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ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT AND GC-MS ANALYSIS OF HEXANE FRACTION FROM *MEZONEURON BENTHAMIANUM* BAILL (CAESALPINICEAE)

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ABSTRACT

The antimicrobial activity of the leaf extract and the gc-ms analysis of the hexane fraction from the extract of *Mezoneuron benthamianum* were examined. The extract showed zone of inhibition of 21 mm against *Escherichia coli*, 18 mm against *Shigella flexnerii*, 11 mm against *Salmonella thyphii* and 10mm against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The gc-ms analysis of the hexane fraction revealed the presence of twenty three compounds. Some of the compounds with reported activities are vitamin E 2.09 %, spathulenol 2.34 %, phytol 17.63 %, 9-Octadecyne 1.12 %, γ-sitosterol 4.73 %, squalene 2.17 %, Hexadecanoic acid methyl ester 19.50 % and linoleic acid ethyl ester 10.00 %. The research effort shows that the plant demonstrated antimicrobial activity which could be related to the individual or additive effect of its active compounds.

KEYWORDS: Antimicrobial activity, GC-MS analysis, *Mezoneuron benthamianum.*

INTRODUCTION

A large proportion of people in developing countries rely on herbal medicines for their healthcare. The efficacy of herbs in treating various ailments has been linked to the therapeutic agents in them. Herbal medicines are affordable, easily accessible and belief to possess less side effect. Wachtel-Galor and Benzie wrote that people use herbal medicine because it corresponds more closely to their ideology. Research into medicinal plants has also

increased due to the trend of antibiotic-resistant of some pathogens. The drug-resistant *Escherichia coli* even protects drug sensitive *Salmonella typhimurium* (Perlin et al, 2009). Medicinal plants are known to be a source of chemical substances with antimicrobial properties (Ibekwe and Ameh, 2014). The combination of medicinal plant extracts of antimicrobial potency with antibiotics is said to be of great value since new mode of action could be developed (Abdallah, 2011). The drug-resistant pathogen may also become susceptible due to immunomodulatory effect of the plant extracts. The report on resistance modifying activities of extracts of *Mezoneuron benthamianum* on *Staphylococcus aureus* by Dickson et al seems to be consistent with this idea.

Mezoneuron benthamianum is widely used in folklore medicine for treating diseases and wounds. The use of the plant in remedying erection impairment was reported by Zamble et al. The anti-diarrhoeal activity of the plant was reported by Mbagwu and Adeyemi. Mbagwu also reported the plant to be analgesic, antipyretic and anti-inflammatory. The resistance modifying activities of extracts from *M. benthamianum* on standard antibiotics against *Staphylococcus aureus* possessing efflux mechanisms of resistance have also been assessed (Dickson et al, 2006). Decoction of the leaves of the plant are used by traditional healers in Guinea to treat malaria (Jansen et al 2017). Osho reported the use of the plant in treating venereal diseases, dysentery, eye inflammation and snake bite. The aim of this study is to investigate the antibacterial activity of the ethanol extract of the leaves of *Mezoneuron benthamianum* and to analyze the phytoconstituents in the hexane fraction of the extracts using GC-MS.

MATERIAL AND METHODS

Plant Collection: The medicinal plant was obtained from Olokemeji Forest Reserve in Oyo state was identified by Mr T. K. Odewo of the Forestry Research Institute of Nigeria, (FRIN), Ibadan.

Plant Extraction: 300 mls of 80 % ethanol solution was added to 60 g of the dried powdered sample of the plant. The mixture was kept at room temperature for 72 h with gentle and intermittent shaking and thereafter was filtered. The filtrate was dried at 42.5^{o} C. Sequential extraction was carried out on the ethanol extract to obtain the hexane fraction.

Test Microorganisms: The test organisms used were *Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhyimurium, Shigella flexnerii, Staphylococcus*

aureus. All the organisms were obtained from the Research Laboratory of the Department of Biotechnology, Nigerian Institute of Medical Research, Yaba, Nigeria.

