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Chemistry

Shea Butter Samples in Nigeria

Phytochemical Study and Analysis

Highlights

Substituted and Fused Pyridines

Heterocycles Bearing Active Methyl

Discovering Thoughts, Inventing Future

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Chemistry

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Phytochemical Study and Analysis of Extracts from Two Plants: *Teucrium Polium* and *Lavandula Stoechas* from the Region of Essaouira, by Gas Chromatography Coupled with Mass Spectrometry (GC/MS)

By Zakya M'hamdi, Oumaima Zargane, Mohamed Elhouri, Maryame Sabiri & Ali Amechrouq

Moulay Ismail University

Abstract- Lavandula stoechas and Teucrium Poliums are two plants that belong to the Lamiaceae family. They are widely used in traditional medicine. They mainly have analgesic, anti-infectious and antispasmodic properties.

For this it is interesting to highlight the extracts of these two plants by carrying out, on the one hand, a phytochemical study and the determination of total polyphenols and, on the other hand, analyzes by gas chromatography coupled with mass spectrometry.

Keywords: teucrium polium, lavandula stoechas, phytochemical, gas chromatography.

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Phytochemical Study and Analysis of Extracts from Two Plants: *Teucrium Polium* and *Lavandula Stoechas* from the Region of Essaouira, by Gas Chromatography Coupled with Mass Spectrometry (GC/MS)

Zakya M'hamdi [α], Oumaima Zargane [σ], Mohamed Elhouri [ρ], Maryame Sabiri [ω] & Ali Amechrouq [¥]

Abstract- ***Lavandula stoechas*** and ***Teucrium Poliums*** are two plants that belong to the Lamiaceae family. They are widely used in traditional medicine. They mainly have analgesic, anti-infectious and antispasmodic properties.

For this it is interesting to highlight the extracts of these two plants by carrying out, on the one hand, a phytochemical study and the determination of total polyphenols and, on the other hand, analyzes by gas chromatography coupled with mass spectrometry.

The phytochemical results showed that ***Lavandula stoechas*** and ***Teucrium Poliums*** are rich in alkaloids, catechetical tannins, flavonoids and saponisides. For total polyphenols The highest concentration was measured in the extract of ***Lavandula stoechas.***

On the other hand, the results of the GC / MS analysis of the majority chemical composition of the extract and of the essential oil are identified by comparison of their spectral data and their retention times with those of the reference compounds and the databases (NIST).

Analyzes by gas chromatography coupled with mass spectrometry of the extract and essential oil of ***Lavandula stoechas*** respectively showed 35 compounds of which (+) - 2-Bornanone is the most predominant (55.68%) and 36 compounds including Cyclohexene, 1-methyl-4- (1-methylethylidene) which very much abandoning (47.57%); while ***Teucrium Polium*** extract showed 10 compounds including Tricyclo [5.1.0.0 (2,4)] oct-5-ene-5-propanoic acid, 3,3,8,8-tetramethyl- (17,53) is the majority.

Keywords: teucrium polium, lavandula stoechas, phytochemical, gas chromatography.

I. Introduction

Morocco, a country in northern Africa, known for its climate (Mediterranean, semi-arid) and the nature of its soils, has a particularly rich and varied flora in medicinal and food plants [1], traditionally used to treat several diseases, including diabetes, cardiovascular diseases, digestive system pathologies and other pathologies [2].

Moroccan flora brings together several species of plants that have not yet been studied or have been studied, but endowed with real pharmacological properties. This wealth must now be exploited [3].

As part of the promotion of aromatic plants from the ESSAOUIRA region, a study of two very well-known medicinal plants widely used in traditional Moroccan medicine known under the name of HALHAL "*Lavandula stoechas*" and JAADIA "*Teucrium Polium*". These plants grown spontaneously possessing mainly analgesic, anti-infectious and antispasmodic properties and can be used as a culinary condiment [4].

II. Botanical Description

a) Lavandula stoechas

The leaves of *Lavandula stoechas* are opposite linear 1-4 cm long, with a rolled margin, to mentose and gray on both sides (Figure 1). While the Flowers are grouped by 6-10 in false quadrangular whorls, very tight 2-3 cm wide, forming false terminal spikes. Rhomboidal-spatulate bracts 4-8 mm long, brownish purple (Figure02); the tallest, sterile, are much larger (1-5cm long) and blue-violet. Tubular calyx 4-6 mm long with 13 veins and 5 teeth, the upper tooth being terminated by an enlarged appendix. Dark violet corolla 6-8 mm long, vaguely bilabiate, with 5 lobes 4 stamens 2 short and 2 long, inhabiting dry locations, rocky, non-calcareous mountain pastures [5].

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Figure 1: Leaves and Flowers of *Lavandula stoechas*

b) Teucrium polium

Teucrium polium is a whitish, perennial herbaceous plant, very fragrant and very polymorphic, where determination of micro morphs is always delicate. It is branched from the base, has linear, greyish-green leaves strongly revolving on the margins, is a perennial plant often taken, covered with woolly hairs which give it a bluish gray color and its size varies between 6 and 25 cm.

The flowers are white or yellowish in dense clusters at the top of the twigs [6]. Their characteristic is to have only one lip, the lower lip, grouping together the 5 fused petals [7].

Figure 2: Flowers and whole plant of *Teucrium Polium*

III. Preparation of Plant Material

The plant material used during this study is the aerial part of *Lavandula stoechas* and *Teucrium Poliums* collected in February 2019 in the region of EL HRARTA province of Essaouira Morocco.

After harvesting, the used parts of the plant (leaves and stems) were dried in a well ventilated place, for one month, at room temperature and protected from light to avoid any modification or degradation of the constituents present.

After drying, these parts were cut into small pieces, then subjected to extractions in order to extract the different classes of chemical compounds contained in our plant for phytochemical tests.

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Figure 3: Geographical location of the harvesting sites of the plants studied

a) Extraction of total polyphenols

There are various extraction methods which are particularly suitable for the extraction of polyphenols, from which the method which was carried out by.

25 g of the aerial part of the plant is subjected to maceration, respectively, with hexane and dichloromethane in order to remove all pigments and lipids.

The marc thus obtained is subjected to maceration for 24 hours in the presence of a mixture (methanol / water / acetone (60/10/30).

The crude extract of total polyphenols is obtained after evaporating the filtrate to dryness in a rotary evaporator at a temperature of 60 ° C (Figure 4).

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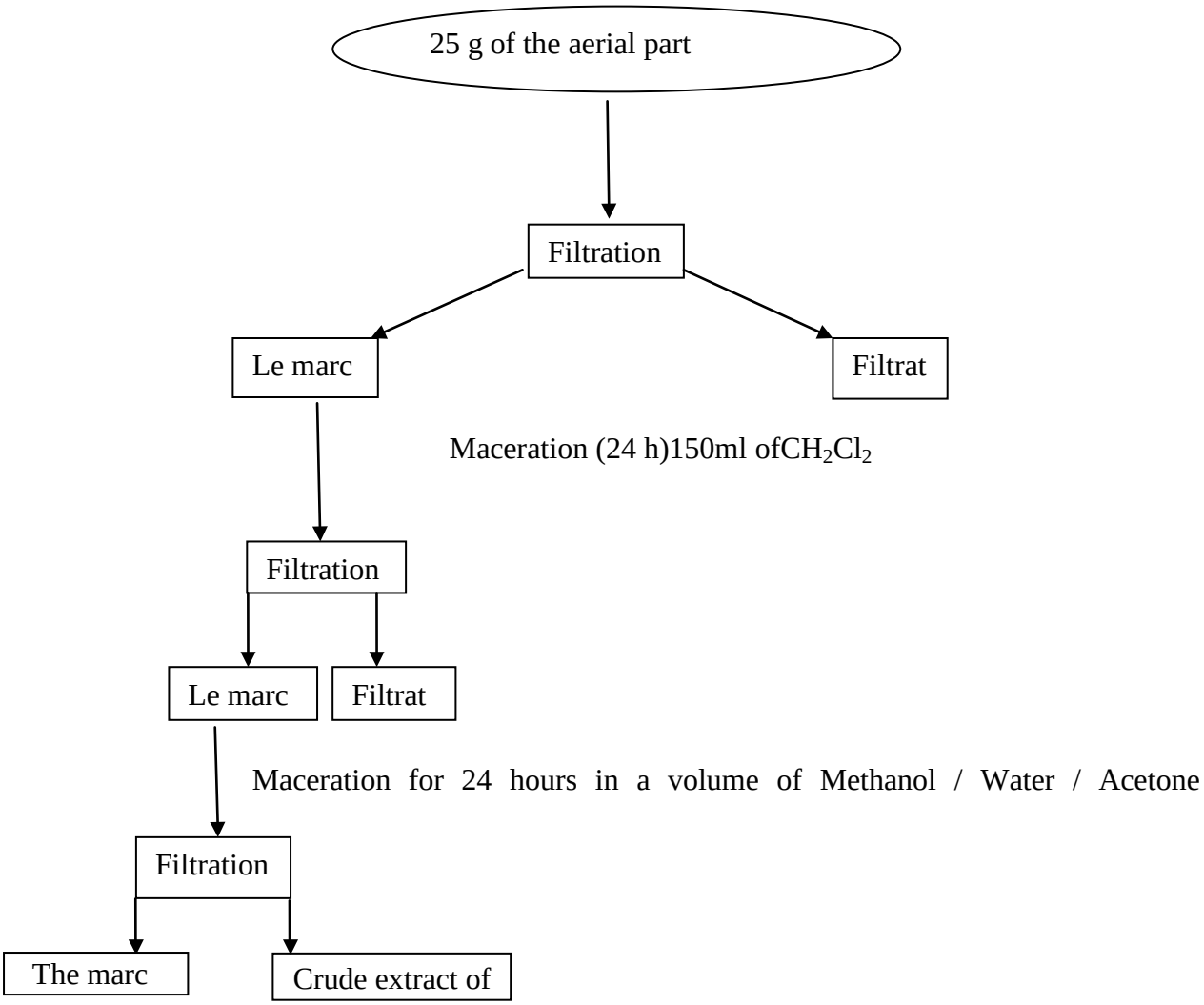

Figure 4: The crude extract of total polyphenols

b) Extraction of essential oils by hydro-distillation

The essential oils of *Lavandula stoechas* and *Teucrium Poliums*were isolated by hydro distillation using a Clevenger type apparatus. It consists of directly immersing the plant material (200 g) to be treated in a flask filled with distilled water (3.5 L) surmounted by a column 60 cm in length connected to a condenser. Everything is then brought to the boil for 3 hours.

c) Soxhlet extraction

The extracts of the two plants studied were obtained by mounting Soxhlet with a continuous solvent of a chemical species contained in a solid powder.

Table 1: Solvent, volume and mass used for the extraction of the two plants

	Lavandula stoechas	*Teucrium Polium*
Solvent	Dichloromethane	Ethyl acetate
Volume	350 ml	350 ml
Masse de la plante	50g	50g

IV. Results and Discussions

a) Moisture level of the two plants

After having treated the two plants *Lavandula stoechas* and *Teucrium Poliums*in an oven at a temperature of 110 ° C for 4 hours, the humidity level corresponds to approximately 52.9% for *Lavandula stoechas* and 2.01% for *Teucrium Polium* distributed as shown in Figures 14 and 15. More than half of the fresh weight of the *lavandula* species is water while the majority of the *Teucrium Polium* species is in the dry form.

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b) *Lavandula stoechas*

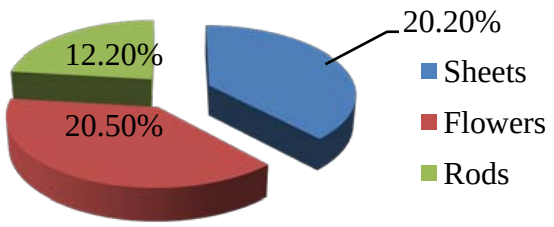

Figure 5: Water content of different parts of *Lavandula stoechas*

c) *Teucrium polium*

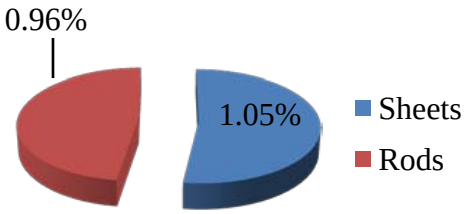

Figure 6: Water content of different parts of *Teucrium Polium*

d) *Extraction yields of essential oils and extracts*

The essential oils were obtained by hydro distillation, and the extracts by the Soxhlet assembly, the yields of different extractions are shown in figure 7.

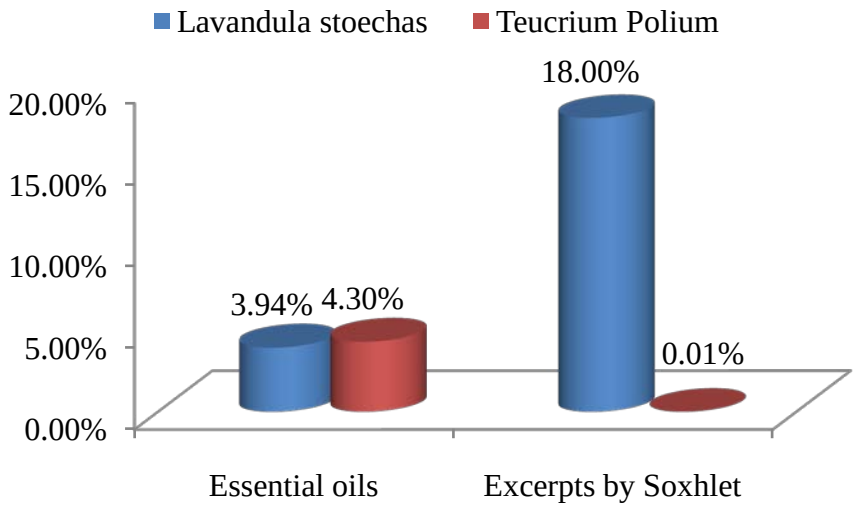

Figure 7: Diagram showing the yields of different extractions of the two plants

The extraction results obtained show that *Lavandula stoechas* gave a very good yield of essential oil (3.94%), this plant also gives a significant amount of extract (≤18%). On the other hand, *Teucrium Polium* has a very low quantity of essential oil (0.01%) but it gives a lesser quantity of extract (4.30%).

e) *Results of the phytochemical tests of the two plants studied*

The phytochemical tests carried out on the different preparation methods made it possible to demonstrate the presence of some secondary metabolites present in the two plants studied by qualitative reactions (precipitation, coloring with specific reagents, or by examination under UV light). The results are summarized in Table 2.

These tests showed the richness of two plants in alkaloids, tannins, flavonoids and saponosides, with the absence of starch, proteins, iridoids and prothocynidols. But *Lavandula stoechas* is richer than *Teucrium Poliumen* in glucosides, sterols and tri-terpenes.

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Table 2: Results of the phytochemical tests of *Lavandula stoechas* and *Teucrium Poliums*

f) Total polyphenol content of the two plants

The estimation of the total polyphenol contents was carried out by the spectrophotometric method and the Folin-Ciocalteu reagent. The results obtained are expressed in milligram gallic acid equivalent per gram of extract (mg GAE / mg extract). The linear regression equation of the plotted gallic acid calibration curve is: (y = 0.005x + 0.9919, r2 = 0.9919) (Figure 8).

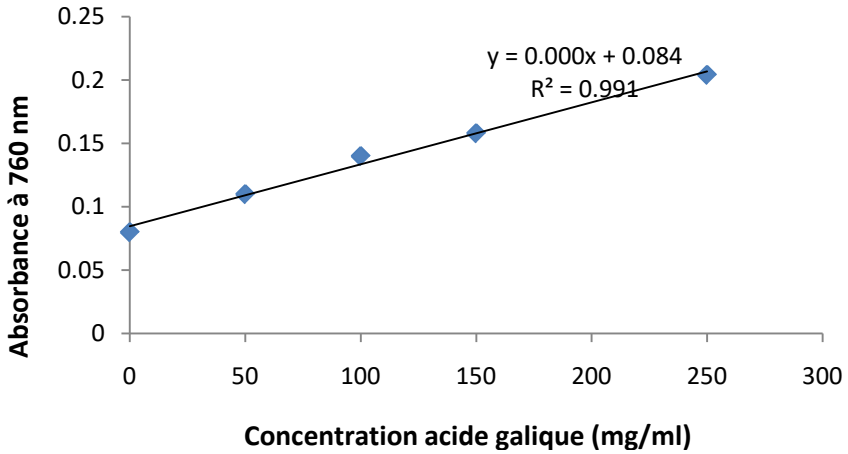

Figure 8: Gallic acid calibration curve for the determination of total phenols

The results obtained are of the order of 95.18 mg EAG / mg extract for *Lavandula stoechas* and 72.26 mg EAG / mg extract for *Teucrium Polium*. The concentration of phenolic compounds is noted in the extract of *Lavandula stoechas*. The highest concentration of phenols was measured in the extract of Figure

V. Gas Chromatography of the Two Plants

a) Lavandula stoechas

Lavandula stoechas essential oil and extract has been identified by GC / MS. Analysis of the *Lavandula stoechas* extract by GC / MS gave the chromatogram shown in Figure 9.

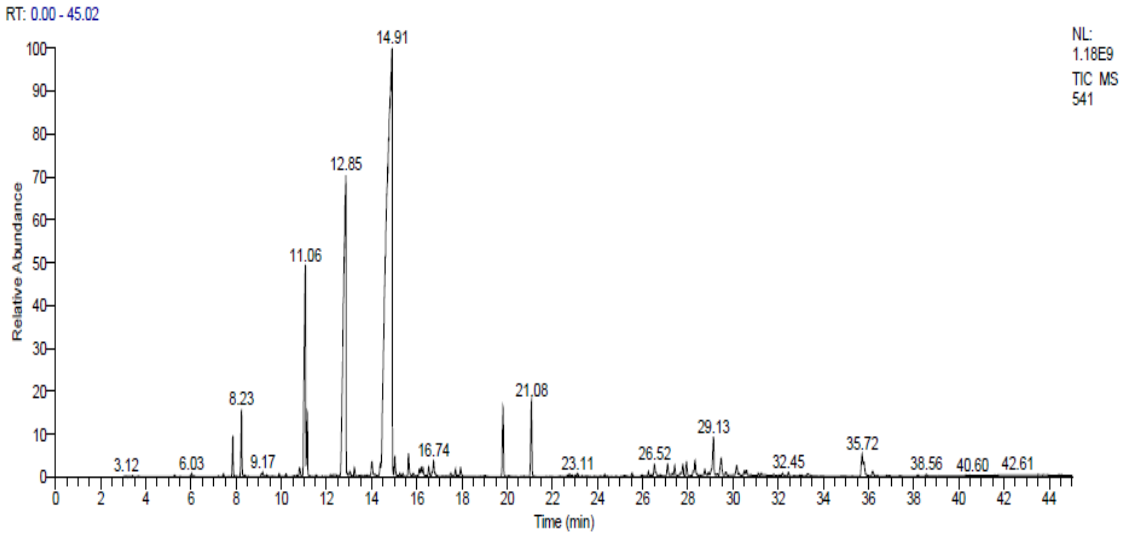

Figure 9: CG / MS chromatogram of *Lavandula stoechas* extract

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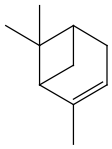
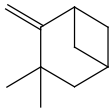
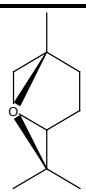
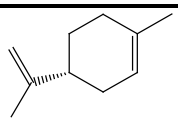
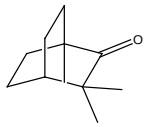
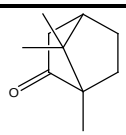
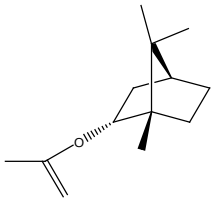
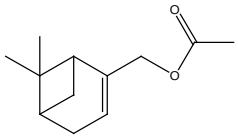
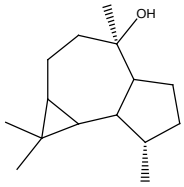
The results of the analysis of the majority chemical composition of the extract and essential oil are identified by comparing their spectral data and retention times with those of reference compounds and databases (NIST).

Analysis of *Lavandula stoechas* extract showed 35 peaks, 9 of which were predominant, of which (+) - 2-Bornanone was the most predominant (55.68%) (Table 3).

Table 3: Major molecules of *Lavandula stoechas* extract

Retention time	Brute formula	Molecules	Molecule structure	Percentage(%)
7.85	$C_{10}H_{16}$	α-Pinene		0,98
8.23	$C_{10}H_{16}$	Camphene		1.66
11.06	$C_{10}H_{18}O$	Eucalyptol		7.86
11.14	$C_{10}H_{16}$	D-Limonene		1.46
12.85	$C_{10}H_{16}O$	Fenchone		17.51
14.91	$C_{10}H_{16}O$	(+)-2-Bornanone		55.68
19.82	$C_{12}H_{18}O_2$	Isobornyl acetate		1.94
21.08	$C_{15}H_{26}O$	Myrtenylacetate		2.18
29.13	$C_{15}H_{25}O$	1HCycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-,[1aR (1aà,4á,4aá,7à,7aá,7bà)]-		1.34

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Likewise, the analysis of the essential oil of *Lavandula stoechas* by CG / MS, gave us the chromatogram shown in figure 10.

