GC-MS Analysis and Antimicrobial Evaluation of Essential Oil from the Epicarp of Nigerian Grown *Afraegle paniculata* (Rutaceae)

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(Received: November 4, 2022; Accepted: May 19, 2023; Published (web): June 21, 2023)

ABSTRACT: Essential oils are used as therapeutic agents in aromatherapy. In this study, the chemical composition and antimicrobial activity of the essential oils from the epicarp of *Afraegle paniculata* fruits from Nigeria were evaluated. The essential oil was obtained via hydro distillation and chemical components of the oils were analysed on Agilent Technologies 7890A gas chromatograph-mass spectrometry system. Antimicrobial assay was performed by disc diffusion method. GC-MS analysis revealed sixteen compounds consisting mainly of oxygenated monoterpene (39.61 %), esters (15.3 %), sesquiterpenes (13.1 %) and sesquiterpene alcohol (9.42 %). The oil showed appreciable activity against all the tested microorganisms except *Aspergillus niger.* At concentration of 1000 µg/ml, the inhibitory effect observed for *S. aureus* was 16 mm followed by 15 mm for *B. subtilis* and 7 mm for *P. aeruginosa.* The findings suggest that essential oil from *A. paniculata* fruits could serve as a valuable raw material for perfumery and cosmetic products.

Key words: *Afraegle paniculata*, Rutaceae, essential oil, GC-MS analysis, antimicrobial activity.

INTRODUCTION

Medicinal plants are useful in the maintenance of human health. Chemical substances from medicinal plants have a definite physiological action on the human body. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms.[1] Essential phytochemical constituents of plants are flavonoids, alkaloids, tannins and phenolic compounds. Plant oils and extracts have been used widely for various purposes over several years. These applications vary from the use of juniper berry oil, lime or fennel in flavouring drinks, to cedar wood and rosewood in perfumery and the preservation of stored food crops with lemongrass oil.[2] Specifically, the antimicrobial activity of plant oils and their extracts has formed the foundation of many applications such as pharmaceutical, alternative medicine, raw and processed food preservation and natural therapies.[3] The antimicrobial properties of some of the oils have been well documented.[4] Essential oils are plant-based volatile oils that are made up of different chemical compounds such as aldehyde, alcohols, phenol, hydrocarbons, ketones and esters; attributing to the major constituents of essential oil.[5] A vast number of interrelated factors; climatic, seasonal and geographical conditions, harvest period and extraction techniques determine the quantity of essential oil to be extracted from plant.[6] The stages of plant growth also affect the yield

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Dhaka Univ. J. Pharm. Sci. **22**(1): 73-80, 2023 (June)
DOI: https://doi.org/10.3329/dujps.v22i1.67097

of oils from plants. Several studies have revealed the efficiency of essential oils in low doses in combatting bacterial pathogens.[7,8] even against multi-resistant bacteria.[9]

