens on Recorded Plants from Indigenous Knowledge in Cameroon

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Keywords: usual, potential, convinced, suspected and threaten medicinal plants.

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I. Introduction

From empirical uses of plants and animals, ethnopharmacological studies have brought to humanity more than 60 % of daily drugs. As a multidisciplinary science, ethnopharmacology has developed original methodologies that combine tradition and modernity and open promising perspectives. The usual medicinal plants are species known by the traditional healers for the treatment of the diseases. The species which successfully treat or relieve a patient are convinced medicinal plants. Meanwhile, some medicinal plants and the environmental threatens that they undergo, are still misunderstood. Traditional healers, especially those of hinterland (Boro or Fulani and Pygmies) do not recognize modern medical terminologies of several diseases. This reason render difficult to carry out an ethnopharmacological survey particularly at sedentary Pygmies of East and South regions of Cameroon, Fulani in mountains and some illiterate old traditional healers. In traditional medicine, the diagnostic is not outright. Nevertheless, traditional healers treat certain pathologies. These treatments are mostly dependent on the experience of the indigenous people who indirectly treat the diseases based on their signs, their symptoms and/or their complications. According to the theory of likeness or aphorisms of positive medicine, some medicinal plants are adopted to the treatment of a specific disease by exploiting the similarity between the form and the color of the plant organs and the patient's color of the eyes and/or of the skin, due to this disease. The interpretation of the diseases' names, of the plants' names, of the plant habitats' names, of the behavior of the animals after consuming a given plant and of the mystic activities, the myths, the histories and the incantations, can permit to identify medicinal uses of plants. In these cases, the species identified are suspected medicinal plants.

More than 200 000 plants species on 300 000 recorded in the world live in tropical countries of America, Africa and Asia. Cameroon, a country of the Congo basin, counts about 10 000 plants species and

only 800 medicinal plants are known (1). The medicinal plants constitute a natural heritage of a great importance for its population health. Since antiquity, man mainly uses plants for his health problems. The exigencies of resistant to be synthetized or resistant to the synthesis like vincristine and vinblastine, the phenomena of microbes' resistance to usual antibiotics and the persistence of incurable diseases reinforce the resort to traditional medicine.

The general objective of the present work was to exploit the strong experience, developed since the antiquity on medicinal plants uses in Cameroon, for their future valorization by the scientific community.

a) Detailed botanical prospection and ethnopharmacological thorough preparation

The survey was conducted nearby 1131 informants from 58 tribes of Cameroon, in a random distribution. Folklore medicinal information on medicinal plants used in the symptomatic treatments of diseases and environmental threatens on the species, were recorded during interviews and discussions, following a semi-structured ethnopharmacological detailed methodology developed in Tsabang N. *et all*. 2015 (2). Samples of recorded plants were collected, dried, identified and confirmed at National Herbarium of Cameroon, and conserved in the Institute of Medical Research and Medicinal Plants Studies. In addition, data for environmental conditions in which lives the recorded species were also collected.

i. Distribution of interviewers

The 1131 informants are distributed as follow, according to some social characters: from their environment: 301 city-dwellers and 830 villagers; from academic standard: 727 illiterates and 404 educated (academic standard ≥ FSLC: Frist School Living Certificate); from purchasing power: 921 poor people and 210 riches [annual income < 370 US$ (World Bank)]; from religion : 738 animists, 313 Christians and 80 Muslims; from sex : 394 men and 737 women; from profession : 70 traditional healers, 10 ethnobotanists or botanists, 05 physicians, 07 nurses, 397 housewives, 502 ethnoveterinarians-farmers, 37 cattle breeders, 21 hunters, 20 organic chemistry, 11 physiologists, 06 pharmacists, 25 sellers, 06 shepherds and 14 others; from familial situation: 1046 married, 42 bachelors et 43 widows and widowers; from age brackets : 91 age between 30 and 40 years, 327 age between 41 and 50, 349 age between 51 and 60, 210 age between 61 and 70, 114 age between 71 and 80, and 40 are between 80 and 90. This sample of interviewers presents all the characteristics susceptible to provide more information.