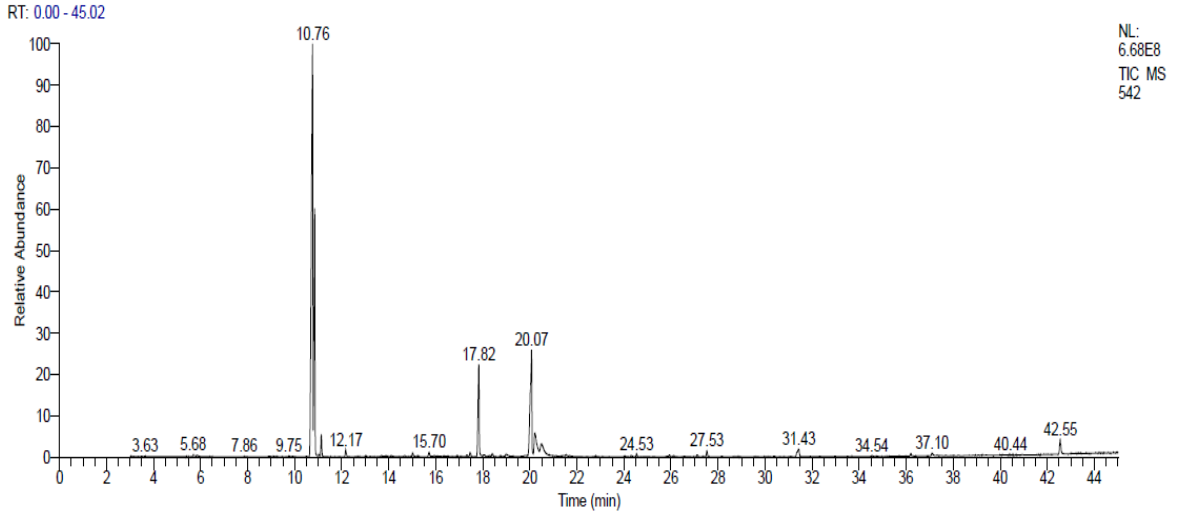
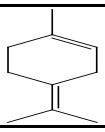
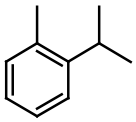
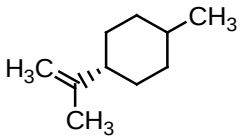
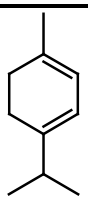
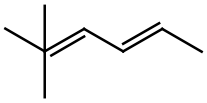


Figure 10: GC / MS chromatogram of *Lavandula stoechas* essential oil

Analysis of the essential oil of *Lavandula stoechas* revealed 36 compounds, 19 of which were predominant, including Cyclohexene, 1-methyl-4- (1-methylethylidene), which very much abandoned (47.57%) (Table 4).

Table 4: Majority molecules of *Lavandula stoechas* essential oil

Retention time	Brute formula	Molecules	Molecule structure	Percentage (%)
10.76	$C_{10}H_{16}$	α-Terpinolene		47.57
10.85	$C_{10}H_{14}$	o-Cymene		18.15
11.13	$C_{10}H_{16}$	D-Limonene		1.52
12.17	$C_{10}H_{16}$	δ-Terpinene		0.53
15.01	C_9H_{16}	6,6-Dimethylhepta-2,4-diene		0.34

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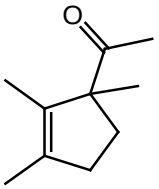
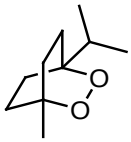
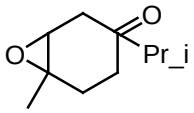
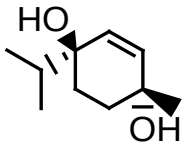
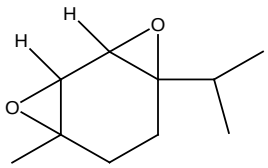
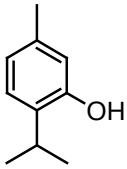
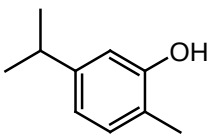
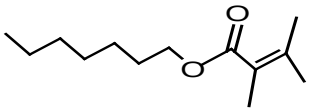
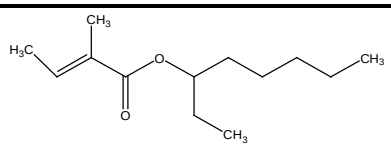
15.70	$C_{10}H_{16}O$	1-(1,2,3-Trimethylcyclop ent-2-enyl)-ethanone		0.45
17.46	$C_{12}H_{18}O_2$	1S,2R,4R,7R)-4 Isopropyl-7-methyl-3,8-dioxatricyclo[5.1.0.02,4]octane.		0.38
17.82	$C_{10}H_{16}O2$	Ascaridole		8.65
18.40	$C_{10}H_{16}O2$	7Oxabicyclo[4.1.0]heptan -2-one,3-methyl-6-(1-methylethyl)-		0.27
18.99	$C_{10}H_{18}O_2$	trans-Ascaridol glycol		0.27
20.07	$C_{10}H_{16}O_2$	Isoascaridol		12,24
20.21	$C_{10}H_{14}O$	Thymol		2.72
20.49	$C_{10}H_{14}O$	Phenol,2-methyl-5-(1-methylethyl)-		1 .27
24.53	$C_{12}H_{22}O_2$	Heptyl(E)-2-methylbut-2-enoate		0.22
27.53	$C_{13}H_{24}O_2$	Octyltiglate		0.51

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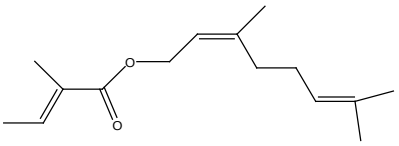
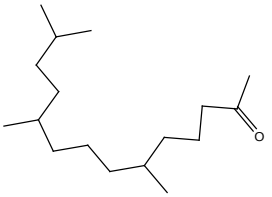
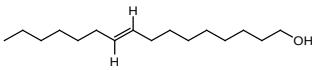
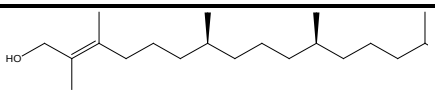
31.43	$C_{15}H_{24}O_2$	Geranylangelate		1 .21
36.20	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl-		0.22
37.10	$C_{16}H_{32}O$	Hexadecen-1-ol, trans-9-		0.32
42.55	$C_{20}H_{40}O$	Phytol		1.39

b) Teucrium polium

Analysis of the *Teucrium Polium* extract by GC / MS gave us the chromatogram shown in Figure 11.

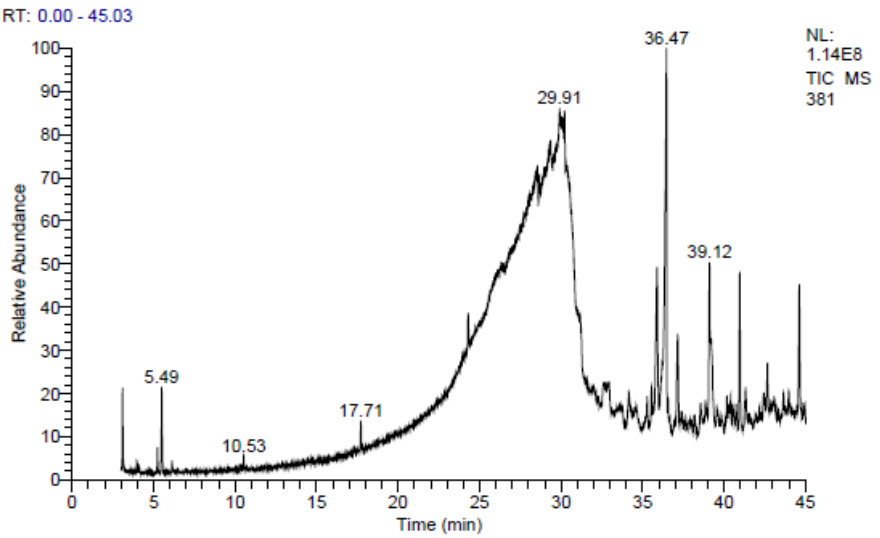


Figure 11: CG / MS chromatogram of *Teucrium Polium* extract

The results of the analysis of the majority chemical composition of the extract are identified by comparing their spectral data and their retention times with those of reference compounds and databases (NIST).

Analysis of the *Teucrium Polium* extract revealed 35 peaks, 10 of which were predominant, including Tricyclo [5.1.0.0 (2,4)] oct.-5-ene-5-propanoic acid, 3,3,8,8- tetramethyl- (17.53) is the most predominant (Table 5).

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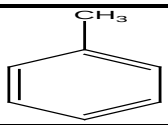
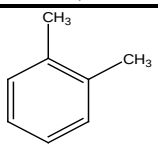
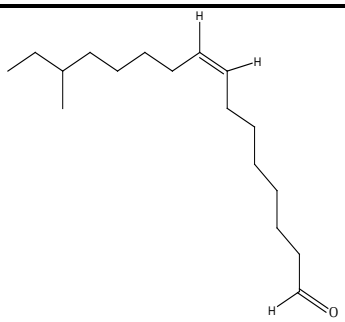
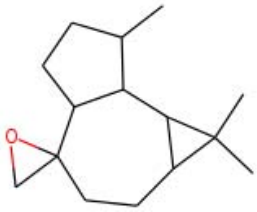
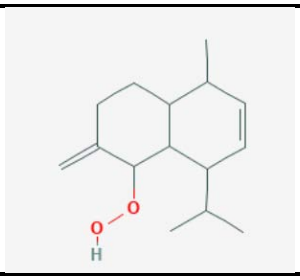
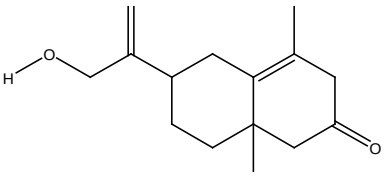
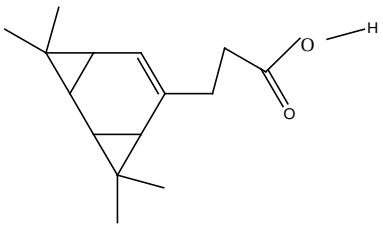
Table 5: Majority constituents of *Teucrium Polium* extract

Retention time	Brute formula	Molecules	Molecule structure	Percentage (%)
3.09	C_7H_8	Toluène		2,37
5.49	C_8H_{10}	o-Xylène		2.93
29.91	$C_{17}H_{32}O$	8-Hexadecenal, 14-méthyl-, (Z)-		3.22
30.08	$C_{15}H_{24}O$	Aromadendrene oxide-(1)		2.76
30.22	$C_{16}H_{34}$	Hexadécane		2.59
31.19	$C_{15}H_{24}O_2$	Murolan-3,9(11)-diene-10-peroxy		3.32
35.90	$C_{15}H_{22}O_2$	6-(1 Hydroxy-methylvinyl)-4,8a-dimethyl-3,5,6,7,8,8 a-hexahydro-1H-naphthal en-2-one		9.37
36.47	$C_{15}H_{22}O_2$	Tricyclo[5.1.0.0(2,4)]oct-5 -ene-5 propanoic acid, 3,3,8,8-tetramethyl-		17.53

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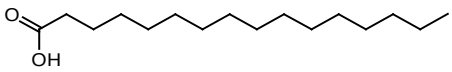
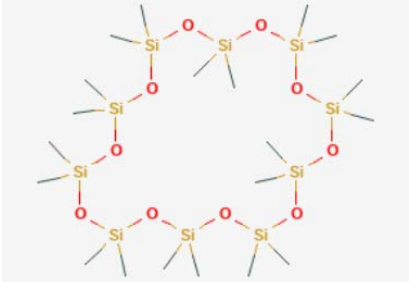
39.12	$C_{16}H_{32}O_2$	n-Hexadecanoicacid		12.61
40.97	$C_{20}H_{60}O_{10}Si_{10}$	Cyclodecasiloxane, eicosamethyl-		6.62

VI. Conclusion

Lavandula stoechas and *Teucrium Poliums*are two plants that belong to the Lamiaceae family. The phytochemical study of the extract from the two plants *Lavandula stoechas* and *Teucrium Poliums* showed the richness in alkaloids, tannins, flavonoids and saponosides, with the absence of starch, proteins, iridoids and prothocynidols. Moreover, *Lavandula stoechas* is richer than *Teucrium Polium* in glucosides and in sterols and tri-terpenes.

Analysis of the chromatogram of the extract of *Lavandula stoechas* revealed 9 major compounds including bornanone which is high proprtion (55.68%), While the *Teucrium Polium* extract contains 10 major compounds including Tricyclo [5.1.0.0 (2,4)] oct-5 -ene-5-propanoic acid, 3,3,8,8-tetramethyl- (17.53%) which in large quantity.

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The Antibacterial Activity against Methicillin-Resistant *Staphylococcus Aureus* from the Ethyl Acetate Extract in *Clinacanthusnutans*

By Kamol Yusook, Wrannawath Inthong & Petaya Panvongsa

Abstract- *Clinacanthusnutans* (CN) plants have been received much interest from phytochemical researchers because of their plentiful bioactive compounds of total phenolic compound (TPC) and total flavonoid compound (TFC). Many of report study exhibit wide varieties of biological activities. In this study, herbal ethyl acetate extracts was isolated from *C. nutans* (CN) leaf, identified by Thin layer Chromatography. Phytochemical screening illustrated to the tannins compound. Crude extracted was freshly prepared by solubilizing in DMSO and immediately diluted in Müller-Hinton broth for antibacterial testing. The data showed that *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) of three strains such as ATCC 29213, MRSA 4738 and MRSA 20649 were susceptible to crude extracts in disc diffusion method.

Keywords: *clinacanthusnutans, antibacterial, MRSA, tannin, TEM.*

GJSFR-B Classification: *DDC Code: 614.44 LCC Code: RA761*

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Kamol Yusook [α], Wrannawath Inthong [σ] & Petaya Panvongsa [ρ]

Abstract- *Clinacanthusnutans* (CN) plants have been received much interest from phytochemical researchers because of their plentiful bioactive compounds of total phenolic compound (TPC) and total flavonoid compound (TFC). Many of report study exhibit wide varieties of biological activities. In this study, herbal ethyl acetate extracts was isolated from *C. nutans* (CN) leaf, identified by Thin layer Chromatography. Phytochemical screening illustrated to the tannins compound. Crude extracted was freshly prepared by solubilizing in DMSO and immediately diluted in Müller-Hinton broth for antibacterial testing. The data showed that *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) of three strains such as ATCC 29213, MRSA 4738 and MRSA 20649 were susceptible to crude extracts in disc diffusion method. Using the two-fold microdilution method, it was found that the extracts possessed antimicrobial activity against MRSA with minimum inhibitory concentration (MIC) of CN extract is 3.125 mg/ml. Minimum bactericidal concentration (MBC) is 6.25 mg/ml. To investigate the mechanism of action of the extracts, transmission electron microscopy (TEM) was performed to observe the ultrastructure of MRSA. The TEM images showed that ruptured the bacterial cell membrane and cell wall. It is suggested that the action of the crude extracts is likely to be the direct disruption of the cell membrane.

Context: *Clinacanthusnutans* belongs to the Acanthaceae herbal family and very common grows in Southeast-Asia. It is traditionally used as a remedy for herpes, snake and insect bites, and inflammation.

Aims: To investigate the antibacterial activity against Methicillin-resistant *Staphylococcus aureus* from the ethyl acetate extract of *Clinacanthusnutans*.

Settings and Design: In vitro study

Methods and Material: Disc diffusion method, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were conducted to evaluate antibacterial activity. Transmission electron microscopy (TEM) was conducted to investigate the ultrastructure of MRSA.

Statistical Analysis used: Means, SD

Results: The data showed that Methicillin resistant Staphylococcus aureus (MRSA) of three strains such as ATCC 29213, MRSA 4738 and MRSA 20649 were susceptible to crude extracts. Using the two-fold microdilution method, it was found that the extracts possessed antimicrobial activity against MRSA with minimum inhibitory concentration (MIC) of CN is 3.125 mg/ml. Minimum bactericidal concentration (MBC) is 6.25 mg/ml. To investigate the mechanism of action of the extracts, transmission electron microscopy (TEM) was performed to observe the ultrastructure of MRSA. The TEM images showed that ruptured the bacterial cell membrane and cell wall.

Conclusions: Tannins is the active compound in the ethyl acetate extract from *C. nutans* leaves. Antibacterial possess against MRSA. It is suggested that the action of the crude extracts is likely to be the direct disruption of the cell membrane.

Keywords: *clinacanthusnutans, antibacterial, MRSA, tannin, TEM.*

Key Messages: The leaves of *C. nutans* are rich in natural antibacterial total phenolic compound such as tannins. It possesses against MRSA. To investigate the mechanism of action of the extracts, transmission electron microscopy (TEM) was performed to observe the ultrastructure of MRSA. The TEM images showed that ruptured the bacterial cell membrane and cell wall.

I. Introduction

Currently, the numbers of nosocomial infections have increased continuously. *Staphylococcus aureus* is a major problem in nosocomial infections disease such as pneumonia, operative wound infections and sepsis [1]. *S. aureus* was caused infections include skin lesions such as boils, furuncles and more serious infections, for example, phlebitis, meningitis, endocarditis and urinary tract infections. The mortality rate for nosocomial endocarditis is found higher than that for urinary tract infection when the pathogen is *S. aureus* [2]. The lesion of staphylococcal infection is the abscess, which consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus containing organisms and leukocytes. The organisms may be disseminated hematogenously, even from the smallest abscess. *S. aureus* has a tendency to spread to particular sites, including the bones, joints, kidneys, and lungs. This may result to virulent sepsis. The presentation of staphylococcal sepsis is similar to that of gram-negative sepsis, with fever, hypotension, tachycardia, and tachypnea. Severe cases progress to many organs dysfunction, lactic acidosis and death.

Many strains of *S. aureus* are developing resistance to available antibacterial agents, creating a serious problem in public health such as methicillin-resistant *S.aureus* (MRSA). The organism may acquire genes encoding enzymes, for example β-lactamase that

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destroys the antibacterial agent before it can have an effect. For these problems, searching and development of novel antibacterial compounds are urgently required.

Clinacanthusnutans are well known and interesting sources for new antibacterial agents. It is usually slithered along other trees, about 1-3 meters tall, The trunk is round, the skin is smooth and green. Propagate with the method of cutting or separating the rhizomes to plant. Grows well in all kinds of soils. There are distribution zones in China, Vietnam, Indonesia, Malaysia and Thailand. In Thailand, it is commonly found in mixed forests in all parts of the country, or grown in common houses. The appearance of the leaves is spear-shaped, oval, narrow, parallel edges. The leaves are dark green and smooth, the weasters bloom at the ends of the branches, each inflorescences have 3-6 flowers, the petals are red-orange, the petals are welded together into tubes. The tip is divided into 2 mouths, the lower and upper mouths have 5 petals, the petals are cylindrical, and the petals are green, equally long, with sticky glands around them. The flowers contain 2 male pollen, while the female pollen is hairless, flowering from around October to January (but rarely flowering). They are ubiquitous in antibacterial compounds and are commonly found in traditional medicine. These compounds have been used in traditional herbal medicine as the principal physiologically active constituents to treat human diseases for centuries. In addition, this class of natural products is becoming the subject of antimicrobial research. Many groups of pure compound possessing antiviral, antifungal or antibacterial activities have been isolated and identified for the structure [3].

Clinacanthus plants have been received much interest from phytochemical researchers because of their plentiful bioactive compounds of flavonoids, betulin, lupeol, n-butanol and glycoglycerolipids. Many of pure compounds exhibit wide varieties of biological activities. For example, botulin and lupeol have been reported to possess anti-action and anti-inflammatory activity [4]. There are several lines of evidence demonstrating its antimicrobial potential, including antiviral, antibacterial and antimycobacterial activities. Several studies have demonstrated the mechanisms of action underlying antimicrobial effects of flavonoids extracted from medicinal plants. Because of a variety of the structures in this phytochemical class, the mechanism of action previously established by researchers varies dramatically. Antimicrobial activities of the plant has been demonstrated, however its mechanism of action has never been documented. Therefore, in the present study, bioactive compounds will be purified from *C. nutans* leaves. Then screening test for antibacterial activities of crude extract against MRSA will be performed. Bacteria which are susceptible to crude extract will be used to investigate its mechanism of action. From the preliminary data, *C. nutans* crude extract showed best activity against the Gram-positive bacteria *S. aureus*. Moreover, the pilot study also revealed that the extract caused damage of bacterial cell wall and/or cell membrane. The data obtained from this study will provide scientific evidence to support the use or development of this compound as antimicrobial agent.

II. Subjects and Methods

a) Chemicals

Hexane, ethanol, methanol, dichrolomethane, ethyl acetate, NaCl, Agar, Mueller-Hinton, 0.5 McFarland, $CDCl_3$, glutaraldehyde, acetone and dimethyl sulfoxide were purchased from Carlo Erba (Italy). Vancomycin was purchased from Sigma-Aldrich (USA).

b) Plant Material and Herbal Crude Extraction

Clinacanthusnutans was collected from Phetchabun province, Thailand. Botanical identification was performed by Dr. Surangrut Punsang, Program of Biology, Phetchabun Rajabhat university (PCRU). A voucher specimen (PCRU-CN-001) was deposited at Program of Public Health, PCRU. The leaves was air dried at room temperature. The leaves was stored at room temperature until used for extraction.