A. paniculata (Schum and Thonn) Engl. is a species of tree in the family Rutaceae. It is found in West Africa from Senegal to Nigeria and is commonly known as Nigerian powder-flask fruit. It is a shaft 8 to 15 m tall, with a trunk diameter between 25 and 40 cm. Its alternating leaves and leaflets are 8 to 16 cm long. Its fruits are globular or ovoid, like a big orange (6-8 cm in diameter at the mature age). *A. paniculata* almonds of the Côte d'Ivoire species have relatively high fat matter (38.4 %), which makes it useful in oil mill.[10] It is cultivated in the villages for its multiple uses; nutritional, medicinal etc. The extract is used for treatment of malaria.[11] hypertension and infertility.[12] Previous studies on the fruit of *A. paniculata* showed that the mucilage of the fruit of the Ghanaian species contains L-arabinose, D-galactose, L-rhamnose and D-glucuronic acid.[13] Quartey (1961) reported the presence of a coumarin and ɣ-sitosterol in the fruit of the species.[14] Phytochemical investigations of *A. paniculata* pericarp extract revealed the presence of several coumarins and quinolone alkaloids.[15] Tsassia *et al.* (2010) isolated 6',7'–Dimethyl-S-trans-marmin and seven other compounds from *A. paniculata* stem bark and investigated their antibacterial, fungicidal, and algicidal properties.[16] Proximate analysis of the fixed oil obtained from *A. paniculata* seeds revealed 28.81 ± 0.02 % crude fat, 25.03 ± 0.12 % crude protein, 10.90 ± 0.03 % moisture, 3.11 ± 0.01 % ash, 25.19 ± 0.02 % crude fibre and 6.96 ± 0.14 % carbohydrate.[17] Chemical composition of the essential oil extracted from the peel of the fruit of *A. paniculata* from Côte d'ivoire revealed 40 compounds including sesquiterpene hydrocarbons (64.49 %), monoterpene hydrocarbons (7.82 %), oxygenated sesquiterpenes (7.60 %) and oxygenated monoterpenes (5.78 %). The major compounds were δ-cadinene (11.71 %), α-selinene (9.01 %), α-cubebene (8.80 %), *o*-menth-8-ene (6.06 %) and β-caryophyllene (5.66 %).[18] Similarly, the major components of the essential oil obtained from the leaves were sesquiterpenoids α-copaene, (*E*)-caryophyllene, δ-cadinene and caryophyllene oxide.[19] The present study aimed at investigating the chemical composition and antimicrobial activity of the essential oil extracted from the epicarp of *A. paniculata*.

MATERIALS AND METHODS

Sample collection and identification. *A. paniculata* fruits were collected from the campus of Ajayi Crowther University, Oyo State, Nigeria. The fruit was identified and authenticated at the Forestry Research Institute of Nigeria, Herbarium (111394FHI).

Sample preparation. The hard epicarp of *A. paniculata* fruits was broken and the brownish mesocarp separated. The numerous white seeds were manually separated from the segments by washing them under water tap in order to remove the mucilaginous substance on the seeds. The epicarp was cleaned to remove any foreign matters and broken into smaller size using a porcelain mortal. It was then kept in the refrigerator until further used.

Hydrodistillation of essential oil. A known quantity of *A. paniculata* epicarp was transferred into 5 litres distillation flask and filled with water to about two-third of the flask. The flask was then placed on a heating mantle and fitted with all glass Clevenger apparatus. Two ml of n-hexane was injected into the water column. The extraction was carried out using hydro distillation method for about 3 hours at thermostat temperature of 80-100 °C. The set-up was closely monitored to ensure that the cold water flows continuously through the condenser. The distillate was collected over n-hexane using a syringe into a weighed vial sample bottle and then reweighed to determine % yield. The essential oil was kept in a refrigerator prior to antimicrobial and GC-MS analysis.

$$\%\,yield = \frac{weight\ of\ the\ essential\ oil \times 100}{weight\ of\ the\ sample}$$

Antimicrobial assay and minimum inhibitory concentration. The antimicrobial activities of the essential oils were performed by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi.[20] Discs containing 20 µl of the essential oil was used and growth inhibition zones was measured in millimetres after 24 h of incubation at 37°C for bacteria and at 25°C for 72 h for fungi. Gentamicin and nystatin were used as standard antibiotics for bacteria and fungus respectively.[21] The concentration of essential oil achieved through serial dilution ranged from 200-1000 µg/mL. The microorganisms tested were *Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Fusarium oxysporum* and *Aspergillus niger*. After incubation, the minimum inhibitory concentrations (MICs) were measured and recorded.[22] The MIC was defined as the lowest concentration with visible inhibition of growth of the microorganism observed. Thereafter, the minimum bactericidal concentration (MBC) was carried out.[23] The MBC is the quadrant with the lowest concentration of the essential oil without bacterial and fungal growth.

