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RESEARCH ARTICLE

Chemical Composition, Free Radical Scavenging and Antimicrobial Activities of Essential Oil of *Mariscus alternifolius* Vahl

Patricia A. Onocha*, Ganiyat K. Oloyede and Jamiu A. Akintola

Natural Products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Oyo State, Nigeria

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Abstract:

Introduction:

The study of medicinal plants in different parts of the world has led to major breakthroughs in drug chemistry. The increased interest in discovering drugs from natural source led to our investigation of the chemical composition, antioxidant and antimicrobial activities of the essential oil of *Mariscus alternifolius* Vahl, used in the treatment of wounds, bacterial and infectious diseases in ethno medicine.

Methods:

The plant essential oil was collected over hexane by Hydro distillation technique and Gas Chromatography-Mass Spectrometry (GC-MS) was employed for analysis. Antioxidant activity of the essential oil was evaluated using the 2,2-diphenylpicrylhydrazyl (DPPH) method while the antimicrobial screening was determined by the agar well diffusion method.

Results:

GC-MS analysis revealed the presence of a total of 10 constituents representing 71.91% with tricosane (19.45%) as the most abundant constituent in the essential oil of the plant while others with relatively high percentages include z-14-nonacosene (13.37%) and octacosyltriflouroacetate (10.91%). The free radical scavenging activity of the essential oil gave percentage inhibition of 97.95% at 5 mg/ml which was comparable to that of the standard antioxidant ascorbic acid (97.88%) used for the assay. The essential oil inhibited the growth of *Staphylococcus aureus, Escherichia coli, Bacillus substilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Candida albicans* and *Aspergillus niger* used in this study. The minimum inhibitory concentration (MIC) of 25 mg/ml was observed for *Staphylococcus aureus* and *Escherichia coli*.

Conclusion:

In summary, the essential oil of *M. alternifolius* Vahl. possesses high antimicrobial and antioxidant activities.

Implications:

This study therefore provide some scientific basis for its utilization in ethno medicine.

Keywords: 2,2-diphenylpicrylhydrazyl, Hydrodistillation, *Mariscus alternifolius*, MIC, Tricosane.

INTRODUCTION

Mariscus alternifolius belongs to a family of monocotyledonous flowering plants known as *Cyperaceae* which resemble grasses otherwise known as sedges found in tropical Asia, Africa and South America. It has been reported to be effective in the treatment of wounds, stomach troubles, diarrhoea, gonorrhea and as an insecticide while the rhizome is aromatic and used as a food-flavouring agent [1 - 7]. The biologically active constituents of medicinal, poisonous and

* Address correspondence to this author at the Department of Chemistry, University of Ibadan, Nigeria; Tel: +2348023144743; E-mail: triciaonocha@yahoo.com

commercial plants, many of which are secondary metabolites: alkaloids, flavonoids, terpenoids, essential oils amongst others have been studied in medicinal chemistry. Artemisinin for instance, is an anti-malarial drug isolated from *Artemisia annua*, sweet wormwood, a Chinese herb [1, 8 - 10].

Essential oils contain interesting natural plant secondary metabolites which possess various biological properties. Some of these secondary metabolites like mono- and sesquiterpenoids, benzenoids, phenylpropanoids, have been reported to exert pharmacological effect as antimicrobial, anti- inflammatory and anti oxidant to mention a few. They have also been used in the food industry as seasoning agents, as scents in soaps, cosmetics or perfumes [11, 12]. Phyto-remediation study of four common Nigerian weeds among which was *Mariscus alternifolius* Vahl has been investigated [13]. However, very little is known in literature about the composition of the leaf essential oil and its antimicrobial and antioxidant properties. Due to the various medicinal applications of essential oils in ethno medicine and coupled with the fact that the crude extracts of *M. alternifolius* have been shown to demonstrate appreciable activities as antimicrobial [2] and antioxidant agents in our previous work on the plant [3], we now report the biological activities of the essential oil of this plant.

MATERIALS AND METHODS

Plant collection and identification

Mariscus alternifolius plant was collected in June 2014 from the Botanical garden of the University of Ibadan and identified by Dr Ayodele of the Botany Department, University of Ibadan.

Reagents

Methanol and hexane obtained from Sigma Aldrich were used.

Test Organisms

Staphylococcus aureus, Escherichia coli, Bacillus substilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Candida albicans, Aspergillus niger, Penicillium notatum, Rhizophus stolonifer.

