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Essential oil constituents and *in vitro* antimicrobial activity of the root of *Mondia whitei* (Hook. F.) Skeels (Periplocaceae)

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The composition of the essential oil of the root of *Mondia whitei* was analysed by gas chromatography-mass spectrometry (GC/MS). The agar-well diffusion technique was used for the antimicrobial assay of the oil against nine clinical pathogenic organisms viz. *Bacillus cereus*, *Escherichia coli, Candida albicans, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Staphylococccus aureus* and *Streptococcus pyogenes*. Twenty-eight compounds representing 99.92% of the essential oil were characterized. The major constituents of the oil were (E)-2-hexen-1-ol (25.96%), heptacosane (20.94%), phytol (15.60%), 1-hexanol (8.94%), (E)-2-hexenal (4.29%) and 2-hydroxy-p-anisaldehyde (4.21%). At 10^6 cfu/ml inoculums concentration, the oil was most active against *E. coli* (50.0 mm) and *S. aureus* (48.3 mm) and least active on *C. albicans* (15.0 mm). Generally, the oil exhibited significant ($P \leq 0.05$) antimicrobial activity against the test organisms. The observed antimicrobial activity justify the ethnomedicinal uses of *M. whitei* in Nigeria.

Key words: Essential oil, *Mondia whitei*, root, (E)-2-hexen-1-ol, pathogenic organisms, antimicrobial assay.

INTRODUCTION

Mondia whitei is a climbing shrub medicinally used in tropical Africa. The root and root bark have a vanilla-like odour. The roots are valuable as aphrodisiac, to prevent premature ejaculation, increase sperm production and generally, to treat sexual weakness. A decoction or infusion of the roots is widely used to treat malaria, gastro-intestinal problems, pains and as restorative and appetite stimulant (Gill, 1992; Burkill, 1997). As food, the pulverised bark of *M. whitei* is eaten with fish or peanuts in Democratic Republic of Congo. The fresh or dried leaves are cooked with peanut butter, and eaten as a vegetable in Central and East Africa. In Nigeria and Uganda, dried powdered leaves are added to food as condiment. Due to the fragrance or vanilla-like odour of the root, the dried powdered roots are used in magico-religious mixture in Gabon (Burkill, 1997).

The *in vitro* antioxidant and antimicrobial activities of the ethanol extracts of the leaf and root of *M. whitei* have

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been earlier reported (Gbadamosi and Erinoso, 2015). Although, the aromatic roots of the plant are extensively used in tropical Africa for therapeutic purposes, there is a dearth of information on its active compounds and bioactivities of such compounds. In view of the ethno-medicinal importance of *M. whitei* and the need to further harness its medicinal potential, this study characterized the essential oil of the plant and screened the oil for antimicrobial activity against nine clinical pathogenic microorganisms.

MATERIALS AND METHODS

Source and identification of plant materials

M. whitei whole plants were collected from a forest near Oyo town, Oyo State, Nigeria. The plant was identified (UIH 22405) at the University of Ibadan Herbarium (UIH). The roots were cut from the whole plant, air dried at room temperature, ground into powder and stored in air tight bottle for further experiments.

Essential oil analysis

The essential oil was extracted from 300 g of the powdered sample by hydrodistillation method in an all glass Clevenger-type apparatus fitted to a 5 L round bottom flask and sitted in a heating mantle for 3 h (British Pharmacopoeia, 1980). The essential oil distilled was collected over water from the transparent side arm of the Clevenger apparatus, dried over anhydrous sodium sulphate and then the oil was stored in a vial and kept refrigerated at 4°C until analysis. The composition of the essential oils was determined by gas chromatography-mass spectrometry (GC/MS) using an Agilent 7890N GC with Agilent mass detector Triple Quad 7000A in EI mode at 70 eV (m/z range 40 – 600 amu) and an Agilent Chem Station data system. The GC column was equipped with an HP-5MS column (30 m x 250 µm x 0.25 µm) a split-split less injector heated at 200°C and a flame ionization detector (FID) at 230°C. The oven temperature was programmed as follows: Initial temperature 40^{o}C for 5 min, increased 5 ^{o}C/min to 180°C for 6 min and then 10°C/min to 280°C for 12 min. Helium was the carrier gas at flow rate of 1 mL/min. The injection volume was 2.0 µL (split ratio 1:20). The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data (Adams, 2004). The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column.

