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# Composition and biological assays of the leaf essential oil of *Asparagus flagellaris* (Kunth) Bak

Oluwakayode O. Odeja<sup>1</sup>, Michael Gabriel Ibok<sup>2\*</sup>  and Ejike O. Okpala<sup>3</sup>

## Abstract

**Background:** *Asparagus flagellaris* leaves are ethnomedicinally used to treat syphilis, gonorrhoea and other sexually transmitted diseases (STDs), with no reports on the volatile constituents. This study was aimed to quantitatively and qualitatively characterise the composition of essential oil, evaluates the free radical scavenging and antimicrobial capacity of the essential oil.

**Methods:** The essential oil was isolated by hydrodistillation method using all-glass Clevenger-type apparatus, while the identification and quantification of constituents were performed by gas chromatography-mass spectrometry (GC-MS) technique. The antioxidant activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated and the oil was also tested against 10 strains of microorganisms consisting of 6 bacteria: *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* and 4 fungi: *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* and *Rhizopus* spp. using broth dilution and surface plate methods, respectively.

**Results:** A pale yellow essential oil with a characteristic scent was obtained, with a yield of 0.80% (w/w). A total of 28 compounds accounting for 97.41% of the total oil contents were identified. The oil was predominated by Thymol and its derivatives, accounting for 57.48%. The most abundance (% area) constituents of the essential oil were 5-Thymyl tiglate (18.49%), Thymyl-2-methyl butyrate (17.34%), Thymol hydroquinone dimethyl ether (10.52%), Thymol methyl ether (9.42%) and 5-Propyl-1, 3-benzodioxole (4.59%). The essential oil showed a significant free radical scavenging activity compared to the standard antioxidant drugs used in this study, with % inhibition varying from  $88.06 \pm 0.0001$  to  $93.05 \pm 0.0006$ . The leaf essential oil exhibited antimicrobial activity on all the tested organisms at 500–125  $\mu\text{g}/\text{mL}$ , with an 18–10 mm inhibitory zone.

**Conclusion:** The leaf essential oil of *A. flagellaris* contains notable chemical compounds responsible for its antioxidant and antimicrobial activities.

**Keywords:** Antioxidant, Antimicrobial, *Asparagus flagellaris*, Chemical constituents, Thymol derivatives

## Introduction

Medicinal and aromatic plants have been used for decades and are still relevant today as alternative therapies. Herbs are used all over the world, especially in developing countries. Historically, herbs represent the route sources of most drugs [1].

*Asparagus flagellaris* (Kunth) Bak. is widespread in Northern and Southern Nigeria [2]. It belongs to the family Asparagaceae. It grows to a height of about 1 m and is the scandent, more or less erect plant with arching spiny branchlet as shown in Fig. 1 [3].

It has a variety of medicinal uses across the continent of Africa. The branchlets (cladodes) are the main ingredient to combat guinea worm and ointment for hair growth [2]. The stem and leaves are used for treating syphilis, gonorrhoea and other sexually transmitted diseases (STDs) [2].

\* Correspondence: [michaelibok@gmail.com](mailto:michaelibok@gmail.com)

<sup>2</sup>Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria  
Full list of author information is available at the end of the article



Fig. 1 Image of *Asparagus flagellaris*

diarrhea and urinary infections [3]. In Unani, the roots are used as laxatives, tonic, aphrodisiac, galactagogue, kidney and liver diseases [4]. Ancient Grecians, Romans, Indians and Chinese used this plant to treat diuretic, cholera and rheumatism [4]. Indians used it to promote fertility, reduce menstrual cramping and increase milk production in

nursing mothers. It acts to increase cellular activity in the kidney and thus, increases the rate of urine production. Chinese pharmacists save the *Asparagus* roots for their families and friends, believing that it will increase compassion and love [4].