100 grams of dried leaves was extracted with 500 ml ethyl acetate, using maceration for 7 days. The total extracts were filtered with Whatman No.1. Then the extracts were rotary evaporated and freeze dry. Thin layer chromatography (TLC) was used to confirm purity of the extract. Crude extract obtained was dissolved in ethanol and 5 μl of the solution was submitted to TLC on silica gel G_{60} F_{254} aluminium plates. Dichrolomethane: Methanol (95:5) was used as the eluent. Spot was detected by UV light at 254 to confirm the purification.

c) Determination of Constituents of Crude Extract by Phytochemical Screening

Phytochemical screening was carried out to identify constituents of *C. nutans* extract. The extract was screened for terpenoids, flavonoids, saponins, tannins and cardiac glycosides. Phytochemical screening was performed based on previously reported methods [5].

d) Antibacterial Assays

i. Disc Diffusion

Bacteria used in this study was obtained from Department of Medical Sciences Thailand (DMST). The antibacterial activities of ethyl acetate crude extract from *C. nutans* was evaluated against *Staphylococcus aureus* ATTC 29213, *Staphylococcus aureus* (MRSA) DMST 20649, *Staphylococcus aureus* (MRSA) DMST 4738. The screening of the antibacterial activity was done using disc diffusion method. Bacterial suspensions was prepared by inoculating one loopful of a pure colony into Mueller-Hinton Broth (MHB), incubated overnight and

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diluted in 0.85% NaCl. Cell suspensions, of which adjusted turbidity equivalent to that of a 0.5 McFarland standard, contains about 10^8 cfu/ml. These was used to inoculate on Mueller-Hinton Agar (MHA) plates by swabbing over the entire agar surface. The CN crude extract (25, 50, 75 µg/disc) was impregnated to filter paper discs (Whatman No.1, 6 mm diameter) and then placed on the previously inoculated agar plate. After 24 h of incubation at 37°C, the antibacterial activity was determined by measuring the diameter of the inhibition zones formed around the disc. Vancomycin and DMSO was used as a positive and vehicle controls, respectively[6].

ii. *Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*

A modified broth microdilution method according to Clinical and Laboratory Standard Institute Guidelines [7] was used to determine MIC and MBC of the extract. It was dissolved in DMSO and two-fold serial dilutions will be made in Mueller-Hinton broth (MHB) using 96-well flat bottom microtiter plate (Corning Life Sciences, USA). Suspension of bacteria in MHB was prepared from the overnight broth culture. The final bacterial cell concentration was adjusted to $5x10^5$ cfu/ml. The final concentration of the CN extract was ranged from 0.25-512 µg/ml. Vancomycin and DMSO was used as positive and negative controls, respectively. The MIC was considered as the lowest concentration of the agents showing no visible growth of microorganism after incubation at 37°C for 24 h. The MBC determination was carried out by subculturing 20 µl from the broth with no growth onto Mueller-Hinton Agar (MHA) plates followed by incubation for 24h at 37°C. The lowest concentration with no visible growth was taken as the MBC. All tests was performed in triplicate independent experiments.

e) Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was used to visualize the change in morphology at the membrane and cell wall ultrastructure of Methicillin resistant *S. aureus* after treatment with the CN extract. TEM preparations were made in accordance with the previously reported method with slight modifications [8].

The bacterial samples were prepared similar to the SEM method. After the extract treatment for 12 h, cells were gently washed with 0.1 M PBS (pH 7.2), fixed with 2.5% glutaraldehyde in PBS and rinsed with PBS. Post-fixation was then carried out with 1% osmium tetroxide (Electron Microscopy Sciences: Hatfield, PA, USA) in 0.1 M PBS for 2 h at room temperature. After washing in the buffer, the samples were dehydrated using sequential exposure for acetone concentrations ranging from 20 to 100%. Subsequently, infiltration and embedding were performed using Spurr's resin (EMS). Finally, the samples were sectioned using an ultramicrotome with a diamond knife and were mounted on copper grids. They were stained with 2% uranyl acetate and lead citrate. The samples were viewed with a JEM-1230 electron microscope (Tokyo, Japan). The morphology of bacterial cells was observed and compared to vancomycin-treated cells as positive control.

III. Results

a) Phytochemical Screening

The result showed that the CN extract exposed tannins compound (Table 1).

Table 1: Phytochemicals screening of ethyl acetate extract from *C. nutans* leaves

Compounds	CN extract
Flavonoid	-
Saponins	-
Cardiac glycoside	-
Tannins	+
Terpenoids	-

+: Presence and -: Absence of metabolites in the extract

b) Thin Layer Chromatography (TLC)

The results of the active compound extraction of *C. nutans* leaves are shown a dark green viscous after maceration. Thin layer chromatography (TLC) used to confirm purity of the extract. Dark green viscous obtained dissolved in ethanol and submitted to TLC on silica gel G_{60} F_{254} aluminium plates that shown in Figure 1. Dichrolomethane:Methanol (95:5) used as the eluent.

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Figure 1: TLC of the CN extract was detected by UV light at 254 nm

c) Antibacterial Activity

By disc diffusion assay, it was found that the CN crude extract can inhibit growth of *Staphylococcus aureus* ATTC 29213, *Staphylococcus aureus* (MRSA) DMST 20649, *Staphylococcus aureus* (MRSA) DMST 4738 at 25, 50 and 75 µg, respectively (Table 2). Whereas, Vancomycin was used as a positive control.

Table 2: Antibacterial activity of the CN crude extract using Disc diffusion

	Diameter of inhibitions zone (mm)			
	CN (µg/disc)			Vancomycin (µg)
Microorganism	25	50	75	30
Staphylococcus aureus 29213	12 ± 0.2	20 ± 0.6	25 ± 0.2	37 ± 0.5
Staphylococcus aureus (MRSA) 4738	10± 0.5	23 ± 0.6	25 ± 0.3	24 ± 0.7
Staphylococcus aureus (MRSA) 20649	10 ± 0.1	23 ± 0.2	26 ± 0.5	28 ± 0.7

Data are means ± SD (*n*=3)

The MIC and MBC values of the CN crude extract obtained using the microdilution method as shown in Table 3. The active compound of *C. nutans* leaves gave MIC and MBC values at 8 and 16 µg/ml against *Staphylococcus aureus* 29213, respectively. In addition, the antibacterial activity showed MIC and MBC values at 3.125 and 6.25 µg/ml against *Staphylococcus aureus* (MRSA) 4738 and *Staphylococcus aureus* (MRSA) 20649 when compared with control cell after 24 h of incubation at 37°C. The assay were carried out in triplicate.

Table 3: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the CN crude extract against MRSA compared with vancomycin

Microorganisms	CN		Vancomycin	
	MIC (mg /ml)	MBC (mg /ml)	MIC (µg/ml)	MBC (µg/ml)
Staphylococcus aureus 29213	8	16	4	8
Staphylococcus aureus (MRSA) 4738	3.125	6.25	4	8
Staphylococcus aureus (MRSA) 20649	3.125	6.25	4	8

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d) Transmission Electron Microscopy (TEM)

TEM analysis was conducted and the data showed that after incubation with MIC for 12 hr, the CN crude extract, as well as vancomycin, obviously ruptured bacterial cell membrane and/or cell wall (Figure 2). Cell death and irregular shape of bacterial cells were seen in the treated groups, the CN extract and vancomycin. Damage of cell wall and cell membrane of dividing cells were observed after 12 h of incubation with the CN extract and vancomycin, compared with control.

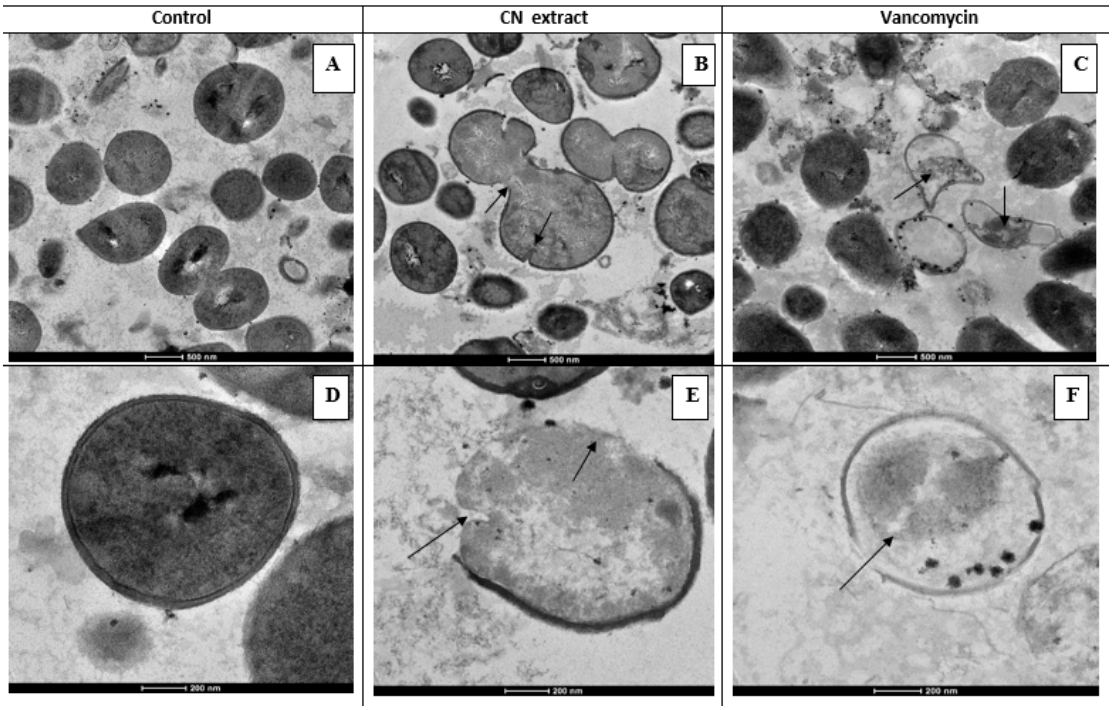

Figure 2: Transmission electron micrograph of MRSA treated with CN extract. A, B and C are overview of control and cells treated with CN extract and vancomycin respectively. Cell death and irregular shape of bacterial cells were seen in the treated groups, CN extract (B) and vancomycin (C). Damage of cell wall and cell membrane of dividing cells (indicated by arrows) were observed after 12 h of incubation with CN extract (E) and vancomycin (F), compared with control (D). Enlargement: bar = 200 nm, and 500 nm

IV. Discussion

According to this research, when extracting substances from the leaves of *C. nutans*, it was found that the extract was coarse. It has a sticky, dark green, pungent and sour odor, which can be an important characteristic of various substances, including flavonoids, which have anti-inflammatory effects, monoglycosyl diglycerides such as 1,2-O-dilinolenoyl-3-O-b-d-glucopyranosyl-sn-glycerol, and glycoglycerolipids, which inhibit the herpes simplex virus [9].

In this study, methicillin resistant *Staphylococcus aureus* (MRSA) was tested for anti-methicillin resistant *Staphylococcus aureus* (MRSA) with Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC). The results showed that coarse extracts can inhibit bacterial growth by causing inhibition zones on MHB compared to vancomycin, with statistically significant differences ($p<0.05$) as shown in Table 2. Antibacterial were identified by using MIC and MBC. The CN extract showed MIC of 3.125 μg/ml and MBC of 6.25 μg/ml against MRSA (Table 3). In addition, the researchers investigated whether all three strains were MRSA based on MIC values of ampicillin found to be greater than 512 μg/ml (data not shown). To investigate the mechanism of action, Transmission electron microscope (TEM), a powerful technique was applied to determine the physiological changes in bacterial cells. TEM is an technique with the change in morphology of extracellular and intracellular bacteria as shown in Figure 2. Moreover, the extract caused membrane bleb, ruffing or detachment, hypodense cytoplasmic release and cell vacuolization compared to control. After damaging the cell membrane, which acts as a barrier for most molecules, bacteria degrade the cell's permeability control, resulting in an increase in intracellular pressure and subsequently destruction of the cell wall [10,16].

It is widely known that total phenolic compounds in plants comprise several groups of phytochemicals such as tannins, terpenoids and flavonoids. Over the years, *C. nutans* have been reported to produce numerous betulin, lupeol, β-sitosterol, flavonoids and glycoglycerolipids compounds[11]. It have been found to possess significant antimicrobial, antiviral, and anti-inflammatory

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in mouse. The leaves of *C. nutans* are rich in natural antibacterial total phenolic compound such as tannins. In the present study, phytochemical screening was identified to flavonoids, saponins, cardiacglycosides, tannins and triterpenoids. It was found that the CN crude extracts exhibited tannins compound (Table 1). In general, tannins are found naturally in various parts of plants. The main chemical properties are antibacterial, antioxidant, anticancer and anti-inflammatory activity [12].

Tannin, sometimes called tannic acid, is a compound derived from phenolic acids and belongs to a group of compounds called polyphenols. Other compounds in the polyphenols group also have the ability to adhere to other molecules such as proteins, cellulose, starch and minerals easily[13], making tannins more insoluble and more resistant to decomposition than other compounds in the same group. As a matter of time, tannins can be found naturally. In different parts of the plant, both edible and inedible parts, such as root trees and bark of plants produce tannins to help protect yourself from natural pests. Foods containing tannins are most commonly found in tea, coffee, chocolate and wine. Tannins are substances that belong to the group of polyphenol compounds. Chemicals with the main properties are antioxidants[14,15]. It helps prevent inflammation and may help against cancer. Eating foods high in tannins can help fight free radicals, one of the major problems that contribute to cell degeneration in the body and can lead to cancer. Antioxidants like tannins can also help prevent and reduce inflammation. This makes the wound regenerate more quickly. In conclusion, one recent study found that *C.nutans* have antimicrobial properties, these medicinal plants can be a source for isolation of anti-MRSA compounds.

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Part-III: Utilities of Active Methylene Compounds and Heterocycles Bearing Active Methyl or having an Active Methine in the Formation of Substituted and Fused Pyridines

By Mohamed Abdel-Megid
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Abstract- This review discusses how we use the most common and novel synthesized active methylene compounds as well as heterocycles having active methyl or methine in the syntheses of a wide variety of substituted and fused pyridines interesting medical and pharmaceutical importance. Many synthetic approaches were used for the preparation of target heterocyclic systems such as cyclocondensation reactions, ring opening-ring closure, cycloaddition, acid- or base-catalyzed reaction, intermolecular cyclization and self-condensation have been reviewed in this paper. Also, the antimicrobial activity carried out on some newly selected synthesized pyridines and their fused derivatives were reported.

Keywords: active methylene, pyridines, thiazolopyridines, indopyridines, pyridopyrimidines, pyridotriazines, pyridotriazepines, antimicrobial activity.

GJSFR-B Classification: LCC Code: E487

PARTIIIUTILITIESOFACTIVEMETHYLENECOMPOUNDSANDHETEROCYCLESBEARINGACTIVEMETHYLORHAVINGANACTIVEMETHINEINTHEFORMATIONOFSUBSTITUTEDANDFUSEDPYRIDINES

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I. Introduction

Pyridine derivatives are important heterocyclic systems whose preparation, reactivity, and properties are of continuing interest. The biological activity associated with naturally occurring and synthetic pyridines has led to the development of pyridine-containing medicinal scaffolds and investigations into their pharmacological properties [1]. Benzopyridines specially quinolinones are the effective moiety in a large number of natural and synthetic heterocyclic compounds that exhibit signi ficant antibiotic activity with a wide variety of significant medicinal, pharmacological, and industrial applications[2]. In continuation to our recent target in setting on some heterocyclic reviews discuss utility of active methylene compounds and heterocycles bearing active methyl or having an active methine in their structures in heterocyclization of five-, six-, and seven-membered heterocyclic systems of important applications in many fields such as medicine, agriculture, pharmacology and pharmaceutical. consequently, we recently publish some reviews [3-6] and this review summarized our work done in the last three decades and it involves the methods developed for the syntheses of substituted and fused pyridine derivatives using the titled compounds and reported the antimicrobial activity of some selected synthesizes compounds.

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II. Formation of Substituted Pyridines

Some active methylene compounds such asmalononitrile, cyanoacetohydrazide, cayno-acetamide, cyanothioacetamide, and an enaminone were used for the synthesis of some pyridines bearing heterocyclic substituents and tested their biological activities.

a) Using Synthesized Enones

α, β- Unsaturated ketones are called chalcones or enones was synthesized via the interaction of aromatic aldehydes with compounds having active methylene or methyl groups in their structures. The activated influence of the carbonyl group on the exocyclic double bond render the enones susceptible to the cycloaddition reaction forming the target pyridines. The preparation of a novel pyridines bearing substituted heterocyclic systems requires the formation of biheterocyclicenones such as the enone (3), which synthesized via Knoevenagel condensation of 3-formylchromone (1) with thiazolidene-2, 4-dione (2) having an active cyclic methylene group [7]. Whereas, the biheterocyclic enone 6 performed by condensation of formyl derivative of pyrazolobenzothieno- pyrimi-dinydine (4) with 4-acetylpyridazinone 5 having an active methyl group [8] (Scheme 1).

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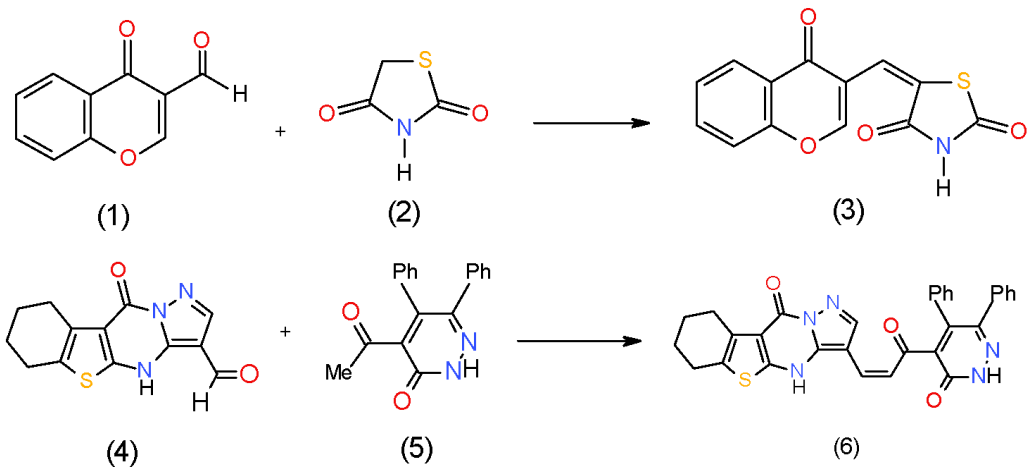
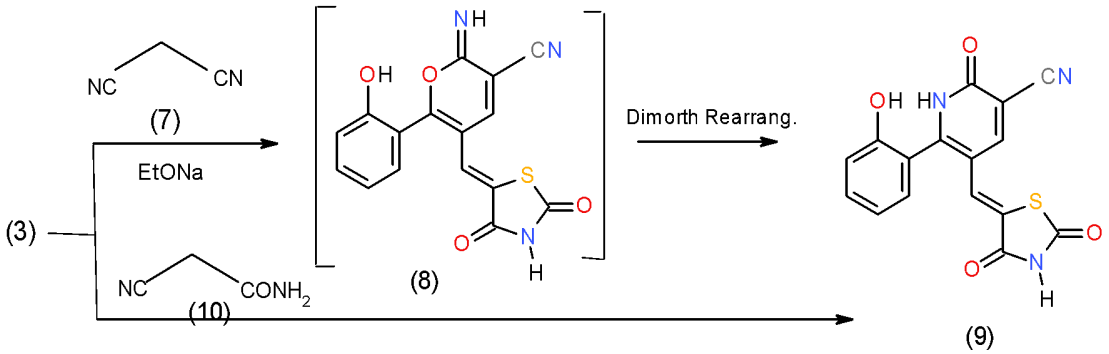


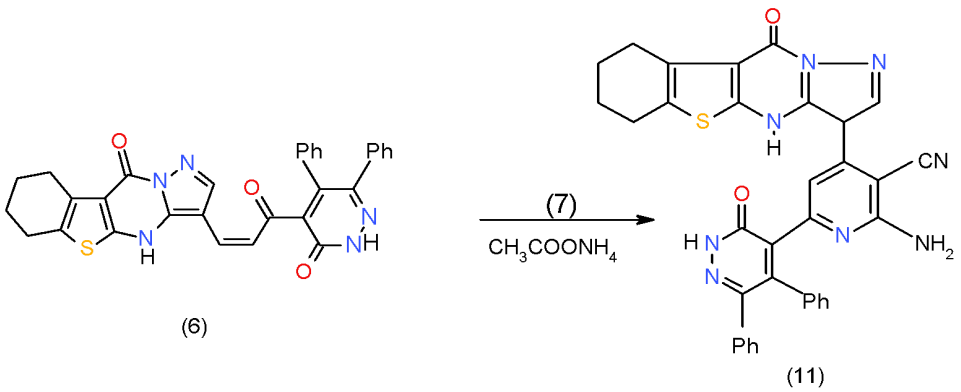
Scheme 1: Formation of bi-heterocyclic enones

The action of malonitrile (7) upon the two enones 3 and 6 showed two different behaviors. The presence of chromone with an active methine at C2 in the enone 3 facilitate the attack by the carbanion at C2 causing ring-opening of the ɣ-pyron ring followed by ting closure producing the target pyridine. Thus, the reaction of chromenylthiazolidine derivative 3 with malonitrile (7) in basic medium afforded the intermediate 8, which underwent Dimorth rearrangement under the reaction condition to yield oxopyridinecarbonitrile 9. Compound 9 also obtained directly by the action of cyanoacetamide (10) upon biheterocyclicenone 3 [7] (Scheme 2).



