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Full Length Research Paper

The chemical composition of essential oil from the root of *Cissampelos owariensis* (p.beauv) and free radical scavenging activities of its extracts

Olajide Olutayo[1], Orishadipe Abayomi[1]*, Fatokun Olakunle[1], Akande Temitope[2] and Iliya Ibrahim[3]

[1]Chemistry Advanced Laboratory, Sheda Science and Technology Complex, Sheda-Abuja, Nigeria.
[2]Nuclear Technology Centre, National Atomic Energy Commission, Sheda- Abuja, Nigeria.
[3]National Institute for Pharmaceutical Research and Development, Idu- Abuja, Nigeria.

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Air dried root of *Cissampelos owariensis* (P.Beauv) on steam distillation yielded 0.82% (w/w) of essential oil. Gas chromatography–mass spectrometry (GC-MS) analysis of the oil resulted in the identification of 11 compounds, among which 1H-cyclopropa[a]naphthalene,1a, 2, 3, 5, 6, 7, 7a, 7b-octahydro-1, 1, 7, 7a-tetramethyl-,(1aR-1aa, 7a, 7aa, 7ba) was found to be the most abundant at 39%. The antioxidant features of the 3 extracts (methanol, chloroform, and n-hexane) of the plant were also evaluated by UV-visible spectrophotometer at 517 nm using inhibition of 2, 2 –diphenyl-1-picrylhyrazyl radical (DPPH) and vitamin C as standard. Out of all the extracts evaluated, only the methanolic extract exhibited potent antioxidant activity that was concentration dependent, followed by chloroform extract as compared with vitamin C.

Key words: *Cissampelos owariensis*, diphenyl-1-picrylhyrazyl radical (DPPH), essential oil, free radical scavenging, inhibition.

INTRODUCTION

Cissampelos owariensis (P. Beauv) is a plant under the menispermaceae family, which comprises of about 450 species found in the Tropical region. It is a climber with twinning spindly hairy leaves and bears small flowers with green leaves. The English name is referred to as velvet leaf and it is known across West Africa by different names. The leaves are used widely for their healing properties; alone they are applied externally to abscess or in some form of preparation to abscess, scabies and sores (Bouquet and Debray, 1974; Watt and Breyer-brandwijk, 1962). When the leaves are crushed up, macerated or cooked, it can be taken internally for diarrhoea, to promote menstrual flow and for painful or irregular menses (Walker, 1953; Bouquet, 1969). The root is very bitter and has many medicinal uses. It aids infertility, assist in difficult pregnancy and prevent threatened miscarriage. Root chewed with Tiger nuts (the rhizome of *Cyperus esculentus* (Linn.) are said to be aphrodisiac (Dalziel, 1937). In fact, rats were administered with ethanolic extract of *C. owariensis* at dose rate of 100 and 200 mg/kg body weight orally for 14 days respectively.

Blood glucose concentration and body weight was measured by ACCU Chek Glucometre test kit and electronic balance, compared with a patent drug glibenclamide at a dose rate of 100 mg/kg body weight. The herbal preparation of this plant significantly increased body weight gain and decreased blood glucose

*Corresponding author. E-mail: oorisat@yahoo.com.

when compared with patent drug (Ekeanyanwu et al., 2012). Previous phytochemical studies from the *Cissampelos* genus described the isolation and characterisation of isochrondrodendrine, berberine and cycleanine from the roots and leaves which are endowed with anti-malarial, anti- bacterial as well as phytotoxic agents (Southon and Buckingham, 1989). Also, the air-dried root of *C. owariensis* was successively extracted with n- hexane and 95% ethanol. The two extracts were chromatographed on a Florisil each to give owarienone and cissampelone respectively.

These two sesquiterpenes showed reasonable activities from 625 to 2500 µg/ml against some human pathogens (Efiom et al., 2009). As part of our investigation on some aromatic and medicinal plants of Nigeria, we report for the first time in this paper chemical composition of the volatile oils isolated from the root of *C. owariensis* and free radical scavenging activities of hexane, chloroform and methanol extracts from Nigeria and newly identified compounds

MATERIALS AND METHODS

Plant materials

The root of *C. owariensis* was collected from the field in Damaturu, Yobe State through the assistance of a traditional herbal dealer. Identification was done by Mrs. Grace Ugbabe, a taxonomist of the Herbarium unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja, where voucher specimens were deposited.

Oil isolation

1000 g of air-dried root sample of *C. owariensis* was pulverised and subjected to steam-distillation for 3 h in a clevenger type apparatus according to the British pharmacopoea specification. The resulting oil was collected, preserved in a sealed sample bottle, and stored in a refrigerator until required for analysis.