Antimicrobial assay: This was carried out according to the method of Sharhidi-Bonjar (2004). The test organisms were subcultured on Blood Agar and Mac-Conkey Agar (Oxoid, UK). Suspensions of the microorganisms in sterile normal saline were adjusted to 0.5 McFarland standards to give suspensions containing approximately 1 x 10^5 CFU cm^{-1}. The medium plates were labeled and each was uniformly seeded with a test organism using sterile swab rolled in the suspensions and streaked on the surface of the plate. Wells of 5 mm in diameter placed about 2 cm apart were punched in the culture media with sterile cork borer. One hundred microlitre of various concentrations of test extracts were dropped into each well (Sharhidi-Bonjar, 2004). Ciprofloxacin, water, hexane and ethyl acetate without test compounds were placed in wells on each plate as control. Each plate was kept in the refrigerator at 4^o C for 1 h before incubating at 37^o C for 24 h. Zones of inhibition around the wells were measured in millimeter and were used as positive bioactivity.

GC-MS Analysis: Constituents in the hexane extracts and hexane fractions of the plant were elucidated using GC-MS performed on Agilent Technologies 7890 A GC coupled with Agilent Technologies 5975 C MS. Helium was used as carrier gas and sample was injected in split less mode at 70 ev in a column HP 5 MS, length 30 meters, internal diameters 0.320 mm, column thickness 0.25µm. The initial temperature was 50^o C, held for 2 minutes, flow rate 10^o/min, final temperature 240^o C, held for 6 minutes. The resulting GC-MS was analyzed using commercially available standards.

RESULTS AND DISCUSSION

The ethanol extract of the leaves of *Mezoneuron benthamianum* showed inhibitory effect on all the microorganisms tested as shown in Table 1. This inhibitory effect showed the zone of inhibition of 11 mm and 10 mm against *Salmonella typhi* and *Staphlocuccus aureus* respectively. Salmonella typhi and Staphylococcus aureus are Gram-positive bacteria. Gram positive bacteria have been reported to be susceptible to plants extracts (González-Lamothe et al, 2009). The extract of *M. benthamianum* showed zone of inhibition of 10 mm, 21 mm and 18 mm against *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella flexneri* respectively. The three bacteria are Gram negative bacteria. Gram –negative bacteria have been reported to offer more resistant to antibiotics. Gao et al reported that the resistance of Gram-negative bacteria towards antibiotics is related to lipopolysaccharides in their outer membrane.

Mixture of compounds in medicinal plants extracts could provide a synergistic antibacterial activity against gram-negative bacteria (González-Lamothe et al, 2009). The extract showed 10 mm zone of inhibition against Candida albicans. The inhibition effect on candida albicans is consistent with anticandida effect reported by Fayemi et al. The resistance modifying activities of extracts of *Mezoneuron benthamianum* on standard antibiotics against Staphylococcus aureus possessing efflux mechanisms of resistance had been assessed (Dickson et al, 2006). Binutu and Cordell reported that Gallic acid and its methyl ester isolated from the plant were weakly active against Gram-positive and Gram-negative bacteria but not the fungus and that some of the compounds were active against the Gram-positive microorganism but inactive against Gram-negative microorganisms and the fungus. The results in this research therefore suggest the additive effect of plants extracts compared to individual effects of the constituents compounds.

Table. 1: Antibacterial activity of ethanol extract of the leaves of *Mezoneuron benthamianum*.

Microorganisms	M. benthamianum
Pseudomonas aeruginosa	10 mm
Salmonella typhii	11 mm
Escherichia coli	21 mm
Staphylococcus aureus	10 mm
Shigella flexnerii	18 mm
Candida albicans	10 mm

The GC-MS analysis of the hexane fraction from the leaves of *M. benthamianum* revealed the presence of twenty three compounds shown in Table 2. Figure 1 represents the chromatogram for the GC-MS analysis. Esters of long chain saturated and unsaturated fatty acids constituted more than 75 % of the total abundance of the constituents. Linoleic acid ethyl ester was 10.00 % Linoleic acid ethyl ester is hypocholesterolemic, antihistaminic and antieczemic (Sudha, 2013) Linoleic acid antibacterial activity against *Staphylococcus aureus* had been assessed by Lee et al and Ohta et al. The presence of hexadecanoic acid methyl ester of 19.50 % could be suggested to contribute to the antioxidant and antifungal property of the plant reported by Fayemi etal. Hexadecanoic acid methyl ester is antioxidant (Sujayil and Dhanaraj, 2016) and antifungal (Chandrasekan et al, 2011).

