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Chemical Constituents and Antimicrobial Activity of Essential Oil of the Whole Plant of *Cleome viscosa* L. (Cleomaceae)

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Abstract *Cleome viscosa* L. (Cleomaceae) is an annual herb that is widely distributed in the tropical and warm temperate regions. It has many ethnomedicinal, ethnobotanical and economic importance. This study investigated the essential oil constituents and antimicrobial activity of *C. viscosa* whole plant due to scarcity of information on these properties. The essential oil from the whole plant was extracted by hydrodistillation and characterised using Gas Chromatography (GC) and Gas Chromatography- Mass Spectrometry (GC-MS) techniques. The components of the essential oil were identified based on retention indices and comparison with published spectra. The agar-well diffusion technique was used for the antimicrobial screening of the essential oil against nine pathogenic organisms obtained via due process from University College Hospital, Ibadan, Nigeria. Data were statistically analysed. 20 compounds representing 94.7% of the essential oil were characterised. The most abundant component of the oil was 1,10-di-epi-cubenol (30.0%); an oxygenated sesquiterpene and 5 out of the 20 components had less than 1.0 % concentration of compound viz. nonanal (0.7%), cis-α-ambrinol (0.9%), calamenene 10,11-epoxide (0.5%), α-calacorene (0.7%) and n-hexadecane (0.8%). At 10^6 cfu/ml, inoculum concentration of organism, the essential oil exhibited significant antimicrobial activity against 6 out of the 9 organisms. The F-value for the antimicrobial assay was significant for the model and organisms. The oil showed remarkable antimicrobial activity against *Salmonella typhi* (47.50 mm), *Candida albicans* (41.00 mm), *Escherichia coli* (39.00 mm), *Klebsiella pneumoniae* (31.50 mm), *Pseudomonas aeruginosa* (31.00 mm) and *Streptococcus pyogenes* (30.00 mm). The bioactivity might be attributed to abundant sesquiterpenes component of the oil. Ethnobotanically, the essential oil could be the treatment of infections such as urinary tract infection, sore throat, typhoid fever and candidiasis. However, toxicological study of the oil will confirm its safety in administration.

Keywords *Cleome viscosa*, volatile oil, terpenes, 1,10-di-*epi*-cubenol, infections, herbal medicine

Introduction

Cleome viscosa a member of the family Cleomaceae is a small size herb with yellow flowers and long slender pods containing seeds. The leaf and the stem surfaces of the plant show presence secretory trichomes with club cylinder and cylinder morphologies [1]. *C. viscosa* is a widely distributed annual, sticky herb that attains a height of up to 120 cm. It is a fodder plant and it has many ethnomedicinal, ethnobotanical and ecological importance [2].

C. viscosa is used traditionally for the treatment of many diseases. The leaves are used for the treatment of fever, malaria fever, skin diseases, diarrhoea, inflammation, bronchitis and other respiratory tract infections. Also used as diaphoretic, rubefacient and vesicant. The leaf juice has therapeutic effect in the treatment of piles, lumbago and earache [3]. The fresh leaves of *C. viscosa* are consumed as vegetable and the seeds are used in curries. The seeds are also added to tobacco to enhance narcotic quality [4]. The seeds contain 18.3% oil and the oil is very similar in fatty acid composition to non-edible oils of rubber, *Jatropha*, safflower, linseed, pongamia and rapeseed edible oils [5].

The plant has various biological activity such as antidiarrheal, antifungal, antiulcer, antidiabetic, antioxidant, antianaemic, insecticidal, anticancer, antiplasmodial, cytotoxic, antitumor, anti-inflammatory and hepatoprotective effects [1, 6-8]. In view of scarcity of information on the essential oil constituents of *C. viscosa* whole plant, this study investigated the essential oil constituents of the plant and screened the oil for antimicrobial activity against nine pathogenic organisms.

Materials and Methods

Analysis and identification of components of essential oil of *Cleome viscosa* whole plant

Extraction of Essential oil: The essential oil of *Cleome viscosa* whole plant was extracted from 300 g of the shredded 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 3h [9]. 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 for further use.

Gas Chromatography (GC): The essential oil was analysed on an Agilent Model 7890A Gas Chromatography equipped with a HP-5MS fused silica capillary column of dimension 30 x 0.25 mm, film thickness 0.25 μm. The analytical conditions were programmed as: Oven temperature: 60 ^{o}C, with 2 minute initial hold, and then to 280 ^{o}C at 4 ^{o}C/min, with final hold time of 10 minutes; helium was used as carrier gas at a flow rate of 1 mL/min. Retention indices were determined with reference to a homologous series of normal alkanes analyzed under the same conditions. Percentage composition of each constituent was calculated by integration of the GC peak areas.

