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TLC analysis and GC-MS profiling of Hexane extract of *Syzygium guineense* Leaf

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Abstract
Introduction: *Syzygium guineense* leaf and bark of are used for the treatment of tuberculosis, chronic diarrhea, cough, dysentery, malaria, amenorrhea, wounds, ulcers, rheumatism and infections.
Material and Method: The various compounds in the n-hexane extract of the leaf were analysed by TLC and identified by GC-MS technique. The TLC results indicated that four (4) terpenes are present in hexane extracts of the leaf of *Syzygium guineense* after treating TLC plates with vanillin-Conc.H_2SO_4.
Results: The results of the GC-MS analysis revealed twelve (12) compounds in the n-hexane extract of *Syzygium guineense* leaf. These are 1-ethyl-2-methylbenzene (2.61%), Ylangene (2.42%), decahydro-4a-methyl-1-methylene-7-(1-methylethynyl)-naphthalene (γ-muurolone) (2.47%), 4-dimethyl-7-(1-methylethenyl)azulene(2.06%), caryophyllene oxide(3.86%), myristic acid (2.11%), n-hexadecenoic acid (11.94%), 9-octadecanoic acid (25.72%), tetratriacontane (31.45%), 1,2-benzenedicarboxylic acid (2.71%), tetratriacontane (6.70%) and pentatriacontane (3.95%). These compounds fall into three classes; terpene/terpenoids, organic acids and hydrocarbons with the major compounds been the organic acids 42.48%. Hydrocarbons constituent 42.1% of the extract while only 0.38% constitute terpenes/terpenoids.
Conclusion: The results of this study offer a basis of using *S. guineense* leaf as an alternative medicinal agent as anti-inflammatory analgesic, antipyretic and platelet-inhibitory actions.

Keywords: *Syzygium guineense* leaf, TLC, GC-MS, Terpenes.

1. Introduction
Plants are described as "nature's chemical factories" which may contain natural substances that exhibit bioactive properties by producing a definite physiological action on the human body when administered [1]. Such derived compounds are reported to be less toxic and even more effective in fighting diseases [2]. For instance, natural compounds have provided the best anti-malarials known to date with quite a number awaiting investigation [3]. Isolating and elucidating structures of different chemical constituents in a plant is a basic task in the drug discovery process [4, 5]. In some cases the crude extract is more effective pharmacologically than the purified bioactive compound from the extract. Synergy between the identified active compounds with other compounds present in seems to add to pharmacological activity [3, 1]. Natural products introduces new chemical entities of wide structural diversity that will are templates for semi-synthetic and total synthetic modification. Apart from plants, other natural sources are yet to be fully tapped from planktonic organisms to mammals. Intensive research is still needed through concerted cooperation to explore the biological activity of all sources of natural products as core scaffolds for future drugs [6]. New approaches to drug discovery, such as combinatorial chemistry, and computer-based molecular modeling design cannot replace the important role of natural products in drug discovery [6].
This research is carried out to analyse and identify the phytochemical constituents of *Syzygium guineense* leaf extracts. The leaf and bark of this plant are used for the treatment of tuberculosis, chronic diarrhea, cough, dysentery, malaria, amenorrhea, wounds, ulcers, rheumatism and infections. The investigation involves extracting the leaves with organic solvents, concentrating the extracts, thin layer chromatography (TLC) analysis of the extracts, and spectra analysis using hyphenated technique of gas chromatography-mass spectrometry (GC-MS). This work seeks to establish a scientific basis for the application of *Syzygium guineense* by herbal practitioners.
Syzygium guineense is a member of the family *Myrtaceae*. It is an evergreen water loving dicotyledon which grows to a height of 8 – 15 metres [23]. In Africa, the plant is distributed in Nigeria, Senegal, Eritrea, Ethiopia, Somalia, Zaire, Rwanda, Zambia, Malawi, Zimbabwe,

Namibia, Uganda, Swaziland, Cameroon, and South Africa. In Nigeria, it is known by different names depending on the dialect such as afour (Afizere/Jarawa), molmol (Hausa) and ori (Yoruba). The root, bark and leaf are used in traditional medicine as remedy for various ill health conditions. A mixture of water and powder made from the bark and roots of the plant when administered act as a purgative [18]. Similar preparation is applied as a remedy for dysentery, diarrhea and treatment of stomach ache [19]. *Syzygium guineense* extract is used against *Naja katiensis* venom [20]. Gastro-intestinal upsets can also be remedied by using this plant [22].

