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Phytochemistry and GC-MS analysis of methanolic leaf extract of *Newbouldia leavis* (Bignonacea)

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Abstract
Newbouldia leavis is a shrub found around Nigeria. It is known as fertility plant. This study covers the phyto-chemical evaluation and GC-MS analysis of the methanolic extract of the leaves of *Newbouldia leavis*. Quantitative phytochemical screening was done using various standard laboratory methods. The result obtained from the phytochemical screening revealed the presence of alkaloids, phenols, oxalate, tannin, saponin among others. The GC-MS analysis showed eight (8) peaks corresponding to eight distinct phytochemicals which include: n-hexadecanoic acid, Phytol, 9, 12-Octadecadienoic acid (Linoleic acid), 13-Octadecenal, Phthalic acid, Oleic acid, Squalene, Stigmasterol. Each of the identified phytochemicals possesses a wide range of Pharmacological activities ranging from antioxidant properties to anti-osteoporotic and to cancer prevention. Most of its constituents possess fertility activity. The methanolic extract of leaves of *Newbouldia leavis* can serve as a useful source of raw material for the synthesis of new drugs in the Pharmaceutical industry.

Keywords: *Newbouldia leavis*, Phyto-chemical, GC-MS, Fertility plant, Alkaloid

Introduction
From ancient times, man had been exposed to a lot of diseases. The need to find a cure or means to manage these cases became paramount to man. Man believed that God created plants and the things around him to help him live well. The practice of the use of herbal preparations began as an empirical activity without the backing of sound scientific proves. *Newbouldia laevis* commonly known as fertility plant is an evergreen plant found commonly in the tropical region of Africa. This plant is known worldwide for its various activities which include antibacterial, antidiabetic, antihypertensive with its fertility property being the most prominent. Fertility plant also called 'tree of life'. A decoction of the leaf is taken by women to treat fertility problems. However, a higher dosage of the leaf decoction has a uterine contraction effect and traditional healers in Nigeria use it to facilitate labor. The young leaf can also be boiled with vegetable oil and is taken during labor to facilitate easy delivery. A decoction of the leaf applied as gargle in the mouth for 4 to 5 minutes continuously is used to treat dental caries. The bark is stomachic and analgesic. A decoction is used in the treatment of dysentery, diarrhea, epilepsy, cough and convulsion. The pounded dried bark combines with *Xylopia sp* and other spices is taken inform of herbal tea to relieve dysmenorrhea. A decoction of the bark is sieved and applied inform of an enema as a treatment for piles and constipation. Dried powdered of the bark combined with alligator pepper (*Aframomum melegueta*) is sniffed inform of snuff to treat migraine and sinusitis. The bark applied externally is use to cure wounds, ulcers and abscesses. A decoction or ethanol extract of the root is used for the treatment of syphilis and intestinal problems such as worms. The decoction of the root is laxative and is used to treat constipation. The maceration of the root is effective for the treatment of arthritis and rheumatic pains. The powdered root combine with chili pepper (*Capsicum frutescens*) are put into a carious tooth to treat toothache. The powdered dry root combine with root of Lopharia sp. are infused inform of ointment and use to massage edema arriving through dietary deficiency. Decoction of the root and leaves are remedy for scrotal elephantiasis, fever and as an aphrodisiac.

Fig 1: Leaves of *Newbouldia leavis* during collection

Materials and Methods

The leaves of Newouldia leavis was identified and collected from the Botanical garden of the Department of Pharmacognosy Faculty of Pharmacy Madonna University Rivers State April 2019. The leaves were authenticated by Pharm. Dr Osuala of the Department of Pharmacognosy Faculty of Pharmacy Madonna University, Nigeria. The collected leaves were garbled after collection and dried under shade for two (weeks) to prevent the loss of useful thermolabile constituents. The dried leaves were pulverized using a mechanical mill. The pulverized leave was macerated using two (2) liters of methanol for three days with intermittent agitation. The extract was obtained by filtering using a tight sieve and purified using whatman`s filter paper. The filtrate was dried over water bath at 45-50 ^{O}C. The dried extract was scrapped and put into sample bottles and refrigerated to preserve it.