Gas Chromatography-Mass Spectrometry (GCMS): GC-MS analyses were performed on an Agilent Model 7890A Gas Chromatography interfaced to an Agilent 7000 GC/MS Triple Quad. The temperature program used for the GC was the same as described above. The MS was operated in EI mode with ionization voltage 70eV and ion source temperature, 250 ^{o}C.

Components identification: The components of the essential oil were identified based on their retention indices. Identification confirmation was by comparison of their mass spectra with published spectra and those of reference compounds from the Library of National Institute of Standard and Technology (NIST) [10] database and literature [11-12].

Results and Discussion

Twenty (20) compounds representing 94.7 % of essential oil of *C. viscosa* whole plant were characterised by GC-MS analyses (Table 1). The essential oil constituents were in the order: 1,10-di-*epi*-cubenol (30.0 %) > δ-cadinene (16.2 %) > Pentadecanal (8.1 %) > caryophyllene oxide (7.1 %) > *(E)*-β-ionone (6.9 %) > humulene epoxide II and T-cadinol (4.6 %) > *cis*-14-muurol-5-en-4-one (3.1 %) > α-muurolol (2.8 %) > *(E)*-geranylacetone (1.5 %) > germacrene D (1.4 %) > *trans*-cadina-1,4-diene (syn. cubenene) (1.3 %) > *cis*-α-ambrinol and *cis*-cadina-1,4-diene (1.2 %) > Tridecanal (1.1 %). 5 out of the 20 constituents recorded < 1.0 % concentration of compounds. Generally, the essential oil was richest in oxygenated sesquiterpenes (52.20 %), followed by sesquiterpene hydrocarbons (22.20 %), non-terpene derivatives (10.7 %) and apocarotenes (9.6 %). No oxygenated monoterpenes was characterised in the essential oil of *C. viscosa* whole plant.

The essential oil of *C. viscosa* showed significant (P<0.05) antimicrobial activity against nine pathogenic organisms (Table 2). It was very active against *Salmonella typhi* (47.50 mm), *Candida albicans* (41.00 mm), *Escherichia coli* (39.00 mm), *Klebsiella pneumoniae* (31.50 mm), *Pseudomonas aeruginosa* (31.00 mm) and *Streptococcus pyogenes* (30.00 mm). The oil was moderately active against *Proteus mirabilis* (19.50 mm) and *Staphylococcus aureus* (11.50 mm). The least antimicrobial activity was recorded against *Bacillus cereus* with 10.00 mm zone of inhibition. The F-value of the antimicrobial assay was highly significant (P<0.05) for the model and organisms used for the experiments (Table 3).

Table 1: Chemical components of essential oil of whole plant of *Cleome viscosa*

S/N	Constituents	l.r.i.	*C. viscosa* Essential oil
1	Nonanal	1102	0.7
2	*cis*-α-ambrinol	1437	1.2
3	*(E)*-geranylacetone	1456	1.5
4	*cis*-muurola-4(14),5-diene	1463	0.9
5	germacrene D	1482	1.4
6	*(E)*-β-ionone	1487	**6.9**
7	calamenene 10,11-epoxide	1495	0.5
8	*cis*-cadina-1,4-diene	1496	1.2
9	Tridecanal	1510	1.1
10	δ-cadinene	1524	**16.2**
11	*trans*-cadina-1,4-diene (syn. cubenene)	1533	1.3
12	α-calacorene	1543	0.7
13	caryophyllene oxide	1582	**7.1**
14	*n*-hexadecane	1600	0.8
15	humulene epoxide II	1607	**4.6**
16	1,10-di-*epi*-cubenol	1615	**30.0**
17	T-cadinol	1641	**4.6**
18	α-muurolol	1646	2.8
19	*cis*-14-muurol-5-en-4-one	1690	3.1
20	Pentadecanal	1716	**8.1**
Oxygenated monoterpenes			0.0
Sesquiterpene hydrocarbons			**22.2**
Oxygenated sesquiterpenes			**52.2**
Apocarotenes			**9.6**
Non-terpene derivatives			**10.7**
Total identified			**94.7**

Table 2: *In vitro* antimicrobial effects of *C. viscosa* essential oil on deleterious human microbes

S/N	Test Organisms	Zone of Inhibition (mm)
1	*Bacillus cereus*	10.00^{e}
2	*Escherichia coli*	39.00^{b}
3	*Candida albicans*	41.00^{b}
4	*Klebsiella pneumoniae*	31.50^{c}
5	*Pseudomonas aeruginosa*	31.00^{c}
6	*Proteus mirabilis*	19.50^{d}
7	*Salmonella typhi*	47.50^{a}
8	*Staphylococcus aureus*	11.50^{e}
9	*Streptococcus pyogenes*	30.00^{c}

Means with the same alphabets down the COLUMN are not significantly different at P<0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means" only. Replicates = 2.