Reference Standards

Antibacterial and antifungal standards: gentamicin® (10 µg/ml) and tioconazole® (70%) respectively, were obtained from the University Medical Hospital, Jaja Clinic Pharmacy. Ascorbic acid obtained from the same place was used as an antioxidant standard.

Major Equipment Used

The following equipment and apparatus were used: heating mantle, Clavenger apparatus, electronic weighing balance (OHAUS), Oven (Carbolite), round bottom flask, syringe, Gas chromatograph/GC-MS Agilent 7890B, CE 2021, 2000 series double beam UV-Vis spectrophotometer.

Isolation of Essential Oil

The Clavenger apparatus and a heating mantle set at a thermo-stated temperature of 100^{o}C were the major apparatus used for extracting the essential oil in accordance with the European Pharmacopoeia, 1996 in a technique known as the hydro distillation method. Freshly collected plant (874 g) of *M. alternifolius* was air dried for 5 days. This yielded 367 g which was transferred into a 10 litres distillation flask and distilled water was added until the sample was completely submerged. The flask was then placed on a heating mantle and fitted with a glass Clavenger. At a thermo-stated temperature of 100^{o}C, hydro-distillation was carried out for a period of 3 hours under close monitoring to ensure continuous flow of cold water through the condenser and to ensure that the distillate (essential oil) was not lost due to its volatile nature. After 3 hours, the essential oil which was made to dissolve in a known quantity of n-Hexane, was collected from the receiving arm of the Clavenger into a vial bottle of known weight and stored at 4^{o}C [14].

Analysis of the Essential Oils

Gas Chromatography–Mass Spectrometry

GC-MS Agilent 7890B - Gas Chromatograph coupled with MS Agilent technologist 5975 series MSD was used to

analyse the essential oil. The capillary column used had 30.0 m column length, 320 µm internal diameter and 0.5 µm film thickness. The carrier gas was helium at a constant flow rate of 1.4123 ml/min and an average velocity of 43.311 cm/sec. The pressure was maintained at 1.5 psi. The initial column temperature was set at 50°C for 5mins at the rate of 5°C/min and increased to 280°C for 9 mins. The volume injected was 3 µl and the split ratio was 50:1 at the split flow rate of 70.615 ml/min.

Identification of Components

Flame Ionization Detector (FID) set at a temperature of 300°C was used to determine the compositions of the various constituent, by recording the peak numbers and relative percentages of the characterized compounds. Identification of individual component was done by comparing their retention time to identical compounds known from literature data and their spectral with NIST 0.8L/Database/chemstation data system [15 - 17]. The peak numbers and relative percentages of the essential oil component are shown in Table **1**.

Table 1. Chemical composition of the essential oil from the *Mariscus alternifolius.**

S/N	RT (Minutes)	Chemical Composition	Molecular Formula	AI	T.P (%)
1	3.235	Octane	C_8H_{18}	0800	2.99
2	13.141	α-Cyperene	$C_{15}H_{24}$	1398	4.73
3	17.023	Decanal	$C_{10}H_{20}O$	1209	2.81
4	18.501	2-Decanone	$C_{10}H_{20}O$	1201	2.46
5	23.920	Octacosyltrifluoroacetate	$C_{30}H_{57}F_3O_2$	-	10.91
6	24.810	Hentriacontane	$C_{31}H_{64}$	3100	2.52
7	25.071	1-Eicosene	$C_{20}H_{40}$	1987	5.71
8	25.214	2-methyl-1-hexadecanol	$C_{17}H_{36}O$	-	5.96
9	25.368	z-14-nonacosene	$C_{29}H_{58}$	2900	13.37
10	25.582	Tricosane	$C_{23}H_{48}$	2300	19.45
				Total%	**71.91**

*RT = Retention Time, AI = Arithmetic retention index, T.P = Total Percentage.

Free Radical Scavenging Activity

The free radical scavenging activity of the essential oil of *M. alternifolius* Vahl, was studied using the DPPH free radical scavenging method [11, 18]. Dilutions (5 mg/ml, 25 mg/ml and 100 mg/ml) of 1.5 ml of the essential oil was mixed with a 0.5 mM methanol DPPH solution (2.0 ml) prepared by dissolving 240 mg of DPPH in 1 L of methanol. This was incubated in the dark for 15 min at room temperature and the absorbance at 517 nm was recorded using CE 2021, 2000 series double beam UV-Vis spectrophotometer. In its radical form, DPPH absorbs, but upon reduction by antioxidant specie, its absorption reduces and is decolorized. A blank experiment was carried out by applying the same procedure but without the test material (DPPH and Methanol) and the absorbance values were recorded. The standard ascorbic acid was also examined for its antioxidant activity using the same method. The free radical scavenging activity of the test oil solutions was then calculated [11, 18].