Scheme 2: Formation of oxopridinecarbonitrie

When biheterocyclic enone 6 was subjected to react with malononitrile (7) in boiling ethanol containing ammonium acetate, cyclocondensation took place at α, β-unsaturated carbonyl part yielding 4, 6-diheterocyclylpyridinecarbonitrile 11[8] (Scheme 63).



Scheme 3: Formation of aminopridinecarbonitrie

Similarly, another two synthesized enones having quinolone in their structures was prepared, the enone 13 obtained by condensation of formylchromone (1) with acetylquinolone (12) while the enone 15 formed through the condensation of formylquinolone (14) with acetylpyridazinone (5). When the reaction between

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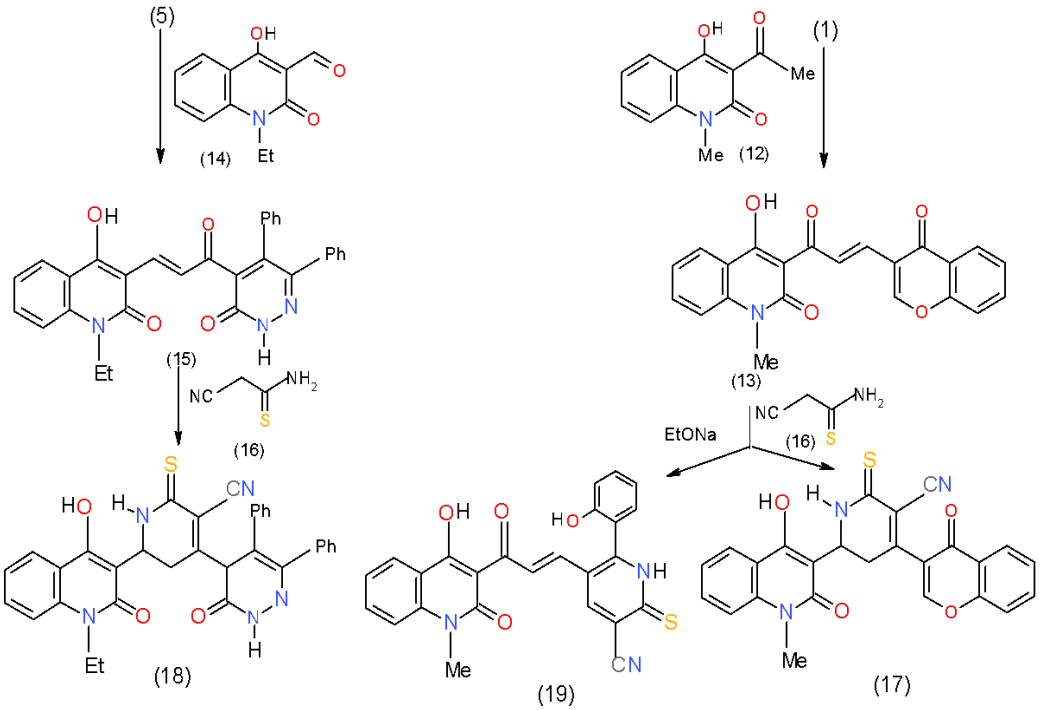
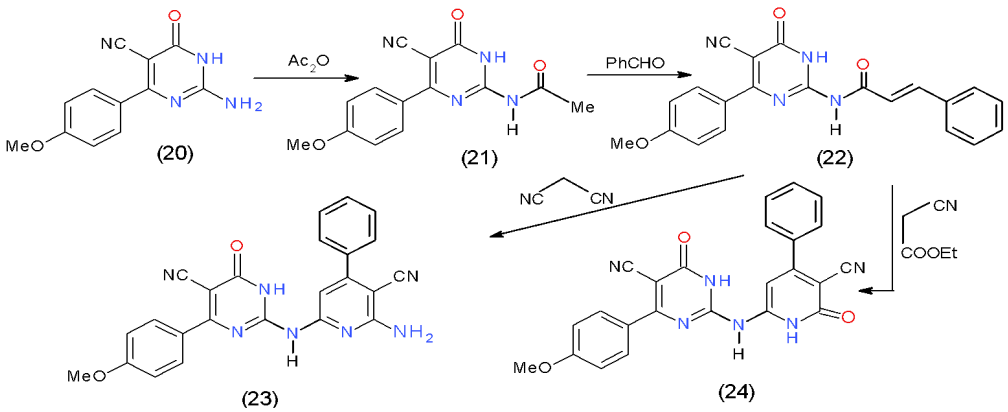
cyanothioacetamide (16) and the enones 13 and 15 was carried out in ethanol containing piperidinium acetate, the respective 4,6-diheterocyclylpyridines 17 and 18, was obtained. But when the reaction between 13 and 16 took place in sodium ethoxide, ring opening of ɤ- pyrone ring followed by ring closure took place giving rise to the thioxopyridinecarbonitrile 19 [9, 10] (Scheme 4).



Scheme 4: Cyanothioacetamide in the formation of thioxopyridinecarbonitries

Moreover, acylation of 2-aminopyrimidinecarbonitrile (20) using acetic anhydride yielded 2-acetamidopyrimidinecarbonitrile (21), which underwent condensation with benzaldehyde in ethanolic sodium hydroxide giving 2-(N-cinnamoylamino) pyrimidine-carbonitrile (22). As reported for α, β-unsaturated ketones, the interaction of compound 22 with active methylene compounds afforded the substituted pyridine. Accordingly, when compound 22 was allowed to react with malononitrile (7) and ethyl cyanoacetate in the presence of ammonium acetate afforded 2-amino-6-pyrimidinylaminopyridinecarbonitrile (23) and 3-cyano-6-pyrimidinylamino-pyridone (24), respectively [11] (Scheme 5).



Scheme 5: Formation of pyrimidinylaminopyridines

b) Using Acetylpyridazine

In addition to the use of acetylpyridazine (5) in the formation of some enones, it could be converted into a new active methylene compound or enaminone and used them in the synthesis of pyridine. Accordingly, synthesized active methylene compound namely, pyridazinyl-butan-1,3-dione 27 obtained from the reaction of 4-acetylpyridazinone 5 with ethyl acetate (25) under clasein condensation [12]. Whereas, the enaminons 28 formed through condensation of 5 with dimethyl- formamide dimethylacetal, DMFDMA, (26) in non-polar solvent [9]. Cyclocondensation of cyano-

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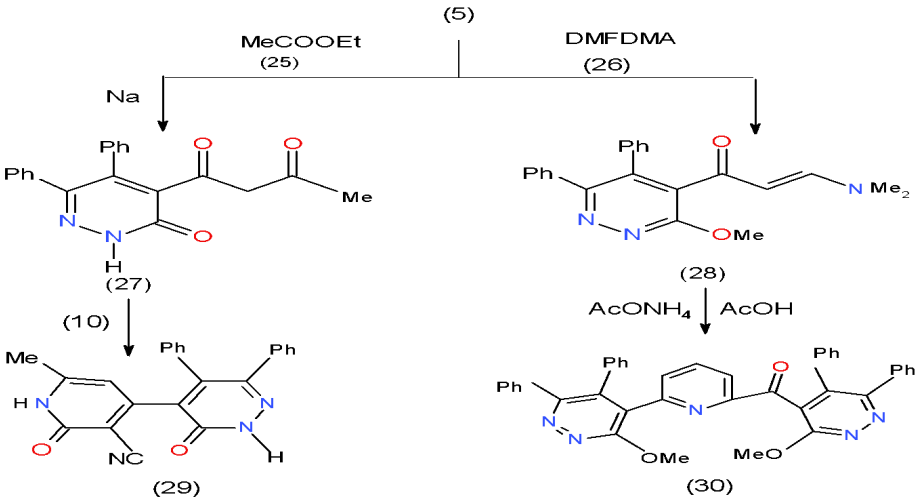
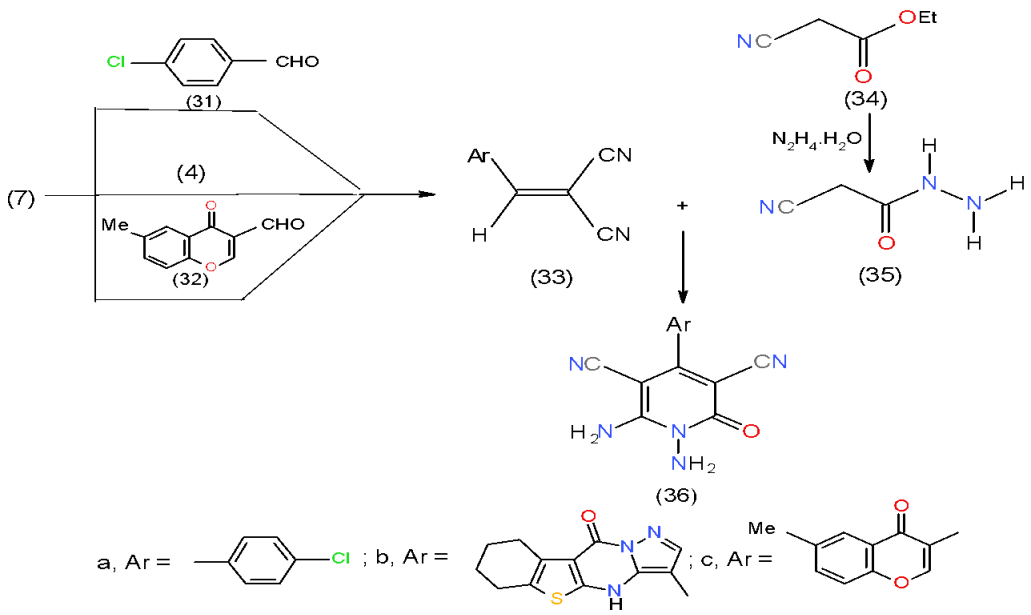
acetamide (10) with compound 27 in refluxing ethanol containing catalytic amount of triethyl amine afforded 4-pyridinylpyridazinone 29 [8]. Whereas, 2-pyridinyl 4-pyridazinyl ketone (30) synthesized on refluxing the enaminone 28 in acetic acid in the presence of ammonium acetate [13] (Scheme 6).



Scheme 6: Formation of pyridazinylpridines

c) Using Arylidene Malononitriles

Condensation of malononitrile (7) with each of p-chlorobenzaldehyde (31), formyl derivative (4) and 3-formyl-6-methylchromone (32) afforded the respective arylidenemalononitriles 33a-c. In refluxing dimethyl formamide containing catalytic amount of piperidine cyanoacetic acid- hydrazide (35), the product of hydrazonolsis of ethyl cyanoacetate, (34) was added to arylidenemalononitriles 33a-c, and yielded the corresponding 1,6-diamino-4-aryl-2-oxo-1, 2-dihydro-pyridine-3,5-dicarbonitriles (36a-c) [14,15] (Scheme 7). Compound 36 was considered as a suitable synthon for many fused pyridines.



Scheme 7: Formation of diaminopridinedicarbonitrile

III. Formation of Fused Pyridines

Incorporating pyridine with other heterocyclic systems such as thiazole, indole, pyrimidine, triazine and triazepines in one molecular frame-work enhance the biological activities of the produced condensed pyridine derivatives. Synthesis of fused pyridine could be carried out using some heterocyclic systems having active methylene such as thiazoldinones, 2-indolinone or heterocycles have active methine like 6-amino-1,3-dimethyluracil and with a heterocycle with a vicinal diamino groups as in compound 36.

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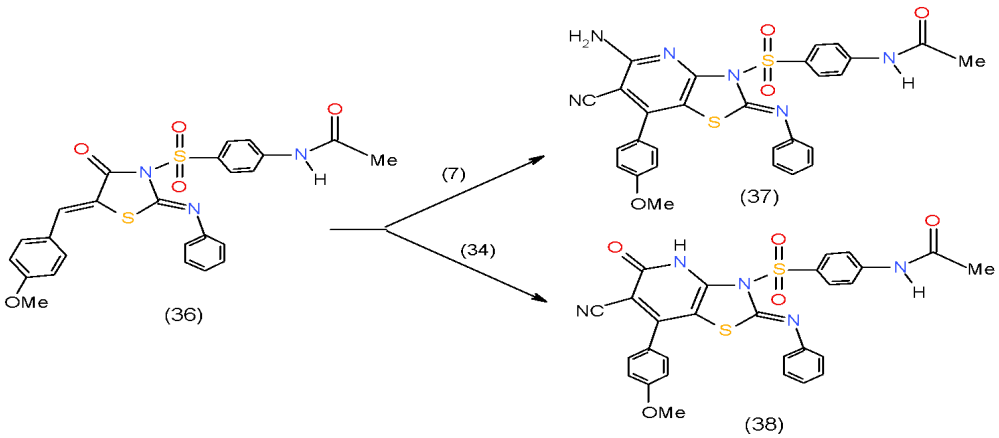
a) Synthesis of Thiazolopyridines

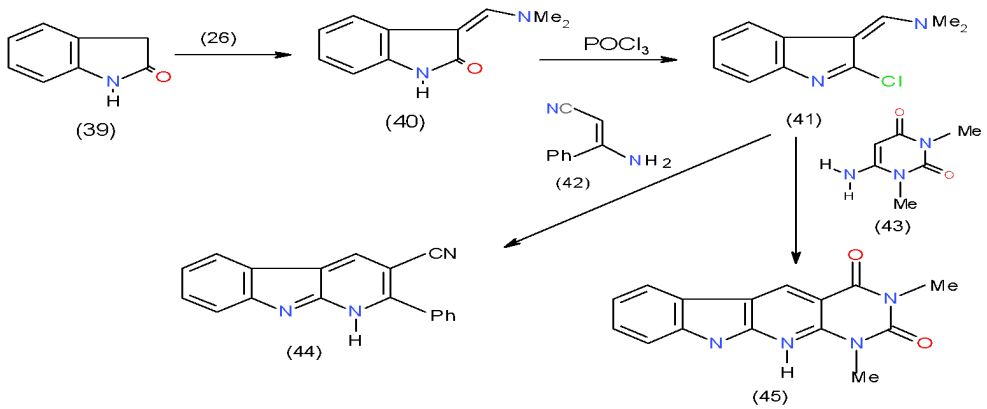
A number of methods to prepare thiazolopyridines have been documented in the literature. For example, methods that construct the bicycle by formation of a thiazole ring, include condensations of 3-amino-2-halopyridine, or 3-amino-2-pyridone derivatives, with thiocyanates, thioamides, or thioesters, condensations with 3-aminopyridin-2-thiones, and reactions of N-(2-pyridone-3-yl) acetamides with phosphorous pentasulfide, the oxidative ring-closure of 3-aminopyridine thioamides or thioureas, [16]. In our work, we annulated pyridineon thiazolidnone derivative using active methylene compounds. Thus, cyclocondensation of 5-arylidenethiazolidinone 36 with malononitrile (7) and ethyl cyanoacetate (34) in the presence of ammonium acetate yielded thiazolopyridine 37 and thiazolopyridone 38, respectively [17] (Scheme 8).



Scheme 8: Formation of thiazolopyrimidines

b) Synthesis of Indolopyridines

In the last 30 years' hundreds of indoloquinoline analogues were synthesized and their biological activities evaluated as scaffolds for drug discovery. This fact aroused us to synthesize some bioactive indolopyridines starting with 2-indolinone (39) was studied. Thus, condensation of 2-indolinone (39) with DMFDMA (26) afforded the respective enaminone (40), which on treating with $POCl_3$ gave 2-chloroindole derivative 41. Cyclocondensation of 41 with two compounds having active methane site in their structures such as, enaminonitrile 42 and 6-aminoaminouracil 43, afforded the indolopyridine-carbonitrile 44 and indolopyridopyrimidinedione 45, respectively [18] (Scheme 9).



Scheme 9: Formation of indolopyridine derivatives

On the other hand, treatment of 40 with 1-naphthylamine (46) yielded 3-N-naphthylamino derivative 47, which on boiling in poly phosphoric acid (PPA), dehydration took place giving rise to indolobenzoquinoline 48. Heating of indolinone (39) with enaminone 40 in acetic acid and ammonium acetate mixture furnished indolopyridoindole (49) [18] (Scheme 10).

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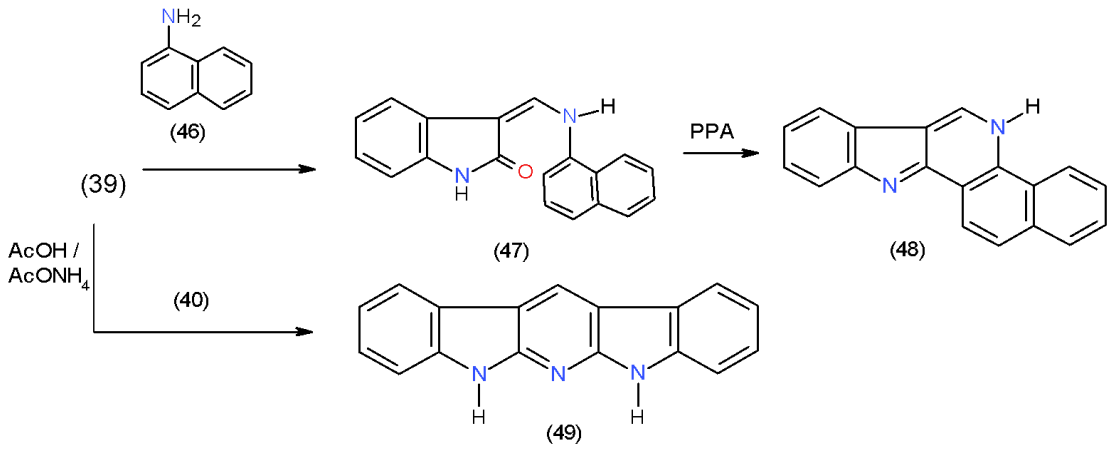
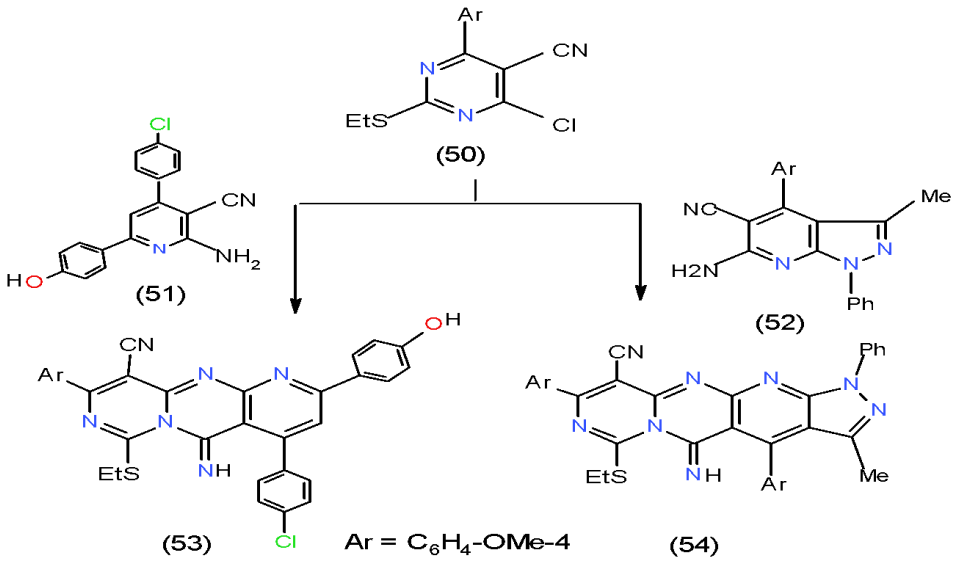


Scheme 10: Formation of poly cyclic system having pyridine

c) Formation of Pyridopyrimidine Derivatives

Pyridopyrimidine derivatives are a privileged bicyclic ring system. Due to its potent and significant biological activities it has great pharmaceutical importance; synthesis of these compounds is considerable interest. The development of a practical method for the synthesis of various pyrido- pyrimidines, in view of their structure relation with peteridine, is of interest in the field of medicinal chemistry [19]. Most preparation of pyridopyrimidines concentered on ring closure reactions of either pyridine or pyrimidine nucleus having appropriate substituents. Accordingly, chloro-pyridinecarbonitrile 50 reacted with some heterocycles having vicinal amino-cyano groups such as 2-aminopyridinecarbonitrile 51 and 3-aminopyrazolo-pyridinecarbonitrile 52 to afford the respective polycyclic systems 53 and 54 having pyridolopyrimidines in their structures [20] (Scheme11).



Scheme 11: Formation of pyridopyrimidopyrimidine derivatives

On the other hand, Condensation of 3-acetyl quinolinone 12 with DMFDMA (26) yielded the enaminone 55. The action of thiourea (56) and cyanoguanidine (57) upon the enaminone 55 gave rise to pyrimidoquinolinones 58 and 59, respectively [21] (Scheme 12).