II. Results

a) Identification of some diseases treated based on their signs, symptoms and complications

Hepatitis, typhoid, appendicitis, etc. are classified in abdominal diseases. Sickle cell anemia, malaria, typhoid fever, etc., are often confounded in traditional medicine. Diabetes and arterial hypertension are unknown in the hinterland. The cancer, gangrenes, elephantiasis, scrotum, etc., were mystified, regarding their extraordinary complications. Table 1 presents the correspondence between signs, symptoms and complications of suspected diseases, described by a physician; some of these manifestations are treated with suspected medicinal plants. Many of these diseases that include malaria, typhoid fever, sickle cell anemia, hepatitis, have common symptoms which render difficult the application of their symptoms for their diagnostic. Therefore traditional healers can easily confound them. But the strong frequencies of these signs, symptoms and complications in the management of certain pathologies sustain their indirect treatment by traditional healers. The recorded suspected plants must be used to treat at least three of these manifestations. The recorded potential medicinal plants, in addition to treat at least three manifestations of diseases, possess isolated actives ingredients and/or extracts.

Table 1 : Correspondence between signs, symptoms and complications of suspected diseases, medicinal plants and treated manifestations.

Signs, symptoms and complications of diseases described by a physician	Diseases' manifestations known and treated by traditional healers	Suspected or potential plants used to treat diseases' manifestations	Suspected diseases treated
Fever 8 to 30 days after infection, headaches, muscles or joints' pains, weakening, vomiting, diarrhea, cough, and typical cycles varying with fever, shivering, cool sweat and intense transpiration: this is an access malaria. *Plasmodiumfalciparum* multiplication and red cells or erythrocytes explosion (anemia), cerebral malaria with the blood vessels irrigating the brain infected by *Plasmodiumfalciparum* that attack the blood red cells. It is often fatal if the treatment is not well follow up.	Headaches, muscles or joints' pains, weakening, vomiting, cough, shivering, cool, sweat and intense transpiration, anemia.	*Annickia chlorantha, Nauclea diderricii* (fever, headaches, shivering, yellow vomiting); *Rauvolfia vomitoria, Nauclealatifolia* (headaches, joints' pains, shivering, vomiting, fever); *Morinda lucida, Azadirachta indica* (fever, yellow vomiting, splenomegalie, anemia)	Malaria
Anorexia, fatigue, mild fever, muscle or joint aches, nausea and vomiting, pain in your belly; some people have other issues, such as: dark urine, light-colored stools, jaundice (yellowing of the skin and whites of the yes), itchy feeling, mental changes, such as stupor (being in a gaze) or coma and bleeding inside your body.	Anorexia, fatigue, mild fever, muscle or joint aches, nausea and vomiting, jaundice, itchy feeling	*Anchomanes diformis* (anorexia, mild fever, muscle and joints aches); *Azadirachta indica*(nausea, vomiting, dark urine, jaundice and anorexia)	Hepatitis
Anemia, Red blood cells usually live for about 120 days before they die and need to be replaced. **Occurrences of varied in intensity pain (**crises**)**, are a major symptom. Pain develops when sickle-shaped red blood cells block blood flow through tiny blood vessels to the chest, abdomen and joints; Pain in bones; **Hand-foot syndrome:** Swollen hands and feet may be the first signs in babies. **Frequent infections:** damage spleen (organ that fights infection). This may make patient more vulnerable to infections, such as pneumonia. **Delayed growth:** A shortage of healthy red blood cells can slow growth in infants and children and delay puberty in adolescents. **Vision problems, Abdominal swelling,Fever that is the** first sign of an infection. **Pale skin or nail beds. Yellow tint** to the skin or whites of the eyes. **Any signs or symptoms of stroke:** one-sided paralysis or weakness in the face, arms or legs, confusion, trouble walking or talking, sudden vision problems or unexplained numbness and a headache.	**Anemia, intensity pain (**crises**)**, Swollen hands and feet, **Delayed growth, Abdominal swelling, Fever,** confusion, trouble walking or talking, sudden vision problems, headache.	*Fagara tessmannii* (anemia, pains, swollen feet and hands, yellow tint)	*Sickle cell anemia*
Earlyillness: fever that starts low and increases daily, possibly reaching as high as 40.5 C, headache, weakness and fatigue, muscle aches, sweating, dry cough, anorexia, weight loss, abdominal pain, diarrhea or constipation, Rash, extremely swollen abdomen. **Laterillness**: patients who don't receive treatment, may become delirious, lie motionless and exhausted with their eyes half-closed in what's known as the typhoid	Subsisted fever, headache, weakness and fatigue, muscle aches, sweating, dry cough, anorexia, abdominal pain, extremely swollen abdomen.	*Gossypium arboreum*, *G. barbadense, G. hirsutum*and *G. herbaceum* (fever, sweating, anorexia, headache, fatigue, diarrhea, abdominal pains)	Typhoidfever