The phytochemical screening of the stem bark and leaves of *A. flagellaris* showed an appreciable amount of flavonoid and a moderate amount of carbohydrate, cardiac glycoside, and saponin reducing sugar, ketones and pentose were detected in trace amount [3]. Mshelia et al. [5] reported the inhibition of six microorganisms viz. *Escherichia coli*, *Corynebacteria*, *Klebsiella*, *Neisseria gonorrhoeae*, *Shigella dysenteriae* and *Candida albicans* at various concentrations of the ethanol extract of *A. flagellaris* grown in Kokona, Nasarawa state - Nigeria, while the aqueous extract was susceptible to five organisms namely *Corynebacteria*, *Streptococcus pyogenes*, *Proteus* spp., *Neisseria gonorrhoeae* and *Treponema pallidum*.

The constituents and biological assay of the leaf essential oil of *A. flagellaris* have not been reported in the literature to the best of our knowledge. Hence, this study was aimed at isolating, qualitative and quantitative

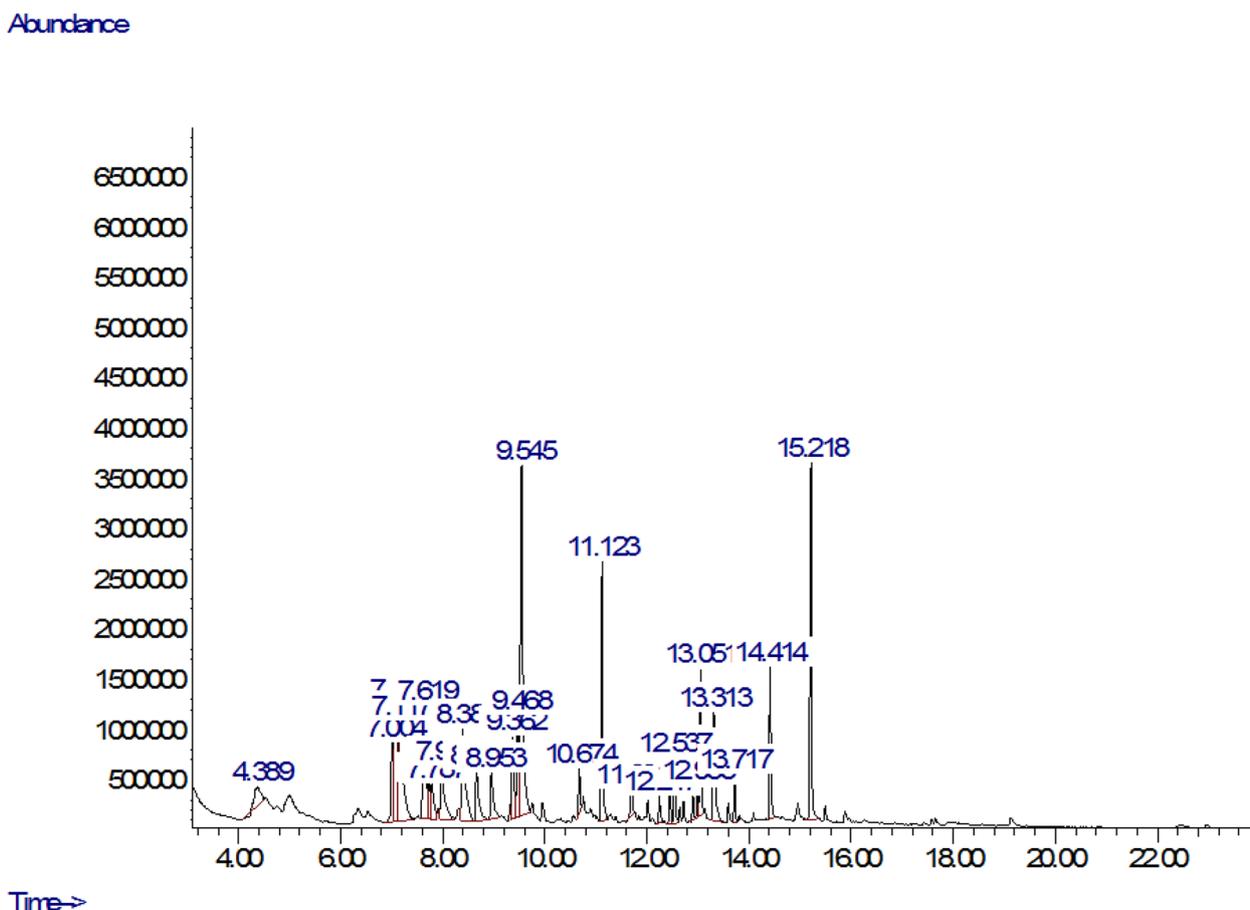


Fig. 2 GC-MS Chromatogram of *A. flagellaris* leaf

analysis, antioxidant and antimicrobial activities of essential oil from the leaves of *A. flagellaris*.

## Material and methods

### Collection and preparation of the plant materials

The leaf of *A. flagellaris* was collected from Efurum town, Delta State, Nigeria in June 2019. It was identified and authenticated at the Herbarium unit of the Botany Department, University of Ibadan, Nigeria. The fresh leaves were air-dried for a week to remove moisture and pulverised. Evaporation of the volatile constituents in the dried plant materials and contamination were avoided by storing the material in air-tight bags before hydrodistillation.

### Isolation of essential oils

Pulverised leaves (720.10 g) of *A. flagellaris* were hydro-distilled for 3 h in an all-glass Clevenger-type apparatus (with a thermo-regulated heating mantle) according to British Pharmacopoeia [6]. The volatile oil distilled over was collected in the receiver arm of the apparatus containing water and analytical grade *n*-hexane (which dissolves the oil). The oil and the hexane were collected into sample bottles. The volatile oils were preserved in a sealed glass vial and stored under refrigeration (at 4 °C) before analysis and biological assays. The % yield of the essential oil was calculated according to the formula below:

