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Phytochemical Profiling of the Hexane fraction of *Crassocephalum crepidioides* Benth S. Moore leaves by GC-MS

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***Crassocephalum crepidioides* is an edible plant which is also used in the ethnomedical treatment of stomach ulcer, indigestion, wounds, boils and burns in Africa and some other parts of the world. This study aims at identifying and characterizing the bioactive compounds present in *C. crepidioides* hexane fraction which may be responsible for the ethnomedicinal uses and reported activities of the plant. The crude extract from the powdered leaves of *C. crepidioides* was obtained with 70% methanol, followed by solvent partitioning with hexane to give the hexane fraction which was subjected to phytochemical profiling using gas chromatography-mass spectrometry (GC-MS). Spectrum interpretation was obtained from the library search of the database of National Institute of Standards and Technology (NIST), while biological activities of compounds identified were predicted based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed the presence of several bioactive compounds with various biological activities including Hexadecanoic methyl ester and α-Linolenic acid with reported hypocholesterolemic properties; Benzofuranone and Benzofuran with anticancer and antiviral activities; phenolic compounds and flavonoids with reported antioxidant, anti-inflammatory and antifungal activities among others. The study showed that *C. crepidioides* contains compounds with important biological activities which provide scientific support for some medicinal uses of the plant.**

Key words: Phytochemicals, *Crassocephalum crepidioides*, gas chromatography-mass spectrometry (GC-MS), ethnomedicinal.

INTRODUCTION

Medicinal plants have been found to be rich sources of secondary metabolites with important biological activities. Many of the active substances found in plants are secondary metabolites called phytochemicals, including phenols, flavonoids, alkaloids, steroidal esters, glycosides, tannins and terpenoids. These bioactive compounds are relevant sources of novel therapeutic agents. Therefore, phytochemical analysis of plants has become increasingly important procedure in phytomedicine and drug discovery. Furthermore,

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identification and knowledge of the chemical constituents of plants are vital for scientific explanation and rationalization of their ethnomedicinal uses.

Crassocephalum crepidioides (Benth.) S. Moore (Henderson, 1973; Lemmens, 2003), commonly called fireweed ragleaf, is an annual edible plant that is widespread in tropical and sub-tropical regions (Rajesh, 2011). It is an erect, sparingly branched herb about 40-100 cm tall. The stem is rather stout, soft, ribbed and apical with short thick hairs (Kostermans et al., 1987), the leaves are lamina elliptic to ovate in outline, and the seeds consist of floating balls of many silky white hairs that can be wind dispersed.

The plant is recognized as a highly invasive weed which have become distributed eastward out of Africa into East Indies, India, East Asia and Philippines (Kiew, 2009; Randall, 2012). It is eaten by humans in many countries of Africa, where the succulent leaves and stems are used as vegetables in soup and stews (Burkill, 1985; Sakpere et al., 2013). The various local names of the plant include: *Ebolo* by Yoruba Southwest, Nigeria (Adams, 1963); *mkpafit* by Efik, Akwa Ibom; and *obuinenawa* by Edo people, South- south, Nigeria (Omotayo *et. al.,* 2015); *gbolo* in Benin republic (Adjatin et al., 2013); *ye tong hao* by the Chinese, Eyukula by the Portuguese, and *benibanaborogiku* by the Japanese (Tomimori et al., 2012). *C. crepidioides* is traditionally used in the treatment of wounds, boils, burns, indigestion, stomach ulcer, nose bleeding, fever, inflammation and edema (Ajibesin, 2012; Aniya et al., 2005; Oyelakin and Ayodele, 2013; Chaitanya et al., 2013; Sakpere et al., 2013).

Scientific investigations have shown *C. crepidioides* to be a useful source of protein in both human and animal diet (Dairo and Adanlawo, 2007). The plant has also been reported to be a good source of vitamins and minerals (Smith and Eyzaguirre, 2007), therefore making it a good source of nutraceuticals in prevention and management of diseases (Adjatin et al., 2013).