state; In addition, life-threatening complications often develop at this time. In some people, signs and symptoms may return up to two weeks after the fever has subsided.			
Headaches, sexual weakness, obesity, dizziness, gangrene, physical asthenias, lumbago, cramps, and reduce visual acuity, excessive transpiration, redoubtable complications like: left ventricular hypertrophy, occlusion of a blood vessel (infarction) and in the brain (cerebral softness), kidney insufficiency, vascular cerebral accidents.	Headaches, sexual weakness, obesity, dizziness, gangrene, physical asthenias, excessive transpiration, cramps, kidney insufficiency	*Laportea ovalifolia* (sexual weakness, dizziness, cramps, obesity) *Morinda lucida* (headache, visual acuteness, kidney insufficiency, physical and sexual asthenias)	Diabetesandhypertension
Hypertension, nocturnal diarrhea, limbs' numbness, calves' lesions, fecal and urinary incontinence, nails' break, hair fall, fungal infections, Physical and sexual asthenias	Hypertension, nocturnal diarrhea, limbs' numbness, fungal infections.	*Momordica charantia* (fecal urinary incontinence, fungal infection,)	Diabetes
Diabetes, abundant micturition, muscles' weakness, cardiac palpitations, ears' buzzing, edema, excess salt, insomnia, dysuria, nasal bleedings,	abundant micturition, , ears' buzzing, edema, excess salt, insomnia, nasal bleedings,	*Hallea inermis*, *Hallea stipulosa*, *Asystasia gangetica* (abundant micturition, edema, insomnia)	Hypertension
Tiredness, breathlessness and looking pale (due to a lack of red blood cells); frequent infections (due to a lack of white blood cells) and unusual bleeding or bruising (due to a lack of platelets).	Tiredness, breathlessness, looking pale and unusual bleeding.	*Aloe vera, Aloe buttner i*(tiredness, looking pale, bleeding)	*Cancer: **leukaemia***
Weight loss, anorexia, feeling very full after a small meal, nausea or vomiting, enlarged liver, felt as a mass under the ribs on the right side, an enlarged spleen, felt as a mass under the ribs on the left side, pain in the abdomen or near the right shoulder blade, swelling or fluid build-up in the abdomen; itching; yellowing of the skin and eyes (jaundice), fever, enlarged veins on the belly, and abnormal bruising or bleeding. In case of chronic hepatitis or cirrhosis patients may feel worse than usual. Hypercalcemia due to hormonal production by liver), nausea, confusion, constipation, weakness, or muscle problems. Low blood sugar levels (hypoglycemia), fatigue or fainting; Breast enlargement (gynecomastia) and/or shrinkage of the testicles in men; high counts of red blood cells (erythrocytes) which can cause someone to look red and flushed and high cholesterol levels.	Anorexia, feeling very full after a small meal, nausea or vomiting, enlarged liver, pain in the abdomen, itching, jaundice, constipation, weakness	*Moringa oleifera*, *Moringa stenopetala* (Anorexia, weight loss, nausea, enlarged abdomen, vomiting, mass in the abdomen, constipation, weakness, fatigue)	Liver cancer