The crude extract of the plant has shown anti- mycobacterium activity and anti-diarrheal activity in tested organisms [18, 23]. The aqueous extract exhibited antibacterial activity against *Salmonella E., Shigella D., Shigella F., E. coli, Enterobacter A.* [21]. Essential oil consituents of the dried leaf include caryophyllene oxide, cadinene, viridiflorol, *epi*-α- cadinol, cadinol, *cis*-calamenen-10-ol, citronellyl pentanoate, caryophyllene and humulene [22]. Betulinic acid, oleanolic acid, 2-hydroxyoleanolic acid, 2-hydroxyursolic acid, arjunolic acid, asiatic acid, terminolic acid, 6-hydroxyasiatic acid, arjunolic acid 28-glucopyranosyl ester and the asiatic acid 28-glucopyranosyl ester were reported [21]. Arabinogalactan polysaccharide was isolated from the Malian leaf [24]. Essential oils extracted from dried leaves of *Syzygium guineense* collected in Benin analysed by GC-MS contain caryophyllene oxide (7%), δ-cadinene (7.5%), viridiflorol (7.5%), *epi*-α-cadinol (9.8%), α-cadinol (12.7%), *cis*-calamenen-10-ol (14%), citronellyl pentanoate (15.2%), β-caryophyllene (20.1%) and α-humulene (39.5%) [22].

Arjulonic acid, Terminolic acid, 2,3,23-Trihydroxy-(2*α*,3*β*,4*α*) olean-11-en-28 oic acid and asiatic acid (Hydroxyasiatic acid) were also isolated and they show antibacterial activity against *B. subtilis, E. coli* and *Shigella sonnei* [21]. Similarly 2*α*, 3β, 24-Trihydroxyolean-12-en-28-oic acid isolated from the same plant were also antibacterial activity against *Planchonia careya* and *Enterococcus vancomycin resistant* [25].

The plants of the Family Myrtaceae are dicotyledonous angiosperm shrubs and trees found in the tropics, sub-tropics and temperate Australia [9]. They are characterised by radially symmetrical flowers with a reduced calyx (sepals) and corolla (petals) and numerous stamens [9]. The sepals and petals number either 4 or 5 or have united to form a cap over the flower (as with the eucalypts) [9]. The fruit is usually a berry or capsule. The leaves generally contain oil glands. There are closely 150 genera in this family. The total number of species seems to be disputable as different literature report gives different number of species [9]. However within *Myrtaceae*, species belonging to the genera *Corymbia*, *Myrtus*, *Psidium*, *Pimenta*, *Eugenia*, *Pseuocaryophyllus*, *Syzygium*, *Eucalyptus*, *Leptospermum*, *Plinia*, and *Malaleuca* are reported to be widespread compared to the other species. Phytochemically, several members of this family mainly accumulate flavonoids, tannins, other phenolic derivatives and Terpenoids [9]. The plant families particularly rich in essential oils are compositae, *matricaria,* Labiatae, *menthe spp;* Myrtaceae, *Eucalyptus; Rutaceae* and *Umbilliferae.* The various compositions of terpenes can be markedly different from one species to another [7]. Currently, there is an increased interest in terpenoids for antibacterial, antineoplastic, and other pharmaceutical functions [8].