Gas chromatography- mass spectrometry (GC-MS) analysis. GC-MS analysis of oil obtained from *A. paniculata* epicarp was performed on an Agilent Technologies 7890A equipped with 5975 mass selective detectors (MSD) with an HP-5MS capillary column (30 m×0.32 mm, 0.25 µm film thickness). The carrier gas used was helium at a constant flow rate of 1.8 ml/min. Injector and detector temperature were set at 200°C and 220°C, respectively. Oven temperature was kept at 90 °C for 2 min, then gradually increased to 220° C at 3° C/min and finally held isothermally for 40 min. One microliter of the diluted sample (1/100 in hexane, *v/v*) was injected manually (split mode, split ratio 1:16). Mass spectra were acquired at 70 eV with mass range of 50–300 m/z. Calculation of peak area percentage was performed using the GC HP-Chemstation software (Agilent Technologies, Santa Clara, CA, USA).

Identification of components. The components were identified by comparing the retention times and mass spectra of the chromatographic peaks with those of standards analysed under the same conditions. Moreover, special software, namely mass lib software (V9.3-106; 1996–2008) was used for processing and interpretation of mass spectra with commercially available libraries included: Wiley Registry of Mass Spectral Data (4th Ed.) and NIST/EPA/NIH Mass Spectral Library (2005).

RESULTS AND DISCUSSION

Antimicrobial assay and minimum inhibitory concentration. The antimicrobial activity of the essential oil was investigated against some selected microorganisms such as *S. typhi, P. aeruginosa, B. subtilis, S. aureus, F. oxysporum* and *A. niger*. It was observed that the antimicrobial activity of the essential oil was concentration-dependent and the standard antibiotics used were Gentamicin and nystatin (Table 1). The essential oil was found to be active against five of the tested organisms whereas no

Table 1. Antimicrobial screening of essential oil of *A. paniculata* epicarp (µg/ml).

Test organisms	1000	800	600	400	200	Negative control	
Bacteria	Zone of inhibition /mm						Gentamicin (10µg/ml)
S. typhi	12.5	10.1	4.0	-	-	-	8.0
P. aeruginosa	7.0	5.5	4.5	-	-	-	6.0
B. subtilis	15.0	11.0	10.0	-	-	-	-
S. aureus	16.0	9.5	7.5	-	-	-	14.0
Fungus							Nystatin (10µg/ml)
F. oxysporum	10.0	9.0	7.5	-	-	-	11
A. niger	-	-	-	-	-	-	6

(-) means no activity

activity was found against *A. niger*. As the concentration decreased, the zone of inhibition decreased. At a concentration of 1000 µg/ml, the zone of inhibition observed for *S. aureus* was 16 mm followed by *B.* subtilis (15 mm) whereas that of *P. aeruginosa* was 7 mm. Similar findings were also reported in the work of Arshad *et al*. (2021) where a minimum inhibitory zone was observed for *P. aeruginosa* and *S. aureus* (7 mm) from the ethyl acetate and butanol extract of *Saara hardwicikii* respectively.[22] Cazella *et al*. (2019) reported *S aureus*, *B. cereus* and *P aeruginosa* as the most susceptible species to essential oil of *B dracunculifolia* with MIC of 0.5, 1.1, and 1.05 mg/ml and MBC of 2.1, 1.5, and 2.1 mg/ml, respectively.[24]

Generally, at high concentration, the activity of the essential oil against the tested microorganisms was observed to be quite higher than that of the standard antibiotics (Gentamicin); this was similar to the findings of Guo *et al*. (2020), where olive oil polyphenolic extract showed strong antimicrobial activity against *Salmonella typhimurium* and *Staphylococcus aureus*.[25] At concentration of 400 - 200 µg/ml, there was no activity against the tested organisms. The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are presented in table 2. MIC is the lowest level of antimicrobial agent that inhibits the growth of the tested organism whereas MBC is the lowest level of antimicrobial agent that results in microbial death.[23] MIC of 600 µg/ml was obtained for all the tested microorganisms with the exception of *A. niger*. Concentration below 600 µg/mL seemed to have little or no antimicrobial activity. The MBC of 1000 µg/ml was obtained for *S. typhi*, *B. subtilis* and *S. aureus*. The observed antimicrobial activity of the essential oil of *A. paniculata* epicarp may be attributed to the high percentage of linalool in the oil which has been reported to have antibacterial properties.[26] and also act as antifungal agent against several fungal strains.[27] Similar findings were observed in the work of Sripahco *et al*.[28] with essential oils obtained from *E. splendens* which showed antibacterial inhibitory activity against *S. aureus*, *S. epidermidis*, and *Propionibacterium acnes.* Besides, antimicrobial activity appeared to be a result of the synergistic effect of the mixture of bioactive compounds present in the volatile oil. The results of our finding indicated that *A. paniculata* epicarp essential oil seemed to be a good candidate for further biological and pharmacological investigations.