Antimicrobial assay of *Mondia whitei* root oil

The antimicrobial assay was carried out using agar well diffusion method (Hood et al., 2003). All overnight cultures of organisms were grown in nutrient broth at 35 ± 2°C for 18 h. 1 ml of the inoculums (10^{6}cfu/ml) was added to 19 mL of sterile nutrient agar. The mixture was poured into Petri-dishes and allowed to solidify. From each plate, two wells were cut using 6 mm cork borer. Each well was filled with 50 µL of the oil or sterile nutrient broth (control). The plates were incubated at 35 ± 2°C for 18 - 38 h. Zones of inhibition were recorded in millimetres (mm). Each experiment was carried out twice.

Statistical analysis

The analysis of variance and comparison of means of data of antimicrobial activity were carried out using Statistical Analysis System (SAS). Means of values were assessed for significance at $P \leq 0.05$ by Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Twenty-eight (28) compounds were detected by GC-SM analysis (Table 1). The identified compounds were characterized by a lofty amount of (E)-2-hexen-1-ol (25.96%), heptacosane (20.94%), phytol (15.60%), 1-hexanol (8.94%), (E)-2-hexenal (4.29%) and 2-hydroxy-p-anisaldehyde (4.21%). Others were linalool (3.02%), cyclohexylmethane (2.09%), 3-octanol (2.68%), 1-octen-3-ol (2.24%), β-ionone (2.1%), m-xylene (1.56%), 3-octanone (1.37%) and 1-octen-3-one (1.25%). 13 out of the 28 compounds recorded <1% concentration of compounds. The two major compounds in the essential oil were (E)-2-hexen-1-ol (25.96%) and heptacosane (20.94%) and the medicinal value of the plant could be attributed to their presence in the oil. (E)-2 hexen-1-ol was identified as one of the major compounds produced as a sex pheromone in the male bug *Pristhesancus plagipennis* which attracts female *P. plagipennis* (James et al., 1994) and has been classified as one of the bioactive compounds which confer medicinal properties on essential oils and is also used in fruit and vegetable flavours and in perfume as it easily isomerizes to (E)-2-hexenal (Valero and Serrano, 2010) while heptacosane (a higher alkane) was identified among compounds which exhibited antibacterial activity (Marrufo et al., 2013; Yogeswari et al., 2012; Mihailovi et al., 2011). It is also possible that addition of one or more of the minor compounds also contribute to the observed bioactivity of the oil since constituents at even very low concentrations have been found to contribute to the aroma, flavour or activity of essential oils. The results could justify the therapeutic used of *M. whitei* root as aphrodisiac and appetizer, as well as remedy for the treatment of an array of diseases.

Most reported bioactivities of *M. whitei* are based on the screening of extracts and active compounds of the plant parts, especially the root with little or no information in literature on the activity of its oil. Watcho et al. (2007) reported the effects of the aqueous and hexane root extracts of the plant on sexual activity of male rats. Also, Patnam et al. (2005) isolated chlorinated coumarinolignan from the roots of *M. whitei*. The plant showed promising *in vitro* antidepressant properties (Pedersen et al., 2008). The non-toxic effect of the plant in mice has also been reported (Kuo et al., 2006).

The essential oil of *M. whitei* root exhibited varied *in vitro* antimicrobial activity against the nine clinical organisms (Table 2). The oil was most active against *Escherichia coli* (50.0 mm) and *Staphylococccus aureus* (48.3 mm) and least active on *Candida albicans* (15.0

Table 1. Components of essential oil of *M. whitei* root.

S/N	Compound	*RI	+RI	Composition (%)
1	Cyclohexylmethane	781	-	2.09
2	Toluene	794	785	0.87
3	(E)-2-Hexenal	814	846	4.29
4	1-Hexanol	862	863	8.94
5	(E)-2-Hexen-1-ol	868	859	25.96
6	m-Xylene	907	893	1.56
7	p-Xylene	907	893	0.77
8	1-Octen-3-one	943	969	1.25
9	3-Octanone	952	962	1.37
10	1-Octen-3-ol	969	974	2.24
11	3-Octanol	979	989	2.68
12	Benzaldehyde	982	999	0.21
13	Linalool	1082	1088	3.02
14	2-Methyl-2-nonen-4-one	1136	_	0.69
15	Fenchol	1138	1147	0.21
16	Safranal	1186	1196	0.39
17	β-Cyclocitral	1204	1200	0.21
18	4,4,7a-Trimethyl-2,4,5,6,7,7a-hexahydro-1H-inden-1-one	1371	_	0.41
19	2-Hydroxy-p-anisaldehyde	1392	_	4.21
20	Megastigmatrienone	1454	1462	0.28
21	β-Ionone	1457	1460	2.1
22	Dodecanoic acid	1570	1550	0.46
23	Apiole	1705	1697	0.48
24	Hexahydrofarnesylacetone	1754	1751	0.62
25	Isobutyl phthalate	1908	_	0.36
26	Phytol	2045	2045	15.6
27	Heptacosane	2705	2705	20.94
28	1,2-Cyclohexanedicarboxylic acid dinonyl ester	2965	_	4.96
	Total			99.92