Eucalyptus species are particularly abundant and have a wider range of distribution than the other myrtaceous genera since they are frequently grown as exotics in commercial plantations [10]. Members of this genus are used in folk medicine as antidiarrheal, antimicrobial, antioxidant, antirheumatic, anti-inflammatory, cleansing agents and are also known to be effective in reducing blood cholesterol [11]. Majority of the plants are also known to produce essential oils, most of which are bacteriostatic, fungistatic, anti-inflammatory and antifungal activities and as such used in creams, soaps and toothpastes [11]. Leaf of *Eugenia uniflora* L analyzed by GC-MS majorly contains atractylone and curzerene. It's essential oils are active towards gram-positive bacteria, *Streptococcus equi* and *Staphylococcus epidermis* [9]. *Plinia trunciflor* leaf contains α-cadinol, apiole and cubenol majorly. The essential oils showed activity towards gram-positive *Streptococcus equi* and *Staphylococcus epidermis* [9]. In *P. cattleianum*, the most prominent compound is caryophyllene oxide [12]. Caryophyllene oxide is the main constituent most *Psidium* species. Where variations exist in oil content and composition, it is attributable to factors related to ecosystem, the environment (temperature, relative humidity, irradiance and photoperiod), genetics, chemotypes and the nutritional status of the plant [12]. Acetylated glycosidic flavonoids in genus *Syzygium* are present in the genera *Eugenia* and *Eucalyptus* (Myrtaceae) [12].

Syzygium australe and *Syzygium leuhmannii* are widespread in tropical and subtropical regions of South-East Asia, Australia and Africa [13]. The use of these plants as medicinal agent is common with Australian aborigines. *Syzygium Cumini* is found throughout India up to an altitude of 1800 meters from Myanmar and to Afghanistan and in other countries like Thailand, Philippines and Madagascar [14]. *Syzygium jambos* (L.) is widespread and traditionally used in sub-Saharan Africa particularly Benin, Democratic Republic of Congo and Cameroon to treat infectious diseases [15]. It has been used in the treatment of pernicious attack, amenorrhea, abdominal pain and diarrhea. This specie of *Syzygium* is also distributed in Reunion Island, Central America (i.e. Guatemala) and Asia (i.e. Malaysia, Nepal) [15]. *Syzygium forrestii* is an evergreen broad-leaved tree distributed on the mountain slopes (altitude range from 800 to 2400 m) endemic to Yunnan Province, in southwest of China [12].

The family *Myrtaceae* is characterised by tannins and flavonols as the main chemical constituents. The isolated flavonoid, myricitrin seems to be the main flavonoid in this family. This same flavonoid is present in *Syzygium levinei* and *Syzygium samarangense* while (-)-epicatechol-3-O-gallate was found from *S. samarangens* [12]. Furthermore, nilocitin, pedunculagin and gemin D, all hydrolyzable tannins are present in *Syzygium aromaticum*. Similar flavonoids and hydrolysable tannins ar contained in *S. forrestii*. Therefore, the flavonoid glycoside myricitrin and the three hydrolyzable tannins can serve as the chemosystematic markers of the genus *Syzygium* [12].

Methanol extracts of *S. australe* showed antimicrobial activity against 73% of the gram-negative bacteria tested and 67% gram-positive bacteria tested had their growth inhibited. The leaf extract showed non-activity against *Enterobacter aerogenes, Escherichia coli, Salmonella salford, Bacillus subtilis Candida albicans, Saccharomyces cerevisiae* [13]. The extract displayed antifungal activity against a nystatin resistant strain of *A. niger* but did not affect the growth of *C. albicans* or *S. cerevisiae* [13]. Acetone and aqueous extracts of *S. jambos* bark showed some activity against *Staphylococcus aureus, Yersinia enterocolitica, Staphylococcus hominis, Staphylococcus cohnii* and *Staphylococcus warneri* [15].