b) Similarities of colors and forms

Due to the yellow color of *Anacardium occidentale* fruits, 33 informants with age between 80 and 90 used them to treat jaundice; the reddish color of tubers and petioles of *Betavulgaris* make this species used by 54 housewives against anemia and the treatment was also known by 10 riches; the twin fruits of *Voacanga africana* because of it similarity in form with the testicles, are used by 39 villagers and 66 citizens to treat the testicular edema; *Schumanniophyton magnificum* because of its names Tsid Modo or Tsid Meki in Ewondo, that means somebody's blood defender, this plant was adopted for malaria treatment. This information was collected nearby 378 informants. The form of snack of *Entada gigas*' stem makes the linkage that was in the origin of its seeds use to prevent and to cure snack bites. This information was given by 16 Pygmies; According to 71 informants, the fruits consumption of *Momordica charantia* by certain pregnant mammals has oriented early people to use them for delivering; For 677 informants, the red color of the decoction of many species that include *Eremomastax speciose, Hibiscus sabdariffa* and *Hypoetes verticillaris* has orientated the indigenous people to use these plants against anemia. The yellow bark of *Annickia chlorantha* and the yellow color of the decoction of *Senna alata* make the two plants used in the treatment of hepatitis by 55 informants.

c) Environmental threatens and benefits

Anacardium occidentale is an important fruit tree in Far North of Cameroon. However, 66 informants recognize that many biotic factors, especially insects threatened its production. Fifty informants say that *Azadirachta indica* presents harmful and beneficial effects on both animal and vegetal biodiversity. Seventy five people know that this plant improves human health. Twenty seven housewives use *Moriga oleifera* seeds to purify well water. According to nine cattle breeders, this species is much resisted to drought and that explains the use of its leaves to feed animals in dry season. *Aloe* spp are planted by 919 indigenous people to fight against drought, because these herbs are xerophytic, succulent and desiccation-tolerant.

The information on the ethnopharmacological data preparation and the precision of plants' habitats, for convinced, usual and suspected or potential medicinal plants are presented in table 2.

Table 2 : Description of all the recipes in the treatment of all the recorded diseases