Extraction/partitioning procedure

The air-dried sample (300 g) was also extracted with 2 L of methanol using Soxhlet apparatus for 18 h. The extract was collected and concentrated with the aid of a Stuart rota-vapor and kept in a refrigerator. The crude methanol extract was then partitioned successively in n-hexane and chloroform. Thereafter, free radical scavenging activities test was carried out on the fractions [scavenging effect on 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH)].

Phytochemical screening

For the purpose of this study, phytochemical screenings were carried out on the crude methanol extract obtained to confirm the presence or absence of the following plant secondary metabolites: alkaloids, tannins, flavonoids, phenols, resin, balsams, saponins, sterols, terpenes, anthraquinones, cardiac glycosides, and carbohydrates (Harborne, 1973; Trease and Evans, 1989).

Alkaloids: 0.2 g of methanolic extract was shaken with 1% HCl for 2 mins. The mixture was filtered and few drops of dragendorff's reagent were added. Formation of a precipitate indicates the presence of alkaloids.

Saponins

0.2 g of methanolic extract was shaken with 5 mls of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins.

Tannins

0.2 g of methanolic extract was stirred with distilled water and filtered. Ferric chloride was added to the filtrate. A blue black, or green or blue green precipitate was taken as an evidence for the presence of tannins.

Test for steroids (Salkowski's test)

0.2 g of the extract was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicates the deoxy sugar characteristics of Cardenolides. A Violet ring may form just above the ring and gradually spread throughout the layer.

Test for cardiac glycoside-(Keller-Killani test)

0.2 g of the methanolic extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycosides.

Test for carbohydrates

5 ml of the methanolic extract was placed in a test tube. Few drops of molisch reagent were poured through the side of the test tube containing 10 ml of sulphuric acid solution held in a slant position. The acid forms a layer beneath the aqueous solution without mixing with it. A reddish brown solution indicates the presence of carbohydrate.

Test for flavonoids (Shinoda test)

A little amount of magnesium powder and a few drops of concentrated HCl were added to 3 ml of the methonolic extract. A red or intense red colouration indicates the presence of flavonones.

Test for resins

5 ml of copper acetate solution was added to 5 ml of the methanolic extract. The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin.

Test for anthraquinones (Born-Trager's test)]

0.2 g of the methanolic extract was shaken with 4 ml of benzene. The mixture was filtered and 2 ml of 10% ammonia solution was

Table 1. Percentage of inhibition at different concentrations (mg/ml) compared with vitamin C.

Parameter	Different concentration						
Extracts/concentration (mg/ml)	0.05	0.1	0.2	0.5	1.0	2.0	5.0
Methanol extract (%)	0.09	1.90	2.75	28.79	35.26	51.64	87.16
Chloroform extract (%)	0.01	0.02	0.05	0.08	10.17	43.97	57.57
Hexane extract (%)	0.00	0.01	0.0	1.02	1.15	2.03	2.41
Vitamin C (standard) (%)	90.19	90.47	90.77	91.32	91.50	90.41	85.00

added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammonical (lower phase) indicates the presence of free anthraquinones.

Test for phenols

0.2 g of methanolic extract was dissolved in $FeCl_3$ solution. A green or dirty green precipitate indicates the presence of phenolic compound.

Gas chromatography

Quantitative and qualitative data were determined by GC-MS respectively. *C. owariensis* oil was injected into a Thermo-Scientific Trace GC ULTRA system coupled to DSQ II mass spectrometer, and equipped with an AS 3000 auto sampler and a split/split-less injector. The column used was an TR-5MS, 30 m × 0.25 mm i. d., 0.25 µm d. f., coated with 5% diphenyl-95% polydimethyl siloxane , operated with the following oven temperature programme: 140°C, held for 1 min, rising at 8°C/min to 300°C, injection temperature and volume,250°C and 1.0 µl, respectively; injection mode, split, split ratio, 15;1; carrier gas ,helium at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280°C. The components of the sample were identified based on the basis of their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 1989) and those of reference compounds from Library of National Institute of Standard and Technology (NIST) database.