The major components in n-hexane extracts of *Syzygium*

aromaticum identified using GC-MS are eugenol and eugenol acetate [16]. The dichloromethane extract of the buds yielded limonin and ferulic aldehyde along with eugenol while the ethanol extract yielded the flavonoids tamarixetin 3-*O*-b-D-glucopyranoside, ombuin 3-*O*-b-D-glucopyranoside and quercetin which showed strong antioxidant activity against 1, 2-diphenyl picrylhydrazyl (DPPH) [16].The leaf of *Syzygium cumini* are particularly rich in acylated flavonol glycosides such as quercetin, myricetin, myricetin 3-O-(4"-acetyl)-α-L-rhamnopyranoside and galloyl carboxylase. The stem bark is rich in the pentacyclic triterpenoid, betulinic acid, friedelin, epi-firedenalol, β-sitosterol, eugenin and fatty acid ester of epi-fierdanalol, quercetin, kaempferol, myrecitin, gallic acid, bergenins and ellagic acid. These phytochemicals are also reported present in the flower of *Syzygium cumini* [14]. β-sitosterol is found in almost all parts of *Syzygium cumini* [14]. Friedelin ($C_{30}H_{50}O$), a pentacyclic triterpenoid and epi-friedelanol ($C_{30}H_{51}OH$) were reported in the plant [14]. Pharmacological studies on activities of *Syzgium cumini* revealed that it is gastro-protective, anti-ulcerogenic, anti-inflammatory, hypoglycemic, a ntioxidant hypolipidaemic, anti-anaemic, antibacterial, and radio-protective [14]. The flowers are rich in kaempferol, quercetin myricetin-(quercetin-3-glucoside), myricetin-3-L-arabinoside, quercetin-3-D-galactoside, dihydromyricetin, oleanolic acid, acetyl oleonolic acid and eugenol [17]. The root is rich in flavonoids glycosides. One of the varieties found in Brazil possesses malvidin-3-glucoside and petunidin-3-glycoside. The purple colour of the fruit is due presence of one or two cyanidin diglycosides [17]. Cyanidin diglycoside are sap pigments and the actual colour the express on the plant depends on the pH. The fleshy pericarp contains sterol [17]. Tannins, cynidin glycoside, oleanolic acid, betulinic acid and friedelin are main constituents of *Syzgium cumini* L. The medicinal value of the plant was attributed to malic acid, oxalic acid and gallic acid. The leaf contains essential oils with pleasant odour which contains limonene, dipentene (20%), sesquiterpenes of cadalane type (40%), and sesquiterpenes of azulene type (10% or less) with yield and physical characteristics of the oil varying according to the season of collection [14]. Major component of the essential oil appears to be triterpene hydroxyl acid, oleanolic acid [14]. The seeds contain tannins, ellagic acid, gallic acid, a glycoside- jamboline, starch, myricyl alcohol in the unsaponified fraction of seeds and a small quantity (0.05%) of pale yellow essential oil [14].

Djipa *et al* reported that different studies between 1982 and 2007 have shown that the aqueous, methanol and ethyl acetate extracts of *S. jambos* Guatemala leaf possess anti-inflammatory activity while the ethanol extract showed antiviral activity [15]. Myricetin and quercetin-3-*O*-β-D-xylopyranosyl-(1-2)-α-L- rhamnopyranosides were isolated from the active extracts [15]. The methanol extract of the leaf contain ellagic acid derivatives: 3, 3', 4'-tri-*O*-methylellagic acid-4-*O*-β-D-glucopyranoside and 3, 3', 4'-tri-*O*-methylellagic acid. Ellagitannins (pedunculagin, casuarinin, tellimagrandin I, strictinin, casuarictin, and traces of tellimagrandin II) were detected, as in several other Myrtaceae, in the extract of *S. jambos* from Japan [15].

2. Materials and Methods

2.1 Plant Material

Syzygium guineense leaves were collected from Shere hills area in Jos Plateau state, Nigeria in August, 2012. The plant was authenticated at Federal College of Forestry, Jos and a herbarium sample deposited with voucher number FHJ 0947 in the herbarium. The leaves of the plant were air dried under shade and stored in an air tight container for subsequent use.

