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Chemical Composition, Antimicrobial and Antioxidant Activities of Root Essential Oil of Nigerian Specie of *Asparagus Flagellaris* (Kunth) Baker

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Federal University of Petroleum Resources, Effurun, Delta State, Nigeria**Abstract:**

Asparagus flagellaris (Asparagaceae) root have a variety of medicinal uses including treatment of syphilis, gonorrhea and other sexually transmitted diseases, with no report on its essential oil composition. This study investigated the quantitative and qualitative characterising of the chemical composition, evaluating the antimicrobial and antioxidant activities of root essential oil from *A. flagellaris*. The essential oil was obtained by hydrodistillation method using Clevenger apparatus, the identification and characterisation were done by gas chromatography-mass spectrometry (GC-MS) technique. Antimicrobial properties were assessed by broth dilution method and the antioxidant activity were evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) method. A pale-yellow essential oil with characteristic aroma was obtained with a yield of 0.44 % (w/w). GC/MS analysis revealed the presence of nineteen (19) compounds representing 97.03 % of the total oil composition. The essential oil was predominated by 84.54 % thymol derivatives. The root essential oil showed moderate inhibitory zone (18 – 10 mm) when compared with the reference standards Gentamicin and Tioconazole for both bacteria (40 – 35 mm) and fungi (28 mm) respectively. The root essential oil can be classified as bacteriostatic on test organisms. The oil exhibited a distinct antioxidant activity due the synergy of the identified constituents and IC₅₀ values of scavenging abilities on DPPH radicals were Ascorbic acid (0.1734 mg/mL) > BHA (0.1954 mg/mL) > essential oil (0.3162 mg/mL). The root essential oil of *A. flagellaris* contains important chemical compounds that are liable for its antioxidant and antimicrobial activities.

Keywords: *Asparagus flagellaris*, root essential oil, antioxidant activity, gc/ms analysis, antimicrobial activity, thymol derivatives

1. Introduction

Asparagus flagellaris (Kunth) Baker is widespread in tropical Africa with characteristics offensive scent. *A. flagellaris* belongs to the family Asparagaceae. It grows to a dimension of about 1 m and is scandent or more/less erect plant with arching spiny branchlets produced from a woody rootstock (Burkill, 1985). The plant is widely used as a food and medicine in Africa. The branchlets (cladodes) are the main adjunct of herbal medicine to treat guinea worm and as a gel for hair growth (Vander Burg, 2004). The root has a variety of medicinal uses; they are added to food or baths for treating syphilis, gonorrhea and other transmitted diseases - either being macerated or decocted. The hot brew is used to cure diarrhea and urinary infections (Burkill, 1985, Vander Burg, 2004). The phytochemical screening of the stem bark and leaves of *A. flagellaris* showed the appreciable amount of flavonoid and moderate amount of carbohydrate, cardiac glycoside and saponin while reducing sugar, ketones and pentose were detected in trace amount (Mshelia et al., 2008). Mshelia and coworkers also reported the inhibition of six organisms viz *Escherichia coli*, *Corynebacteria*, *Klebsiella*, *Neisseria gonorrhoeae*, *Shigella dysenteriae* and *Candida albicans*, at various concentrations of the ethanol extract, while the aqueous extract was susceptible to five organisms namely *Corynebacteria*, *Streptococcus pyogenes*, *Proteus* specie, *Neisseria gonorrhoeae*, and *Treponema palladium* (Mshelia et al., 2008).

The study of aromatic and medicinal plants enable finding producing effective essential oils that have a considerate level of application both in food and medicine. A good number of them are biologically active against a wide range of organisms such as bacteria, fungi, viruses and protozoa. In recent time, a good number of essential oils and their components have been invested for their antioxidant and antimicrobial properties against bacteria and fungi (Kalemba and Kunicka, 2003). Hence, this paper focuses on the chemical compositions, anti-oxidant and anti-microbial activities of the root essential oil from *A. flagellaris*.

2. Materials and Methods

2.1. Plant Materials

A. flagellaris root was collected in June 2019 at Efurum, Delta State, Nigeria. Identification and authentication were carried out at the Herbarium Unit, Department of Botany, University of Ibadan, Nigeria.