$$\% \text{Yield} = \frac{\text{Weight of essential oil}}{\text{Weight of Sample}} \times 100$$

Gas Chromatography/Mass Spectrometry (GC-MS).

The essential oil was subjected to GC-MS analysis on an Agilent 7809A Gas Chromatography hyphenated with an Agilent mass detector having split/splitless injector interfaced with mass selective detector operating at 70 eV. The ion source temperature was set to 200 °C over a mass spectral range of *m/z* 50–700 at a scan rate of 1428 amu/sec. The column of the GC used was HP-5MS with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was programmed as follows: initial temperature of 80 °C for 2 min, increased at 10 °C/min to a temperature of 240 °C for 6 mins. Helium was used as the carrier gas 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 their retention indices as determined with

respect to a homologous series of *n*-alkane as well as by comparison of their mass spectral fragmentation patterns (NIST data/base/chemstation data system) with the data previously reported in the literature [7, 8]. The relative percentages of the essential oil components were obtained by FID peak-area normalisation, all relative response factors being taken as 1.

### Antioxidant assay

The antioxidant potential of leaves essential oils of *A. flagellaris* was studied using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method described by Brand-William et al. [9] with some modifications. Various concentrations (1000, 500, 250, 125 and 62.5) µg/mL of the essential oil were mixed with 100 µM methanolic DPPH solution (2.0 mL). The mixture was shaken vigorously and left to incubate for 30 min in the dark at room temperature. The absorbance at 517 nm was recorded as *A<sub>s</sub>* and *A<sub>b</sub>* for the sample and blank, respectively using a GS UV-12, UV-Vis spectrophotometer. A blank experiment was carried out applying the same procedure but without essential oils (DPPH + Methanol) and the absorbance was recorded as *A<sub>c</sub>*. The antioxidant activity of Ascorbic acid and Butylated hydroxyanisole (BHA) were used as standards for comparison. Each experiment was carried out in triplicates and the free radical scavenging activities of the essential oils were calculated as percentage inhibition according to the formula:

$$\% \text{Inhibition} = \frac{A_b - A_s}{A_b} \times 100$$

Where *A<sub>b</sub>* - Absorbance of blank, *A<sub>s</sub>* - Absorbance of Sample/Reference.

