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Research Article

Antibaterial activity of the GC-MS characterized essential oil from the leaves of *Crassocephalum crepidiodes* from North Central Nigeria

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Abstract

This study was carried out to isolate and evaluate the antibacterial activity of the leaf essential oil of *Crassocephalum crepidiodes* against selected bacteria. Hydrodistillation of 500 g of sample of the plant's leaves (for four hours) gave a yield of 0.10 %(w/w) of volatile oil. The oil was characterized using GC-MS and the result showed abundant of monoterpenoids in the oil and D-limonene (77.1 %) was the predominant constituent. Meanwhile, linalool (2.6 %), α-pinene (5.5 %), β-caryophyllene (2.4 %), and α-terpineol (2.6 %) were minor relative to the amount of limonene but are significant constituents of the oil. The agar well diffusion method was used to determine the antibacterial activity of the oil against *Enterococcus faecalis, Bacillus cereus, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi* and *Salmonella* sp. Gram-positive bacteria were more susceptible to the oil than Gram-negative bacteria. *S. aureus* was the most susceptible to inhibition at the lowest concentration (25 %) used in this study. Therefore, the oil has applications for the treatment of infections caused by the Gram-positive bacteria, especially *S. aureus*.

1. Introduction

Crassocephalum crepidioides of the family Asteraceae is found commonly in Australia, Africa and Asia. In south-western Nigeria, where its succulent leaves and stems are eaten as vegetables, the plant is known as "ebolo". It is also used in many folk medicines to treat indigestion, stomach aches, nose bleeds epilepsy, and wounds. Solvent extracts from the leaves of this plant inhibited *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa* and established its antimicrobial activity [1–3]. Alkaloids, flavonoids, coumarins, tannins, saponins, quinones, and phenolic compounds were confirmed in the crude extracts of the plant [4, 5]. Despite claims of its wound healing capability, the antibacterial activity of extracts from *Crassocephalum crepidioides* has not been extensively studied. However, many previous studies have attributed its wound healing capability to its antioxidant properties [6].

There are several reports on the characterization of volatile oils from various parts of *C. crepidiodes* of

different origin in the world. For example, Prevost *et al.*, characterized the stem and flower volatile oils of *C. crepidiodes* grown in Cote d'Ivoire [7]. The flower oil contained higher quantities of myrcene, limonene and β-pinene. The three compounds accounted for 95.32 % of the oil. Myrcene, terpinolene, limonene and β-bourbonene were the major constituents in the stem oil. Myrcene was found to be the most predominant of the ethereal oils from the leaf, stem, and flower parts of *C. crepidiodes* from Vietnam. Other constituents present in considerable amounts in the leaf extract include: cryptone, β-phellandrene, limonene and perillene while α-copaene, bornyl acetate, β-caryophyllene, β-phellandrene, α-humullene, β-farnesene, hummullene oxide and caryophyllene epoxide were other principal constituents in the oil of the stem [7]. The flower oil of the plant contained β-phellandrene, cryptone, α-copaene, α-humullene, and humulene epoxide in appreciable amounts [8]. Joshi, investigated the chemical compounds that constituted the ethereal oil from the roots of *C. crepidiodes* of Indian origin and the result revealed the predominance of β-farnesene, α-humullene, β-caryophyllene, β-guaiene and α-burnesene in the oil [9]. α-Pinene and myrcene were the constituents found in greater percentages in the leaf essential oil of the Chinese grown *C. crepidiodes* [10].

The contribution of essential oil constituents to the antimicrobial activity of aromatic plants has been established. This was invoked by Owokotomo and Owokotomo, [3], in their study on the effects of ethereal oils from the leaves and stems of *C. crepidiodes* against Gram-positive bacteria (*Salmonella typhi, Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). From their results, the stem and the leaf oils inhibited all the tested bacteria. However, the stem oil had a lower inhibitory concentration and showed higher potency than the leaf oil. The higher potency of the oil could be due to the abundance of oxygenated compounds, most notably thymol, in the oil as compared to the leaf oil. To the best of our knowledge, there is no report on the characterization and antibacterial activity of ethereal oil from the leaves of *C. crepidiodes* from north-central, Nigeria. Therefore, our study provides the chemical profile and antibacterial activity of volatile oil from the leaves of *C. crepidiodes* against strains of bacteria.