2.2. Isolation of Essential Oils

The oil was obtained from the chopped fresh root (467.44 g) of *A. flagellaris* by hydro-distillation on a Clevenger type apparatus for 4 hours following the British pharmacopeia specifications (British Pharmacopoeia, 1981). 2 mL of hexane was intermittently added to aid the trapping of the oil during the process. The essential oil was stored at 4 °C erstwhile analysis.

The % yield of the oil was calculated using:

$$\% \text{Yield} = \frac{\text{Weight of Essential Oil}}{\text{Weight of Sample}} \times 100 \quad (1)$$

2.3 Identification and Quantification of the Essential Oil Constituents

The root of *A. flagellaris* essential oil subjected to GC-MS analysis on an Agilent 7809A Gas Chromatography hyphenated with an Agilent Mass Detector having split/splitless injector interfaced to mass selective detector operating at 70 eV. The ion source temperature was set to 200 °C above a mass spectral range of 50-700 (m/z) at a scan rate of 1428 amu/sec. The column of the GC used was HP-5MS with a length of 30 m, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was automated as follows: initial temperature 80 °C for 2 min, increased at 10°C/min to a temperature of 240 °C for 6 minutes. The carrier gas (Helium) was used at a flow rate of 1 mL/min. Injection volume; linear velocity and pressure were adjusted at 1.0 µL, 362 cm/s and 56.2 KPa respectively. The oven temperature was set at 60 °C, hold for 1 min to 180 °C for 3 min at 10 °C/min, the final temperature was 280 °C for 2 min at 10 °C/min both the injector and detector temperatures were fixed at 250 °C. Identification of the essential oil components was based on comparison of their mass spectral fragmentation patterns (NIST database 14.L/Chemstation data system) with the data previously reported in the literature (Adams, 2001).

2.4. Anti-Microbial Activity on *A. Flagellaris* Root Essential Oil

Root essential oil of *A. flagellaris* was tested against 10 strains of micro-organisms consisting 6 bacteria; 3 Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*), 3 Gram-positive (*Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus subtilis*) and 4 fungi (*Candida albicans*, *Penicillium notatum*, *Aspergillus niger* and *Rhizopus* spp.). All microbes were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria. Broth dilution and surface plate methods were employed for antibacterial and antifungal activities, respectively (Mshelia et al., 2008, Bauer and Kirby, 1966.). Five (5) concentrations of the essential oil were prepared, 62.50, 125, 250, 500 and 1000 µg/mL and 1 mL aliquot of each concentration of the essential oil was tested for bioactivity. DMSO was used as a negative control, Gentamicin as a positive control for bacteria and Tioconazole as positive control for Fungi. All test and analysis were carried out in duplicates. Observed zones of inhibition of growth were measured and recorded in millimeter (mm).

2.5. Antioxidant Activity on *A. flagellaris* Root Essential Oil

Free radical scavenging activity on 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) was carried out using the method described by Brand-William et al. (1995) to evaluate the antioxidant activity of root essential oil of *A. flagellaris*. A solution of 100 mM (DPPH) in methanol was prepared. Five (5) concentration (1.0, 0.5, 0.25, 0.125 and 0.0625) mg/mL was prepared to determine free radical scavenging activity of the oil at by measuring the decrease in absorption after 30 minutes of incubation at 517 nm in a UV/visible spectrophotometer. A decrease in absorption was measured against that of the control. The same experiment was repeated on ascorbic acid and butylated hydroxyanisole (BHA), well known antioxidant agents. All test and analysis were carried out in triplicates. The activities were also determined as a function of their % Inhibition which was calculated using the formula:

$$\%I = \frac{(Ab - As)}{(Ab)} \times 100 \quad (2)$$

Where Ab: Absorbance of blank. As: Absorbance of Sample. %I: Percentage of Inhibition.

2.6. Statistical Analysis

All results were conveyed as means±standard deviation. 50 % inhibition concentration (IC50) of the samples and Pearson r was used to determine any significant difference (p<0.05) between groups using the statistical analysis software package GraphPad 5.0. All graph/chart was drawn with GraphPad 5.0.