Phytochemical Screening

This was done to determine the constituents of methanol extracts of the leaves of fertility plant using established methods (Trease and Evans, 2009).

Test for Tannins: 0.5 g of the methanol was stirred with 10 ml of distilled water and filtered. 2 drops 5% ferric chloride was added to the filtrate. A green precipitate was formed which indicated presence of tannins.

Test for Phlobatannins: 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 1% hydrochloric acid. There was no reaction which indicated absence of phlobatannins.

Test for Saponins: 0.5 g of the extract was shaken vigorously with distilled water in a test tube. There was no frothing and this indicated absence of saponins.

Test for flavonoids: 0.5 g of the extract was dissolved in 2 ml of distilled water and filtered. Two drops of 5% dilute hydrochloric acid was added to mixture and a yellow color was seen and this show a positive test.

Test for Alkaloids: 0.5 g of the extract was stirred with 5 ml of hydrochloric acid on a water bath and then filtered. 1 ml of each filtrate was treated with few drops of Dragendoff's reagent and a reddish-brown precipitate was seen which indicated a positive test. A second 1 ml was similarly treated with Mayer's reagent and a cream-colour precipitate was formed which indicates a positive test. The third 1 ml was treated with few drops of Hager's reagent and a yellow colour precipitate was seen which indicated a positive test.

Test for Anthraquinone: 1g of the plant extract was shaken with 10 ml of chloroform and filtered. To the filtrate, 10% ammonia solution was added and the mixture shaken. There was no reaction and this indicated absence of Anthraquinone.

Test for Carbohydrates: 0.1g of the extract was dissolved in water. To 2ml of this mixture, 2 drops of 10% alcoholic solution of α-naphthol were added in a test tube. The test tube was inclined at angle 45 and 2ml of concentrated sulphuric acid (H_2SO_4) was carefully added. A deep violet ring at interface indicated the presence of carbohydrates.

Test for Phenols: 1 g of the extract was taking with 1 ml of water in a test tube and 1 to 2 drops of iron III chloride ($FeCl_3$) was added. A blue, green, red or purple color is positive test.

GC-MS Analysis

The concentrated methanol extracts were re-dissolved, whirled and filtered. 1 mcg aliquot of the sample was injected into the GC-MS equipment.

GC-MS analysis was carried out on a GC-MS (Model: QP2012 plus Shimadzu, Tokyo, Japan) which comprises of AOC-20i auto-sample and gas-chromatography inter-phased to a mass spectrometer(GC-MS) instrument equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 m film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.58 ml/min. The injector and mass transfer line temperature were set at 250-200°C respectively, and an injection volume of 1 mcg was employed. The oven temperature was programmed for 80°C, with an increase of 10°C/min to 200°C for 4mins, 10 °C/min to 280 ° ending with a 5-minute isothermal at 280°C. The MS operating parameters were as follows: Ionization energy, 70 eV; Ionization temperature, 200 °C.

The relative amount of each phyto-component was computed by comparing the average peak to the total areas. The detection made use of the NIST Version 2.0 year 2007 library. The compound prediction was based on Dr. Duke's Phytochemical and Ethno-botanical Databases by Dr. Jim Duke of Agricultural Research Service/USDA (Dr. Duke Database, 2014). Interpretation of GC-MS was conducted using the data of NIST having more than 62,000 patterns. The spectrum of unknown phyto-components was compared with spectrum of known components stored in the NIST library. The name and molecular weight of the test were ascertained.

The Gas Chromatograms of the different extracts of *N. leavis* leaves show relative concentration of various compounds getting eluted as a function of retention time (RT). The height of the peak indicates relative concentrations of the components present. The result pertaining to GC-MS analysis of phyto-components shows the retention time, name of the compound, molecular formula, molecular weight and percentage (%) peak area.