2. Materials and methods

2.1 Sample collection

Leaves of *C. crepidiodes* were collected in a farmland in Offa, Kwara State, Nigeria. Voucher specimens [UILH/001/1779/2023] of the plant were deposited at the University's Herbarium in the Department of Plant Biology, University of Ilorin, Nigeria, after its identification. Clinical isolates of the bacteria used in this study were collected from the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria.

2.2 Essential oil isolation

The hydrodistillation method was used to isolate essential oil from the leaves in a Clevenger-type apparatus, as documented by British Pharcomacopia [11]. In this method, 500 g sample of blended leaves of *C. crepidiodes* was hydrodistilled for four hours. A sealed sample tube was used to collect the oil and later stored at 4 ^{o}C until the period of analyses.

2.3 Gas chromatography – mass spectrometry (GC-MS) characterization of the oil

A quadruple focusing mass spectrometer (433HP-5) that was coupled with an Agilent gas chromatograph of the model 19091S was used to characterize the oil. Helium gas (flow rate of 1.5 mL/min) was used as a carrier gas. The GC instrument was fitted with a fused silica capillary column (30 m by 0.25 mm). The fitted GC was coated with phenyl methyl siloxane and the split ratio was 1:50. The thickness of the film was 0.25 µm while the operating temperature of the oven was kept at 100 °C initially for 5 min. and later increased to 150 °C at a rate of 4 °C/min. for 8 minutes and then to 250 °C at a rate of 20 °C/min. Electron impact ionization mode was 70 eV and the mass detector temperature was 300 °C. The areas of GC peaks were used to estimate the percentage composition of the oil's constituents.

2.4 Identification of phytochemicals in the oils

To identify the constituents in the oil, a homologous series of n-alkanes with carbon atoms ranging from C_7 to C_{30}, (Supelco Bellefonte, PA, USA) under identical experimental conditions were used to estimate their retention indices (RI), after it was co-injected with standards and the result was compared with the data

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from the libraries of NIST 08 and Wiley 275. The fragmentation pattern of each compound in the mass spectra was also compared with the data from NIST 08 and Wiley 275 libraries [12–14]. The percentage composition of each phytochemical was estimated from the area of the GC peak (FID response) and no correction factor was used.

2.5 Antibacterial analysis

The volatile oil of the plant was tested for antibacterial activities against Gram-positive (*Enterococcus faecalis, Staphylococcus aureus, Streptococcus pneumoniae,* and *Bacillus cereus*) and Gram-negative (*Salmonella sp. and Salmonella typhi*) bacteria using the method of agar well diffusion [15]. Briefly, test organisms of 0.5 McFarland were inoculated on Mueller Hinton agar plates by spreading; wells measuring 6.0 mm were bored in the seeded agar; and 50 µL of the extracts were filled into the wells. Two drops of molten agar were immediately added to seal the wells before incubation at a temperature of 37 ^{0}C. The plates were examined for growth of test organisms and inhibition zones around the loaded wells after 24 hours of incubation. The diameter of inhibition zones around wells was measured in mm using the meter rule. The extract was diluted two folds to obtain 25, and 50% diluents using Tween 80 to assay for the minimum inhibitory concentration (MIC). Tween 80 and streptomycin (25 µg), 50 µL were each loaded into separate 6 mm wells as the negative and positive controls, respectively.

2.6 Statistical analysis

The antibacterial test was carried out in triplicates and the values were used to obtain the mean values. The data obtained from the various antimicrobial parameters was expressed as mean ± SD (n = 3) and was compared using a one-way analysis of variance (ANOVA) test. This was followed by Dunnett multiple comparison tests with tests equal sample size test. Statistically, values were said to be significant only at $p < 0.05$. Non-linear regression was used to calculate the IC_{50} values from the mean values. SPSS for Windows version 10 was used to carry out the statistics.