### Antimicrobial assay

#### Test organisms

Ten strains of microbes consisting of six bacteria; four Gram-negative: *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and two Gram-positive: *Staphylococcus aureus* and *Bacillus subtilis*, with four fungi: *Candida albicans*, *Penicillium notatum*, *Aspergillus niger* and *Rhizopus* spp. were used in this study. All microbes were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria.

#### Preparation of sample solution [10]

Oil samples were prepared such that 1 mL of the oil was regarded as 1000 µg/mL; 0.5 mL of this essential oil was taken into 0.5 mL of methanol to give 500 µg/mL. More serial dilutions gave different concentrations such as 250 µg/mL, 125 µg/mL and 62.50 µg/mL. The 6th, 7(a)

and 7(b) th test tubes were negative control (DMSO), positive controls; Gentamycin for bacteria and Tioconazole for fungi, respectively.

#### **Antibacterial assay – broth dilution method**

Overnight cultured of microorganisms were prepared by taking two loopful of already grown organisms from the stock and inoculating each into the sterile nutrient broth of 5 mL each for 18–24 h at 37 °C. The inoculation of six strains from standardised suspensions was made using a Sterr multi-inoculator using suspensions standardised at 0.5 MacFarland, with a second dilution performed onto BHI inoculum - an approximate concentration of  $10^5$ – $10^6$  CFU/mL according to the method described by Andrade et al. [11]. From the overnight culture, 0.1 mL of each organism was added into 9.9 mL of sterile saline (0.85%) to get  $10^{-2}$  dilution of each microorganism. From the diluted microorganism, 0.2 mL was taken into the prepared sterile nutrient agar at 45 °C. This was aseptically poured into the concentrations of each essential oil and controls. The plates were incubated for 18–24 h at 37 °C. Each experiment was carried in duplicates. The antimicrobial activity was evaluated by measuring the diameter of the inhibition growth zone in millimeters (mm) for the test organisms compared with the control. Gentamicin (10 µg/mL) was used as a positive control.

#### **Antifungal assay via surface plate method**

The sterile Sabouraud Dextrose Agar (62 g/L) was prepared accordingly and aseptically poured into the sterile plates and allowed to set; 0.2 mL of  $10^{-2}$  of the fungus was spread on the surface of the set agar using a sterile spreader to cover all the surface of the agar. The wells were made using a cork borer of 8 mm diameter according to the concentrations and the standard. The essential oil and control were put inside the well as well as the control. The plates were incubated for 48 h at 28 °C. Tioconazole (0.7 mg/mL) was used as positive control.

#### **Statistical analysis**

All data represent the means of two or three replicates ± standard deviation. Results were subjected to analysis of variance (ANOVA) and the mean comparisons were performed by Bonferroni's Multiple Comparison Test using the statistical analysis software package (Graph-PadPrism 5). Differences between means were considered significant at  $P$ -value < 0.05.

#### **Results**

Hydrodistillation of 720.10 g air-dried leaves of *A. flagellaris* produced 5.74 g pale yellow essential oil with a yield of 0.80% (w/w) calculated on a dry weight basis. The compositions of the oil is presented in Table 1 and

Figure 2. Twenty-eight (28) compounds were identified from the GC-MS analysis, accounting for 97.41%. The essential oil is composed of a mixture of terpenes, terpenes derivatives and non-terpenes. The DPPH free radical scavenging activity was determined based on % inhibition of the essential oil to the reference standard. The concentration of the oil and the reference drugs are given in Table 2. Table 3 shows the inhibition zones observed in leaves of *A. flagellaris* essential oil at various concentrations (1000–62.5 µg/mL) compared to the standard drugs to the well diameter.