Plants, families, Common names, Vernacular names and dialect, Types of plants and Morphological type of plant	Part used	Methods of preparation	Route of administra-tion	Posology, duration of treatment and secondary effects
1- *Rauvolfia vomitoria* (Apocynaceae) Menzanga-Menzanga (Beti); Aton (Bamileke-Yemba), Sebal (Fufulde, Fulani); Supected medicinal plant; Shrub in mountain forests, Tree in fallow lands of forest regions.	Root bark	Decoction of 1.5 g of root bark per kg of body weight in 3 liters of water for 15 min.	Orally	**Malaria**: Take 250 ml of decoction, 2 times a day, for 5 days. The long time use and the strong doses can provoke gastric ulcers (10).
2- *Nauclealatifolia* (Rubiaceae); Gueleden (Bafia), Usual, suspected and convinced medicinal plant; Savana shrub	Leaves	Decoction of 100 g of leaves in 3 liters of water, for 25 min.	Orally	**Malaria**: Take 250 ml of decoction three times a day, for a week.
3- Naucleadiderricii (Rubiaceae); Akodok (Bety), Ntomba (Pygmies Baka); Suspected medicinal plant; Secondary forest tree.	Bark	Decoction of 40 g of powder of bark, in 1.5 liter of water, for 10 min	Orally	**Malaria**: Drink 250 ml of decoction, 3 times a day.
4-Morinda lucida (Rubiaceae) Nime (Medumba, Nde), Akeng (Ewondo); andIkeng (Bassa), Akyang (Fang); Kikengue, Koua Kengue (Baya); Potential and convinced medicinal plant (3); Tree of secondary forest and old fallow lands.	Leaves	Decoction of 1,5 g of dry leaves and 1,5 g per kg body weight in 4 liters of water, for 15 min.	Orally	**Malaria (leaves), diabetes and Hypertension (bark)**: Drink 250 ml of decoction 3 times daily, for a week.
5-and 6 -*Aloe buttneri or Aloe barteri*(Liliaceae); Lelang (Yemba-Dschang), Ladiecheu (Fefe), Niate (Bassa); Suspected medicinal plants; Cultivated Herb.	Leaves	Infuse 200 g of bulb of onion, cut into small pieces, in 2 liters of water, for 24 hours.	Orally	**Leukemia**: Drink 250 ml 'infusion, 4 times a day, for a week.
7- *Zanthoxylon tessmannii* (Rutaceae); Bongo (Bety), Bolongo (Pygmies Baka), Djou Souatomo (Badjoué); Usual and suspected medicinal plant; Secondary forest tree.	Bark	Decoction of 100 g of bark, in 3 liters of water, for 15 min.	Orally	**Sickle cell anemia**: Drink 250 ml of decoction, 3 times daily, for 7 days.
8- Moringa oleifera or *Moringa stenopetala* (*Moringa*ceae); Common name: Moringa (French); Suspected medicinal plant (4) Cultivated small tree.	Leafy stems or leaves	Decoction of 100 g of leafy stems or leaves, in 2 liters of water, for 15 min.	Orally	**Liver cancer**: Drink 250 ml of decoction, 3 times daily, for 7 days.
9-Hallea inermis (Rubiaceae**);** Koli, Harhandelo (Fululdé), Har (Kotoko), Kabé, Diaye, Diéya (Haoussa); Usual and Potential medicinal plant (5); Steppe and Savannahs Sudano Sahel Shrub	Bark	Decoction of 200 g of stem bark in 3 liters of water, for 20 min.	Orally	**Hypertension:** Drink 250 ml of decoction, 3 times a day, for a week.
10- Halleastipulosa (Rubiaceae); Adjobojan, Afopzam (Boulou), Ohambé (Bassa), Elolom (Ewondo), Etokakpa (Ejagham, Balong and Oroko);Suspected medicinal plant;Tree of swamping forest.	Stem Bark	.Decoction of 200g of stem bark in 4 liters of water, for 30 mn.	Orally	**Hypertension:** Drink 250 ml of decoction, 3 times daily, for 7 days.
11 et *12-Gossypium arboreum*, *G. barbadense* and *G. hirsutum* (Malvaceae); Tree cotton (English); Usual and suspected medicinal plants; Shrub planted in Gardens.	Stem bark	. Decoction of 200 g of stem bark in 4 liters of water, for 30 mn.	Orally	**Typhoid fever**: Drink 250 ml of decoction, 3 times daily, for 7 days.
13- Anacardium occidentale (Anacardiaceae); Cashew tree (English); Suspected medicinal plant; Soudano-Sahel Tree (Far North)	Stem Bark	. Decoction of 200g of stem bark in 4 liters of water, for 30 mn.	Orally	**Jaundice**: Drink 250 ml of decoction, 3 times daily, for 7 days.
14- *Voacangaafricana* (Apocynaceae); Common name: Voacanga (French);EyollaNjongi (Douala)Suspected medicinal plant; Savannah shrub.	Stem Bulb	Decoction of 100 g of stem and 100 g of cut bulb in 4 liters of water, for 15 min.	Orally	**Testicular edema**: Drink 250 ml of decoction, 2 times daily, for a week.