3. Results and Discussions

3.1. Essential Oils Isolation

467.44 g of freshly chopped root *A. flagellaris* sample gave 2.08 g essential oil via hydro-distillation with light yellow colour and characteristics aroma. The percentage yield (w/w) obtained was 0.44%. The yield is an indication that *A. flagellaris* is rich in essential oil content.

3.2. Chemical Composition of the Root *A. Flagellaris* Essential Oil

The qualitative and quantitative data on the volatile metabolites of the essential oil from the root of *A. flagellaris* are summarised in Table 1. The preliminary GC analyses showed the presence of nineteen (19) compounds (representing 97.03 % of the oil) were identified. The identification of the individual GC peaks was by comparison of their relative retention time (tR) with those of authentic samples on the HP-5MS packed column and the computer searching followed by matching the mass spectral (MS) data with those held in the NIST database 14.L/Chemstation data system. The essential oil was predominantly in nature (84.54 %) thymol derivatives (thymol methyl ether, thymol hydroquinone dimethylether, thymol isobutyrate, 8, 9-dehydro-4-hydroxy thymol dimethylether, thymyl-2-methylbutyrate and thymyl tiglate). 3.64 % oxygenated monoterpenes (Durenol and 1-(2, 5-dimethoxyphenyl)-Ethanone), 3.10 % hydrogen sesquiterpenes (Cis- β -Farnesene, α -Santalene, α -Humulene, β -Bisabolene and δ -Cadinene), 1.36% oxygenated sesquiterpenes (α -Bisabolol) and 10.99% non-terpenes/non-terpenoids. Willuhn (1972) reported 5 thymol derivatives as the major constituents of the oil of underground organs of *A. montana*, i.e. 4-hydroxythymol dimethyl ether (46%), 4-hydroxy-8, 9-didehydrothymol dimethyl ether (18%), thymol methyl ether (12%), 8, 9-didehydrothymol methyl ether (3%) and thymol isobutyrate (8%). A similar trend was observed by Weremczuk-Jeżyna et al. (2011) in hairy roots and plant roots of *Arnica montana* L. essential oils that contained mainly of bi-functional thymol derivatives and sesquiterpene hydrocarbons. The most abundance constituents observed in the root essential oil of *A. flagellaris* are as follows thymyl tiglate - 34.73%, thymol methyl ether - 25.80%, thymol hydroquinone dimethylether - 12.72%, thymyl-2-methylbutyrate - 5.47%, Durenol - 3.29. The structures of six (6) major thymol derivatives identified in this study are presented in Fig. 1. Possibly, most of the reputed medicinal properties of *A. flagellaris* in folk medicine are due to the presence of relatively high amount of thymol derivative in the essential oil. The strong antiseptic, anti-inflammatory, antibacterial, antifungal and flavor properties as well as antispasmodic activities of thymol derivatives are well documented in the literature (Sticher, 1977). The occurrence of other minor constituents of the essential oil is of phytochemical interests due to their synergetic action in the inhibition of the biological activities observed in this studied.

Figure 1: Structures of Thymol Derivatives from Root Essential Oil of *A. Flagellaris*

S/N	Compounds Identified	% Composition
1	Durenol	3.29
2	Thymyl-2-methylbutyrate	5.47
3	α -Santalene	0.8
4	n-octylesterundecanoic acid	0.98
5	Isophosphinoline	0.76
6	Thymol methyl ether	25.8
7	2'-Hydroxy-1'-acetoneaphthone	1.77
8	Euparin	0.64
9	Thymolhydroquinone dimethylether	12.72
10	2-isopropenyl-(+)-2-carene	0.24
11	Cis- β -Farnesene	0.26
12	8,9-dehydro-4-hydroxy thymol dimethylether	1.49
13	α -Humulene	0.37
14	Thymolisobutyrate	4.33
15	β -Bisabolene	0.88
16	δ -Cadinene	0.79
17	1-(2,5-dimethoxyphenyl)-Ethanone	0.35
18	Thymyl tiglate	34.73
19	α -Bisabolol	1.36
	Total	97.03

Table 1: Chemical Composition of the Root Essential Oil of *A. Flagellaris*

3.3. Antimicrobial Activity of Root Essential Oil of *A. flagellaris*

Experimental data obtained from the antimicrobial activity are reported in Table 2. The effect of the root essential oil of *A. flagellaris* was compared with reference positive control, for the same volumes against selected bacteria and fungi (see Fig. 2 and 3).