#### **Discussion**

The leaves essential oil of *A. flagellaris* was dominated with 57.48% thymol and its derivatives: 5-thymyl tiglate (18.49%), thymyl-2-methyl butyrate (17.34%), thymol hydroquinone dimethyl ether (10.52%) and thymol methyl ether (9.42%) as shown in Table 1. However, Willuhn [12] reported five thymol derivatives as the major constituents of the oil of underground organs of *A. montana*, thymol methyl ether inclusive. A similar trend was observed by Weremczuk-Jeżyna et al. [13] where fifty-six compounds were identified in the hairy roots and plant roots of *Arnica montana* L. oils that consisted mainly of bi-functional thymol derivatives and sesquiterpene hydrocarbons. Hence, thymol and its derivatives are well-known antioxidants and also inhibited interesting potential against most microorganisms [14].

The leaf essential oil also contains 12.99% oxygenated sesquiterpenes that are of pharmacological interest, such as 6-(3-Isopropenyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanol, 7-epi-cis-sesquisabinene hydrate, Germacrene-4(15), 5,10(14)-trien-1 $\alpha$ -ol, Longiverbenone, 8-Cedre-13-ol and cis-Farnesol. 15.04% hydrocarbon sesquiterpenes (Germacrene D,  $\beta$ -Copaene,  $\gamma$ -Muuroolene,  $\beta$ -Bisabolene,  $\delta$ -Cadiene,  $\gamma$ -Cadine-1,4-diene,  $\alpha$ -Muuroolene, Valence,  $\alpha$ -Patchoulene and 8,9-dihydro-cycloisolongifolene), 6.62% oxygenated monoterpenes (Bornyl acetate, 5-Propyl-1,3-benzodioxol, 2-Allyl-p-cresol and (*E*)-Cinnamyl alcohol), 0.75% oxygenated diterpene (phytol) and 4.55% non-terpenes/non-terpenoids.

However, the constituents of the leaves essential oil of *A. flagellaris* with interesting biological activities such as thymol methyl ether, bornyl acetate, thymol and  $\beta$ -bisabolene were also identified as the most abundant constituents in the root essential oil of *Chaerophyllum villosum* that inhibited significant antioxidant and antimicrobial activities [15, 16]. Germacrene D, farnesol, bornyl acetate,  $\beta$ -bisabolene have been reported to be responsible for the antimicrobial and antioxidant of *Eupatorium adenoporum* essential oil [17]. Dob et al. [18] reported the following constituents; thymol, thymol methyl ether,  $\gamma$ -Muuroolene, Germacrene D and  $\alpha$ -Muuroolene in *Thymus fontanesii* are susceptible against all filamentous fungi (*F. oxysporum* f. sp. *albedinis* and