Antimicrobial Screening

M. alternifolius Vahl has been reported to be used locally as an antimicrobial agent. Agar well diffusion method was used to establish this fact [14, 18]. The essential oil (0.50 ml) was dissolved into 5 ml of hexane to give 100 mg/ml. Hexane (2.5 ml) was poured into five other test tubes to get the stock solution which was serially diluted until concentration of 6.25 mg/ml was achieved in the fifth test tube. Two other test tubes representing the sixth and seventh test tubes contained hexane and gentamicin/tioconazole as the negative and positive controls for bacteria/fungi, respectively. An overnight culture (0.2 ml) of test micro-organisms was added to 20 ml of cooled molten agar and maintained for 8 hours on nutrient agar slants at 12°C, sub cultured in nutrient broth and preserved at 4-5°C prior to each antimicrobial assay. Pour plate method for bacteria and surface plate method for fungi were employed in the antimicrobial screening [14, 18].

In the Agar diffusion pour plate method for anti-bacterial screening, molten sterile agar prepared according to the manufacturer's standard was poured (pour plate) asceptically into sterile petri dishes in triplicate and allowed to set on the bench for 45 minutes. Inoculation of the test organisms from the broth culture into the agar prepared plate was

achieved with the aid of flamed and cooled wire loops. The inoculated plates were allowed to solidify. Thereafter, 9mm wells were asceptically bored into the solid nutrient agar using a sterile cork borer. The test solutions of the oil at various concentrations (6.25 - 100 mg/ml) were introduced into each of the designated wells with the aid of sterile syringes in each plate ensuring that no spillage occurred. The same amount of the solvent (hexane) and standard antibacterial agent (gentamicin) were introduced into the 6th and 7th wells to act as negative and positive controls, respectively. The plates were left at room temperature for two hours, to allow diffusion into the medium, turned upside down and thereafter, incubated at 37°C. After 24 hours, the plates were removed from the incubator and diameter of clear zones of inhibition of test organisms and control were measured in millimeters (mm) using a transparent well calibrated ruler (Table **2**).

Table 2. Antimicrobial activity of Essential oil of *Mariscus alternifolius.**

Conc. (mg/ml)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Kles*	*Sal*	*C.a*	*A.n*	*Pen*	*Rhiz*
100	28	22	20	18	14	18	16	14	-	-
50	24	18	18	14	12	14	14	12	-	-
25	20	16	14	12	10	12	12	10	-	-
12.5	18	12	12	10	-	10	10	-	-	-
6.25	14	10	10	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	40	38	40	38	40	38	28	28	26	28

*Conc. (mg/ml): represents the Essential oil of *Mariscus alternifolius* at various concentrations (6.25-100). *S.a- Staphylococcus aureus, E.c- Escherichia coli, B.Su- Bacillus substilis, Ps.a- Pseudomonas aeruginosa, Sal- Salmonella typhi, Kles- Klebsiella pneumoniae, C.a- Candida albicans, A.n- Aspergillus niger, Pen-Penicillium notatum, Rhiz- Rhizophus stolonifer*. -ve = negative control (Hexane); +ve = positive control (Gentamicin at 10µg/ml for Bacteria or Tioconazole (70%) for Fungi); - = no inhibition.

In the Agar diffusion surface plate method for antifungal screening, sterile sabourand dextrose agar (SDA) prepared according to the manufacturer's standard was poured asceptically into sterile petri dishes in triplicate and allowed to cool down for 45 minutes. 0.2 ml of the fungal strain from the broth culture was spread on the surface (surface plate or spread drop) of the prepared SDA plate using a sterile spreader and allowed to dry and solidify. Subsequently, the same procedure described for antibacterial assay above was carried out. All the plates for the antifungal assay were incubated at 27°C. After 72 hours, the plated were again removed from the incubator and clear zones of inhibition were measured and recorded using the same method as described in the case of antibacterial assay (Table **2**).

Minimum inhibitory concentration was determined for all the tested organisms.