Result and Discussion

Table 1: Percentage yield of the methanol extracts of *N. leavis*

Solvent used	Weight of extract(g)	Weight of pulverized leaves of *N. leavis* (g)	Percentage yield (%)
Methanol	123	1000	12.3

Table 2: Result of preliminary screening of Primary & Secondary metabolites of methanolic extract of *N. leavis*

Phytochemical	Inference
Alkaloid	+
Carbohydrate	+
Phlobatannins	-
Saponins	+
Oxalates	+
Flavonoids	-
Cardiac Glycosides	+
Phenols	+
Quinones	+
Anthraquinones	-
Terpenoids	+
Tannins	+

Gas Chromatogram of Methanolic leaf extract of Newbuoldia leavis

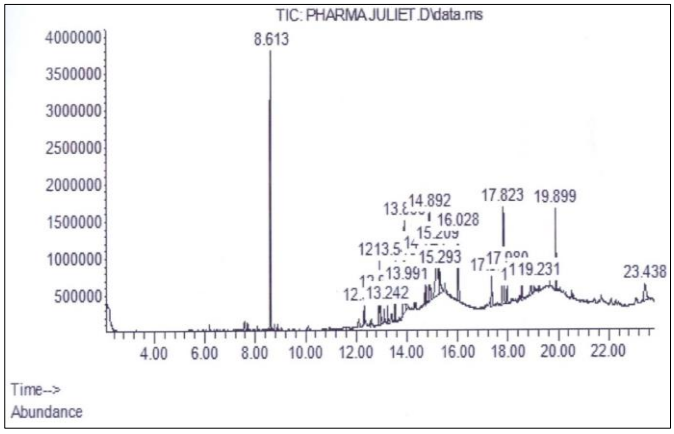


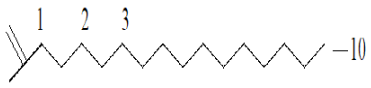
Fig 2: The Gas Chromatogram of ethyl-acetate leaf extract of *Newbouldia leavis* shows 8 prominent peaks corresponding to 8 different Phyto-chemical compounds

Table 3: Phyto-components identified in the Methanol leave extract of *Newbouldia leavis* by GC-MS

S/N	Retention time (min)	Name of Compound	Molecular Formula	Molecular Weight (gmol-1)	Peak area (%)
1	13.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.17
2	14.9	Phytol	$C_{20}H_{40}O$	128.2	6.9
3	14.7	9,12-Octadecadienoic acid (Linoleic acid)	$C_{18}H_{32}O_2$	280.5	15.35
4	17.8	13-Octadecenal	$C_{18}H_{34}O$	266.5	10.98
5	18.6	Phthalic acid	$C_8H_6O_4$	166.1	.82
6	18.9	Oleic acid	$C_{18}H_{34}O_2$	282.47	14.68
7	19.9	Squalene	$C_{29}H_{48}O$	412.68	0.96
8	23.4	Stigmasterol	$C_{29}H_{48}O$	412.69	11.12

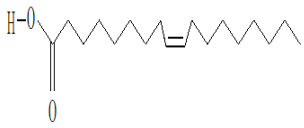
Some Chemical Structures of Phytochemical Principles revealed from the methanol extracts of Newbodia leavis

Squalene

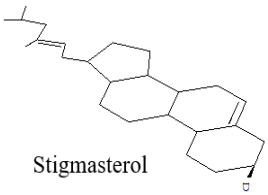


9, 12- Octadecadienoic acid

n-Hexadecanoic acid



Oleic Acid



Stigmasterol

Table 4: Pharmacological activities of some Phytochemical Compounds revealed from the methanol extract of N. leavis