The volatile nature of the sample was the cause of limitation observed during this study. Some of the components of the essential oil might have escaped during long hours of incubation thereby reducing its potency.

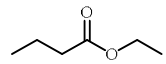
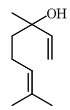
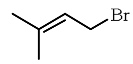
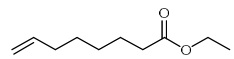
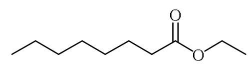
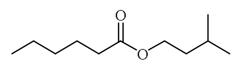
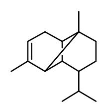
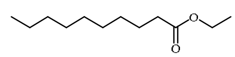
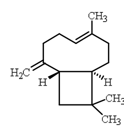
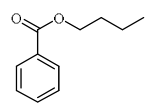
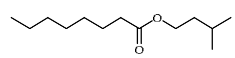
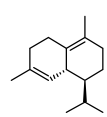
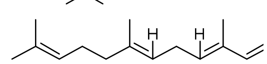
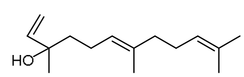
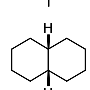
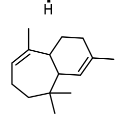
Table 2. Minimum inhibitory concentration and minimum bactericidal concentration.

Test organisms	MIC (µg/ml)	MBC (µg/ml)
S. typhi	600	1000
P. aeruginosa	600	-
B. subtilis	600	1000
S. aureus	600	1000
Fungus		
F. oxysporum	600	8
A. niger	-	-

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Chemical composition of essential oil of *A. paniculata* epicarp. Essential oil with a pale-yellow colour and a pleasant odour was obtained from *A. paniculata* epicarp. The major components of the sixteen compounds found in the oil include linalool (39.61 %), 1-bromo-3-methyl-2-butene (16.53 %), trans-nerolidol (9.42%), naphthalene, decahydro-, cis- (6.04%), octanoic acid ethyl ester (5.60%), δ-cardinene (3.96%) and α-copaene (3.51%) (Table 3). This composition is different from the data reported for the Cote d'Ivoire species (Table 4). The main components of the Cote d'Ivoire species were reported to be δ-cadinene (11.71%), α-selinene (9.01%), α-cubebene (8.80%), o menth-8-ene (6.06%), β-caryophyllene (5.66%) and octanoic acid ethyl ester (4.64%). However, linalool, octanoic acid ethyl ester,

Table 3. Chemical composition of essential oil from *A. paniculata* epicarp.