Further review of Ethnopharmacological reports on *C. crepidioides* showed that the plant possesses anti-helminthic, antibacterial, anti-inflammatory, antidiabetic, and acetyl cholinesterase inhibitory properties (Bahar et al., 2017; Bogning et al., 2016; Chaitanya et al., 2013; Joshi, 2014; Owokomoto et al., 2012; Tomimori et al., 2012). The antioxidant, cytoprotective (Odukoya et al., 2007; Wijaya et al., 2011), cancer chemoprotective and anti-tumor activities (Chia-chung et al., 2007; Chaitanya et al., 2013) of the plant have also been demonstrated. The *in vitro* anticoagulant activity of the plant leaf methanol extract and fractions was recently reported (Ayodele et al., 2019). Therefore, with such great medicinal value being suggested, a detailed analysis to identify and characterize the phytochemical compounds in the plant is very much needed. However, few reports are available on the bioactive compounds present in the plant. Reports on preliminary phytochemical screening of *C. crepidioides* methanol extract have revealed the presence of alkaloids, glycosides, tannins, flavonoids, phenols, saponins and ascorbic acid (Arawande, 2013; Bahar et al., 2017). The essential oils of *C. crepidioides* from south western Nigeria and western Ghats region of India were found to mainly consist of α-caryophyllene, thymol, α-farnasene, β-cubebene and 4-cyclohexybutyramide, thus concluding that *C. crepidioides* may be a natural source of thymol, with established the antimicrobial activity (Owokomoto et al., 2012; Rajesh, 2011).

Over the years, gas chromatography-mass spectrometry (GC-MS) has become an established technique for secondary metabolite profiling in plants. Therefore, the present study aims at identifying and characterizing the volatile bioactive compounds present in *C. crepidioides* hexane fraction which may be responsible for some of the reported activities, using Gas Chromatography- Mass Spectrometry analysis.

MATERIALS AND METHODS

Plant collection and preparation

C. crepidioides was obtained from farms in Ilisan-remo, Ogun State, South Western Nigeria, and identified at the IFE herbarium, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria where a voucher specimen with the voucher specimen registration No: IFE 17634, was deposited. The plant leaves were oven-dried at 40°C and ground into powder using an electric blender and stored in the refrigerator at 4°C until use.

The crude methanol extract was obtained by soaking the powdered leaves in 70% methanol (1:8, w/v) for 48 h at room temperature accompanied by intermittent shaking (Handa et al., 2008). This was followed by sequential filtering through a fine muslin cloth and Whatman No 1 filter paper. The crude extract was then evaporated under reduced pressure at 40°C using a rotary evaporator, and further dried to completion in a hot air- oven at 40°C. The dried methanol extract was reconstituted in distilled water, (2:1, w/v) and subjected to solvent partitioning ratio in 1:1 (v/v) with Hexane (Otsuka, 2006).

GC-MS analysis

Phytochemical characterization of the plant hexane fraction was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) using Agilent® 19091J-413; 3324.57048 with HP-5MS capillary column (30 m length × 320 µm diameter × 0.25 µm film thickness). The carrier gas was helium at constant flow rate of 1.573 ml/min and average velocity of 45.933 cm/s at a pressure of 2.84psi. The initial oven temperature was 60°C held for 1min, then increased at 4°C/min to 110°C for 3 min, and then at 8°C/min to 260°C for 5 min. The temperature was further increased at 10°C/min to 300°C and held isothermally for 12 min. The sample was reconstituted in hexane (5% w/v), and 1 µl was injected in the spitless mode.

Identification of compounds

The total chromatogram was auto integrated by ChemStation and spectrum interpretation was done using the database of National Institute Standard and Technology (NIST). The spectrum of the

unknown components was compared with the spectrum of the known components stored in the NIST library. The systemic names, molecular formulae and structures of the identified components were obtained. The biological activities of compounds were largely predicted on the basis of Duke's Phytochemical and Ethnobotanical Databases (Duke, 2013, 2016).

RESULTS

GC-MS analysis of the hexane fraction of *C. crepidioides* leaves led to tentative identification of the components as shown in Table 1, while the chromatogram is shown in Figure 1. The results revealed 64 bioactive compounds, including n-hexadecanoic acid and its methyl ester (S/N 49 and 50) which are known to possess hypocholesterolemic properties; coumarin-related compound Benzofuranone (coumarin-3-one; S/N 22) which has anti-inflammatory and anticancer activities; α-Linolenic acid (9,12,15-Octadecatrienoic acid; S/N 55) which possesses antiaggregant, anti-hypertensive, immunostimulant, anti-leukotriene, and cancer-preventive activities among others.