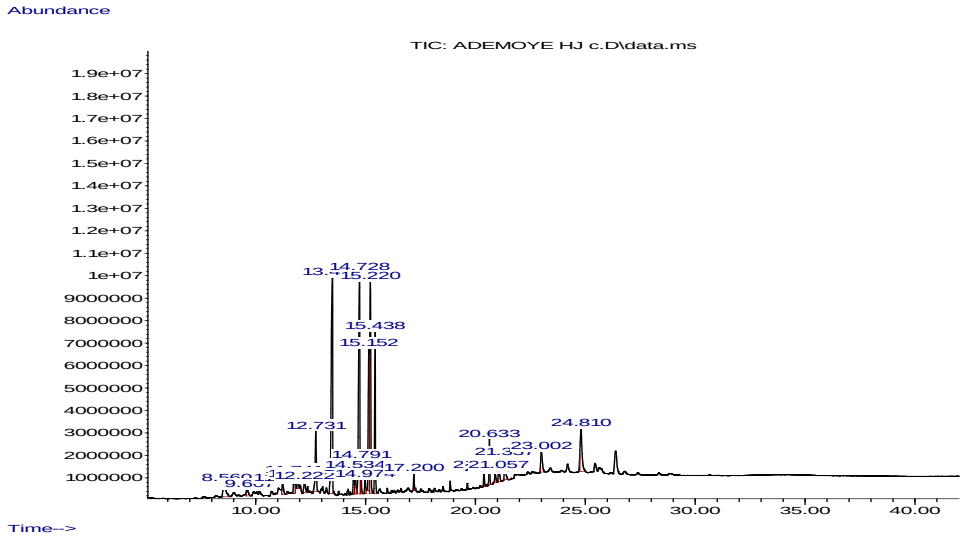
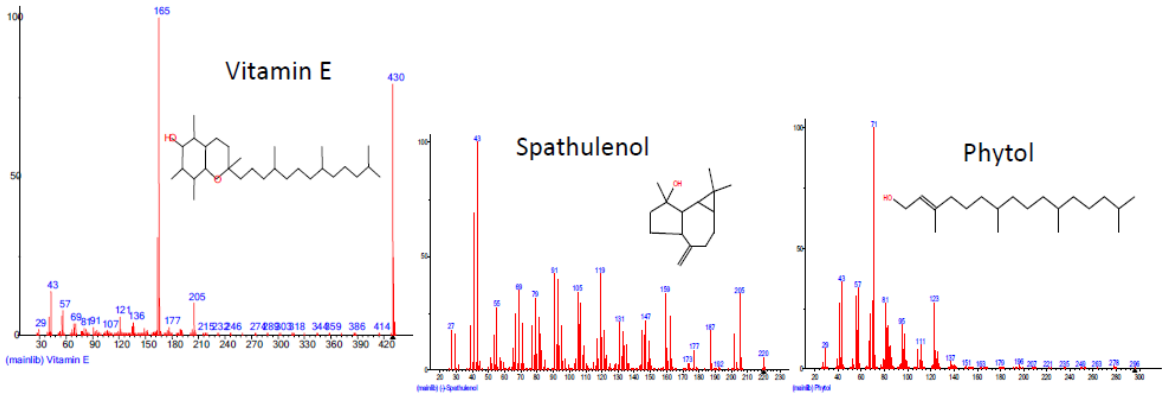


Figure. 1: Chromatogram of Hexane fraction from the ethanol extract of the leaves of *M. benthamianum*.

Phytol, a diterpenoid alcohol, is 17.63 % relative abundance. Phytol is anti-tuberculosis (Rajab et al, 1988), antioxidant and antinociceptive (Santos et al, 2015), antimicrobial (Pejin et al, 2014), antischistosomal (De Moraes et al, 2014), antidepressant, immunoadjuvancy, anticonvulsant, antspasmodic (Islam et al, 2015). Pereira Costa et al did a review on Pharmacological applications of Phytol on central nervous system.