Phyto-Components.	Nature of Compounds	Pharmacological Activities ***
n-Hexadecanoic acid	Saturated fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
9,12-Octadecadienoic acid (Linoleic acid)	Unsaturated fatty acid	5-Alpha reductase inhibitor, antiandrogenic, Antiarteriosclerotic, Anticoronary, Antifibrinolytic, Antihistaminic, Antiinflammatory, antileukotrienic, anti prostatic, hepatoprotective, carcinogenic, immunomodulator, antieczemic, hypocholesteramine.
Oleic acid	Unsaturated fatty acid	5-Alpha-Reductase Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene, Cancer-Preventive, Choleretic, Dermatitigenic FLavor, Hypocholesterolemic. Insectifuge, Irritant, Percutaneostimulant, Perfumery Propecic
Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant, Lipoxygenase-Inhibitor, Perfumery, Pesticide, Sunscreen
Stigmasterol	Phytosterol	Reduction of Atherosclerosis, Cardioprotective, Cholesterolytic agent.

**** Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases [Online database]. ***.

GC-MS Spectra of Some Phytochemical compounds revealed from extract of *N. leavis*

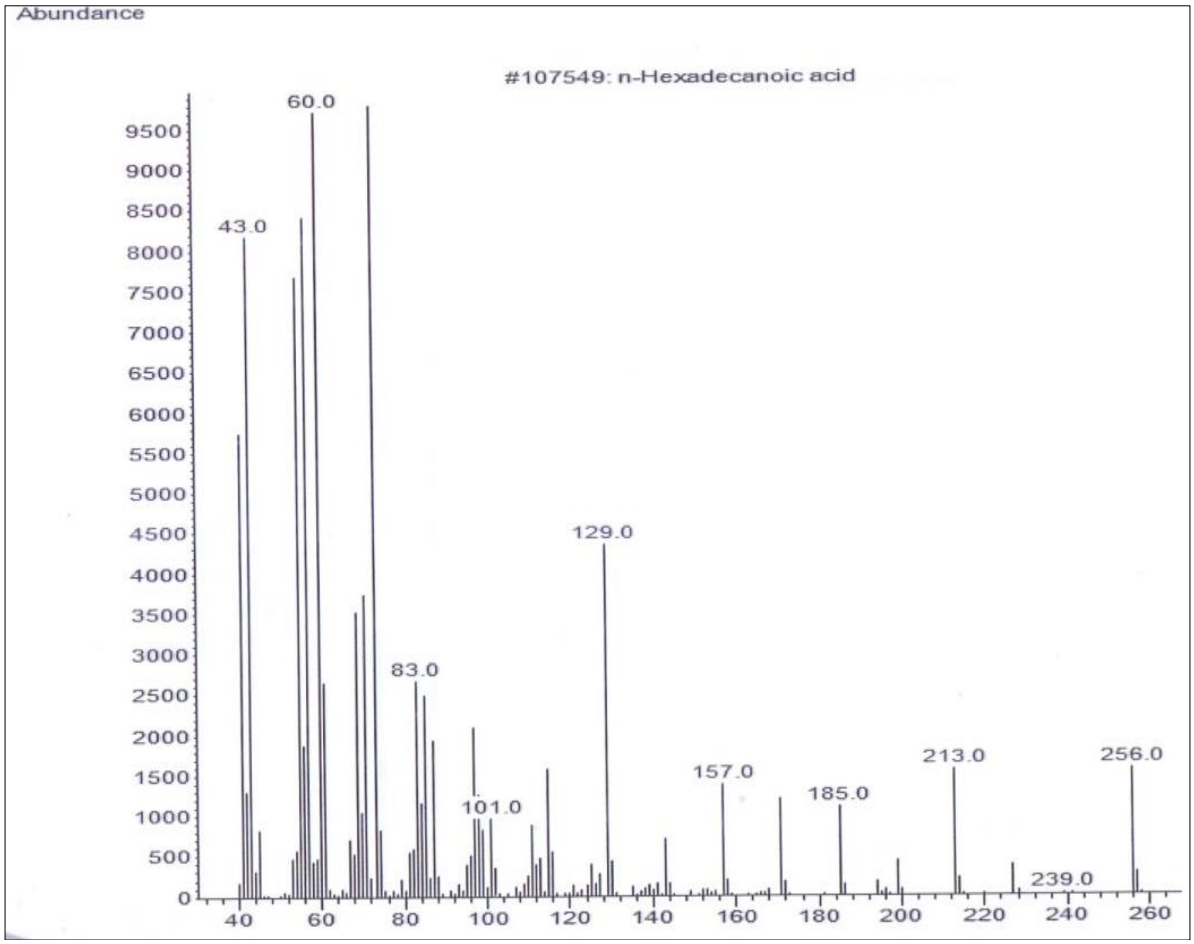


Fig 3: Spectral Analysis of N-Hexadecanoic acid

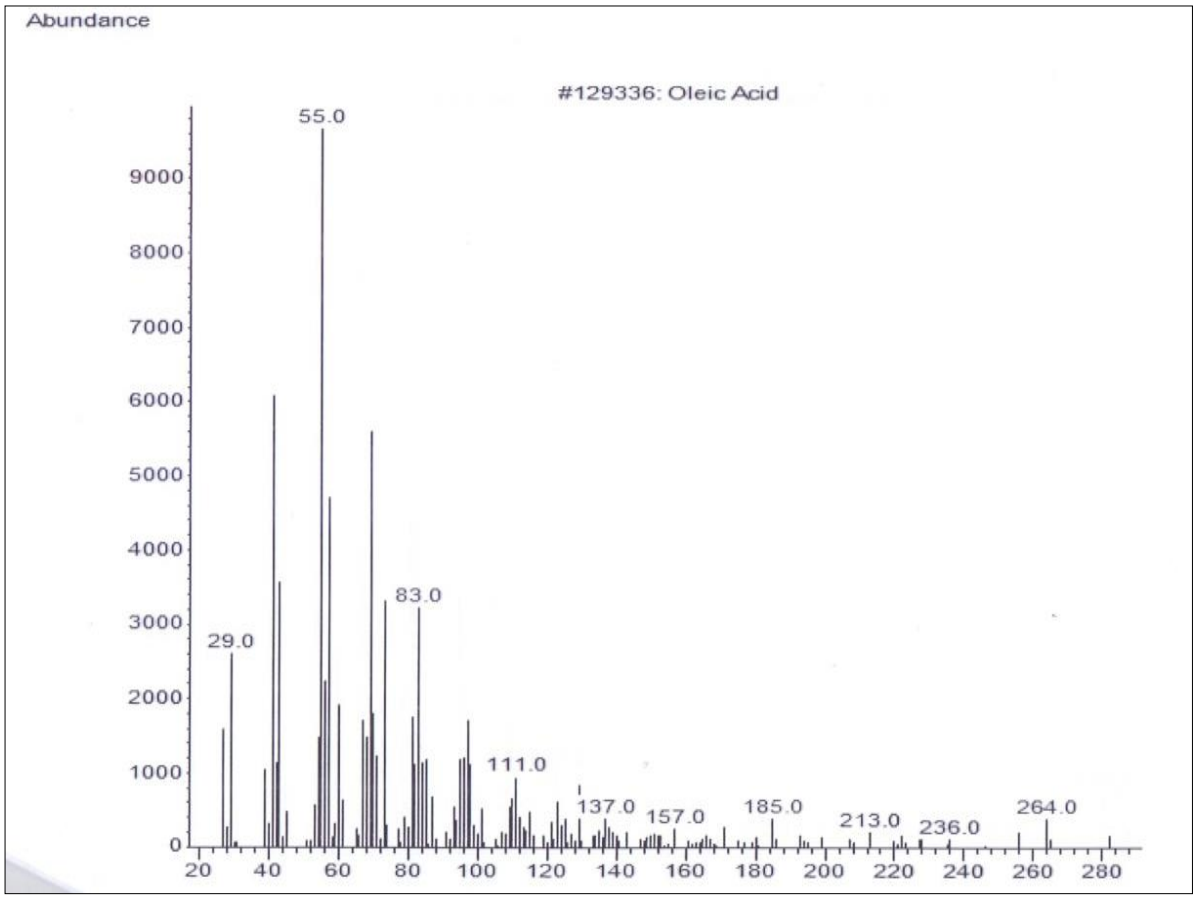


Fig 4: Spectral Analysis for Oleic Acid

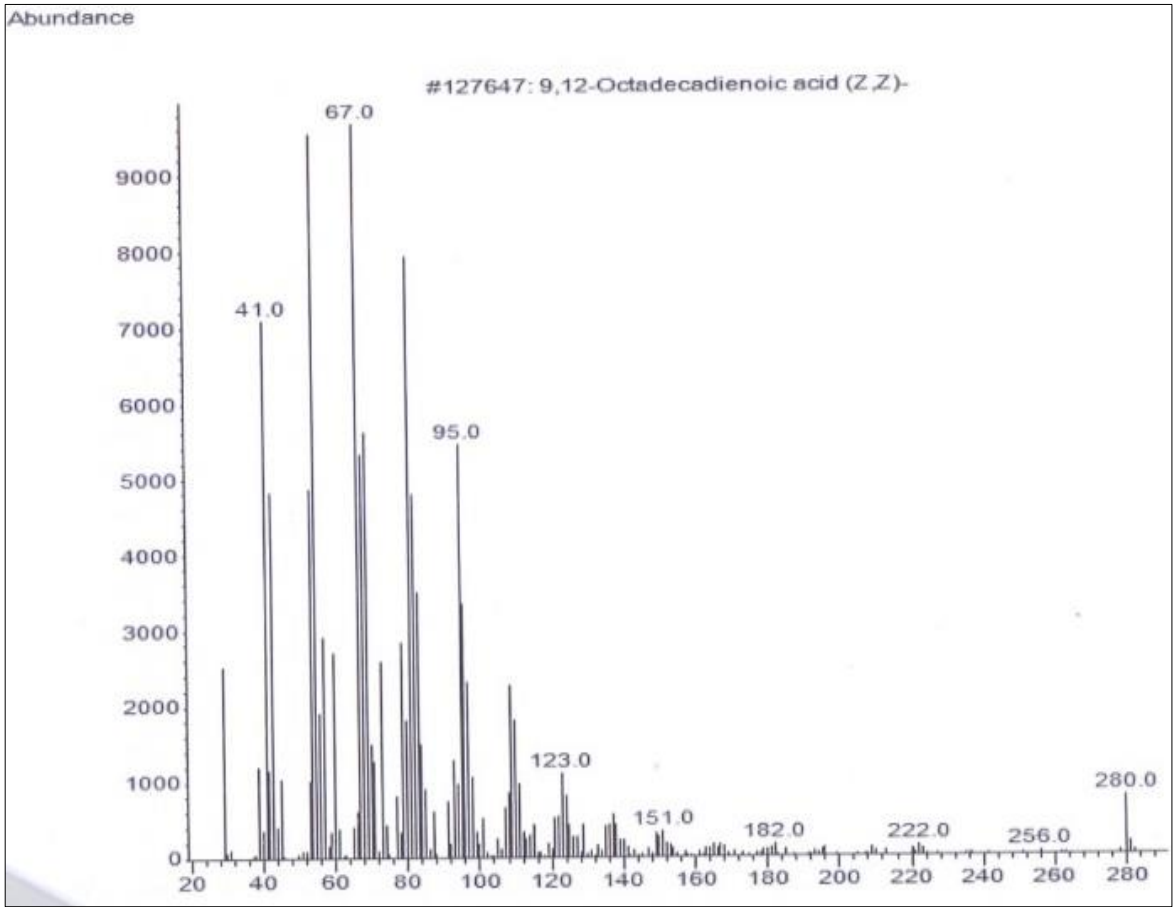


Fig 5: Spectral analysis of 9,12-octadecadienoic acid

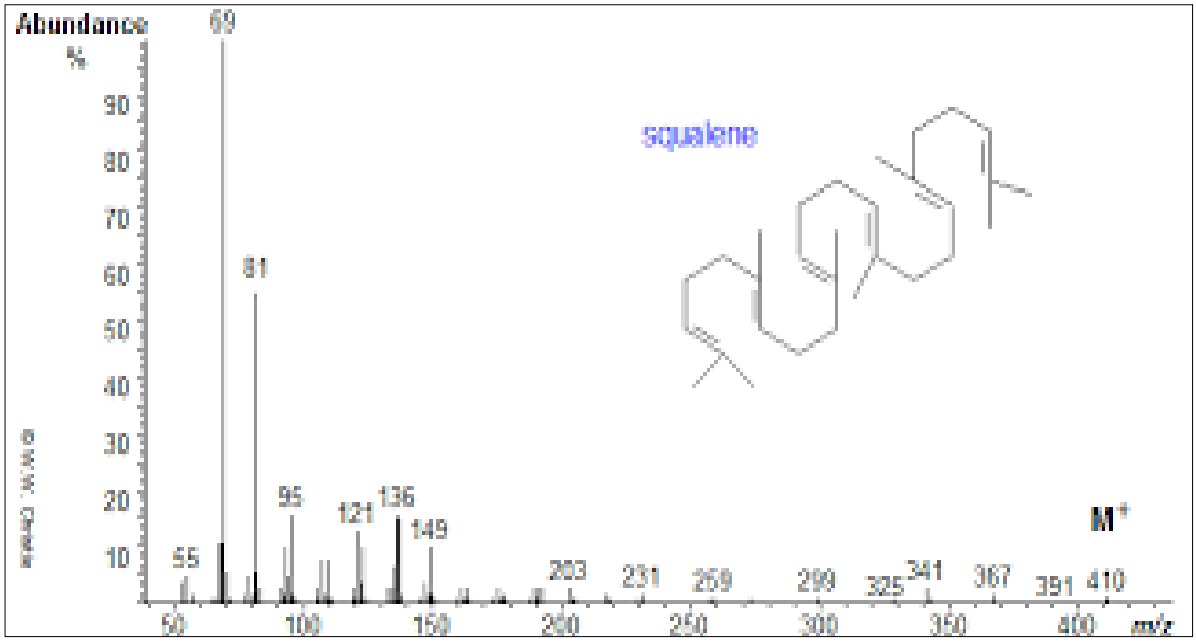


Fig 6: Spectral analysis of squalene

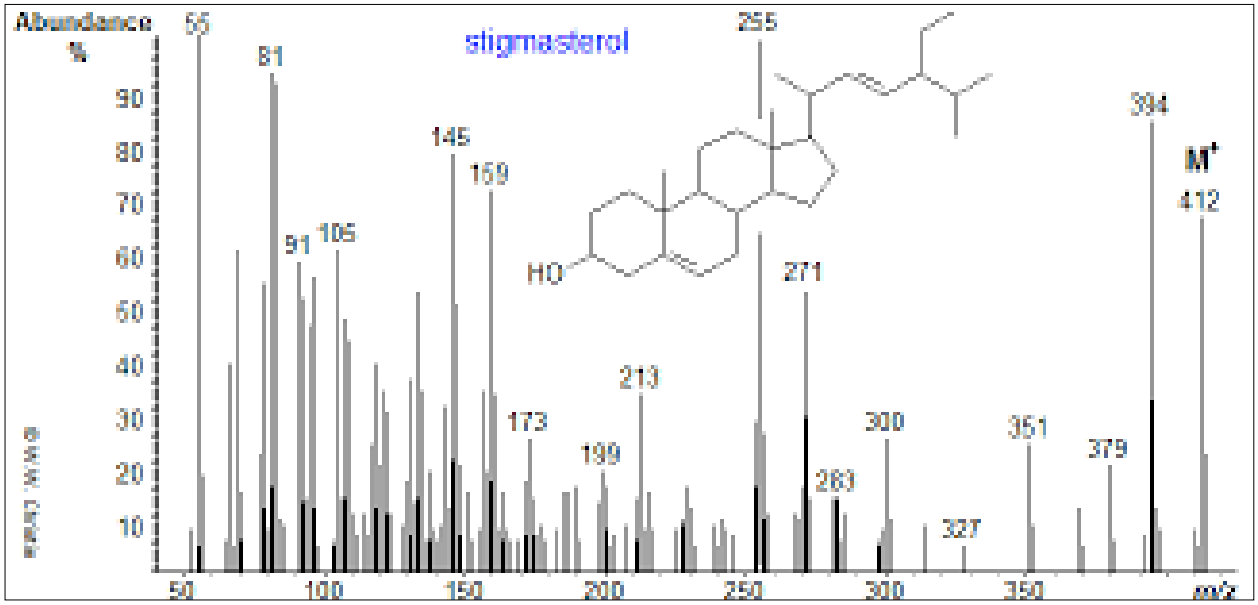


Fig 7: Spectral analysis of stigmasterol

Discussion

Herbal preparations have been used for the treatment of various diseases not only by locals but also internationally. Herbal crude drugs have been used for the development of new and Novel drug products having a variety of pharmacological activities (Pan *et al*., 2013). The Preliminary phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Cardiac glycosides, Phenols, saponins, Tannins, Terpenoids, Quinones and oxalates. Phlobatannins, flavonoids and Anthraquinones were absent.