3. Results and discussion

3.1 Yield of essential oil and its chemical composition

The leaves of *C. crepidiodes* gave 0.10 % (w/w) of ethereal oil after distillation. Lower yield was recorded for the leaves of the same plant native to south-west Nigeria [16]. The higher quantity of oil from this study can be linked to the presence of more secretory cells in the leaves of the plant in this work as compared to the leaves of the plant from south-western region of the country. Table 1 shows the chemical composition of the volatile oil from the leaves of *C. crepidiodes*.

Fourteen compounds that constituted 98.62 % of the oil are shown in Table 1 (The GC-MS chromatogram of the oil is shown in *Supplementary Fig S1).* Hydrocarbon monoterpenoids constituted the oil in higher abundance (86.9 %). The relative percentage of oxygenated monoterpenoids was 5.2 %. Hydrocarbon and oxygenated sesquiterpenoids constituted 3.2 % and 2.9 % respectively. D-limonene (77.1 %) was the most abundant terpenoid in the oil. Terpenoids that were detected in significant amounts include; α-pinene (5.5 %), linalool (2.6 %), α-terpeneol (2.6 %), β-caryophyllene (2.4 %, guaiol (1.8 %), β-pienene (1.7 %), o-cymene (1.5 %) and globulol (1.1 %). The oil contained alloaromadendrene (0.6 %) and 2-carene (0.9 %) in appreciable amounts. Terpenoids such as γ-terpinene (0.2 %), α-cubebene (0.1 %) and α-farnesene (0.1 %) were found in minor quantities.

The chemotype of the oil was D-limonene since the terpenoid constituted the highest percentage in the oil. However, β-cubebene was the chemotype of the volatile oil in the leaves of the plant grown in south-west Nigeria [16]. The chemotypic differences between volatile oils from leaves of *C. crepidiodes* from the two locations in Nigeria may be attributed to differences in agroclimatic conditions which in turn affect the physiological state of the plant.

Terpene synthases have been established as enzymes that usually aid the biosynthesis of terpenoids in plants [17, 18]. The synthetic routes involve the transformations of the isoprenoid's precursors to different compounds via carbocationic intermediates. The activity of the synthases determined the stability of the various carbocation intermediates formed from the ionization of the precursors which in turn determined the type of terpenoids formed after deprotonation or hydration of the ion [19–21].

Thus, the formation of the terpenoids in the leaves of the plant was therefore facilitated by the synthases of

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Table 1. Chemical composition (%) of essential oil from leaves of *Crassocephalum crepidiodes*

S/N	Compounds	Composition (%)	RI[a]	RI[b]	Mass Spectra Data
1	α-Pinene	5.5	939	933	135,105, **93**, 67, 55
2	β-Pinene	1.7	980	982	136,121,93,79
3	2-Carene	0.9	1001	1001	150,121, **93**, 67, 53
4	o-Cymene	1.5	1019	1019	134, **119**, 91, 77, 65
5	**D-Limonene**	**77.1**	**1031**	**1027**	136,107, 93, **68**, 53
6	γ-Terpinene	0.2	1062	1057	136,105, **93**, 77, 65
7	Linalool	2.6	1098	1098	136, 121, **93**, 71,55
8	α-Terpineol	2.6	1189	1188	43,**59**,81,93,107
9	α-Cubebene	0.1	1341	1351	105,119,**161**,189,204
10	β-Caryophyllene	2.4	1418	1418	204, 133, **93**, 79,41
11	α-Farnesene	0.1	1443	1456	**69**,79,93,107,133
12	Alloaromadendrene	0.6	1461	1460	204, 147,**105**,93,41
13	Globulol	1.1	1576	1582	204, 161, 111, **93**¸67
14	Guaiol	1.8	1595	1598	119, 149, **161**, 189, 222
	Total	**98.2**			
	Compound Classes				
	Hydrocarbon Monoterpenoids	86.9			
	Oxygenated Monoterpenoids	5.2			
	Hydrocarbon Sesquipenoids	3.2			
	Oxygenated Sesquiterpenoids	2.9			