The in-vitro antimicrobial activity of the root *A. flagellaris* essential oil against the microorganisms employed and its activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameters values in comparison with the standard antimicrobial drugs Gentamicin and Tioconazole. The result revealed that the root essential oil showed moderate inhibitory zone (18 – 10 mm) when compared with the reference standards for both bacteria (40 – 35 mm) and fungi (28 mm). No effect was detected against *Pseudomonas aeruginosa* and *Salmonella typhi* at all tested concentrations. It can be said the essential oil is more active against gram-positive aerobic organisms at all tested concentration for this study. *Escherichia coli* (16 – 12 mm) and *Klebsiella pneumonia* (14 – 10 mm) showed inhibition at higher concentration (1000 – 250 µg/mL), but no effect was observed at lower concentration as shown in Table 2. A similar trend was observed for the fungi (*Penicillium notatum* and *Rhizopus spp.*), showing inhibition at higher concentration (1000 – 250 µg/mL), but no effect at lower concentrations (125 – 62.5 µg/mL). The root essential oil was susceptible on the following tested organisms, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* and *Rhizopus spp.*

Hence, the moderate activity of the essential oil could be attributed to the synergetic action of the volatile metabolites identified. Although, the absence of antimicrobial active compounds such as linalool, linalyl acetate, α-pinene, terpineol and camphor, credited with potent antimicrobial and antifungal effects (Dorman and Deans, 2000, Magiatis et al., 2002, Soković et al., 2010). The activity of this essential oil can be described as 'bacteriostatic antibiotic' - is assumed to require phagocytic cells to clear bacteria and are therefore thought to be less effective without an efficient immune response. This result correlates with the earlier report by Marchese et al. (2016) that described all the thyme essential oils as bacteriostatic against the microorganisms.

Figure 2: Antibacterial Inhibitory Zone of the Root Essential Oil *A. Flagellaris* against Test Organisms with Standard

Figure 3: Antifungal Inhibitory Zone of the Root Essential of *A. Flagellaris* against Test Organisms with Standard

The result correlated to the antibacterial activity of the crude extracts of *A. flagellaris* stem, bark and leaf, that reported ethanol extract inhibition on the growth of six organisms viz *Escherichia coli*, *Corynebacteria*, *Klebsiella*, *Neisseria gonorrhoea*, *Shigella dysenteriae* and *Candida albicans*, at various concentrations, while the aqueous extract were susceptible on five organisms namely *Corynebacteria*, *Streptococcus pyogenes*, *Proteus spp.*, *Neisseria gonorrhoea*, and *Treponema pallidum* (Mshelia et al., 2008).

Test Organisms	Negative Control	Positive Control		Concentration ($\mu\text{g/mL}$)				
		Gentamicin	Tioconazole	1000	500	250	125	62.5
<i>Escherichia coli</i>	(-)	35	N/A	16 $\pm$ 0.00	14 $\pm$ 0.00	12 $\pm$ 0.00	(-)	(-)
<i>Pseudomonas aeruginosa</i>	(-)	38	N/A	(-)	(-)	(-)	(-)	(-)
<i>Klebsiella pneumonia</i>	(-)	38	N/A	14 $\pm$ 0.00	12 $\pm$ 0.00	10 $\pm$ 0.00	(-)	(-)
<i>Staphylococcus aureus</i>	(-)	36	N/A	17 $\pm$ 1.41	15 $\pm$ 1.41	13 $\pm$ 1.41	11 $\pm$ 1.41	10 $\pm$ 0.00
<i>Bacillus subtilis</i>	(-)	38	N/A	17 $\pm$ 0.00	15 $\pm$ 0.00	12 $\pm$ 0.00	11 $\pm$ 0.00	10 $\pm$ 0.00
<i>Salmonella typhi</i>	(-)	40	N/A	(-)	(-)	(-)	(-)	(-)
<i>Candida albicans</i>	(-)	N/A	28	18 $\pm$ 0.00	16 $\pm$ 0.00	14 $\pm$ 0.00	12 $\pm$ 0.00	10 $\pm$ 0.00
<i>Penicillium notatum</i>	(-)	N/A	28	14 $\pm$ 0.00	12 $\pm$ 0.00	10 $\pm$ 0.00	(-)	(-)
<i>Aspergillus niger</i>	(-)	N/A	28	17 $\pm$ 1.41	15 $\pm$ 1.41	13 $\pm$ 1.41	10 $\pm$ 1.41	(-)
<i>Rhizopus spp.</i>	(-)	N/A	28	14 $\pm$ 0.00	12 $\pm$ 0.00	10 $\pm$ 0.00	(-)	(-)