**Table 1** Constituents of Essential oil from the leaf of *A. flagellaris*

| S/N | Identified Compounds   | Retention Time | Composition (%) |
|-----|--|----------------|-----------------|
| 1   | Thymol methyl ether <sup>f</sup>   | 5.425          | 9.42            |
| 2   | Bornyl acetate <sup>a</sup>  | 6.192          | 0.44            |
| 3   | Thymol <sup>f</sup>  | 6.387          | 1.71            |
| 4   | Thymol hydroquinone dimethyl ether <sup>f</sup>                                | 8.092          | 10.52           |
| 5   | Germacrene D <sup>b</sup>  | 8.849          | 3.83            |
| 6   | β-Copaene <sup>b</sup>   | 8.906          | 0.87            |
| 7   | β-Bisabolene <sup>b</sup>  | 9.439          | 0.92            |
| 8   | δ-Cadinene <sup>b</sup>  | 9.558          | 2.69            |
| 9   | cis-Farnesol <sup>c</sup>  | 9.592          | 1.51            |
| 10  | γ-Cadine-1,4-diene <sup>b</sup>  | 9.616          | 0.37            |
| 11  | α-Murolene <sup>b</sup>  | 9.635          | 0.83            |
| 12  | Valencene <sup>b</sup>   | 10.035         | 1.39            |
| 13  | Thymyl-2-methylbutyrate <sup>f</sup>   | 10.035         | 17.34           |
| 14  | α-Patchoulene <sup>b</sup>   | 10.273         | 2.13            |
| 15  | Thymyl tiglate <sup>c</sup>  | 10.449         | 18.49           |
| 16  | 6-(3-Isopropenyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanol <sup>c</sup> | 10.873         | 3.4             |
| 17  | 7-epi-cis-sesquibinene hydrate <sup>c</sup>                                    | 11.063         | 5.46            |
| 18  | γ-Murolene <sup>b</sup>  | 11.116         | 2.01            |
| 19  | Germacre-4(15),5,10(14)-trien-1α-ol <sup>c</sup>                               | 11.225         | 0.31            |
| 20  | 8,9-Dihydro-cycloisolongifolene <sup>b</sup>                                   | 11.249         | 0.39            |
| 21  | 2-Ethylbenzimidazole <sup>e</sup>  | 11.363         | 1.64            |
| 22  | 3-Chloro-4-fluorophenol <sup>e</sup>   | 11.697         | 2.91            |
| 23  | 5-Propyl-1,3-benzodioxole <sup>a</sup>   | 12.111         | 4.59            |
| 24  | Longiverbenone <sup>c</sup>  | 12.344         | 2               |
| 25  | 2-Allyl-p-cresol <sup>a</sup>  | 13.125         | 0.37            |
| 26  | (E)-Cinnamyl alcohol <sup>a</sup>  | 13.644         | 1.22            |
| 27  | Phytol <sup>d</sup>  | 15.268         | 0.75            |
| 28  | 8-Cedre-13-ol <sup>c</sup>   | 15.797         | 0.31            |
|     | <b>Total</b>   |                | <b>97.82</b>    |

a = Oxygenated monoterpenes; b = Sesquiterpenes hydrocarbon; c = Oxygenated sesquiterpenes; d = Oxygenated diterpene; e = Non-terpenes, f = Thymol and derivatives

*M. ramanianus*), all yeast (*S. cerevisiae* and *C. albicans*). Thymol and thymol methyl ether were among the major components identified in the essential oil of *Thymus vulgaris* that demonstrated antioxidant and antimicrobial activities [19].

However, the biological activities could be associated to the synergistic or antagonistic effect due to complex interactions between the identified chemical components in the essential oil. The ethnomedicinal application of this plant in the treatment of syphilis, gonorrhoea and other

**Table 2** Antioxidant Activity of the leaves essential oil of *A. flagellaris* showing % Inhibition at different concentrations and reference standard

| Test samples  | % Inhibition at each Concentration (µg/mL) |                |                |                |                |
|---------------|--|----------------|----------------|----------------|----------------|
|               | 1000.0                                     | 500.0          | 250.0          | 125.0          | 62.50          |
| APAF          | 91.04 ± 0.0000                             | 90.74 ± 0.0001 | 90.59 ± 0.0000 | 89.40 ± 0.0000 | 88.06 ± 0.0001 |
| Ascorbic acid | 93.05 ± 0.0006                             | 92.87 ± 0.0006 | 92.78 ± 0.0012 | 90.49 ± 0.0001 | 90.40 ± 0.0000 |
| BHA           | 92.60 ± 0.0006                             | 91.96 ± 0.0000 | 91.41 ± 0.0000 | 91.32 ± 0.0000 | 90.68 ± 0.0012 |

APAF: Leaf essential oil of *A. flagellaris*, BHA: Butylated hydroxyanisole, ± = Standard deviation

**Table 3** Antimicrobial Activity of the Leaves Essential Oil of *A. flagellaris* showing zones of inhibition of the isolates at different concentrations compared to the Standard drugs (with reference to Well diameter of 100 mm)