Table 3: Cumulative *in vitro* inhibitory effects of *C. viscosa* essential oils on test organisms

Source	DF	SS	MS	F-value	Pv > F
Model	8	2710.00	338.75	41.20	0.0000***
Organisms	8	2710.00	338.75	41.20	0.0000***
Error	9	74.00	8.22		
Corrected Total	17	2784.00			

*** Highly significant at P<0.05.

C. viscosa essential oil had 30.0% 1,10-di-epi-cubenol content. This same compound was reported as part of essential oil constituent of *Ageratum conyzoides* and the essential oil of *A. conyzoides* showed moderate activity against *Staphylococcus aureus* and *Enterococcus faecalis* [13]. It is important to note that the essential oil of *C.*

viscosa also showed a moderate antibacterial activity against *Staphylococcus aureus* (11.50 mm) in the present study.

The caryophyllene oxide (CPO) constituent of *C. viscosa* oil was 7.1%. The antitumour effect of β-caryophyllene oxide isolated from essential oils of guava (*Psidium guajava*), oregano (*Originum vulgare*), cinnamon (*Cinnamomum* spp), clove (*Eugenia caryophyllus*) and black pepper (*Piper nigrum*) has been reported by previous authors [14]. The results suggested CPO as a potential therapeutic candidate for both prevention and treatment of cancer.

According to Ambrož *et al.* (2019) [15], CPO is a potential anti-proliferative agent in colon cancer. The action could be due to its ability to disrupt the mitochondrial membrane potential and to activate initiator caspases.

β-ionone has anti-proliferative and apoptotic effects on K562 cells, and could be used for the treatment of some leukemia sub-types [16]. The presence of CPO and β-ionone in *C. viscosa* essential oil presents the plant as a potent anti-cancer agent. Caryophyllene oxide showed antioxidant and anti-inflammatory activities in mice. It was effective against paw edema, exudates volume and leucocyte migration and ear edema [17]. CPO showed gastroprotective activity in rats [18]. Inferably, *C. viscosa* could have anti-inflammatory and gastroprotective potential.

In this study, the essential oil of *C. viscosa* contained 74.4% sesquiterpenes in the form of sesquiterpene hydrocarbons, 22.2% and oxygenated sesquiterpene 52.2%. Essential oil terpenes generally, are phytochemical compounds produced in different biological pathways as pollinator attraction signals, the compounds also act as defense mechanisms against insect pests, microorganisms, competing plants and herbivores, thereby protecting plants against parasites and microbial infestation. Previous authors have reported the antifeedant effect and phytotoxic activity of essential oil terpenes [19]. Diterpenes are expectorants, antifungal and antiviral. Some are purgative, while some influence hormonal system [20]. Triterpenes have been reported to possess antitumour, insecticidal and antimicrobial activity [8]. The high sesquiterpenes constituent of *C. viscosa* might be responsible for the observed antimicrobial activity of its essential oil against the pathogenic organisms especially *Salmonella typhi*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The results showed that the essential oil could be explored for the treatment of infections associated with actively susceptible organisms as follows: *Escherichia coli* (urinary tract infection, wounds, meningitis, bronchitis, otitis and sinusitis), *Streptococcus pyogenes* (sore throat, wounds, fever, pneumonia, rheumatism, arthritis and tonsillitis/pharygngitis), styphi (typhoid fever), *Pseudomonas aeruginosa* (Urinary tract infection, athlete infection, wound infection and secondary bone and joint infection), *Klebsiella pneumoniae* (Pneumonia, otitis, urinary tract infection, cholectytitis, osteomyelitis and infantile enteris) and *Candida albicans* (Candidiasis) [13, 21]. Also, Sesquiterpene hydrocarbons are used, therapeutically as anti-inflammatories, sedatives, anti-spasmodics, anti-allergenics and decongestants (anti-phlogistics) [20]. Generally, essential oil constituents of plants have been reported to possess antifungal activity [22], antidermatophytic activity (especially sesquiterpenes) [23] as well as antioxidant activity [24-25].

Conclusion

The essential oil of *Cleome viscosa* whole plant displayed varied antimicrobial activity against nine pathogenic organisms. It showed remarkable antimicrobial activity against 6 out of the 9 organisms. The bioactivity could be attributed to sesquiterpenes which was the major constituent of the essential oil. This study justifies the ethnobotanical uses of *C. viscosa* for the treatment of typhoid bronchitis, fever, urinary tract infections, respiratory tract infections, arthritis and rheumatism. However, toxicological study of the oil will confirm its safety in administration.

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