The Gas Chromatography- Mass Spectroscopy analysis of the methanolic leaf extract of *N. leavis* revealed eight (8) prominent peaks which corresponded to eight different phytochemicals. Palmitic acid, or hexadecanoic acid in IUPAC nomenclature, is the most common saturated fatty acid found in animals, plants and microorganisms (Gunstone *et al*, 2007) [29]. Researches have shown that Palmitic acid possesses antioxidant and anti-inflammatory properties (Chandra *et al*, 2011) [30]. It also possess other pharmacological properties such as Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor. Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid and is one of two essential fatty acids for humans, who must obtain it through their diet (Simopoulos *et al*, 2008). It is colorless or white oil that is virtually insoluble in water. May Reduce Risk of Cardiovascular Disease, Promotes Healthy Brain Function, Supports Skin and Hair Health, Improves Reproductive Health, Boosts Immune Function, Protects Bone Density (Harris *et al*, 2009). According to Dr. Duke's Phytochemical and Ethnobotanical Databases Linoleic acid also possess 5-Alpha reductase inhibitor, antiandrogenic, Antiarteriosclerotic, Anticoronary, Antifibrinolytic, Antihistaminic, Antiinflammatory, antileukotrienic, anti-prostatic, hepatoprotective, carcinogenic, immunomodulator, antieczemic, hypocholesteramine.

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odorless, colorless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18:1 *cis*-9. It has the formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ (Thomas *et al*. 2000). According to Dr. Duke's Phytochemical and Ethnobotanical Databases, oleic acid possess 5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene, Cancer-Preventive, Choleretic, Dermatitigenic FLavor, Hypocholesterolemic. Insectifuge, Irritant, Percutaneostimulant, Perfumery Propecic pharmacological activities. Phthalic acid is an aromatic dicarboxylic acid, with formula C_6H_4 $(CO_2H)_2$. It is an isomer of isophthalic acid and terephthalic acid (Peter M *et al*, 2007). Stigmasterol a plant sterol (phytosterol) – is among the most abundant of plant sterols, having a major function to maintain the structure and physiology of cell membranes (Ferrer *et al*, 2017) In the European Union, it is a food additive listed with E number E499, and may be used in food manufacturing to increase the phytosterol content, potentially lowering the levels of LDL cholesterol (Caprel *et al*, 2017).

Conclusion

The methanol leave extract of *Nebouldia leavis* contains phytochemicals such as Anthraquinones, oxalates, phenols, quinones and a host of many other classes of phytochemicals. The GC-MS revealed the presence of 8 peaks which indicated the presence of chemicals such as oleic acid, palmitic acid, stigmasterol and others. These chemicals possess pharmacological activities that are useful to man. Hence, further research can be performed on the extract to isolate and purify the different compounds which can serve as a useful prospect for drug development.

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