Table 2: Antimicrobial Activity of Root Essential Oil of *A. Flagellaris*
Inhibitions Are Expressed In Mm. (-): No Activity. N/A: Not Applicable, $\pm$ = Standard Deviation

3.4. Antioxidant Activity of the Root Essential Oil of *A. Flagellaris*

The percentage inhibition and IC_{50} of root *A. flagellaris* essential oil compared with reference standard are shown in Table 3, while % Inhibition of root essential oil at different concentration compared with standards was presented in Fig. 4. According to Ruberto and Baratta (2000) reported that phenolic compounds are effective antioxidants; thus, thymol derivatives, oxygenated monoterpenes and oxygenated sesquiterpenes molecules are indeed responsible for the antioxidant activity of this essential oil. The significant antioxidant activity observed in this study in the DPPH reduction can be explained by the ability of the identified compounds to donate a hydrogen atom. Since ascorbic acid is considered as polar compounds, the result of antioxidant activity of the essential oil was not only compared to it, so that the activity observed would not be considered as weak antioxidants, butylated hydroxyanisole (BHA) was used as the second reference standard. The potency of antioxidant activity and scavenging ability of the essential oil are inversely correlated with their IC_{50} values, i.e. the activities of the essential oil were as follows: Ascorbic acid (0.1734 mg/mL) > BHA (0.1954 mg/mL) > RTAF (0.3162 mg/mL).

Test samples	% Inhibition at each Concentration (mg/mL)					IC_{50}
	1	0.5	0.25	0.125	0.0625	
RTAF	90.04 $\pm$ 0.0000	85.01 $\pm$ 0.0000	73.95 $\pm$ 0.0012	61.43 $\pm$ 0.0000	50.73 $\pm$ 0.0006	0.3162
A. acid	93.05 $\pm$ 0.0006	92.87 $\pm$ 0.0006	92.78 $\pm$ 0.0012	90.49 $\pm$ 0.0001	90.40 $\pm$ 0.0000	0.1734
BHA	92.60 $\pm$ 0.0006	91.96 $\pm$ 0.0000	91.41 $\pm$ 0.0000	91.32 $\pm$ 0.0000	90.68 $\pm$ 0.0012	0.1954

Table 3: Antioxidant Activity of the Root Essential Oil of *A. Flagellaris*
RTAF: Root Essential Oil of *A. Flagellaris*. A. Acid: Ascorbic Acid
Butylated Hydroxyanisole. $\pm$ = Standard Deviation

It was observed that the essential oil in the study is concentration dependence, showing a competitive activity with ascorbic acid and BHA at a higher concentration as shown in Fig. 4. Statistical analysis (Pearson r) of the data showed that there was significantly different ($p < 0.005$) between the essential oil and Ascorbic acid/BHA at 0.5 – 0.0625 mg/mL, but there was no different between Ascorbic acid, BHA and the essential oil at 1 mg/mL.

Figure 4: % Inhibition of Root Essential Oil of *A. Flagellaris* Antioxidant Activity Compared with the Reference Standard

4. Conclusion

This study revealed the potency of root essential oil of *A. flagellaris* as antioxidant agent with IC₅₀ value of 0.3162 mg/mL. The essential oil was predominated by thymol derivative (84.54%), 3.64 % oxygenated monoterpenes, 3.10 % hydrogen sesquiterpenes, 1.36 % oxygenated sesquiterpenes and 10.99 % non-terpenes/non-terpenoids. The oil with characteristics odour recorded susceptibility against some bacteria and fungi strains - *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* and *Rhizopus* spp respectively. Hence the biological activities of this study can be attributed to the synergy of the chemical constituents.

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