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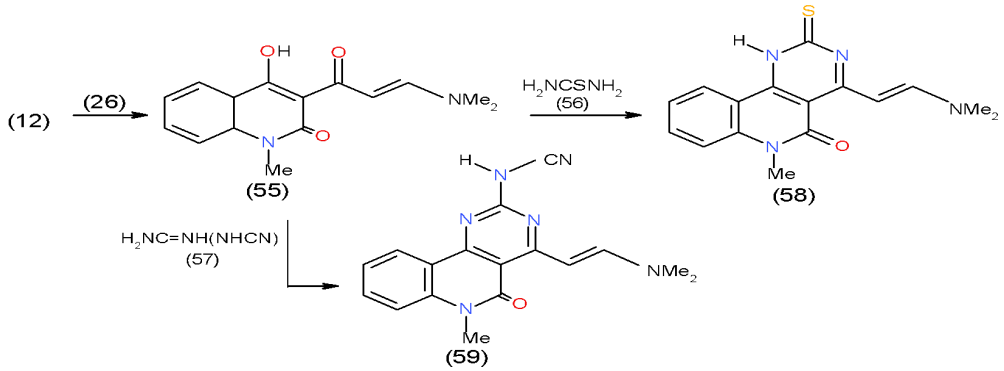
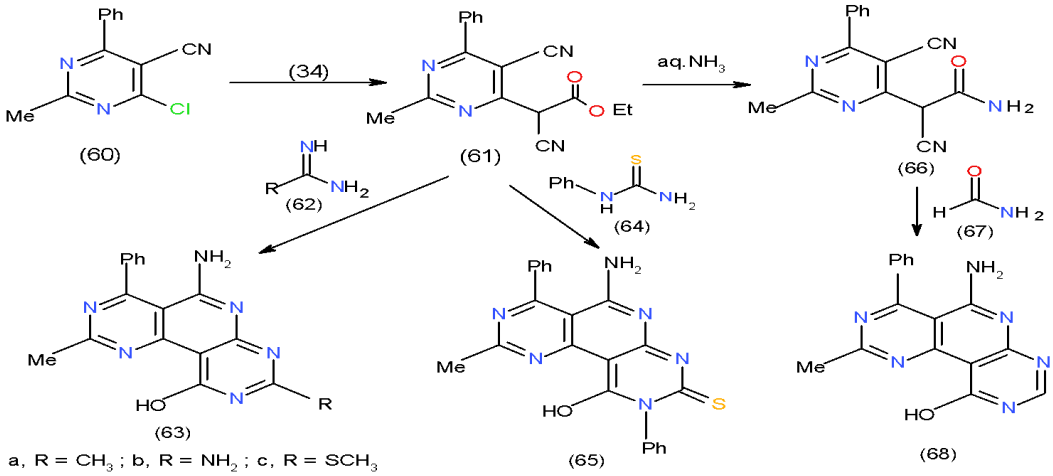


Scheme 12: Formation of pyrimidoquinolinones

Nucleophilic substitution of the chlorine atom in chloropyrimidinecarbonitrile (60) using ethyl cyano-acetate (34) under basic condition afforded pyrimidinyl cyanoacetate derivative (61). Compound 61 is considered a good synthon for some interesting tri-nitrogenous heterocyclic systems having pyridine nucleus in their structures. Thus, cyclocondensation of 61 with acetami- dine hydrochloride (62a), guanidine hydrochloride (62b) and S-methylthiourea sulphate (62c) in sodium ethoxide solution furnished substituted pyrimidopyridopyrimidines 63a-c respectively. Similar behavior was observed when compound 61 also reacted with N-phenylthiourea (64) to afford pyrimido-pyridopyrimidinethione 65. Moreover, treatment of 61 with aqueous ammonia at room temperature gave the corresponding amide 66, which reacted with formamide (67) in DMF to yield the substituted pyrimido-pyridopyrimidines 68 [22] (Scheme 13).



Scheme 13: Formation of pyrimidopyridopyrimidines

Recently, an analogous method for the synthesis of pyridopyrimidines was proposed via the reaction of 6-amino-2-thiouracil with activated olefinic system possessing a leaving group such as dimethyl-amino [23]. Motivated by this fact, we allowed ethyl cyanoacetate (34) to react with both 3,5-dimethyl-pyrazole (69) and tiethylorthoformate (70) to afford 3,5-dimethylpyrazol-1-yloxo) acetonitrile (71) and ethyl ethoxymethylenecyanoacetate (72), respectively. Treatment of 71 with DMFDMA (26) yielded the enaminone 73, which reacted with 6-aminouracil 43 to give the pyridopyrimidine 75 via non-separable intermediate 74. Compound 75 was also obtained from the reaction of 6-aminouracil 43 with compound 72[24] (Scheme 14).

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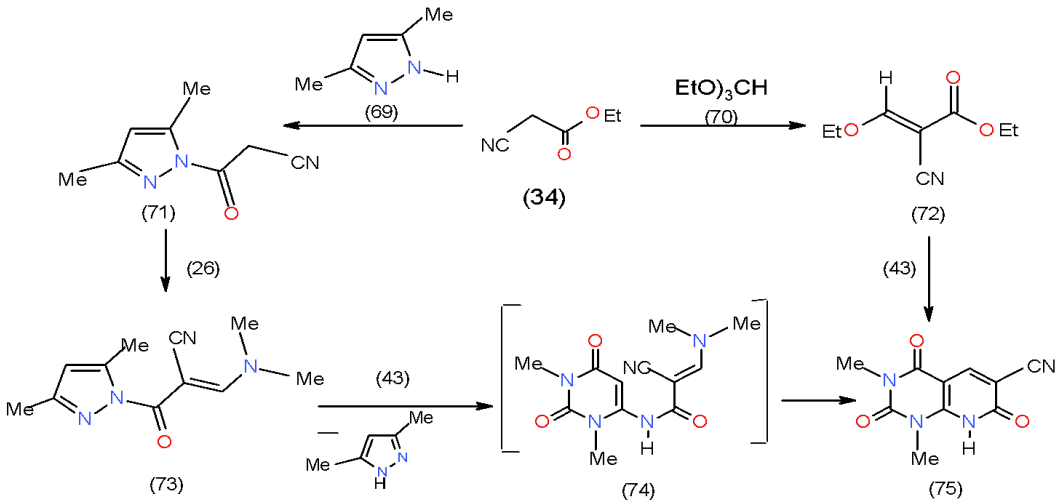
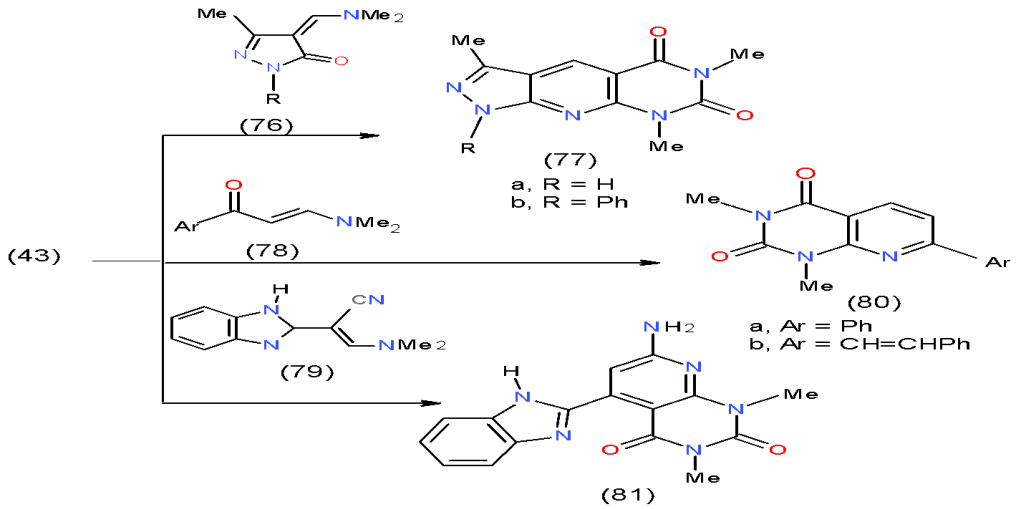


Scheme 14: Formation of pyrimidopyridines

In conjunction to our interest in the chemistry of the enaminones, pyrazolopyrido- pyrimidindiones 77a, b synthesized on treatment of 6-aminouracil 43 with the cyclic enaminones 76a, b, while the pyrido-pyrimidinediones 80a, b and benzimidazolylpyrido-pyrimidinedione 81 obtained from the reaction of compound 43 with two enaminones 78a, b and enamine 79, respectively [25] (Scheme 15).



Scheme 15: Formation of pyridopyrimidinediones

Moreover, 6-amino-1,3-dimethyluracil (43) having an active methine group at position-5, reacted with some bifunctional heterocyclic systems having vicinal chloro-cyano groups in their structures such as 2-chloropyridinecarbonitrile 82, 3-chloropyridazinecarbo-nitrile 83 and 4-chloropyrimidinecarbonitrile 60 and afforded the novel triheterocyclic systems having pyrido-pyrimidines in their structures 84-86, respectively [25] (Scheme 16).

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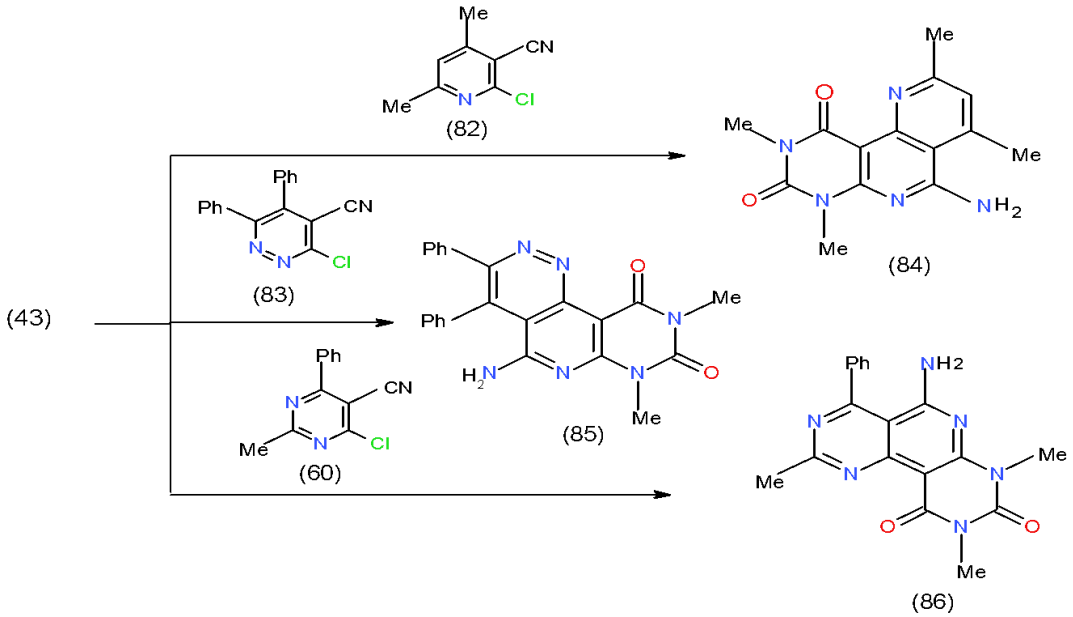
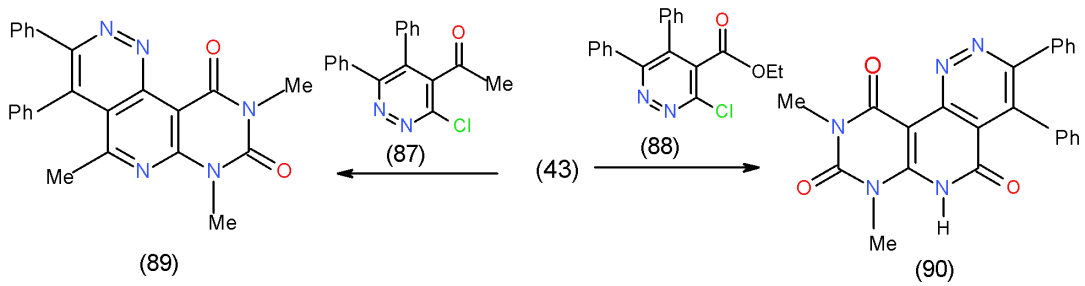


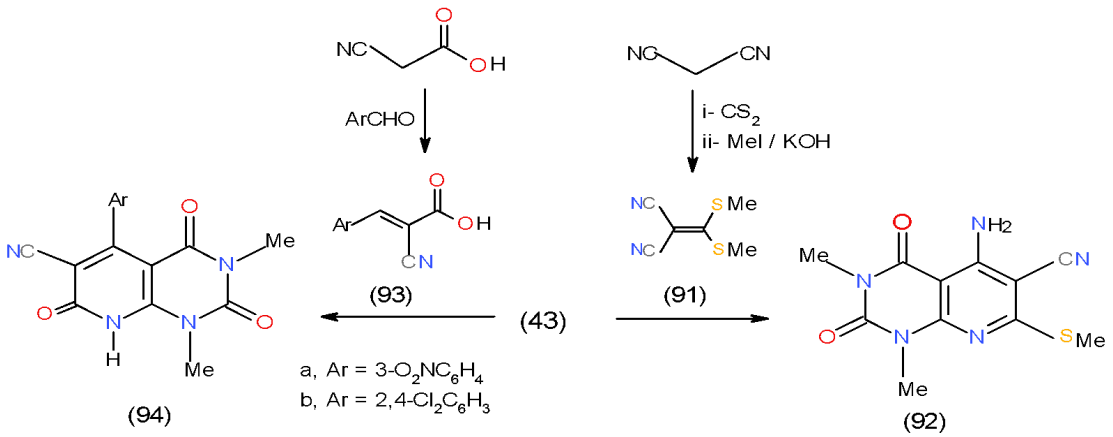
Scheme 16: Formation of pyridine in triheterocyclic systems

Furthermore, bifunctional heterocyclic system having vicinal chloro-acetyl groups (4-acetyl-3-chloro-pyridazine 87) or vicinal chloro-ethoxycarbonyl groups (4-carbethoxy-3-chloropyridazine 88) reacted with 6-amino-1,3-dimethyluracil (43) to yield the corresponding pyrimidopyrido- pyridazines 89 and 90 [25] (Scheme 17).



Scheme 17: Formation of pyrimidopyridopyridazines

On the other hand, the reaction of malononitrile (7) with carbon disulphide in basic medium followed by addition of methyl iodide afforded 2-cyano-3,3-bis (methylthio) acrylonitrile (91) [26], which reacted with 43 to yield pyridopyrimidinedione derivative 92 while, the reaction of 43 with arylidenecyano acetic acids 93a, b, obtained from the condensation of cyanoacetic acid with aromatic aldehydes afforded pyrido- pyrimidine-triones 94a, b [20] (Scheme 18).



Scheme 18: Aminouracil in the formation of pyrimidopyridines

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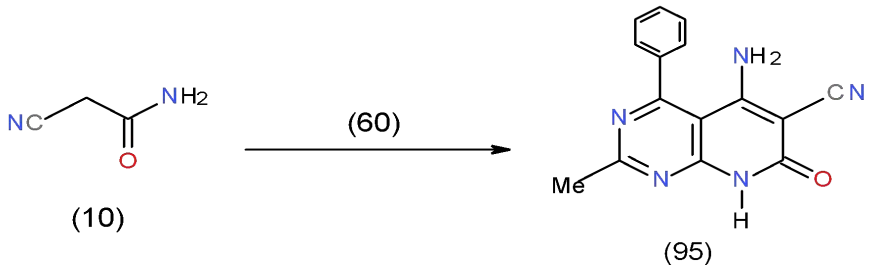
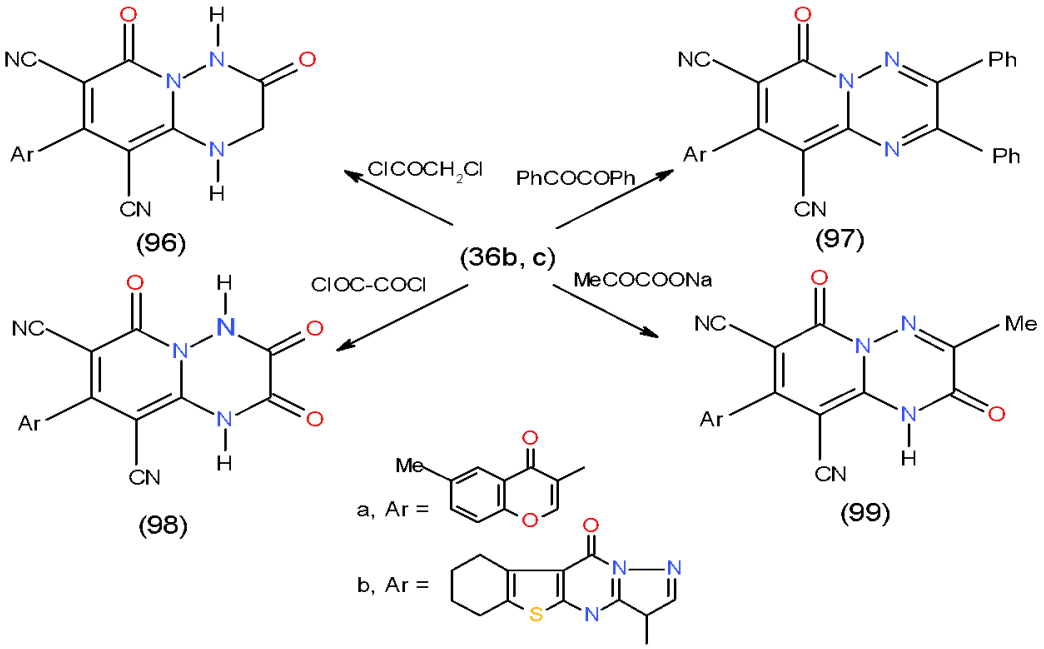
Also, the reaction of 4-chloropyrimidine-carbonitrile 60 with cyanoacetamide (10) in boiling dimethyl formamide gave aminopyridopyrimidinecarbo-nitrile 95 [27] (Scheme 19).



Scheme 19: Formation of aminopyridopyrimidinecarbonitrile

d) Formation of Pyridotriazines Derivatives

The bicyclic heterocycles such as pyridotriazine and pyridopyridazine are useful for treating cell proliferative disorders, such as cancer, atherosclerosis, restenosis, angiogenesis, diabetic retinopathy, psoriasis, and endometriosis and immunological disorders [28]. Motivated by these facts we succeeded in the preparation of some pyridotriazines with the help of 1,6-diamino-4-aryl-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (36a-c) as starting intermediate. Thus, the action of α, β- bifunctional electrophilic reagents such as chloroacetyl chloride, benzil, oxalyl chloride and sodium pyruvate upon compounds 36b, c was studied and yielded the corresponding nitrogen bridgehead pyrido 1,2,4-triazinones 96a, b- 99a, b, respectively, via cyclocondesation process [14,15] (Scheme 20).



Scheme 20: Formation of pyrido1,2,4-triazinones

Moreover, the action of ethyl α-cyano-α-phenylazoacetate 101, obtained via the condensation of ethyl cyanoacetate (34) with benzenediazonium chloride 100 upon diaminopyridinedicarbo- dicabonitrile 36a afforded pyrido 1,2,4-triazinone 104 instead of amino-pyrido 1,2,4-triazine 105. The formation of 104 could be explained via non-separable intermediate 102 not 103 [11] (Scheme 21).