Monoterpene hydrocarbons	0.00
Oxygenated monoterpene	3.62
Sesquiterpene hydrocarbon	0.00
Oxygenated sesquiterpene	2.59
Diterpene	15.60
Non-terpenes	78.12
Total	99.92

Identification of compounds was from gas chromatography-mass spectrometry (GC-MS) spectra, using retention time and mass spectrum. *RI = Retention indices on HP-5 ms capillary column. +RI = Retention indices from literature (Adams, 2004) on DBS capillary column.

mm). There was no significant difference in the activity of the oil against *E. coli*, *S. aureus* and *Streptococcus pyogenes*. Also, there was no statistical variation in the oil inhibitory effect against *K. pneumoniae* (36.66 mm), *P. mirabilis* (35.00 mm) and *P. aeruginosa* (35.00 mm) at 10^6 cfu/ml inoculum concentration. The cumulative effect of the oil of *M. whitei* root against nine pathogenic organisms is presented in Table 3. The oil showed significant ($P<0.05$) inhibitory effect against all the isolates.

There is little information in literature on the antimicrobial effects of *M. whitei*. However, the present study shows that the antimicrobial effect of the root oil against organisms conforms to previous reports on the anti-infective potential of the extracts of the plant (Gbadamosi and Erinoso, 2015; Okitoi et al., 2007; Fankam et al., 2011). When compared with the findings of Gbadamosi and Erinoso (2015), the antimicrobial

Table 2. *In vitro* antimicrobial activity of *Mondia whitei* root oil.

S/N	Organism (10^6 cfu/ml)	Inhibitory zone (mm)
1	*Escherichia coli*	50.00[a]
2	*Staphylococcus aureus*	48.33[a]
3	*Streptococcus pyogenes*	46.66[a]
4	*Klebsiella pneumoniae*	36.66[b]
5	*Proteus mirabilis*	35.00[b]
6	*Pseudomonas aeruginosa*	35.00[b]
7	*Bacillus cereus*	25.00[c]
8	*Salmonella typhi*	20.00[cd]
9	*Candida albicans*	15.00[d]

Values are mean of 2 readings. Diameter of the cork borer = 6 mm. Means with the same letter in each column are not significantly ($p \leq 0.05$) different from one another.

Table 3. Cumulative inhibitory effect of *Mondia whitei* oil against test organisms.

Source	DF	SS	MS	F-value	Pv > F
Model	10	3809.26	380.93	21.23	0.001**
Organisms	8	3796.30	474.54	26.45	0.0001**
Replicates	2	12.96	6.48	0.36	0.7023
Error	16	287.04	17.94		
Corrected Total	26	4096.30			

**Highly significant; $p \leq 0.05$.

effect of the extracts (water and ethanol) and oil of *M. whitei* is in the order: root oil > root water extract > root ethanol extract. The root oil could be the most active antimicrobial agent. The antimicrobial activity exhibited by the oil is an indication that the oil could be valuable in the treatment of infectious diseases, especially infections associated with the test organisms used in the present study. The oil could have therapeutic values in the management of urinary tract infections (*E. coli*, *P. aeruginosa* and *K. pneumoniae*), wound infections (*E. coli*, *P. aeruginosa* and *S. pyogenes*), meningitis (*E. coli*, *S. aureus*, *S. pyogenes* and *K. pneumoniae*), ear, nose and throat infections (*E. coli*, *S. aureus* and *S. pyogenes*), dysentery (*E. coli*), fever (*S. typhi* and *S. pyogenes*), pneumonia (*K. pneumoniae* and *S. pyogenes*), septic arthritis (*S. pyogenes* and *P. aeruginosa*) and skin infections (*C. albicans*, *S. aureus* and *S. pyogenes*) (Neugebauer, 1993; Gbadamosi and Oyedele, 2012).

Conclusion

From the results of this study, it is concluded that the volatile oil of *M. whitei* root is very rich in (E)-2-hexen-1-ol (25.96%) and heptacosane (20.94%), the active compounds might be responsible for the strong antibacterial activity and weak antifungal activity of the oil observed in this study. The findings of the present study could form basis for future research on bioactivities of volatile oil of *M. whitei*, especially on its therapeutic values in the management of infectious diseases such as diarrhoea, bronchitis, urinary tract infection, sexually transmitted infections and skin infections.

Conflict of interests

The authors have not declared any conflict of interests.

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