Anti-oxidant activities of *C. owariensis* extracts

Scavenging effect on 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH)

The radical scavenging activities of the plant extracts against DPPH (Sigma-Aldrich) were determined by UV-Visible Spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described (Brand-Williams et al., 1995; Ayoola et al., 2006). The following concentrations of the extracts were prepared, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol (Analar grade). Vitamin C (Ascorbic Acid) was used as the antioxidant standard at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml.1 ml of the extract was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol and thereafter the decrease in absorption was measured on a UV-Visible Spectrophotometer 10 minutes later. A blank / control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against that of the control and the percentage inhibition was calculated. All test and analysis were run in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation below:

$$\%\ Inhibition = \left\{\frac{A_b - A_a}{A_b}\right\} \times \frac{100}{1}$$

Where, A_b is the absorption of the blank sample (without the extract) and A_a is the absorption of the extract.

RESULTS AND DISCUSSION

The methanol extract of *C. owariensis* roots was found to contain alkaloids, tannins, flavonoids, resins, balsams, saponins, sterols, terpenes, cardiac glycosides, phenols, and carbohydrate. These secondary metabolites are known to exhibit various biological activities such as antimicrobial and anti-oxidant activities which have been associated with their intrinsic reducing capability as pro-oxidants. The presence of these secondary metabolites especially alkaloids and flavonoids justify the use of *C. owariensis* in ethno-medicine.

Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions. Oxidative stress is the excess formation or incomplete removal of highly reactive molecules such as reactive oxygen species (ROS). Antioxidants may play a role on prevention of diseases such as cancer, cardiovascular disease by scavenging free radicals (Aruma, 1998). Methanol, Chloroform, and hexane were used as the solvents for the extraction according to their polarity (polar to non polar).

Out of all the extracts evaluated, only methanol extract exhibited potent antioxidant activity at higher concentration (5 mg/ml), followed by chloroform extract when compared with vitamin C (Ascorbic acid), the presence of flavonoids and tannins in crude methanol extract is likely to be responsible for the free radical scavenging effects observed in Table 1 and Figure 1. Even at higher concentrations, hexane extract is not a good antioxidant. Flavonoids and tannins are phenolic compounds and plant phenolic is a major group of compounds that act as primary antioxidant or free radical scavengers (Polterait, 1997). The biological functions of flavonoids include protection against allergies, inflammation, free radicals scavenging, platelets

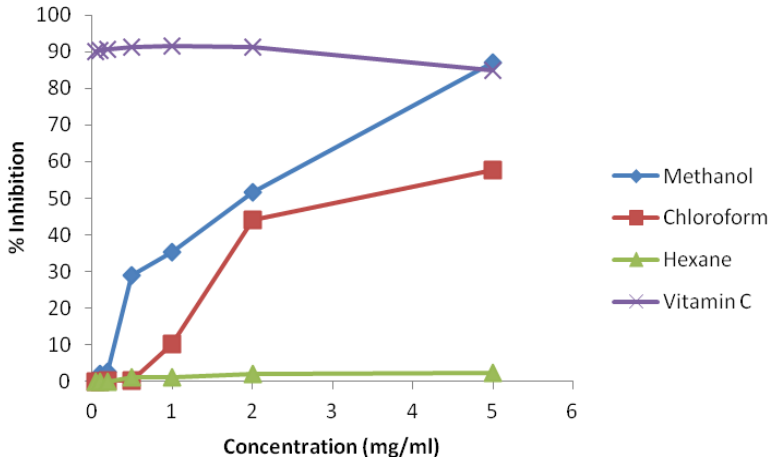

Figure 1. Antioxidant activity curve for methanol, chloroform, and hexane extracts of *C. owariensis* and vitamin C standard.

aggregation, microbes, ulcers, hepatoxins, viruses, and tumours (Ayoola et al., 2008). The DPPH test provides information on the reactivity of the test compounds with stable free radical and it gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and the degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The results suggest that the antioxidant activity of this plant may contribute to their claimed anti-malarial property. However, from our analysis, a larger percentage of the extracts showed the ability to scavenge the free radical used in a concentration dependent manner as shown in the Table 1.