2.2 Preparation of Extracts

Crude extract of n-hexane was prepared by soaking 500grams of coarsely pulverized leaf of the plant in 2 litres of n-hexane. This mixture was intermittently agitated for 72 hours at room temperature. After the 72 hours, the extract was decanted, filtered and concentrated using a rotary evaporator to give 7.0grams of the crude n-hexane extracts and the yield calculated and the n-hexane crude extract was kept in the refrigerator for analysis.

2.3 TLC Analysis of Hexane Extract

TLC analysis of hexane extract was carried out in the solvent mixtures

1. 100% Hexane
2. Hex: EtOAc (3:1)
3. Hex: EtOAc (2:1)

The TLC analysis using 100% hexane didn't resolve the components. Analysis with Hex: EtOAc (2:1) gave three components with R_f values 0.13, 0.23 and 0.84 while Hex: EtOAc (3:1) gave seven components with R_f values of 0.07, 0.13, 0.23, 0.32, 0.40, 0.54 and 0.81. Conc. H_2SO_4 was sprayed on the plates eluted in solvent mixture Hex: EtOAc (3:1). After heating the plates for 5mins, five coloured components were obtained with R_f values of 0.06, 0.12, 0.19, 0.32 and 0.75. Treatment with vanillin-Conc.H_2SO_4, gave four components with R_f 0.06, 0.12, 0.19 and 0.75 were obtained.

2.4 Gas Chromatographic – Mass Spectroscopic (GC/MS) Analysis

The crude n-hexane extract was analyzed using GC–MS-QP2010 system (Shimadzu, Kyoto, Japan) with split mode (1:0) and the purge flow of 3 mL/min. The injector temperature was 250 ˚C. Helium with constant flow of 1.5 mL/min served as carrier gas. The oven was programmed at the following rates; the initial temperature of the column was 80 ˚C (2 min hold) followed by 200 ˚C (4min hold) and finally at 280 ˚C (5min hold). The mass spectrometer conditions were as follows: electron impact ionization (EI); interface temperature, 250 ˚C; ion source temperature, 200 ˚C; the detector voltage, 1 kV; solvent delay, 1.5 min. All data were obtained by collecting the full-scan mass spectra within the scan range of m/z 30 – m/z 800 over 30min.

2.5 Identification of Phytocompounds

The chemical compositions of the hexane extract of *S. guineense* leaf were investigated using Gas Chromatography-Mass Spectrometry while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library along with other libraries. The identity of the components in the extract was assigned by comparing their retention indices and mass spectra fragmentation patterns with those stored in the computer library. Interpretation of Mass-Spectrum was conducted using the database of National Institute Standard and Technology (NIST) having 191, 436 general compounds and the Wiley library containing 310, 000 general compounds. The spectrum of the unknown was compared with the spectrum of known components stored in the NIST libraries and were used for matching the identified components from the plant material. The name, molecular weight and structure of the components of the tested samples were ascertained.

3. Results and Discussion

The extraction using n-hexane yielded 1.4% of the leaf extract. The TLC analysis showed four terpenes are present in the extract when plates were treated with vanillin-Conc.H_2SO_4. But after heating the plates for 5mins, five coloured components were obtained with R_f values of 0.06, 0.12, 0.19, 0.32 and 0.75. The R_f values of the four components are 0.06, 0.12, 0.19 and 0.75. The five (5) compounds may likely be corresponding with the compounds 1-ethyl-2-methylbenzene, Ylangene, decahydro-4a-methyl-1-methylene-7-(1-methylethynyl)-naphthalene, 4-dimethyl-7-(1-methylethenyl)azulene and caryophyllene oxide not necessary in that order, thereby asserting the relationship between the two chromatographic methods employed for the analysis.

The studies on the principles in the leaf of *Syzygium guineense* hexane extract by TLC and GC-MS analysis clearly showed the presence of twelve compounds. The compounds, their structures, class and concentration are presented in Tables 1, 2 and 3.