Determination of Minimum Inhibitory Concentration

Dilution method [19] was used for the determination of the minimum inhibitory concentration (MIC) of the essential oil of *M. alternifolius*. Tubes of nutrient broth containing various concentrations of volatile fractions mentioned in Table **3** were inoculated with microbial strains from the broth cultures. They were incubated in a shaker (120rpm) at 37°C for 24 hours and 27°C for 72 hours for bacteria and fungi, respectively. Control tubes without tested samples were assayed simultaneously. All samples were tested in triplicate. The MIC is defined as the lowest concentration of the test samples at which the organisms do not demonstrate visible growth.

Table 3. Minimum Inhibitory Concentration (MIC) of Essential oil of *Mariscus alternifolius.**

Conc. (mg/ml)	*S.a*	*E.c*	*B.su*	*Ps.a*	*Kles*	*Sal*	*C.a*	*A.n*
100	-	-	-	-	-	-	-	-
50	-	-	-	+	+	-	-	+
25	-	-	+	+	+	+	+	+
12.5	±	+	+	+	+	+	+	+

*Conc. (mg/ml): represents the Essential oil of *Mariscus alternifolius* at various percentage concentrations (6.25-100). *S.a- Staphylococcus aureus, E.c- Escherichia coli, B.Su- Bacillus substilis, Ps.a- Pseudomonas aeruginosa, Sal- Salmonella typhi, Kles- Klebsiella pneumoniae, C.a- Candida albicans, A.n- Aspergillus niger;* - = no growth of organism i.e. inhibition; ± = growth of organism not prominent i.e. partial inhibition; + = growth visible *i.e.* no inhibition.

RESULTS AND DISCUSSION

GC-MS Analysis of Essential Oil from *Mariscus alternifolius*

M. alternifolius Vahl essential oil contained 10 constituents totalling 71.91% (Table **1**). The percentage yield of the essential oil was 0.47%. Tricosane (19.45%) was the most abundant primary active constituent. Other primary active constituents with relatively high percentages include (Z)-14-nonacosene (13.37%) and Octacosyltriflouroacetate (10.91%). Some derivatives of these primary active constituents and a number of the minor constituents of the essential oil of *M. alternifolius* have been reported to possess antimicrobial, antioxidant and other properties. The most abundant constituent tricosane, is one of the major constituents of the essential oil of *Rosa damascena* which has been shown to have strong radical scavenging effect [20]. Nonacosane, a saturated derivative of (Z)-14-nonacosene, another major active constituent of the essential oil has been reported to possess antibacterial activity [21]. Nonacosane is also one of the major constituents of the seed hexane extract of *Hypericum scabrum* L [22]. and essential oil from the leaves of Moringa oleifera [23] which have been shown to exhibit high radical scavenging activity. Octacosyltriflouroacetate, one of the primary active constituents of the essential oil of *M. alternifolius* is the trifluoroacetate derivative of n-octacosane. N-octacosane has been confirmed to have significant antioxidant activity [24, 25]. Another derivative cyclooctacosane on the other hand, has been reported to exhibit significant antibacterial activity [26]. 2-Decanone, one of the constituents of the study plant has been shown to possess antifungal activities [27] while hexadecane, a derivative of the constituent 2-methyl-1-hexadecanol has been reported to be responsible for the anti-bacterial and antioxidant properties of the essential oil of *Cestrum noctrunum* [28] and *Monochaetia kansensis* [29]. Eicosane, a derivative of the constituent 1-Eicosene is reported to be one of the main constituents in the Aloe vera extract responsible for high antimicrobial activity against clinical pathogens [30] while 9-Eicosene has been shown to have potent antimicrobial property [31]. The presence of these constituents in the essential oil justifies the ethno medicinal uses of the plant.

Some of the constituents have also been shown to possess other medicinal properties beyond the generic anti-microbial and free radical scavenging activities. Tricosane is known to provoke behavioral response in several insect predators and parasitoids while octacosane and (Z)-14-nonacosene (a component of the sex pheromones) play an important role in the control of insects [32]. Nonacosane has been reported to possess nematicidal activity [21], antihypertensive, vasodilatory, angiotensin AT2 receptor antagonist and saluretic activities [33]. Hentriacontane, a constituent of *M. alternifolius* has also been shown to possess anti-cancer, anti-inflammatory [34], anti-tuberculosis [35] and weak cytotoxic activities [36].