Essential oils are complex mixtures comprising of many single compounds, each of the constituents contributes to the biological effects of these oil (Buchbauer, 2000). The yield of the volatile oil obtained by steam distillation of the root of *C. owariensis* was 0.82% (w/w). The oil was light yellow in colour but it solidifies after sometimes. It was soluble in petroleum ether and analyses permitted the identification of 11 compounds, accounting for about 91.8% of the whole volatiles. It is worth mentioning that compounds such as ethyl iso-allocholate, 3-ethy-5(2-ethylbutyl) octadecane, acetic acid thiocyanato- 1,7,7-trimethylbicyclo[2.2.1] hep-2-yl ester, methyl- 20 methyl heneicosanoate, docosahexaenoic acid 1,2,3 propanetriyl ester, 2,2,7,7-tetramethyltricyclo[6.2.1.0 (1,6)] undec-4-en-3-one, methyl 18- propylhenicosanoate, have not been reported previously as part of the constituent of this plant. These two compounds, 5(1H) - azulenone-2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(l-methyl ethylidene) and 2H-cyclopropa(a) naphthalene-2-one,11a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl have been isolated and reported earlier from the n–hexane and 95% ethanol extracts of this plant (Efiom et al., 2009). As shown in Table 2, a total of 11 compounds were identified from the retention indices and mass spectra data. Which were also confirmed by the spectral data in Figure 2. They were aromatic compounds, hydrocarbon monoterpenes and oxygenated sesquiterpenes. In this present work, some common compounds such as α- terpineol, limonene, 1, 8-cineole, β-pinene, α-pinene, globulol and linalool are all absent. Geographical and climatic conditions have been implicated as factors responsible for this. Other factors may include the age of the plant, time of harvest and method of extraction. Though, steam and hydro-distillations are the most prominent technique for essential oil extraction. Demerit of this technique includes modification of components by auto-oxidation during distillation (Asekun and Ekundayo, 2003).

Conclusion

The extraction and chemical composition of the essential oil of *C. owariensis* roots was evaluated. The presence of some new compounds in this study showed that, the *C. owariensis* of Nigeria may suggest the existence of a new chemo type of the plant family. Further investigation of *Cissampelos* essential oil from other geographical/ecological regions will help in this regard.

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Table 2. Chemical constituents of the essential oil of *C. owariensis* through GC-MS.

Compound	Retention time	% Yield	Mass spectra
Docosahexaenoic acid 1, 2, 3, propane triyl ester	4.84	3.93	57,93,121, 276, 451, 630
Acetic acid, thiocyanato-, 1,7,7, trimethylbicyclo[2.2.1]hep-2-yl ester	5.43	2.41	57, 82, 95, 179,565, 649
3H-3a, 7-methanoazulene 2, 4, 5, 6, 7, 8- hexahydro- 1,4,9, 9-tetramethyl-[3aR-(3aa,4a,7a)]	7.53	5.05	55,91, 204,491, 589, 567
1H-cyclopro[e] azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl- [1aR-(1aa,4a,4aa,7ba)]	7.75	4.94	55, 79, 105,207,573,583.
1H-cyclopropa(a) naphthalene, 1a, 2,3,5,6,7,7a,7b- octahydro-1,1,7,7a-tetramethyl-,[1aR-(1aa,7a,7aa,7ba]	7.99	39.07	79, 91, 161, 189,207, 598
2,2,7,7-tetramethyltricyclo[6.2.1.0 (1,6)]undec-4-en-3-one	15.92	23.20	67,77,161,218,430, 579
Methyl 20- methylheneicosanoate	25.31	4.45	74, 87,129,207,486,621
Methyl 18-propylhenicosanoate	26.60	1.25	51, 57, 74,344,383,613
Ethyl iso-allocholate	26.66	1.87	51,69,87,318,626,647
3-ethyl-5-(2-ethylbutyl) octadecane	27.96	1.38	57,85,207,281,571,626
9,12,- octadecadienoic acid (z,z)-2,3-bis[(trimethylsilyl)oxy]propylester	28.17	1.73	57, 73, 85, 281,460,649

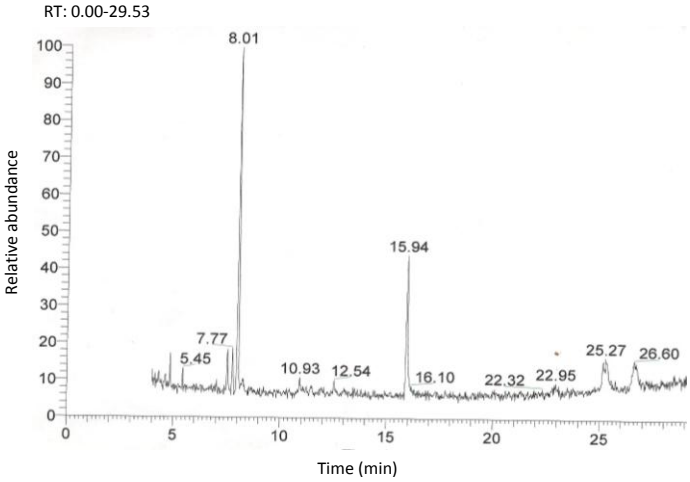

Figure 2. Total ions chromatogram of root essential oil of *C. owariensis.*

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