Phenol (S/N 15) and Phenolic compounds including Eugenol (S/N 16) with anticoagulant and antiaggregant activities, Vanillin (S/N 24), Citronellol (S/N 28), Orcinol (S/N 42), Thumbergol (S/N 48) and Catechol (S/N 62) which possess antifungal, antiseptic, antibacterial, keratolytic and insecticidal activities; Thujone (S/N 14) with antiplatelet activity; Flavonoids and other compounds with anti-inflammatory, antioxidant, antiviral, anesthetic, anti-psychotic, antinociceptive and antimicrobial properties were tentatively identified. Compounds that can be used as food flavour enhancer, in cosmetics and 1,9-octadecadiene (S/N 27) with no reported activity were identified. Most of the activities associated with the identified compounds were obtained from Duke's phytochemical and Ethnobotanical databases (Duke, 2013, 2016) unless otherwise stated in the Table 1 and Figure 1.

DISCUSSION

Medicinal plants have become vital to major populations of the world for treatment and management of diseases. Identification and isolation of phytochemical constituents of plant and testing them for biological activities will provide a great insight to the nature of these components, their pharmacological action and potency. The GC-MS characterization of the hexane fraction of *C. crepidioides* leaf indicated the presence of several bioactive compounds with various biological activities.

These include n-Hexadecanoic acid, Hexadecanoic methyl ester (palmitic acid) and α-Linolenic acid (ALA) with reported hypocholesterolemic and other lipid-lowering properties. Salisu et al. (2019) similarly reported high quality match palmitic, oleic and linoleic acids as major components out of eight compounds identified from GC-MS analysis of n-hexane extract of *C. crepidioides* leaf. However, this present study identified 64 compounds of phytochemical importance in *C. crepidioides* leaf hexane fraction obtained from the methanol (crude) extract.

Compounds with antioxidant properties identified include Benzene acetaldehyde, Erythritol, Phytol, N-hydroxylamine, Aromadendrene oxide and Hydroquinone. These buttress the report of Bahar et al. (2016) on *in vitro* antioxidant activity of *C. crepidioides* in which preliminary phytochemical screening of the plant methanol extract indicated the presence of alkaloids and flavonoids. Bioactive compounds identified with anti-inflammatory properties include Indole, phytol, phenol, Ledol and Benzofuran. These tentatively identified compounds may be responsible for the plant local use in the treatment of stomach ulcer, swollen lips and edema. Karmakar et al. (2018) reported the presence of phenolic compounds, among others, implicated in anti-inflammatory activity of *C. crepidioides* methanol extract.

Identification of Benzofuranone, Benzofuran, Semicarbazone, 2-benzothiozolamine, and Glutaric acid with anticancer, and antiviral activities may corroborate the cancer chemopreventive and antitumor action of *C. crepidioides* essential oils reported by Thakur et al. (2018) and Tomimori et al. (2012). Eugenol, other phenolic compounds and flavonoids commonly possessing antimicrobial, antifungal activities were also tentatively identified. Bahar et al. (2017) and Arawande et al. (2013) reported that preliminary qualitative phytochemical screening of the methanol extract of *C. crepidioides* revealed the presence of alkaloids, glycosides, tannins, flavonoids, phenolic compounds, saponins and ascorbic acid; while Owokomoto et al. (2012) reported α-caryophyllene and β-cubebene as the most abundant constituents identified from GC-MS analysis of the leaf essential oils of *C. crepidioides*.

The reported activities of the phenols and flavonoids identified in the plant may be the rationale for its local use in treatment of wounds and boils. Alpha-linolenic acid has been reported to act as an antiaggregant, Eugenol was reported as an anticoagulant agent in *Cinnamomum cassia* (Kim et al., 2010), while thujone and flavonoids have also been reported to inhibit platelet aggregation (Formica and Regelson, 1995; Cordier and Steekamp, 2011). Ayodele et al. (2019) similarly reported Thujone, Eugenol, α-linolenic acid, and coumarin-related compounds; Benzofuran, Benzofuranone and Benzene acetaldehyde as possible anticoagulant agents in *C. crepidioides* leaf methanol extract and fractions.