Compounds are based on the order in which they are eluted from coated fused silica capillary column with CP-Sil 5; RIa = Retention Indices from Literature, RIb = Retention Indices calculated; Name bolded = Chemotype; Classes of Compounds

D-limonene and β-caryophyllene since both compounds were the most prominent mono- and sesquiterpenoids in the oil. The biogenesis of the terpenoids is shown in the *Supplementary File.* A comparison of the phytochemical profile of the oil from this study and that of the leaves of the plant that was indigenous to south-west Nigeria revealed both qualitative and quantitative variations. For instance, monoterpenoids that constituted the oil of this study were α-pinene, β-pinene, 2-carene, α-terpineol, D-limonene, linalool and γ-terpinene. The biosyntheses of the above named terpenoids were catalyzed by D-limonene synthase. Interestingly, none of these monoterpenoids was reported in the leaf oil of the plant from south-west. This suggested that the activity of the synthase of myrtenol (the enzyme that aided the formation of the monoterpenoids in the leaves of the plant from south-west) did not favour the biosynthesis of the above named compounds in the leaf oil of the south-western grown plant. Meanwhile, myrtenol synthase was able to aid the formation of myrtenol, *p*-myrcene, Z-ocimene and thymol in the leaf oil of the plant indigenous to south-west, Nigeria. Surprisingly, the isoprenoids were not detected in the oil of this study.

The β-caryophyllene synthase aided the biosynthesis of α-cubebene, globulol, alloaromadendrene and guaiol in the oil of this study but the sesquiterpenoids were not found in the other oil. The activity of the β-cubebene synthase (the enzyme that facilitated the formation of the sequiterpenoids identified in the leaf oil of the plant from south-west Nigeria). On the other hand, favour the biosynthesis of copaene, artemisia triene, β-elemene, α-caryophyllene, β-farnesene, α+β-caryophyllene, δ-cadinene, γ-elemene, caryophyllene oxide, humulene epoxide and α-santalol in the oil. Meanwhile, the oil of this study did not contain any of those sesquiterpenoids mentioned above. The activities of the synthases of D-limonene, myrtenol, β-caryophyllene and β-cubebene were affected by agroclimatic conditions in both locations that subsequently influenced the physiological conditions of the plant and then accounted for the absence of some terpenoids in the respective oils [22].

The environmental conditions of both locations favour the activities of the synthases of β-caryo-

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Table 2. Mean inhibition zones (mm ± SD) of test organisms and the MIC by the leaf essential oil of *Crassocephalum crepidioides*

Crude Extracts*	Conc. (%)	Diameter of zone of inhibition of test bacteria (mm)					
		EF	SP	SA	BC	ST	SSP
CCESO	25	0	0	9.67±0.58[a]	8.00±1.00[a]	10.33±0.58[a]	10.00±0.00[a]
	50	0	14.33±1.53[a]	11.33±0.58[b]	12.00±1.00[b]	12.00±1.73[b]	11.67±0.58[b]
	100	15.67±0.58[b]	17.33±0.58[c]	14.00±1.00[c]	12.67±0.58[b]	14.33±0.58[b]	13.67±0.58[c]
Tween 80 (negative control)		0	0	0	0	0	0
Strept. (positive control)		28.33±0.58[a]	34.67±1.53[a]	35.67±0.58[a]	28.00±1.00[a]	36.33±0.58[a]	22.33±0.58[a]

CCESO – Leaf Essential Oil of *Crassocephalum crepidioides*. Test organisms - *Enterococcus faecalis,* EF; *Streptococcus pneumoniae* SP; *Staphylococcus aureus*, SA; *Bacillus cereus,* BC; *Salmonella typhi,* ST; *Salmonella sp.* SSP

phyllene and β-cubebene and the enzymes were able to facilitate the biosynthesis of α-farnesene in the two oils. Meanwhile, the compound was synthesized in higher quantity in the oil of the plant from south-west than the oil in this study. This may be due to the premature termination of the precursor of the compound in the oil of this study as a result of unfavourable agroclimatic conditions [23].