| Test Organisms                | Negative Control | Positive Control |             | Inhibitory Zone (mm) at different Concentration ( $\mu\text{g/mL}$ ) |               |               |               |               |
|-------------------------------|------------------|------------------|-------------|--|---------------|---------------|---------------|---------------|
|                               |                  | Gentamicin       | Tioconazole | 1000   | 500           | 250           | 125           | 62.5          |
| <i>Escherichia coli</i>       | (-)              | 35               | N/A         | (-)  | 14 $\pm$ 0.00 | 12 $\pm$ 0.00 | 12 $\pm$ 0.00 | 10 $\pm$ 0.00 |
| <i>Pseudomonas aeruginosa</i> | (-)              | 38               | N/A         | (-)  | 14 $\pm$ 0.00 | 12 $\pm$ 0.00 | (-)           | (-)           |
| <i>Klebsiella pneumonia</i>   | (-)              | 38               | N/A         | (-)  | 16 $\pm$ 0.00 | 13 $\pm$ 0.00 | (-)           | (-)           |
| <i>Staphylococcus aureus</i>  | (-)              | 36               | N/A         | (-)  | 18 $\pm$ 0.01 | 15 $\pm$ 0.10 | 13 $\pm$ 0.10 | 11 $\pm$ 0.01 |
| <i>Bacillus subtilis</i>      | (-)              | 38               | N/A         | (-)  | 17 $\pm$ 0.00 | 15 $\pm$ 0.00 | 13 $\pm$ 0.10 | 11 $\pm$ 0.01 |
| <i>Salmonella typhi</i>       | (-)              | 40               | N/A         | (-)  | 15 $\pm$ 0.00 | 12 $\pm$ 0.00 | 10 $\pm$ 0.00 | (-)           |
| <i>Candida albicans</i>       | (-)              | N/A              | 28          | (-)  | 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          | (-)  | 12 $\pm$ 0.00 | 10 $\pm$ 0.00 | (-)           | (-)           |
| <i>Rhizopus</i> spp.          | (-)              | N/A              | 28          | (-)  | 12 $\pm$ 0.00 | 10 $\pm$ 0.00 | (-)           | (-)           |

(-): no activity, N/A: not applicable, Mean  $\pm$  Standard deviation

sexually transmitted diseases (STDs) [2], diarrhea and urinary infections [3] might, therefore, be attributed to the chemical components present in the leaf *A. flagellaris*.

In radical form, DPPH absorbs, but upon reduction by antioxidant species, its absorption reduces by hydrogen donating compound, leading to its change in colour, from deep violet/purple to light yellow, which can be monitored spectrophotometrically. It is reported that phenolic compounds are effective antioxidants [20]. Thus, the essential oil in this study contained thymol and its derivatives, oxygenated monoterpenes and oxygenated sesquiterpenes molecules as presented in Table 1, which are undeniably responsible for the antioxidant activity of this essential oil (see Table 2). The significant antioxidant activity observed in this study using DPPH reduction can be explained by the ability of the identified compounds to donate a hydrogen atom or an electron leading to the formation of DPPH-H or to stabilise the free radical, respectively.

It was observed that the % inhibition of essential oil of *A. flagellaris* leaves at all tested concentrations possess a significant scavenging power from 91.04% - 88.06%, which were competing with the % inhibition of two of the standards, ascorbic acid (93.09% - 90.40%) and BHA (92.60% - 90.68%) used in this study. It was also observed that the antioxidant activities were concentration-dependent. The antioxidant activity observed in this study might be attributed to the chemical components in the essential oil. However, the analysis of variance showed no significant ( $P < 0.05$ ) difference in antioxidant between the essential oil and the reference standards (Ascorbic acid and BHA).

The antimicrobial activity of the *A. flagellaris* leaves essential oil and reference standards assessed against 10 pathogenic organisms at different concentrations (1000–62.5  $\mu\text{g/mL}$ ). The antimicrobial property was evaluated