Gamma sitosterol is 4.73 %.The methanolic extract of the barks of *Aegle marmalos* (L.) Correa containing 71.19 % gamma sitosterol was active against Ampicillin resistant Pseudomonas aeruginosa (Nemkul et al, 2018). Gamma sitosterol is anti-diabetic, antimicrobial, anti-inflammatory, antidiarrhoeal and antiviral (Raman et al, 2012).



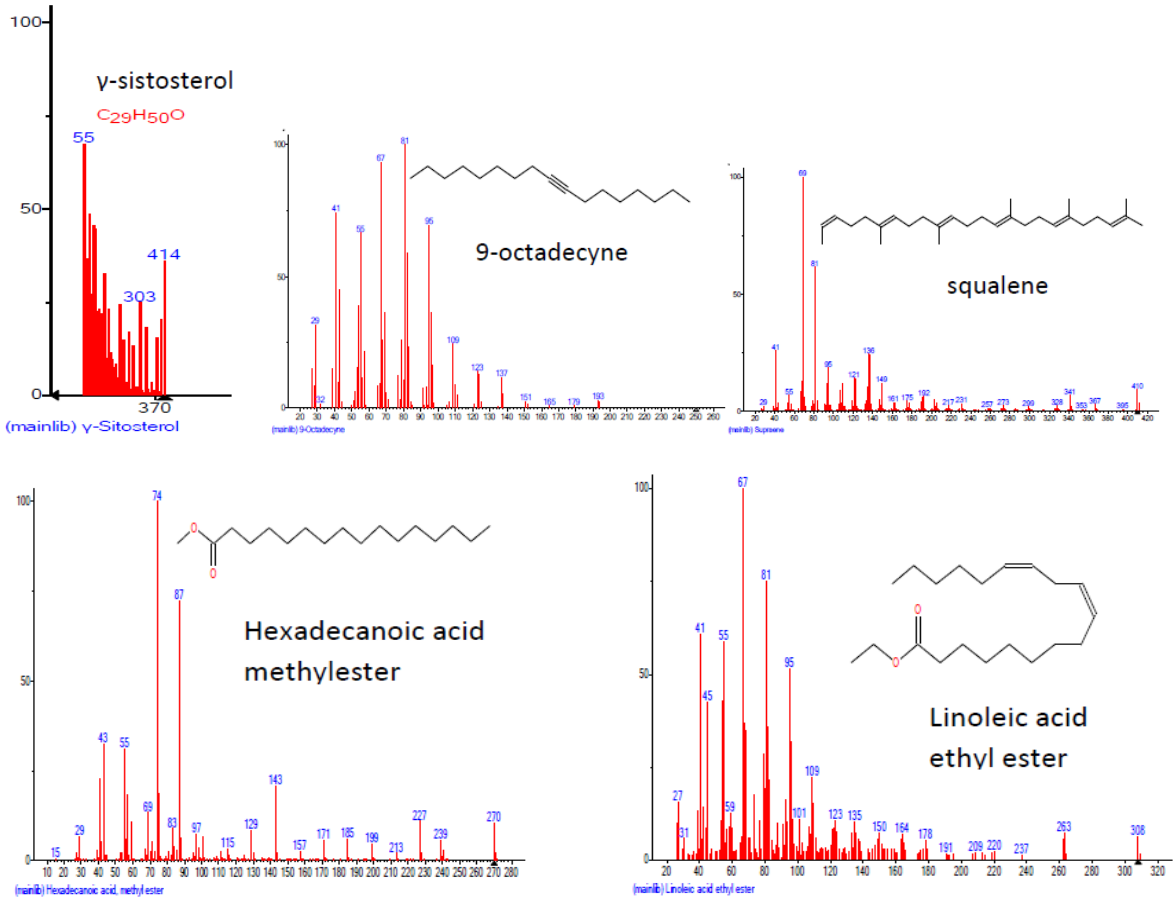


Figure. 2: Mass spectra of some compounds in *Mezoneuron benthamianum*.

Table. 2: Compounds from GC-MS analysis of Hexane fraction of ethanol extract of *Mezoneuron benthamianum*.