15- Schumanniophyton magnificum (Rubiaceae); Suspected medicinal plant; Tsid Modo (Ewondo), small secondary tree of forest.	Stem Bark	. Decoction of 200g of stem bark in 4 liters of water, for 30 mn.	Orally	**Malaria:** Drink 250 ml of decoction, 2 times daily, for a week.
16-Laportea ovalifolia (Tiliaceae); Vernacular names: Tololi, Itoil (Oroko), Sasalako (Bassa), Sassangulu (Pygmies),Kinhiemou (Widekum), Kinshei (Banso), Dandi (Bagweri); Potential and convinced plant (6); Understored herb of open forests distributed from South region to North-West region	Aerial parts	Decoction of 100 g of aerial part in 2 liters of water, for 15 min.	Orally	**Diabetes and Hypertension:** Drink 250 ml of decoction, 2 times daily, for a week.
17-Momordica charantia (Cucurbitaceae); Lebokenan (Bamileke); Usual, Potential and convinced medicinal plant (7-8); Post-cultivated Creeping Herb	Aerial parts	Decoction of 100 g of aerial part in 2 liters of water, for 15 min.	Orally	**Diabetes**: Drink 250 ml of decoction, 2 times daily, for a week. **Delivering**: A pinch of fresh seeds pasta introduced in the vagina.
*18- Asystasia gangetica (*Acanthaceae) Common name: Nelanelli; Usual and convinced medicinal plant (9); Post-cultivated Herb.	Aerial parts	Decoction of 100 g of aerial part in 2 liters of water, for 15 min.	Orally	**Hypertension**: Drink 250 ml of decoction, 2 times daily, for a week.
19- Entada gigas (Mimosaceae); Common names: monkey-ladder, sea bean or Sea Heart (English); Suspected medicinal plant; forests woody big liana.	Seeds	Macerate 80 g of fresh pounded seeds in 4 liters of water.	Orally	**Snack bite**: Drink 250 ml of macerate, 4 times a day for one week.
20- *Vernonia glabra* (Asteraceae); Anfugsa (Kom);Suspected medicinal plant; Woody herb of Mountains from Limbe to Ngaoundere	Leaves	Maceration of 100 g of leaves in 3 liters of water, for at least 2 hours.	Orally	**Diabetes**: Drink 250 ml of maceration, 3 times a day, for 5 days.
21- *Hibiscus sabdariffa* (Malvaceae) Common name: Roselle Vernacular names: Folere (Moufor, Fufulde); Potential medicinal plant (10);Cultivated Herb of Sudano Sahel	Leaves	Boil 50g of leaves in 1 liters of water, for 15 mn.	Orally	**Anemia**: Drink 250 ml of decoction, 3 times per day, for 3 days.
22, 23;24 & 25- Eremomastax speciosa, *Hypoetes verticillaris, Annickia chlorantha* (Annonaceae) and *Sennaalata*; Respective vernacular names : Apouetzem (Bamilileke-Dschang), Ndouetlefo (Bamileke), Npol, Nfol (Eton and Ewondo), Ngom N'tann (Beti); Usual medicinal plants;	Leaves Leaves Bark Leaves	Decoction of 1.5 g of plant 22 leaves, of plant 23 leaves, of plant 24 bark and of plant 25 leaves, per kg of body weight in 12 liters of water for 20 min.	Orally	**Anemia and Hepatitis**: Take 250 ml of decoction, 2 times a day, for 5 days for Anemia and 4 times per day for 3 weeks for Hepatitis. The long time use and the strong doses can provoke gastric ulcers.
26-*Moringa oleracea* (Moringaceae) Common name: Murungai. Usual, Potential and convinced medicinal plant (11); Shrub to small tree of Sahel zone	Leaves or stem back	Decoction of 1,5 g of leaves or 1,5 bark stem bark per kg of body weight in 3 liters of water for 20 min.	Orally	**Hypertension**: Take 250 ml of decoction, 2 times a day, for 5 days.
27-Voacanga thouarsii(Apocynaceae) Eyolla njongi (Douala); Potential medicinal plant (12); Shrub of savannahs and small tree in forests	Stem bark	.Decoction of 200g of stem bark in 4 liters of water, for 30 mn.	Orally	**Hypertension**: Drink 250 ml of decoction, 3 times daily, for a week.
28-*Anchomanes diformis* (Araceae); Kabad (Ewondo)Suspected medicinal plant; Understore Herb	Tuber	Decoction of 1,5 g of tuber per kg of body weight in 3 liters of water for 20 min.	Orally	**Hepatitis**: Take 250 ml of decoction, 2 times a day, for 7 days.
29-Azadirachta indica (Meliaceae) Nom common: Nime (French); Vernacular name; Nim (Moundang), lim (Toupouri); Convinced and potentialmedicinal plant (13); Tree native of India	Stem bark	Decoction of 1.5 g of stem bark per kg of body weight in 3 liters of water for 20 min.	Orally	**Malaria, Diabetes, Hepatitis**: Take 250 ml of decoction, 2 times a day, for 7 days.
30-Phyllanthus amarus (Phyllanthaceae); Aloum (Ewondo); Potential and convinced plant (14-15); Tree of South to North-West region of Cameroon forests	Aerial parts	Decoction of 1.5 g of aerial parts per kg of body weight in 3 liters of water for 20 min.	Orally	**Diabetes**: Take 250 ml of decoction, 2 times a day, for 7 days.