Table 1: Terpenes Identified in the leaf of Syzygium guineense

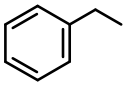
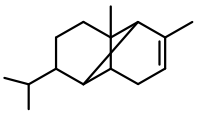
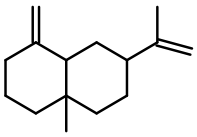
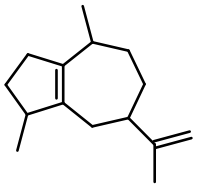
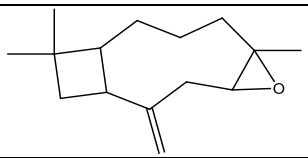
No.	Compounds	Structure	Molecular Weight	Concentration (%)
1	1-ethyl-2-methyl benzene C_9H_{12}		120	2.61
2	Ylangene $C_{15}H_{24}$		204	2.42
3.	γ-muurolone $C_{15}H_{24}$		204	2.47
4.	Azulene $C_{15}H_{24}$		204	2.06
5.	Caryophyllene oxide $C_{15}H_{24}O$		220	3.86

Table 2: Hydrocarbons Identified in the Leaf of *Syzygium guineense*

	Compounds	Molecular Weight	Concentration (%)
1	Tetratriacontane $C_{34}H_{70}$	478	6.70
2	Pentatriacontane $C_{35}H_{72}$	492	3.95

Table 3: Organic Acids and Fatty Acids Identified in the Leaf of *Syzygium guineense*

	Compound	Molecular Weight	Concentration (%)
1	Tetradecanoic acid (Myristic Acid) $C_{14}H_{28}O_2$	228	2.11
2	n-hexadecanoic acid (Palmitic acid) $C_{16}H_{32}O_2$	214	11.94
3.	Trans-Octadec-9-enoic acid (Elaidic acid) $C_{18}H_{34}O_2$	282	25.72
4.	1,2-benzenedicarboxylic acid (Pthalic acid) $C_{24}H_{38}O_4$	390	2.71

The compounds identified by the mass spectroscopy are presented. While the major components in n-hexane extracts of *Syzygium aromaticum* identified using GC-MS are eugenol and eugenol acetate, the major components in *Syzygium guineense* are 9-octadecanoic acid (25.72%) and tetratriacontane (31.45%). The three major phytochemical components in the n-hexane extracts are n-hexadecenoic acid (11.94%), 9-octadecanoic acid (25.72%) and tetratriacontane (31.45%). The other nine (9) components put together makes up only 26.42% and these are 1-ethyl-2-methylbenzene (2.61%), Ylangene (2.42%), decahydro-4a-methyl-1-methylene-7-(1-methylethynyl)-naphthalene, 4-dimethyl-7-(1-methylethenyl)azulene (2.06%), caryophyllene oxide(3.86%), myristic acid (2.11%), 1,2-benzenedicarboxylic acid (2.71%), tetratriacontane (6.70%) and pentatriacontane (3.95%). This study is reporting two acids present in the leaf of *syzygium guineense* not reported in the literature reviewed. These acids are myristic acid and 1,2-benzenedicarboxylic acid.

According to Pubmed data base (http://www.ncbi.nlm.nih.gov), azulene shows anti-inflammatory actions as well as analgesic, antipyretic, and platelet-inhibitory actions. It acts by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase. One mechanism by which it does this is through inhibition of prostaglandin synthesis and this account for their analgesic, antipyretic, and platelet-inhibitory actions. Myristic acid is active in 7 of 711 bioassays, elaidic acid in 2 of 11, palmitic acid is 21 in 381, pthalic acid active in 3 of 645 bioassays. Tetratriacontane is inactive in 6 tested bioassays. The results of this study offer a basis of using *S. guineense* as an alternative medicinal agent.

4. Conclusion

In the present study twelve (12) phytochemical constituents have been identified from hexane extract of *Syzygium guineense* leaf by Thin Layer Chromatography (TLC) and Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of the various compounds particularly the bioactive ones i.e. azulene, tetradecanoic acid (myristic acid), and trans-octadec-9-enoic acid (elaidic acid) justifies the use of the leaf against various ailments by traditional practitioners.

5. Acknowledgement

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6. Competing Interest

Authours have declared that no competing interests exist.

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