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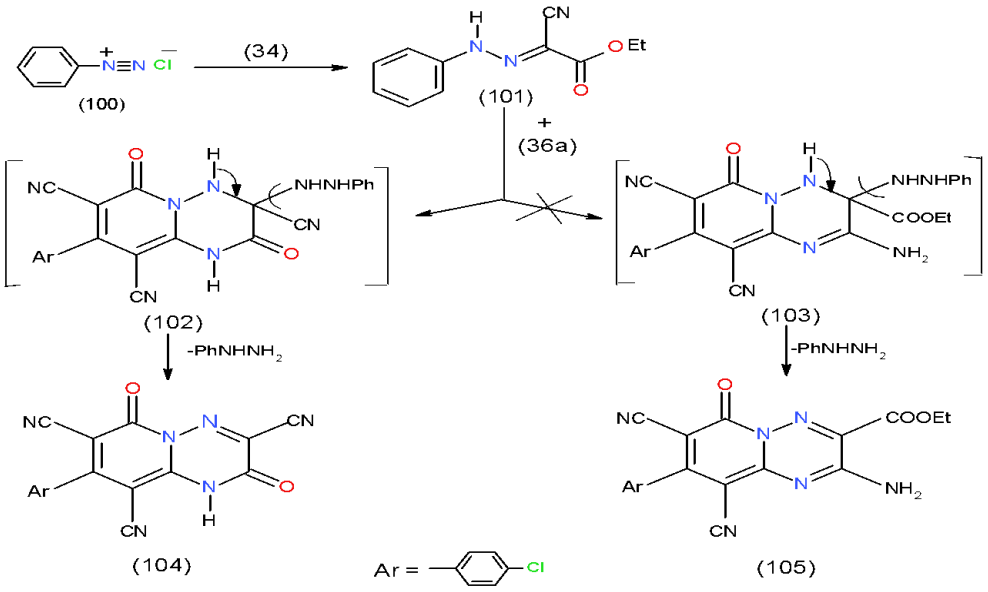
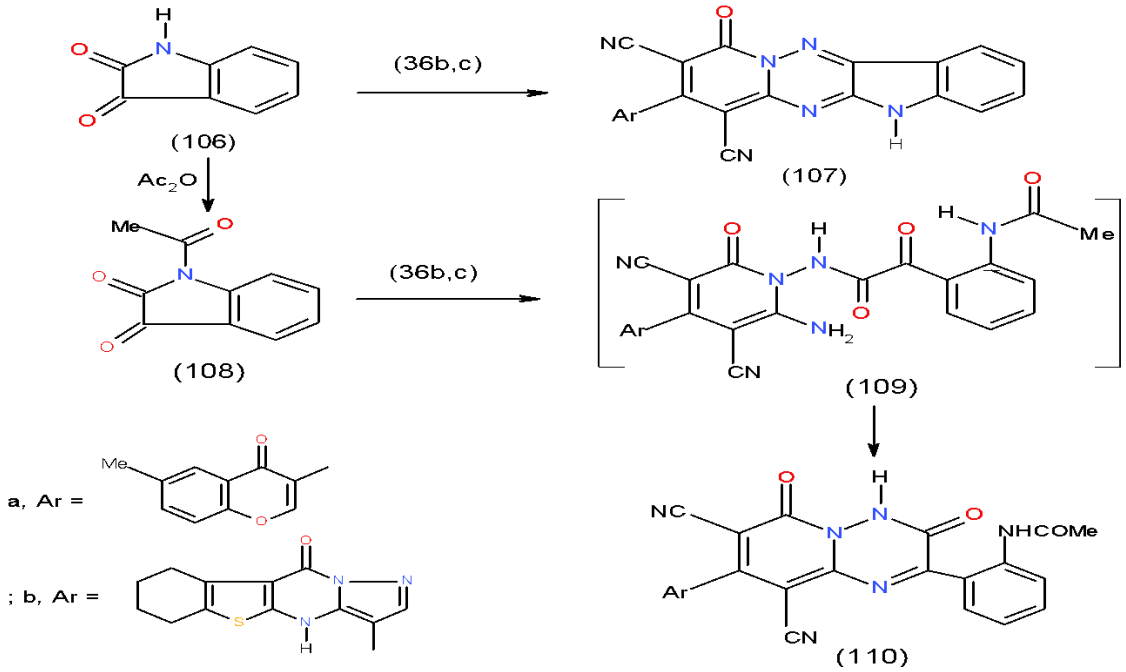


Scheme 21: Formation of pyrido1,2,4-triazinone

Also, condensation of 2,3-indoledione (isatin) (106) with diaminopyridonedicarbonitrile 36b, c in glacial acetic acid containing freshly fused sodium acetate afforded indolopyridotriazine derivative 107a, b. Whereas, a different behavior was obsedved on treating 36b, c with N-acetylisatin 108 in glacial acetic acid as it furnished pyridotriazinone 110a, b. The formation of 110 took place via the intermediate 109 [14] (Scheme 22).



Scheme 22: Formation of pyrido1,2,4-triazinone derivatives

On the other hand, for the preparation of pyridine bearing indotriazine we carried out the condensation of 2,6-diaminopyridine (111) with 2,3-indoledione (106) afforded 2,6-bis (indol- imino) pyridine (112). Alkylation of 112 with phenacyl bromide and chloro acetone gave 2,6-bis (1-substitutedindolimino) pyridine (113a, b), respectively. Hydrazinolysis of 113a, b with hydrazine hydrate yielded the respective 2,6-bis (1,2,4-triazinoindolimino) pyridine 114a, b, whereas, alkylation of 112 with chloroacetamide and ethyl bromoacetate furnished 2,6-bis(1-substitutedindolimino) pyridine (113c, d), which on fusion with hydrazine hydrate produced 2,6-bis(1,2,4-triazinoindolimino) pyridinone 115 [29] (Scheme 23).

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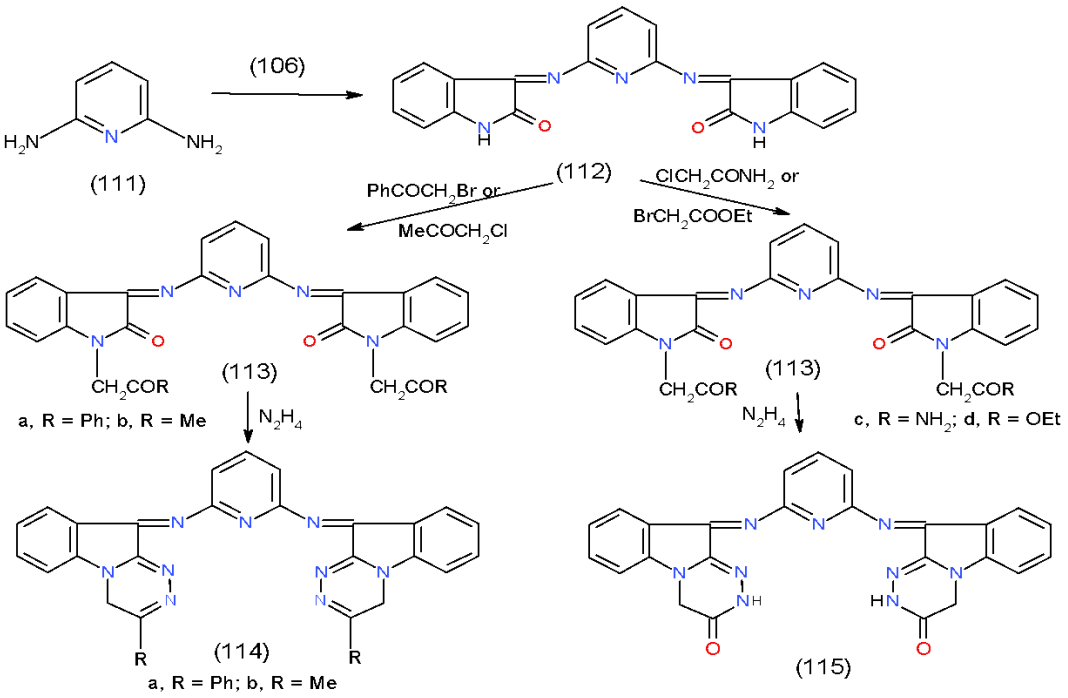
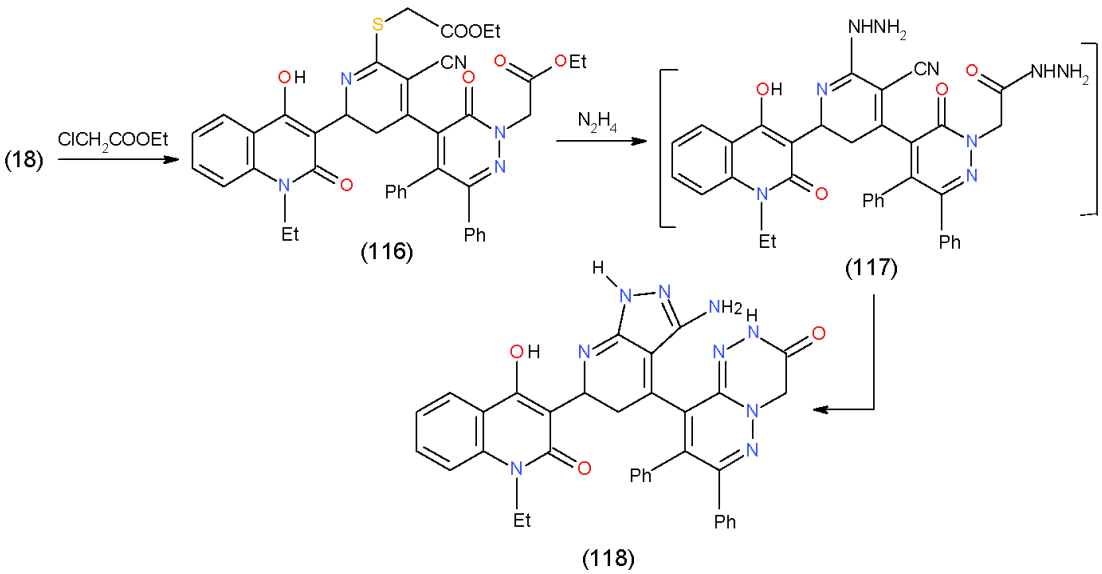


Scheme 23: Formation of bis indolo1,2,4-triazinone

Moreover, the formation of pyrazolopyridine in addition to pyridazinotriazine in one molecular frame was carried out by alkylation of compound 18 with ethyl chloroacetate to afford S- and N- alkylated product 116. Treatment of 116 with hydrazine hydrate produced pyridazino1,2,4-triazinone 118 through the non-separable intermediate 117 [9]. (Scheme 24).



Scheme 24: Formation of and pyridzino1,2,4-triazinones

e) Formation of Pyrido 1,2,4- Triazepines

The literature survey of 1,2,4-triazepines, synthesis and reactions of monocyclic and fused heterocycles incorporating 1,2,4-triazepines as well as their biological evaluation and synthetic applications was described [30]. As a part of our program directed for the synthesis of new polynuclear bioactive heterocyclic systems, one of our synthetic strategy is designed to utilize of 1,6-diamino-4-aryl-2-oxopyridine-3,5-dicarbonitrile 36a-c as a suitable synthon for the synthesis of nitrogen bridge-head pyrido[1,2,4]-triazepines. Accordingly, treatment of 36a with diethyl malonate (119), diazotized acetylacetone (120), dimethyl acetylenedicarboxylate (121) and dehydroacetic acid (122) furnished substituted pyrido1,2,4-triazepines 123-126, respectively [31] (Scheme 25).

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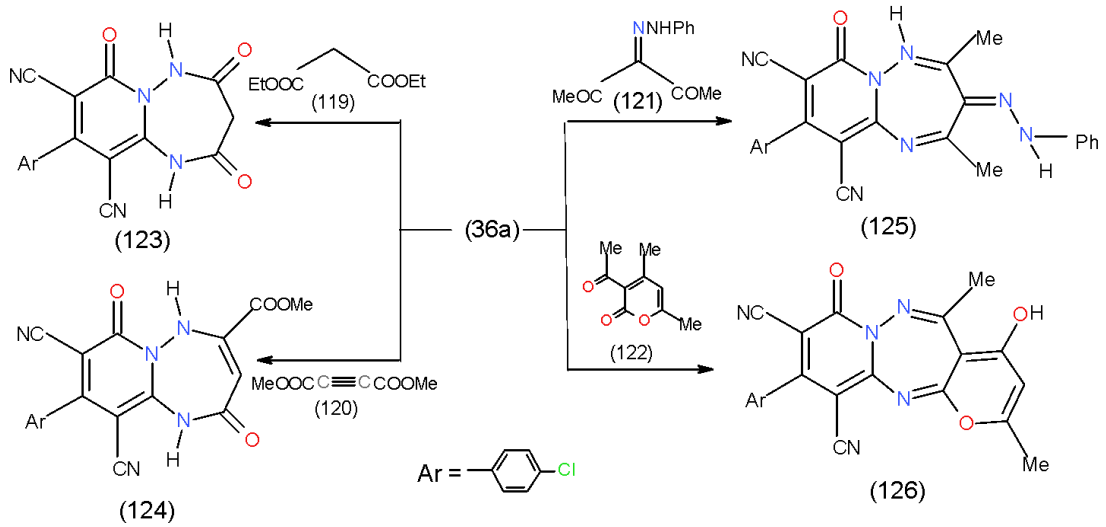
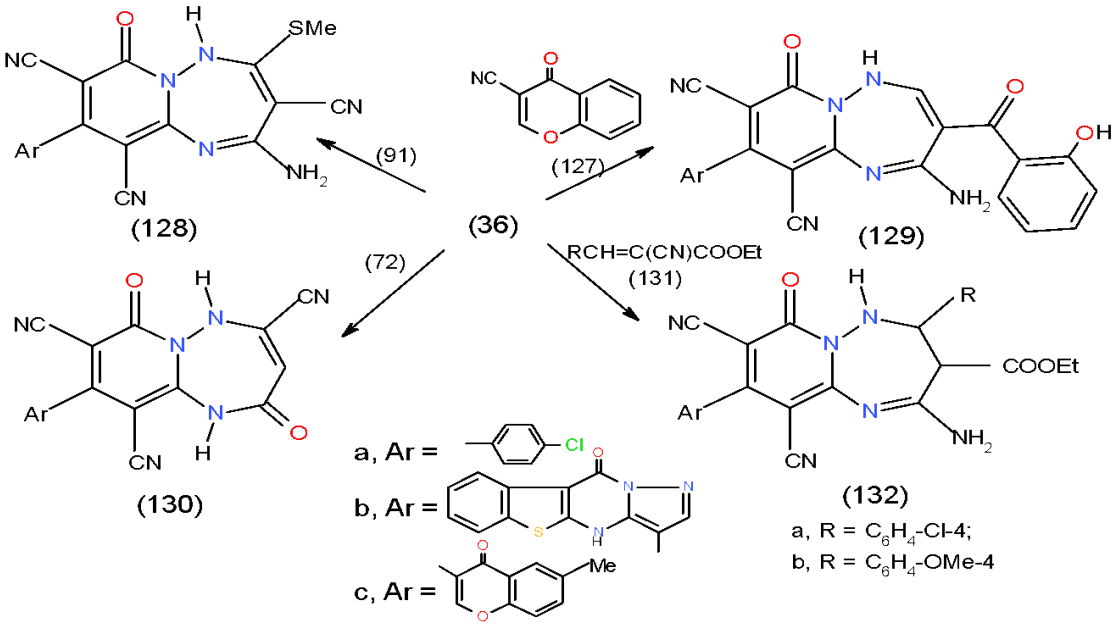


Scheme 25: Formation of pyridotriazepine derivatives

Moreover, the action of 2-cyano-3,3-bis (methylthio)acrylonitrile (91) and chromone-3-carbonitrile 127 upon 36a-c yielded pyrido1,2,4-triazepines 128a-c and 129a-c, respectively. Also, Treatment of 36a with ethyl exothymethylenecyanoacetate (72) gave pyrido1,2,4-triazepine- tricarbonitriles 130 while, the reaction of 36b, c with arylidene cyanoacetate (131) produced aminopyrido1,2,4-triazepinedicarbonitriles 132a, b, respectively [14-31] (Scheme 26).



Scheme 26: Formation of substituted pyridotriazepine

Furthermore, cyclocondensation of 36b, c with arylidedmalonitrile (133), 2-cyano-3,3-bis(methyl thio) prop-2-enamide (134) and o-chloroaldehyde derivative 135 gave the corresponding pyrido- 1,2,4-triazepines 136a, b, 137a, b and 138a, b, respectively [14,15] (Scheme 27).

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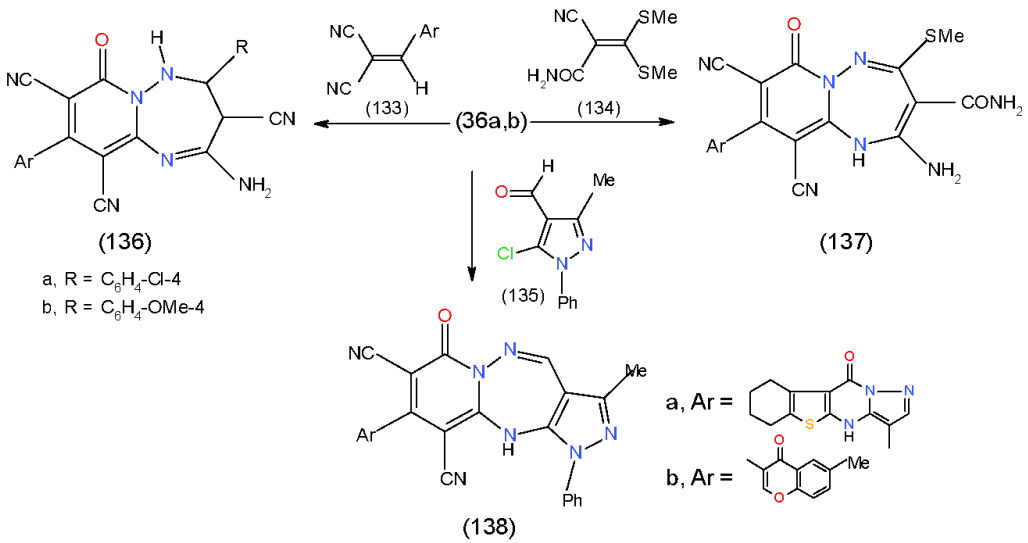
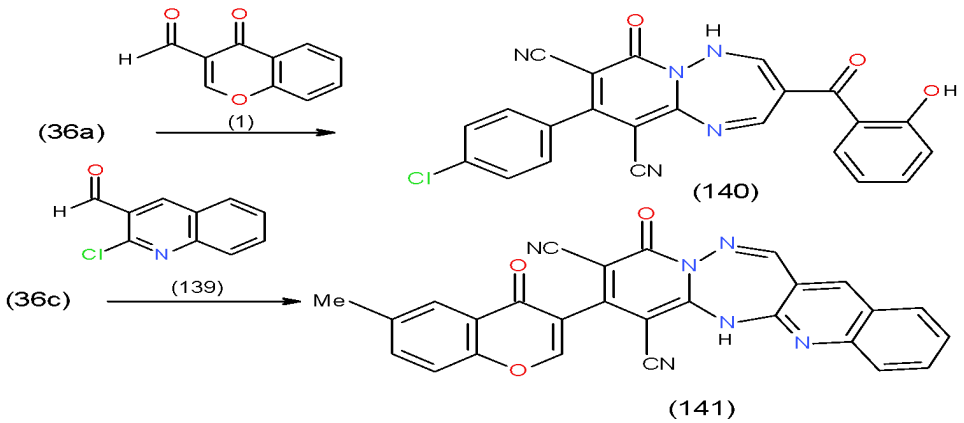


Scheme 27: Formation of substituted and condensed pyridotriazepines

On the other hand, the action of 3-formylchromone (1) upon 36a and the effect of 2-chloro-3-formylquinoline (139) on 36c was studied and furnished the respective pyrido1,2,4-triazepines 140 and 141 [14,31] (Scheme 28).



Scheme 28: Formation of pyridoquinolinotriazepine

IV. Biological Importance

a) Antimicrobial Activities

Some synthesized heterocyclic compounds were tested their antimicrobial activity against some Gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus and Bacillus cereus* and some Gram-negative bacteria namely, *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa* and *Proteus vulgaris* and some fungi for examples *Candida albicans, Aspergillus fumigatus* using standardized disc agar diffusion method [32] or Vincent filer paper disc method [33] with taken some antibiotics as reference. Herein we recorded the compounds exhibited strong inhibition effect against a certain microorganism.

i. *Action on Gram-positive bacteria*

a. *Bacillus subtilis*

A Gram-positive Bacterium *Bacillus subtilis*, like many microorganisms, can form most of the enzymes needed for the biosynthesis of the amino acid tryptophan. The primary role of tryptophan within living organisms is as a novel residue within many proteins. The efficient production of secreted enzymes B. subtilis is considered a key drivers of the successes in the enzyme industry [34]. The selected synthesized heterocyclic compounds were tested against *Bacillus subtilis*. The results showed that pyrido1,2,4-triazepine-tricarbonitriles 130b, which carrying pyrazolobenzo-thienopyrimidinyl nucleus [15] exhibited the highest antimicrobial activity against *Bacillus subtilis* compared with the tested compounds in the study. (Bio-1 scheme).

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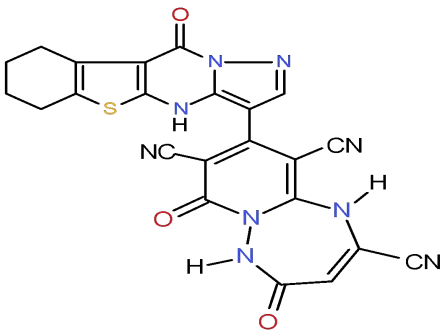
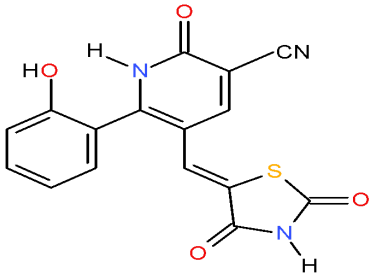


(130b)

Bio-1 scheme: The highest antimicrobial activity one against *Bacillus subtilis*

b. *Staphylococcus areus*

It is a Gram-positive, omnipresent bacterial pathogen that have the ability to adapt and live in various states. *S. aureus* is one of the major causes of spreading of the clinical infection such as bacteraemia and infective endocarditis, osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [35]. The results showed that pyrazolopyridotroazepine 138a, which having pyrazolobenzothienopyrimidinyl moiety exhibited the highest antimicrobial activity against *Staphylococcus areus* [15]. Also, the pyridine carrying thiazolindione nuclei exhibited higher inhibition effect to *Staphylococcus areus* as in oxopyridine-carbonitrile 227 [30] (Bio-2 scheme).