S/N	Compound	RT	%
1.	1H-Cycloprop[e]azulen-7-ol,decahydro1,1,7-trimethyl-4-methylene-[1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha]- (Spathulenol)	8.650	2.34
2.	1H-3a,7-Methanoazulen-5-ol,octahydro-3,8,8-trimethyl-6-methyleneLongifolenaldehyde	9.607	0.81
3.	Tetradecanoic acid, ethylester	11.221	0.98
4.	Bicyclo[3.1.1]heptane,2,6,6-trimethyl.(1.alpha.,2.beta.,S.alpha)	11.741	1.49
5.	(-)-Neoclovene-(1),dihydro	11.907	0.80
6.	9-Octadecyne	12.222	1.12
7.	Hexadecanoic acid, methyl ester	12.731	19.50
8.	Hexadecanoic acid, ethyl ester	13.498	19.50
9.	Heptadecanoic acid, ethyl ester	14.459	1.37
10.	10-Octadecenoic acid, methyl ester	14.534	1.57
11.	Phytol	14.728	17.63
12.	Octadecenoic acid, methyl ester	14.791	1.44
13.	Cyclopentane,1,2,3,4,5-pentamethyl	14.974	0.84
14.	Linoleic acid ethyl ester	15.52	10.00
15.	Ethyl oleate	15.220	13.55
16.	Octadecanoic acid ethyl ester	15.438	8.01

17.	Methyl 19-methyleicosanoate	17.200	0.77
18.	Ethyltetracosanoate	20.387	0.83
19.	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-(all E)-	20.633	2.17
20.	1-pyrrolidinebutanoic acid 2-[(1,1-dimethylethoxylcarbonyl]-alpha.-nitro-,2,6-bis(1,1-dimethyl)-4-methoxyphenylester[S-(R*R^{o})]-	21.057	1.63
21.	trans-3-Hydrocotininetmssilicic acid,tetrakis(1-methylethyl)ester	21.337	1.79
22.	Vitamin E	23.002	2.09
23.	Gamma-Sitosterol	24.810	4.73

The compound 2,6,10,14,18,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl-(all E)- is squalene and its relative abundance in this analysis is 2.17 %. Squalene was initially obtained from shark liver oil (hence the name squalus, genus of sharks). It is an unsaturated hydrocarbon, a triterpene, a precursor for synthesis of sterols and it has antioxidant, chemopreventive and antitumor activities (Spanova and Drum, 2011).Vitamin E is 2.09 % relative abundance. Vitamin E is analgesic, antidiabetic, antidermatitic, vasodilator, antispasmodic (Narayanamoorthi et al, 2015). 9-Octadecyne, a long chain unsaturated hydrocarbon with relative abundance of 1.12 %, was reported to posseess good solubility, good inhibition ability, good penetration potential, low binding energy to target protein and was also recommended to be a drug molecule (Upgade et al, 2014). Spathulenol is 2.34 %. It is a tricyclic sesquiterpene alcohol. Spathulenol has capacity to inhibit proliferation of lymphocytes (Ziaei et al, 2011). It is a good candidate in combination therapy of MDR cancer (Martins et al, 2010). *Mezoneuron benthamianum* has application in treating venereal diseases (Osho 2014) and the compound spathulenol could be suggested to contribute to this therapeutic effect by inhibiting proliferation of infected cells.

From the above discussion it could be acknowledged that some of the phytoconstituents of *Mezoneuron benthamianum* have biological and pharmacological activities relating to the medicinal uses of the plant. Thus, *Mezoneuron benthamianum* promises to be a medicinal plant with active ingredients that could serve as antimicrobial agents.

CONCLUSION

Mezoneuron benthamianum demonstrated activity against the tested Gram-negative and Gram-positive bacteria and against a fungus Candida albicans. The GC-MS analysis of its hexane fraction revealed the presence of high concentration of esters of long chain saturated and unsaturated fatty acids of biological activities. Other compounds of reported activities from the analysis are phytol, squalene, vitamin e, sitosterol, spathulenol and 9-octadecyne. Mezoneuron benthamianum possesses antimicrobial activity due to its active phyto-

constituents. Further research effort on the use of the plant extract on various infectious diseases could result in therapeutic effect.

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