S/N	RT	Compound	MF	MM	PA*	Structure	Bioactivity
1	4.684	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144	1.25		Antidiabetic activity, anticancer activity [45]
2	6.806	Linalool	$C_{10}H_{18}O$	154	39.61		Inflammatory, anticancer, antimicrobial[26, 46]
3	7.156	1-bromo-3-methyl-2-butene	C_5H_9Br	149	16.53		Precursor for terpenes synthesis[47]
4	9.096	7-octenoic acid, ethyl ester	$C_{10}H_{18}O_2$	170	1.48		Insect repellent [48]
5	9.348	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	5.60		Flavouring and flagrances [38]
6	11.050	Isopentyl hexanoate	$C_{11}H_{22}O_2$	186	2.81		Flavours [43]
7	15.733	α-copaene	$C_{15}H_{24}$	204	3.51		Antimicrobial activity[39]
8	16.263	Decanoic acid ethyl ester	$C_{12}H_{24}O_2$	200	1.33		Flavouring agent[44]
9	17.376	β-Caryophyllene	$C_{15}H_{24}$	204	1.21		Anticancer, Antioxidant and Antimicrobial properties[40]
10	17.978	Butyl benzoate	$C_{11}H_{14}O_2$	178	1.39		Food additive and flavouring agent[43]
11	18.152	Isopentyl octanoate	$C_{13}H_{26}O_2$	214	1.44		Food additive and flavouring agent[43]
12	21.192	δ- cadinene	$C_{15}H_{24}$	204	3.96		Antimicrobial and antioxidant properties[41]
13	21.613	(z,z)-alpha-farnesene	$C_{15}H_{24}$	204	1.44		Flavouring agent[43]
14	22.674	Trans-nerolidol	$C_{15}H_{26}O$	222	9.42		Antimicrobial activity[42]
15	22.797	Naphthalene, decahydro,cis-	$C_{10}H_{18}$	138	6.04		No activity reported
16	25.125	γ-Himachalene	$C_{15}H_{24}$	204	2.98		Antioxidant and anticancer activities [29]
TOTAL					**100**		

S/N: Serial number, RT: Retention time, MF: Molecular formula, MM: Molecular mass, PA*: Percentage abundance (%)

Table 4. Comparison of the major components of essential oil obtained from Nigeria and Cote d'Ivoire species of *A. paniculate* epicarp.

S/N	Compound	Nigeria specie	Cote d'Ivoire specie [18] (Konan *et al*., 2011)
1	Linalool	39.61	1.74
2	1-bromo-3-methyl-2-butene	16.53	-
3	trans-nerolidol	9.42	-
4	cis- decahydro naphthalene	6.04	-
5	octanoic acid ethyl ester	5.60	4.64
6	δ-cadinene	3.96	11.71
7	α-selinene	-	9.01
8	α-cubebene	-	8.80
9	o menth-8-ene	-	6.06
10	β-caryophyllene	1.21	5.66

δ-cadinene and β-caryophyllene were identified in the two oils but in different proportions. The differences in the composition of these oils (*A. paniculata* epicarp from Nigeria and Cote d'Ivoire) probably attributed to differences in geographical location[29,30], plant chemotypes, harvesting seasons[31-33], drying methods[34] and extraction methods.[35,36] Some similarities were also observed between volatile compounds found in *A. paniculata* epicarp and those reported for citrus peel (Family: Rutaceae). These include linalool, α-farnesene, β-caryophyllene, δ-cadinene and (*E*)-nerolidol.[37] The observed antimicrobial activity of the essential oil could be traced to the presence of linalool, octanoic acid ethyl ester, α-copaene, β-caryophyllene and δ- cadinene, trans-nerolidol which had been reported to exhibit antimicrobial activity.[26,27,38-42] These compounds accounted for 63.31% of the total composition of the essential oil from *A. paniculata* epicarp. Among the sixteen compounds, octanoic acid, ethyl ester, isopentyl hexanoate, decanoic acid ethyl ester, butyl benzoate, isopentyl octanoate and (z,z)-alpha-farnesene are used as preservative and food flavouring agents.[38,43-44]

CONCLUSION

This study highlighted antimicrobial activity of *A. paniculata* epicarp essential oil which might be due to its high content of oxygenated monoterpene hydrocarbons. The oil has potential of finding application in the production of topical antiseptic products for treatment of various types of skin disorders and also as perfumes since oxygenated terpenes are used in perfumery due to their expressive odour. However, numerous investigations should be carried out on their mode of action and their probable toxicological effects in order to optimize this potential. Sample collection hindered the progress of study because *A. paniculata* is gradually becoming an endangered plant species.

Conflict of interest

The authors have no conflict of interest to declare.

ACKNOWLEDGEMENT

The authors are grateful to Chemistry Department, University of Ibadan, Ibadan, Nigeria for the enabling environment provided for carrying out the study.

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