Free Radical Scavenging Activity

M. alternifolius Vahl displayed a higher free radical scavenging activity at 5 mg/ml than ascorbic acid when absorbance measurement was done at 517 nm. Fig. (**1**) shows the % inhibition of the sample and standard as calculated from the absorbance results obtained [18]. At 5 mg/ml, 97.95% inhibition was observed for the plant essential oil. This was slightly higher than that of ascorbic acid (97.88%). However, at higher concentrations of 25 mg/ml and 100 mg/ml tested, the plant oil exhibited % inhibition of 97.85 and 97.92 respectively. These slightly lower values contradict the usual trend of high % inhibition with high concentration. Percentage inhibition of Ascorbic acid on the other hand, increased with increasing concentration. Nevertheless, the fact that the plant oil exhibited a higher % inhibition than the standard ascorbic acid used at a low concentration (5 mg/ml) is a clear indication of its high free radical scavenging activity.

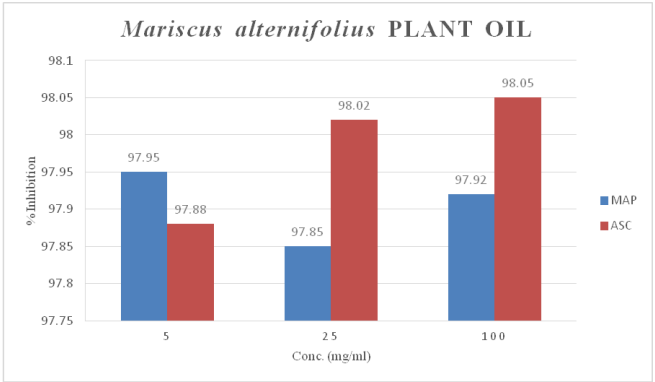

Fig. (1). DPPH Scavenging Activity of *Mariscus alternifolius* Plant (MAP) Oil and Ascorbic acid (ASC).

The essential oil of *M. alternifolius* Vahl as revealed in this study possesses antioxidant activity. Antioxidants are compounds that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition [37 - 39]. They have been reported to have curative effect on Parkinson's disease, cancer, coronary and other heart related conditions. They also find application in the industry as preservatives in food and cosmetics [11, 12, 40].

Antimicrobial Screening of the Essential Oil of *Mariscus alternifolius*

Zones of inhibition of the essential oil of *Mariscus alternifolius* measured during the antimicrobial screening are presented in Table **2**.

Minimum Inhibitory Concentration (MIC) of Essential Oil of *Mariscus alternifolius*

The result of the antimicrobial screening of the essential oil of *M. alternifolius* revealed its inhibitory effect on the growth of all the bacteria: *S. aureus, E. coli, B. substilis, P. aeruginosa, S. typhi, K. pneumoniae* and on two of the fungi: *C. albicans* and *A. niger* used in this assay. No inhibitory effect was observed for *P. notatum* and *R. stolonifer.* The minimum inhibitory concentration (MIC) (Table **3**) varied with organism: 25 mg/ml for *S. aureus and E. coli,* 50 mg/ml for *B. substilis, S. typhi* and *C. albicans* and 100 mg/ml for *P. aeruginosa, K. pneumoniae* and *A. niger* and are comparable to essential oils of other origins [28, 41, 42]. These results indicate that the essential oil of the plant *M. alternifolius* possesses antibacterial activity.

CONCLUSION

The essential oil of the plant *Mariscus alternifolius* is a rich source of hydrocarbons: Octane, α-Cyperene, Decanal, 2-Decanone, 1-Eicosene, 2-methyl-1-hexadecanol, z-14-nonacosene, Hentriacontane, Octacosyltrifluoroacetate and Tricosane. So it can be correlated that the hydrocarbons found in the essential oil of *M. alternifolius* could also exhibit anti microbial and antioxidant activity, as demonstrated in other essential oils [20 - 31].

Free radical scavenging activity was observed at 5 mg/ml to exhibit 97. 95% inhibition. The essential oil showed free radical scavenging activity comparable to ascorbic acid (97.88%), a known antioxidant agent and also inhibited different strains of gram positive and negative bacteria and fungi.

Thus, this plant is a rich source of plant secondary metabolites of medicinal importance and the results of this study provide some scientific basis for the utilization of the plant in ethno medicine as an insecticide and for the treatment of wounds, bacterial and infectious diseases.

Furthermore, the presence in the essential oil of constituents with proven anti cancer, anti inflammatory [34], anti tuberculosis [35], nematicidal [21], antihypertensive, vasodilatory, angiotensin AT2 receptor antagonist, saluretic [33] and insecticidal [32] capabilities gives further added value to the essential oil of *Mariscus alternifolius* and suggests new practical uses of the essential oil.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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