III. Discussions

Previous studies on many of the recorded plants have confirmed their traditional uses and/or their local people's traditional knowledge on environment. On *Anacardium occidentale*, 262 insect species were recorded and identified. The most important insects attacking this plant are *Apate terebrans*, *Eteoryctis gemoniella*, *Helopeltis schoutedeni* and *Helopeltis anacardii,* which are respectively wood-borer, leaf-miner, and mirid-bugs and distortion of young leaves. Fortunately beneficial insect species that are predators, parasitoids, pollinators and vertebrate predators live also in *A. occidentale* trees (16). *Azadirachta indica* trees are bioactive for man diseases (17) and possess beneficial and harmful effects on biodiversity. For beneficial effects, *A. indica* trees are much ameliorated plants by its valued nitrogen-fixing role. Also, the crop fields where these trees are planted, various insect-pests are destroyed. The bioactive compounds accomplish beneficial effects which interrupts the life cycle of handful living organisms. But the bad consumption of seed oil affected dangerously children by provoking nausea, diarrhea, vomiting, drowsiness, respiratory difficulty, seizures, enlarged liver, general discomfort and die (18-19). Sheep, goats, guinea pigs, avian and aquatic species are also intoxicated by neem (20-23).

Moringa oleifera plays important roles in the environment such as cyanobacterial removal, purifying water, crop fertilizers, and possible toxicity in it medicinal uses. In the natural water treatment processes seed powder is flocculants which remove color, turbidity and organic matter; the seeds are also coagulants which remove cyanobacteria. The sludge left over from the water purification can be used as a bio-compost for other crops. On the contrary to artificial coagulants and flocculants, the seeds of *M. oleifera* plant are non-toxic, biodegradable and therefore less handful to the environment. In a dry area of Far North, *Moringa oleifera* tree by growing fast and well, plays a role in the fight against desertification that is partially caused by climate change. The presence of long taproot makes this plant resistant to the drought condition of this region. It is also used to combine soil erosion in the region where strong winds and long dry spells occur simultaneously (24-26). *Aloe buttneri* plants possess fat water-storing leaves. A particularly devastating form of human usage of inselbergs is large-scale extracting due to an increasing demand for granite, iron and gneiss for construction purposes. *Aloe buttneri* can lead to their complete eradication at the landscape level as it is observed around Yaounde town on hills where the extinction of this species was rapid (27).

IV. Conclusion

Six plants have beneficial and/or harmful effects on the environment. Suspected and potential medicinal plants represent respectively thirty-seven percent (37 %) and three percent (3 %) of recorded medicinal plants. The application of indirect methods of identification of medicinal plants has permitted to add 40 % of new medicinal uses for this study. Therefore the results of this study represent an important baseline data for the design and implementation of strategies for plants protection and their sustainable uses. The thorough application of these methodologies can reveal important suspected and potential medicinal plants in several sociocultural groups of Africa. Further work is however required to fully understand the similarities of color and/or forms of plant organs and human organs. Increasing methods on how to collect indigenous environmental knowledge in the field is demonstrating a solid base from which successful environmental threats' fight should be achieved.

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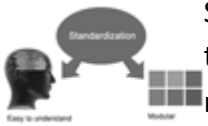

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TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

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21. 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 to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After 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 to 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 in your research.

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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 criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the progression.

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To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

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The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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 a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
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- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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 available information
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Topics	Grades		
	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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