3.2 Antbacterial activity of the oil

The leaf essential oil of *Crassocephalum crepidioides* inhibited all the test organisms with zone of clearance ranging from 13.67 to 17.33 mm (Table 2). *Staphylococcus aureus* was highly susceptible to the essential oil with inhibition at the lowest concentration (25%) used in this study, and an indication that the MIC could be much lower. This corroborates the folklore use in wound treatment since *S. aureus* is a well-known infectious organism in wounds. Additionally, the inhibition of *S. aureus* both in vitro and on wounds had been studied [1, 2, 4]. *Bacillus cereus* also showed high susceptibility to the essential oil at 25%, an indication that its MIC might be at lower concentration as it is with *S. aureus*. Similarly, the Gram-negative test organisms used, *Salmonella typhi* and *Salmonella* sp. were highly susceptible to the essential oil with inhibition at 25% and indicating that the MIC could also be lower. In a similar study, Owokotomo and Owokotomo, also reported inhibition of *S. aureus*, *B. cereus* and *S. typhi* by the ethereal leaf oil of *C. crepidioides* and concluded that the antibacterial activities of the oil support its use for medicinal purposes in south west Nigeria. The strong susceptibility of the oil against Gram-positive bacteria may be linked to the predominance of D-limonene in the oil [3]. The compound was reported to show relevant clinical antibacterial properties against *S. aureus* and *E. coli* [4].

Enterococcus faecalis was inhibited by the essential oil only at 100% showing weak susceptibility. The MIC of S*treptococcus pneumoniae* was 50% which is also high and an indication of weak susceptibility. Moreover, there is no report on the antibacterial activity of the volatile oil from the leaves of *C. crepidioides* against these organisms to the best of our knowledge. The oil may therefore not be suitable for infections caused by the Gram-positive bacteria used in this study.

4. Conclusions

This study is the first report on the antibacterial activity of volatile oil from the leaves of *Crassocephalum crepidiodes* grown in north-central Nigeria. The bacteria studied against the volatile oil were *Enterococcus faecalis, Streptococcus pneumoniae, Bacillus cereus*, *Staphylococcus aureus, Salmonella typhi* and *Salmonella* sp. The oil was extracted and characterized as D-limonene chemotype. Other principal constituents were α-pinene, linalool, α-terpineol and β-caryophyllene. The oil showed greater activity against all the Gram-positive bacteria as compared to the Gram-negative bacteria and *Staphylococcus aureus* was the most susceptible. The oil is therefore recommended for the management of infections that are caused by the Gram-positive bacteria used in this study. However, the oil may not be suitable for the treatment of diseases caused by the two Gram-negative organisms reported.

Authors' contributions

Conceptualization, I.R.O.; Methodology, I.R.O.,

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L.A.U., S.B.K., O.M.A., H.F., E.D.A., A.O.F.S.; Software, S.B.K., H.F.;Validation, O.M.A., E.D.A.; Formal analysis, I.R.O., L.A.U., S.B.K., H.F.; Investigation, I.R.O., S.B.K.; Resources, L.A.U., S.B.K., A.O.F.S., E.D.A.; Data curation, H.F., A.O.F.S.; Writing – original draft preparation, I.R.O., S.B.K.; Writing – review & editing, I.R.O., S.B.K., L.A.U., H.F.; Project administration, L.A.U., S.B.K., H.F., O.M.A.

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Availability of data and materials

All data will be made available on request in accordance with the journal policy.

Conflicts of interest

The authors declared no conflict of interest.

Supplementary materials

Supplementary contents, schemes (1 &2) and Fig. S1

Supplementary material to this article can be found online at https://www.currentsci.com/images/articlesFile/supplementary.1740149136.pdf

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