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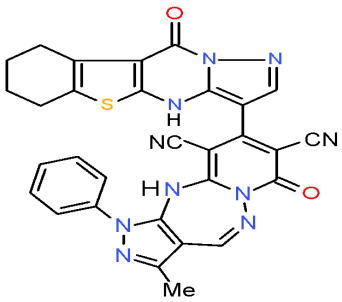


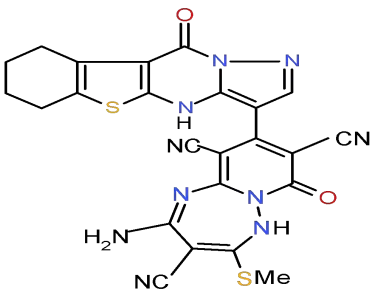
(138a)

Bio-2 scheme: Compounds could use as antimicrobial agents against *Staphylococcus aureus*

ii. *Action on Gram-Negative Bacteria*

a. *Escherichia coli*

Escherichia coli is a large and various group of bacteria that is found naturally in the intestines of healthy humans and animals. Most types of *Escherichia coli* are harmless or cause relatively brief diarrhea, but some of the *Escherichia coli* can cause a disease for people which can be done by creating a toxin known as Shiga Toxin [36]. It can be seen clearly that pyridotriazepine 128b, which having pyrazolobenzothienopyrimidinyl moiety exhibited the highest antimicrobial activity against *Escherichia coli* compared with the tested 77 investegated compounds [8]. (Bio-3 scheme).

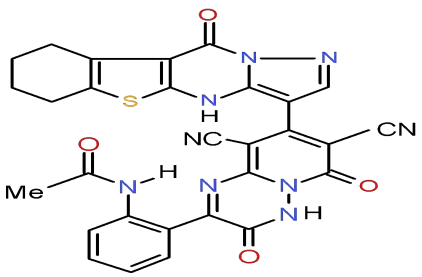


(128b)

Bio-3 scheme: The highest antimicrobial activity compound against *Escherichia coli*

b. *Salmonella typhimurium*

Infection of humans by the enteric pathogen *Salmonella typhimurium* generally results in severe abdominal cramping and diarrhea. These symptoms may largely result from the mucosal immune response elicited by this pathogen. Specifically, colonization of the human intestine by *S. typhimurium* leads to infiltration of polymorphonuclear leukocytes (PMNs) into the intestinal epithelium culminating in the formation of an intestinal crypt abscess [37]. Results obtained disclosed that pyrido1,2,4-triazinedione 110b [15], which having pyrazolobenzothieno- pyrimidinyl moiety exhibited the highest antimicrobial activity against *Salmonella typhimurium* compared with the tested compounds [15] (Bio-4 scheme).



(110b)

Bio-4 scheme: One of the highest antimicrobial activity against *Salmonella typhimurium*

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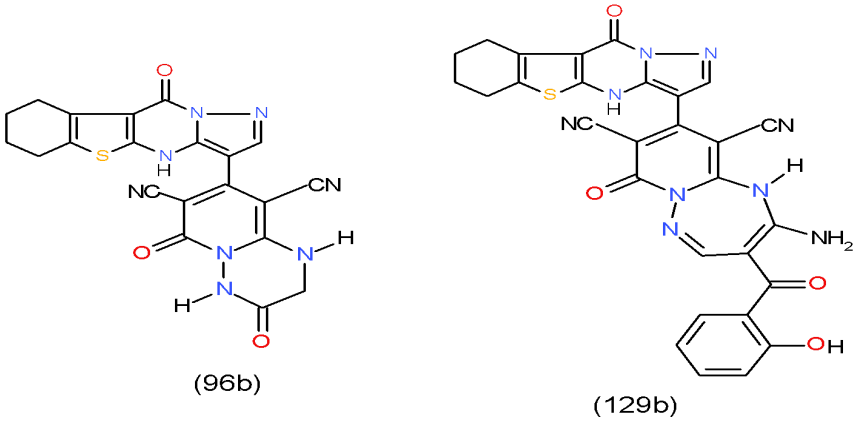
iii. *Action on Fungi*

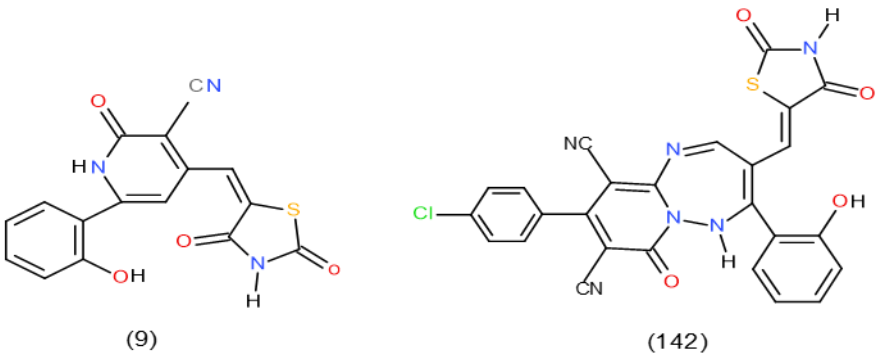
a. *Candida albicans*

Candida albicansis is one of the very few fungal species causing disease in humans—millions of others do not. It is a member of the healthy microbiota, asymptomatically colonizing the gastrointestinal (GI) tract, reproductive tract, oral cavity, and skin of most humans [38]. The results showed that pyrido1,2,4-triazinedione 96b and aminopyrido1,2,4-triazepine 129b that having pyrazolobenzothienopyrimidinyl moiety in their structures exhibited the highest antimicrobial activity against *Candida albicansis* compared with the tested compounds [15] (Bio-5 Scheme).



Bio-5 scheme: Compounds with the highest antimicrobial activity against *Candida albicansis*

Also, the compound carrying thiazolidinedione nucleus in their structures such as pyridinecarbonitrile 9 and pyrido1,2,4-triazepine 142, obtained from reaction of the enone 3 with diaminopyridinedicarbonitrile 36a, may be used as antimicrobial agent against *Candida albicansis* from tested compounds [36] (Bio-6 scheme).



Bio-6 scheme: Compounds may be used as antimicrobial agent against *Candida albicansis*

b. *Aspergillus fumigatus*

Aspergillus fumigatus is an opportunistic fungus causing allergic and invasive aspergillosis in humans and animals. It secretes an array of complex biologically active glycoprotein antigens and allergens, that have been implicated in human respiratory allergic disorders [39]. The compounds carrying pyrazolobenzothieno-pyrimidinyl moiety in their structures such as pyrido 1,2,4-triazepine 136a and pyrazolopyrido1,2,4-triazepine 138a may be used as antimicrobial agent against *Aspergillus fumigates* as they shown the highest inhibition zones [15] (Bio-7 scheme).

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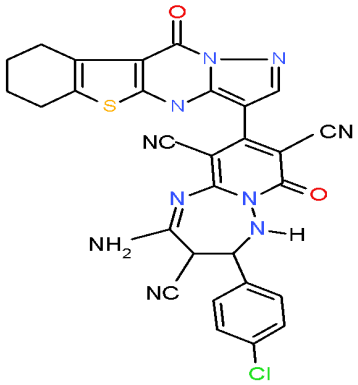
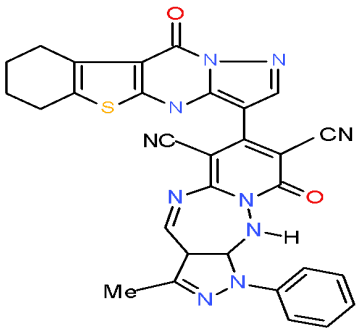


(136a)



(138a)

Bio-7 scheme: Compounds with the highest antimicrobial activity against *Aspergillus fumigatus*

V. Conclusion

Pyridine derivatives are very important chemicals with tremendous biological application. In this study effort to optimize the synthetic procedures for the preparation of various bioactive heterocycles. Herein, we explain how active acyclic and cyclic methylene compounds as well as heterocyclic having active methyl or methine sites were used to synthesize wide varieties of pyridines and its fused systems by choosing certain starting materials and different synthesizing reagents. This study will help researchers in the fields of organic and medicinal chemistry to design and implement new procedures for the constructions of novel biological components.

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Cardiac Oxidative Status in CCl_4-Exposed Rats Treated with Extracts of *Dialium guineense* Stem Bark

By Abu O. D., Iyare H. E. & Ogboi K. U.
University of Benin

Abstract- The present study investigated cardiac oxidative status in carbon tetrachloride (CCl_4)-exposed rats treated with aqueous and ethanol extracts of *Dialium guineense* stem bark. Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg body weight, bwt. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated in heart homogenate.

Keywords: *cardioprotection, dialium guineense, heart, lipid peroxidation, oxidative stress.*

GJSFR-B Classification: *DDC Code: 338.4766288 LCC Code: HD9502.5.B543*

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Cardiac Oxidative Status in CCl_4-Exposed Rats Treated with Extracts of *Dialium guineense* Stem Bark

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Abstract- The present study investigated cardiac oxidative status in carbon tetrachloride (CCl_4)-exposed rats treated with aqueous and ethanol extracts of *Dialium guineense* stem bark. Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg body weight, bwt. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated in heart homogenate. The results showed that there were no significant differences in the concentrations of cardiac total protein (TP) among the groups ($p>0.05$). The activities of the antioxidant enzymes and level of reduced glutathione (GSH) were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract treatment ($p<0.05$). However, the level of cardiac malondialdehyde (MDA) increased by CCl_4 intoxication was significantly reduced after treatment ($p<0.05$). These results indicate that aqueous and ethanol extracts of *D. guineense* stem bark may enhance antioxidant defense in rats hearts exposed to CCl_4.

Keywords: cardioprotection, dialium guineense, heart, lipid peroxidation, oxidative stress.

I. Introduction

Carbon tetrachloride (CCl_4) is a colorless liquid with a "sweet" smell that can be detected at low levels [1]. Its production has steeply declined since the 1980s due to environmental concerns and the decreased demand for chlorofluorocarbons (CFCs), such as the Freons dichlorodifluoromethane (F-12) and trichlorofluro- methane (F-11), which are used primarily as refrigerants [2]. It is also used in petroleum refining, pharmaceutical manufacturing, as an industrial solvent, in the processing of fats, oils, and rubber, and in laboratory applications [3]. Currently, CCl_4 is not permitted in products intended for home use. The primary routes of potential human exposure to CCl_4 are inhalation, ingestion, and dermal contact. The general population is most likely to be exposed to CCl_4 through air and drinking water[4 – 6]. In humans and animals, CCl_4 is rapidly absorbed by any route of exposure. Once absorbed, it is widely distributed among tissues, especially those with high lipid content, reaching peak concentrations in <1– 6 h, depending on exposure concentration or dose. The compound is metabolized in the body, primarily by the liver, but also in the kidney, lung, and other tissues containing cytochrome P450 (CYP450). The fraction of the compound that is metabolized varies with dose [7, 8].

The heart is a muscular organ which pumps blood through the blood vessels of the circulatory system [9]. Blood provides the animal's body with oxygen and nutrients as well as assist in the removal of metabolic wastes. In humans, the heart is located between the lungs in the middle compartment of the chest [10 - 12]. The heart is effectively a syncytium, a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges [13 - 15].

Plants are at the center of Traditional Medicine. Their use in disease management is as old as man [16, 17]. Medicinal plants serve as cheap alternative to orthodox medicine since they are readily available [18 - 20]. *Dialium guineense* is a medicinal plant used in folklore medicine for the treatment of infections such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids [21, 22]. At present not much is known about the potential of extracts of *D. guineense* stem bark to protect against CCl_4-induced cardio-toxicity in rats. The aim of this study was to investigate cardiac oxidative status in CCl_4-exposed rats treated with aqueous and ethanol extracts of *D. guineense* stem bark.

II. Materials and Methods

a) Chemicals

All chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

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b) Collection of Plant Material

The stem barks of *D. guineense* were obtained from Auchi, Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330).

c) Plant Preparation and Extraction

The stem bark was washed and shade-dried at room temperature for a period of two weeks and crushed into small pieces using clean mortar and pestle. Aqueous and ethanol extracts of the stem bark were obtained using cold maceration method as described previously [23].

d) Experimental Rats

Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65% humidity and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for one week. The study protocol was approved by the University of Benin Faculty of Life Sciences Ethical Committee on Animal Use.

e) Experimental Design

The rats were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg bwt [23]. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days.

f) Tissue Sample Collection and Preparation

At the end of the treatment period, the rats were euthanized and their hearts excised, and used to prepare 20% tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain supernatant which was used for biochemical analysis.

g) Biochemical Analyses

The activities of catalase, SOD and GPx were determined [24 - 26]. Levels of total protein, MDA and GSH were also measured [27 - 29]. The activity of GR was determined using a previously described method [30].

III. Results

a) Effect of Extracts of D. guineense Stem Bark on Relative Organ Weight

As shown in Table 1, there were no significant differences in relative organ weight among the groups ($p > 0.05$).

Table 1: Relative Organ Weights of Rats

Group	Relative organ weight x 10^{-2}
Normal Control	3.34± 0.54
CCl_4 Control	3.02± 0.10
Silymarin	3.17± 0.16
Aqueous Extract	3.52± 0.24
Ethanol Extract	2.96± 0.14

Data are relative organ weights and are expressed as mean ± SEM (n = 5).

b) Effect of Extracts of D. guineense Stem Bark on Oxidative Status in Rat Heart

There were no significant differences in the concentrations of cardiac TP among the groups ($p>0.05$). The activities of the antioxidant enzymes and level of GSH were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract treatment ($p<0.05$). However, the level of cardiac MDA increased by CCl_4 intoxication was significantly reduced after treatment ($p<0.05$). These results are shown in Figures 1 to 3.

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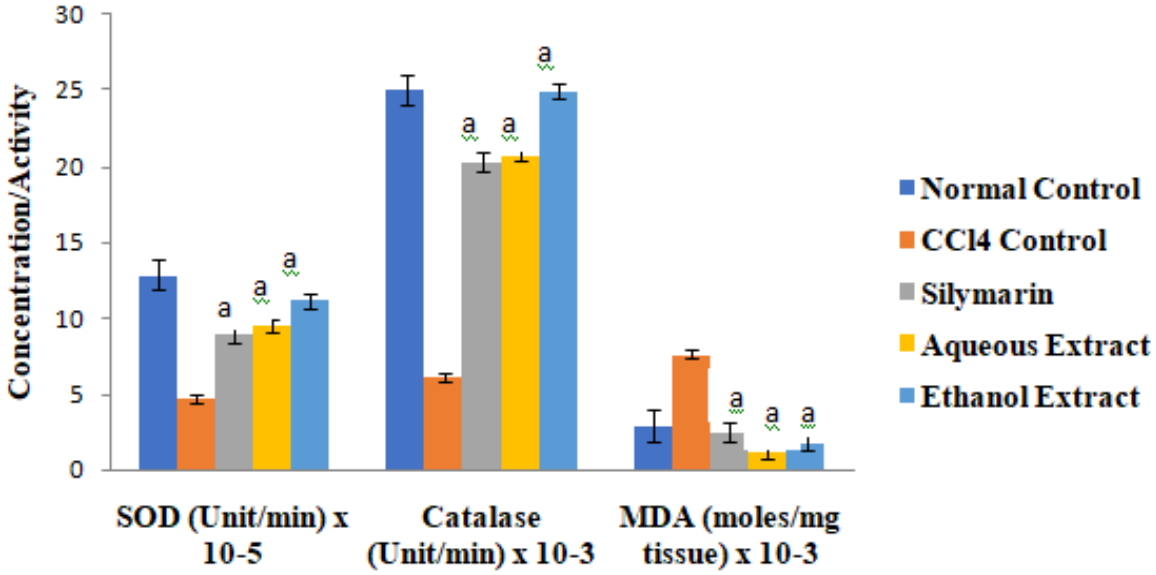

Figure 1: Effect of Extracts of *D. guineense* Stem Bark on Markers of Oxidative Stress in Rat Heart

Data are oxidative stress markers, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

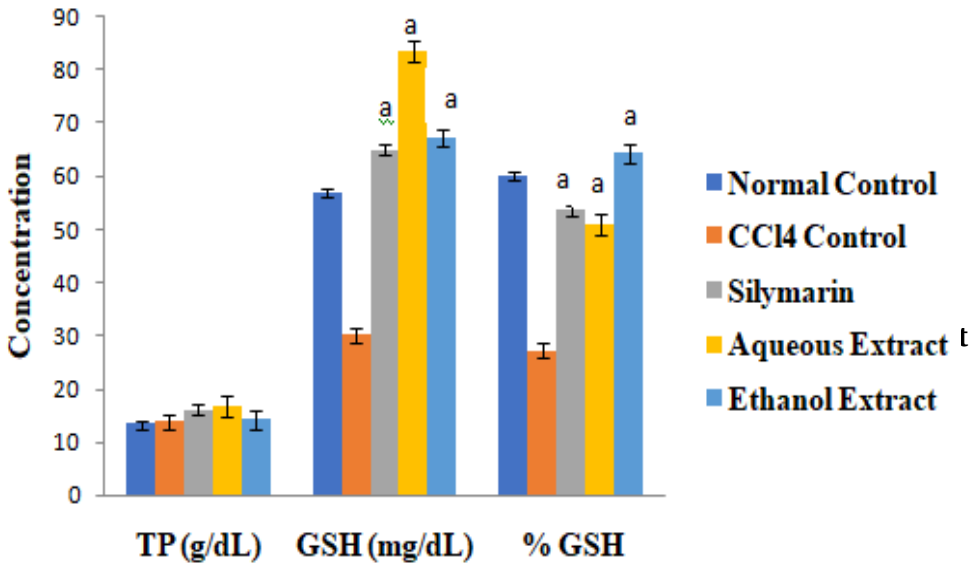

Figure 2: Effect of Extracts of *D. guineense* Stem Bark on Some Oxidative Stress Parameters

Data are oxidative stress markers, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

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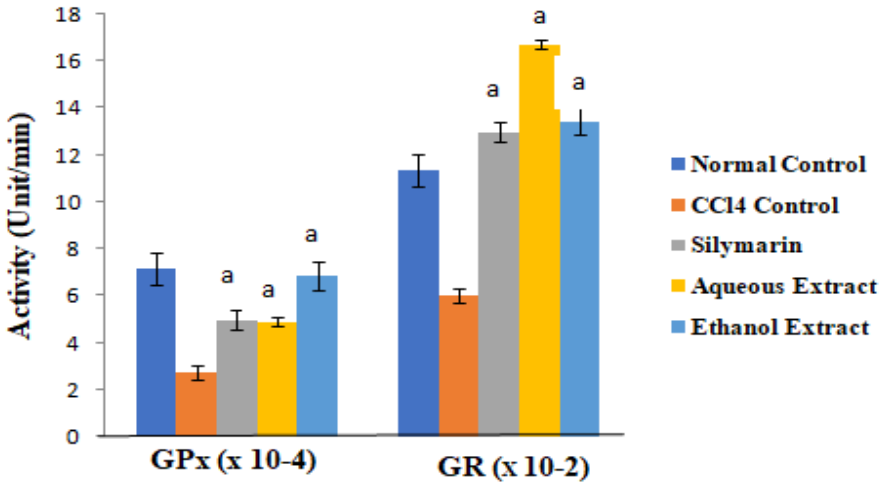

Figure 3: Effect of Extracts of *D. guineense* Stem Bark on Rat Oxidative Status

Data are oxidative stress parameters, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

IV. Discussion

In animals, CCl_4 is rapidly absorbed via any route of exposure. Once absorbed, it is widely distributed among tissues, especially those with high lipid content. It is metabolized in the body, primarily by the liver, but also in the kidney, lung, and other tissues containing CYP450.The poison reaches its maximum concentration in the liver within 3 h of administration, thereafter it falls and by 24 h it is completely cleared from the organ [7, 8].

Tissue injury produced by CCl_4 is mediated by two major processes resulting from bioactivation in the endoplasmic reticulum (ER) and mitochondria of centrilobular hepatocytes [31]; haloalkylation of cellular macromolecules by reactive metabolites such as trichloromethyl free radical or trichloromethyl peroxyl free radical [32–34]; and lipid peroxidation [35].

Reactive oxygen species (ROS) and oxidative stress have been shown to play an important role in the etiopathogenesis of tissue injury. The role of oxidative stress in cardiac hypertrophy and remodeling has been demonstrated. An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis [36, 37]. The ROS cause damage to cellular structures within the vascular wall, thereby triggering several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile. Animal models of atherosclerosis demonstrate the involvement of ROS in atherosclerosis by the accumulation of lipid peroxidation products and induction of inflammatory genes and activation of matrix metalloproteinases [38, 39]. The ROS and reactive nitrogen species (RNS) produced by the endothelium promote oxidative modification of low-density lipoprotein-cholesterol (LDL-C) in the phase that precedes the transfer into the subendothelial space of the arterial wall, where they initiate atherosclerosis [40].

This study investigated cardiac oxidative status in CCl_4-exposed rats treated with extracts of *Dialium guineense* stem bark. The results showed that the activities of the antioxidant enzymes measured as well as level of GSH were significantly lower in CCl_4 control group than in normal control group, but these parameters were increased by extract treatment. However, the level of cardiac MDA increased by CCl_4 was significantly reduced after treatment. These results suggest that extracts of *D. guineense* stem bark may enhance antioxidant defense in rat heart exposed to CCl_4. The capacity of extracts of the medicinal plant to potentiate natural antioxidant defense system has been reported [41-43]. Plants rich in polyphenols are reported to possess good antioxidant capacity [44-46]. Plants with cardioprotective potential have been shown to contain a variety of bioactive compounds, such as diosgenin, isoflavones, sulforaphane, carotenoids, catechins, quercetin, allicin, cardiac glycosides, saponin-shatavarins 1-1V, cyclovirobuxine D and triterpenes/triterpenoids [47–49].