by measuring the inhibition zones around the well of the essential oil shown in Table 3. The result indicated that the essential oil did not show any inhibition against all tested organisms at 1000  $\mu\text{g/mL}$ . No inhibition zone was observed in 125 and 62.5  $\mu\text{g/mL}$  against *K. pneumonia* and *P. aeruginosa* and no inhibition at 62.5  $\mu\text{g/mL}$  against *S. typhimurium*. No inhibition zones at 62.5  $\mu\text{g/mL}$  against all the tested fungi and *A. niger* and *R. spp.* at 125  $\mu\text{g/mL}$ . The leaf essential oil shows moderate inhibition zones at 500–250  $\mu\text{g/mL}$  against all tested organisms for bacteria and fungi. The lowest inhibitory zones were observed in *R. spp.* at 250  $\mu\text{g/mL}$  (10 mm) but no activity at 125–62.5  $\mu\text{g/mL}$ , *A. niger* at 250  $\mu\text{g/mL}$  but no inhibition at 125–62.5  $\mu\text{g/mL}$  while *C. albicans* and *P. notatum* show the lowest inhibition at 125  $\mu\text{g/mL}$  and no activity at 62.5  $\mu\text{g/mL}$ . The difference in antimicrobial activities of *A. flagellaris* leaves essential oil with respect to reference standards (Gentamicin and Tioconazole) and concentrations were statistically significant ( $P < 0.05$ ).

However, the insensitivity of the oil against tested microorganisms at a higher concentration (1000  $\mu\text{g/mL}$ ) might be that only a small amount is needed to attack a specific site in the organism and high concentration will cause accumulation and blockage of the sensitive site, thereby cause no activity [21]. Also, it has been reported that only the non-protein-bound fraction of an antimicrobial is microbiologically active [22], this premise might be attributed to be responsible for the oil in this study to form resistance to the tested microorganisms at the highest concentration assuming it to be protein-bound at higher concentration and this was reduced as the concentration of the oil decreases. The level of antimicrobial activity witnessed in the study might be due to the synergy or antagonism of the constituents' presence in the essential oil of the leaves and thus, these chemical

components can affect multiple target sites against the bacterial cells [23]. This essential oil activity can be described as ‘bacteriostatic antibiotic’ since it limits the growth by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. This group of bacteriostatic antibiotic includes tetracyclines, sulfonamides, spectinomycin, etc. [24]. This result correlates with the earlier report by Marchese et al. [25] that described all the thyme essential oils as bacteriostatic against the microorganisms and the one earlier reported on the leaves extracts [5].

## Conclusion

The leaves essential oil chemical composition, antioxidant and antimicrobial activities of *A. flagellaris* is being reported for the first time. Twenty-eight compounds were identified in the oil sample: mainly oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and oxygenated diterpene, thymol and its derivatives. The most prominent of the leaf's essential oil are 5-thymyl tiglate, thymyl-2-methyl butyrate, thymol hydroquinone dimethyl ether, thymol methyl ether and 5-propyl-1, 3-benzodioxole. The essential oil exhibited significant antioxidant activity (with % inhibition of 88.06–91.04) compared with the standard drugs (with % inhibition of 90.40–93.05) used in this study. The inhibition zones (10–18 mm) obtained from the antimicrobial assay shows that the essential oil at a concentration between 500 and 125 µg/mL exhibited moderate activity against all the tested microorganisms when compared with the standard drugs (Gentamicin; 10 µg/mL and Tioconazole; 0.7 mg/mL) which gave 26–40 mm inhibition zones. Chemical constituents and the bioactivities of the leaves essential oil of *A. flagellaris* support the ethnomedicinal uses of this plant in treating syphilis, gonorrhoea and other sexually transmitted diseases (STDs).

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## Authors' contributions

OOO carried out the sample collection, took part in the laboratory analysis and write up. EOO was involved in the antioxidant and write up. MGI was involved in all the laboratory work, conceptualisation, result interpretation and writing (review and editing). All authors read and approved the final manuscript.

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## Ethics approval and consent to participate

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## Consent for publication

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## Competing interests

Oluwakayode O. Odeja, Michael Gabriel Ibok and Ejike O. Okpala declare that they have no competing interests.

## Author details

<sup>1</sup>Department of Chemistry, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria. <sup>2</sup>Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria. <sup>3</sup>Department of Chemistry, Federal University Lokoja, Lokoja, Kogi State, Nigeria.

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