The cardioprotective effect of medicinal plants may involve attenuation of the damage in cardiac muscle cells, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and macrophages and monocytes. In cardiomyocytes, cardioprotective agents may promote the opening of K_{ATP} channel, increased secretion of atrial natriuretic peptide, as well as the regulation of cardiac hypertrophy, oxidative stress, and apoptosis [50, 51].

V. Conclusion

The results of this study suggest that aqueous and ethanol stem bark extracts of *D. guineense* enhance

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antioxidant defense in rat heart exposed to CCl_4. Their bioactive molecules may exert cardioprotective function via suppression of specific factors, regulation of key enzymes, and scavenging of oxygen-free radicals.

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Physicochemical and Fatty Acid Evalution of Some Shea Butter Samples in Nigeria

By Stella A. Emmanuel, Idoko Owoicho, Onyinye Aguzue, Olajide O. Olutayo, Adedirin Oluwaseye, Sallau A. A., Ibrahim I. D., Buga M. L., A. I. Okereke, Ozonyia G. N. & Alabi F. M.

Abstract- Shea butter is a significant source of fat in the diet of many rural dwellers in Nigeria. It is produced from the seeds of *Vitellaria paradoxa L* tree. Its suitability as dietary fat or use in cosmetic industry is greatly influenced by its physicochemical properties and fatty acid composition. This study aimed at determining the physicochemical properties and fatty acid profile of shea butter found in Nigeria as a possible source of industrial raw material for the production of stearic acid. The physicochemical analyses results showed that the saponification value of the shea butter samples ranged from 174.55 mg/KOH/g - 200.97 mg/KOH, while iodine value is from 42.22 g/100g - 98.46 g/100g, the unsaponifiable matter ranged from 2.32 - 10.55 % and peroxide value ranged from 8.14 meq/kg - 19.66 meq/kg.

Keywords: shea butter, physicochemical, fatty acid and vitellaria paradoxa.

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Physicochemical and Fatty Acid Evalution of Some Shea Butter Samples in Nigeria

Stella A. Emmanuel [α], Idoko Owoicho [σ], Onyinye Aguzue [ρ], Olajide O. Olutayo [ω], Adedirin Oluwaseye [¥], Sallau A. A. [§], Ibrahim I. D. [χ], Buga M. L. [ν], A. I. Okereke [Θ], Ozonyia G. N. [ζ] & Alabi F. M. [£]

Abstract- Shea butter is a significant source of fat in the diet of many rural dwellers in Nigeria. It is produced from the seeds of *Vitellaria paradoxa L* tree. Its suitability as dietary fat or use in cosmetic industry is greatly influenced by its physicochemical properties and fatty acid composition. This study aimed at determining the physicochemical properties and fatty acid profile of shea butter found in Nigeria as a possible source of industrial raw material for the production of stearic acid. The physicochemical analyses results showed that the saponification value of the shea butter samples ranged from 174.55 mg/KOH/g - 200.97 mg/KOH, while iodine value is from 42.22 g/100g - 98.46 g/100g, the unsaponifiable matter ranged from 2.32 - 10.55 % and peroxide value ranged from 8.14 meq/kg - 19.66 meq/kg. From the result of the GC-MS analysis, the dominant unsaturated fatty acids observed are: oleic, linoleic, vaccenic and gamma linolenic, gondoic and palmitoleic acids. While the saturated fatty acids found are stearic, palmitic, arachidic, behenic and lignoceric acids. The data indicate that the Shea butter obtained from the Federal Capital Territory (FCT), Abuja, and Niger state may be good sources of raw material for the production of stearic acid.

Keywords: shea butter, physicochemical, fatty acid and vitellaria paradoxa.

I. Introduction

Plant oil and fats are natural sources of stearic acids. These include cotton seed oil, coconut oil, palm kernel, castor beans oil, rapeseed, soybeans, sunflower and shea butter among others. Plant fats are said to contain low quantity of stearic acid when compared with the animal fats. They are however, increasingly becoming important in nutrition and commerce because they are sources of dietary energy, antioxidants, biofuels and raw material for industrial application (Luis Spitz, 1990 and Okullo *et al,* 2010).

The shea trees seeds (*Vitellaria paradoxa*) are source of vegetable oil/fat used in many food products. Both the kernels and butter are used in health products and confectionery industries (Luis Spitz, 1990) *Vitellaria paradoxa* seed oil has been reported to contain stearic acid with percentage composition ranging between 26 - 48% depending on the growing conditions, climate, as well as the botanical variant. In some parts of Africa, shea butter is more liquid than others due to lower levels of stearic. It is reported that the extent of viscosity and texture of Shea butter depends on the level of stearic acid content. This is because stearic acid is considered essential for the structure of the butter (Realize Ed, 2016).

Nigeria is said to be one of the major producers of Shea in Africa (Collin Nnabuife, 2018). Shea trees are grown in significant quantities in three states of the federation, namely: Niger, Kwara and Kebbi states (Daniel Essiet, 2018). However, this potential has not been fully harnessed (Anonymous). This study therefore seeks to analyze some of the variants of shea butter in some states in Nigeria with the intent of providing information on the plant's potential as a possible source of raw material for producing plant-based stearic acid for industrial applications.

II. Material and Methods

a) Sample Collection and Preparation

Samples of Shea butter were purchased from local markets in four different states (Kwara, Kebbi, Nassarawa, and Niger states) and FCT, Abuja, Nigeria. They were stored in polytene containers for further analyses in the laboratory.

b) Physicochemical Characterization

Physicochemical analyses such as acid values, saponification values, unsaponifiable matter, peroxide values, iodine values, acidity, moisture content, texture, melting point, and colour was carried out using standard procedures (Akpan et al., 2006).

c) Determination of Fatty Acid Composition

Fatty acid percentage composition of the samples was determined using a Gas Chromatography-Mass Spectrometer (GC-MS) machine (Shimadzu GC-17A). The samples were esterified using the method described by Abayomi and Co (Abayomi et al., 2012) before GC-MS analysis. Identification of fatty acid composition was carried out by comparing the Mass Spectrometry data of individual analyte with those of standards in machine library.

III. Result and Discussion

a) Physicochemical Parameters

The results of the physicochemical analyses of the Shea butter samples are presented in Table 1. The moisture content of the Shea butter samples analyzed in this study fell within the range 0.16-11.30%. By using the Grading method of West African regional standard using

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major oil parameters, the Shea butter samples were divided into Grade 1 (Shea butter that can be used by cosmetics and pharmaceutical industries), Grade 2 (Shea butter that are for making confectionaries, Chocolates and margarine) and Grade3 (Shea butter used for production of soap). According to this grading method, the moisture content of Grade 1 range from 0% to 0.05%, Grade 2 ranged from 0.05 % to 0.2 % and Grade 3 ranged from 0.2 % to 2% (Munir et al., 2012). Most of the samples fell within Grade 2 and 3 based on their moisture content, except for KE2 (Kebbi sample with grey coloration) with moisture content 11.30 % and NA (Nasarawa sample) with moisture content 3.96% (Table 1).

Table 1: Physico-chemical parameters of Shea butter oil from selected state in Nigeria

Samples/ Parameters	AB1	AB2	KE1	KE2	KW1	KW2	NI1	NI2	NA
Acid value mg KOH/g)	2.24	23.56	17.95	6.73	28.17	17.39	11.97	1.65	2.73
Iodine value (g/100g)	70.94	70.94	92.27	91.76	60.09	98.46	49.95	42.22	56.03
Peroxide value (meq/Kg)	18.48	38.29	15.05	19.55	39.87	8.14	15.74	18.04	14.53
SV (mgKOH)	174.55	200.92	179.20	189.94	164.29	189.20	180.96	168.30	200.97
FFA (%)	1.12	1.78	8.98	3.37	14.09	8.69	0.98	0.82	1.58
Moisture (%)	0.97	2.00	0.27	11.30	0.59	0.23	0.42	0.16	3.96
USM (%)	3.45	10.50	3.24	8.10	10.55	3.25	8.72	5.01	2.32
Color	Grey	White	Grey	White	Cream	Cream	Cream	Cream	Grey
Texture	S	S	S	S	S	S	S	S	S

AB = Abuja; KE = Kebbi; KW= Kwara; NI= Niger; NA = Nasarawa; SV= saponification value; USM = unsaponifiable matter and S = smooth

Table 2: Saturated fatty acid composition of Shea butter from selected states in Nigeria

Name IUPAC	Myristic acid Tetradecanoic acid (%)	Palmitic acid Hexadecanoic acid (%)	Isopalmitic Acid 14-methyl Pentadecanoic (%)	Stearic acid Octadecanoic acid (%)	Arachidic acid Icosanoic acid (%)	Behenic acid Docosanoic acid (%)	Lignoceric acid Tetracosanoic acid (%)
Ratio	C14:0	C16:0	C16:0	C18:0	C20:0	C22:0	C24:0
AB1	0.71	24.50		19.20	1.60	1.19	-
AB2	-	-	0.89	45.01	0.99	-	0.1
KE1	0.71	3.27		13.20	3.89	4.72	-
KE2	0.15	3.02		0.09	0.12	0.23	-
KW1	-	7.13	15.76	11.36	1.06	0.32	0.13
KW2	-	17.13	15.76	12.11	-	0.32	-
NI1	0.10	8.72	7.38	29.18	1.78	0.19	0.14
NI2	0.14	0.02	2.10	11.86	0.35	0.07	0.14
NA	0.37	12.63	19.14	13.08	0.85	2.84	0.37

AB = Abuja; KE = Kebbi; KW= Kwara; NI= Niger; NA = Nasarawa

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Table 3: Unsaturated fatty acid composition of Shea butter from selected states in Nigeria

Name IUPAC	Oleic acid (Z)-octadec-9-enoic acid (%)	Vaccenic acid (E)-octadec-11-enoic acid (%)	Linoleic acid (9Z,12Z)-octadeca-9,12-dienoic acid (%)	Gamma-Linolenic acid (Z,Z,Z)-6,9,12-Octadecatrienoic acid	Gondoic acid 11- Eicosenoic acid (%)	Palmitoleic acid 7-Hexadecenoic acid
Ratio	C18:9	C18:11	C18: 2,6	C18:5,8,11	C20:11	C16:7
AB1	8.61	-	1.48	1.27	1.12	0.06
AB2	31.49	--	1.18	-	0.28	-
KE1	9.95	-	-	4.82	12.69	0.45
KE2	0.35	-	0.10	0.17	0.03	0.05
KW1	-	-	-	-	-	-
KW2	12.34	19.23	1.65	-	1.06	-
NI1	64.93		2.38	-	0.08	-
NI2	29.38	1.39	8.02	-	1.78	-
NA	16.88	6.02	0.16	-	0.85	-

AB = Abuja; KE = Kebbi; KW= Kwara; NI= Niger; NA = Nasarawa

Generally, high moisture content in oil is known to support the growth of microbes and speed up rate of rancidity (Alirezalu et al., 2011). Enweremdau and Alamu, 2010, reported that Shea butter with 0.036% moisture gave good yield during trans-esterification leading to production of biodiesel. It is observed that for industrial applications, some of the Shea butter sold in Nigerian markets may require further drying.

The acid values of Shea butter analyzed in this study ranged from 2.73 mgKOH to 23.56 mgKOH as presented in Table1. Acid value is a measure of the amount of potassium hydroxide (mgKOH) required to neutralize the free acids in l gram of oil/fat.

Free fatty acids (FFA) values of the samples analyzed in this study ranged from 1.12 % to 3.24 %. These values were within the range for Grade 2 (1.0% to 3 %) and Grade 3 (3.0 % to 8.0%) according to West African regional standard (Munir et al., 2012). FFA are produced by the hydrolysis of oil and fats as a result of time, temperature and moisture content (Mahesar et al., 2014). They are less stable than neutral oil and thus more prone to oxidation and turning rancid. Factors that affect FFA content of an oil are duration of storage, packaging material, processing, moisture content, and general climatic conditions (Mahesar et al., 2014; Okullo et al., 2010).

The peroxide values of the samples are in the range 15.0 meq/Kg to 50.0 meq/Kg as indicated on Table 1. Literature report that peroxide is the initial product of unsaturated fat oxidation and that fresh oil has peroxide values below 10 meq/kg while rancid oil gives peroxide values between 20 and 40 meq/kg (Kirk & Sawyer, 1991). According to West African regional classification based on peroxide value (Munir et al., 2012), the samples in the study may be considered to fall within the Grade 3 range with 15.0 meq/Kg to 50.0 meq/Kg.

The saponification values ranged from 164.29 mgKOH/g to 200.97 mgKOH/g, these values fall within the required range of 180 – 360 mgKOH/g reported (Munir et al., 2012). Saponification value has been described as a measure of the alkali-reactive groups in fats and oil (Shahidi & Ambigaipalan, 2005). It is defined as the number of milligrams of potassium hydroxide (mgKOH) required to neutralize the fatty acids in l gram of fat or oil. High saponification value indicates the suitability of the oil for soap production.

The iodine value of studied samples ranged from 42.22 g/100g to 92.27 g/100g. These values are within the acceptable range (58 - 72 g/100g) of iodine value for shea butter at international level (Samuel et al., 2017) except for KE1 (92.27 g/100g), KE1 (91.76 g/100g) and KW2 (98.46 g/100g). Iodine value is a measure of degree of unsaturation in an oil sample. It can be defined as the number of grams of iodine that can be added to 100 g of oil. It is reported that shea nut fat with low iodine value is indicates its richness in saturated fatty acids and this ensures stability against oxidation; thus making it a good source of oil (Samuel et al., 2017). The obtained values are lower than 100 thus, shea butter is a non-drying oil (Warra, 2015). The results from the physicochemical analysis indicates that the samples may be categorized into Grade 2 and 3 oil.

The fatty acid prolife was analyzed by GCMS. Seven saturated fatty acids and six unsaturated fatty acids identified in Shea butter as reported by (Di Vincenzo et al., 2005) were found in considerable quantities in the analyzed samples. Their identity and percentage composition are presented in Table 2 for saturated and Table 3 for the unsaturated fatty acid. The dominant saturated fatty acid includes myristic acid C14, palmitic acid C16, stearic acid C18, Arachidic acid C20, Behenic acid C22 and lignoceric acid C24.

Stearic acid content was found to be higher than other saturated fatty acids in the samples. Its percentage composition ranged from 0.09% (KE2: Kebbi white) to 45.01 % (AB2: Abuja white) as presented

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in Table 2. Figure 1 showed that percentage composition of stearic acid was the highest in AB2 (Abuja white); KE1 (Kebbi grey) and NI1 (Niger grey sample). The value obtained for AB2 is close to the value reported for Shea butter (49.7%) obtained from Jalingo, Taraba state, Nigeria (Ugese et al., 2010). Therefore, AB2, KE1, and NI1 samples could be a good source for stearic acid production. Furthermore, all other samples contain a considerable amount of stearic acid, as depicted in figure 1.

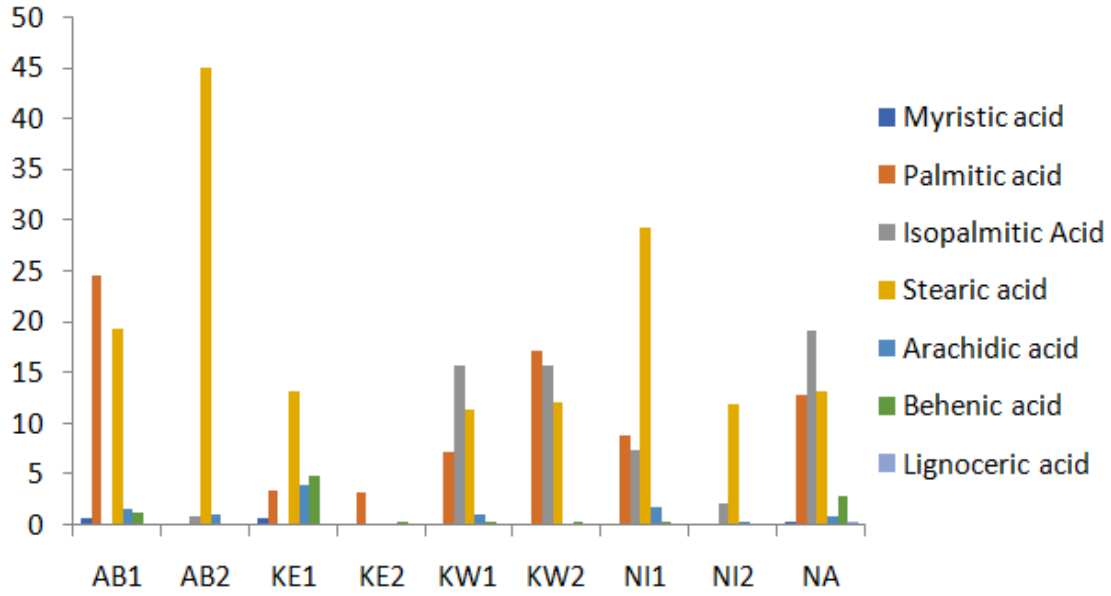

Figure 1: Saturated fatty acid percentage composition of shear butter samples

The predominant unsaturated fatty acids in the samples are the C18 family such as oleic acid C18: 9, vaccenic acid (18:11), linoleic acid C18:2 and six, and the gamma-linolenic acid C18: 5,8,11 as shown in Figure 2. As presented in Table 3, NI1: Niger grey has the highest percentage composition of 64.93%, followed by AB2: Abuja white at 31.49%. This unsaturated C18 family can be converted to a saturated C18 family and hence they could be a good source for the production of stearic acid. Also found in significant quantities in the analyzed samples is Gondoic acid C20:11 and palmitoleic acid C16:7 (Table 3). The result obtained in this study is consistent with the report that the predominant fatty acid in Shea butter are stearic and oleic acid and it determines the consistency of the butter and their end uses (Maranz et al., 2004).

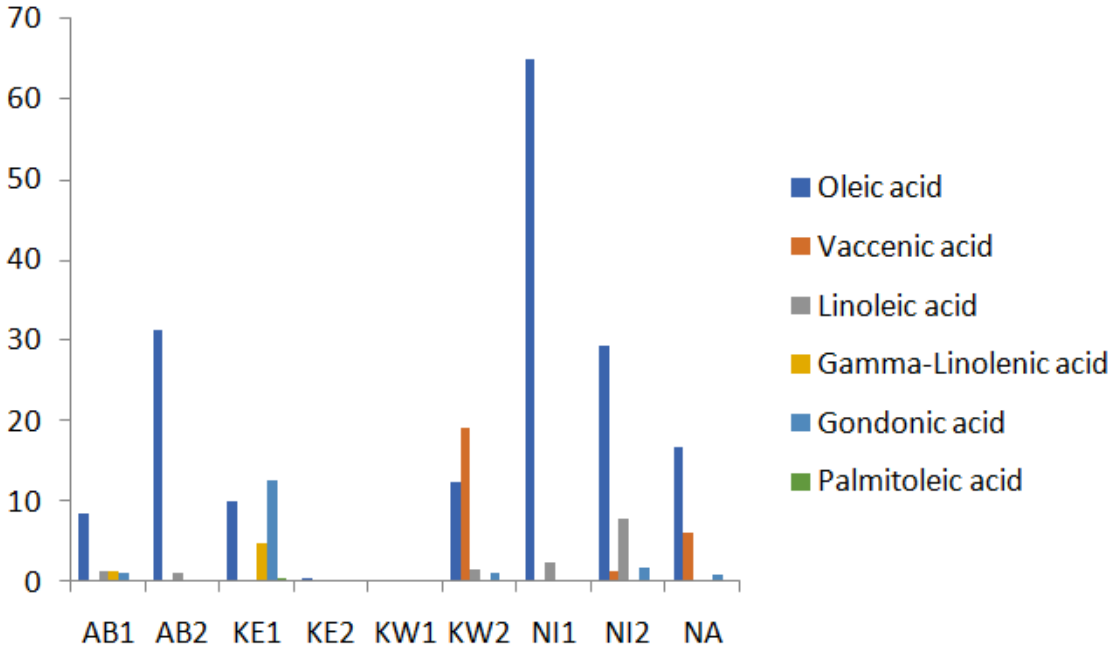

Figure 2: Unsaturated fatty acid percentage composition of shear butter samples

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IV. Conclusion

The physicochemical analyses of Shea butter samples indicate that the samples may be graded into the Grade 2 and 3 oils according to West African regional standards and they are rich in unsaturated fatty acids. The fatty acid profile of the samples indicated that sample from Abuja, mainly the Abuja grey sample is very rich in stearic acid composition, and samples from Niger, especially the Niger white sample is rich in oleic acid. The result indicates that the Shea butter samples from the four states, and FCT, Nigeria, could be good sources for producing Stearic acid.

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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 Science Frontier Research Paper

Techniques for writing a good quality Science Frontier 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 science frontier 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.

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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.

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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.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

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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