The heterogenous compounds identified from the present study may therefore be responsible for the ethnomedicinal uses of the plant. Thus, the GC-MS phytochemical characterization of *C. crepidioides* has revealed the presence of various bioactive compounds with different chemical structures which can be utilized in

Table 1. GC – MS Identified Phytochemical components of the Hexane fraction of *C. crepidioides* leaf extract.

S/N	Retention time (mins)	Name of compound (Library ID)	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Reported biological activity
1	3.586	Butyrolactone	$C_4H_6O_2$	86.09	0.98	Antimicrobial. Central nervous system depressant (CNS) and hypnotic. Anaesthetic.
2	5.449	Benzene acetaldehyde	C_8H_8O	120.15	1.11	Antioxidant Antibacterial, Anaesthetic.
3	5.568	1-methyl, 2-Pyrrolidinone	C_5H_9NO	99.13	2.69	Surfactant, Antifungal Antioxidant, Antibacterial Anticancer, Anticonvulsant (Hosseinzadeh et al., 2017).
4	6.161	Erythritol	$C_4H_{10}O_4$	122.12	1.01	Osmoprotectant Antioxidant Insecticidal (Scanga et al., 2018)
5	6.678	dl-Threitol	$C_4H_{10}O_4$	122.12	0.50	Cryoprotectant (antifreeze agent
6	7.046	Glycerin	$C_3H_8O_3$	92.09	0.61	Antibacterial
7	7.188	Phenylethyl Alcohol	$C_8H_{10}O$	122.16	2.00	Antibacterial
8	9.319	Phthalic acid	$C_8H_6O_4$ $C_6H_4(COOH)_2$	166.14	0.84	Pesticidal
9	10.286	Benzofuran	C_8H_6O	118.10	1.43	Antidepressant, Anticancer, antiviral, antifungal, antioxidant, anti-psychotic, anti-inflammatory. (Asif, 2016)
10	12.215	Indole	C_8H_7N	117.15	0.76	Anti-inflammatory, Anti-tumor, Antimicrobial, Antineoplastic.
11	12.761	N-hydroxylamine	H_3NO_2	33.03	0.49	Antioxidant
12	12.910	Glutaric acid	$C_5H_8O_4$	132.12	1.72	Virucidal (Khurt et al., 1984)
13	13.497	Mequinol	$C_7H_8O_2$	124.14	0.46	Anti-inflammatory, Antimicrobial
14	13.640	Thujone	$C_{10}H_{16}O$	152.23	0.56	Antibacterial, Antifungal, Antinociceptive, Insecticidal, Anthelmintic Antioxidant (Duke, 2013), Antiplatelet (Cordier and Steekamp, 2011).
	13.640	Phytol	$C_{20}H_{40}O$	128.17	0.56	Anti-inflammatory, Antioxidant, Antinociceptive (Santos et al., 2013)
15	14.055	Phenol	C_6H_6O	94.11	0.41	Antioxidant, Antimicrobial
16	14.180	Eugenol	$C_{10}H_{12}O_2$	164.20	4.43	Anti-inflammatory, Antiseptic (Bendre et al., 2016), Anticoagulant, Antiaggregant (Kim e. al., 2010).
17	15.569	1,7-Nonadiene,4,8-dimethyl--	$C_{11}H_{20}$	152.28	2.76	Anti-inflammatory, Anti-cancer,
18	17.249	Isocyclocitral	$C_{10}H_{16}O$	304.50	1.40	Deodorant
19	18.406	2,4 Dimethylanisole	$C_9H_{12}O$	136.19	0.56	Food flavoring agent
20	18.821	2,4,6-Trimethyl-2-(4-methyl-pent-3-enyl) 2H-pyran	$C_{14}H_{22}O$	206.32	0.83	Fragrance in cosmetics
21	19.273	2-Tridecanone	$C_{13}H_{26}O$	198.35	2.44	Food flavoring agent
22	19.795	Benzofuranone	C_8H_6O	134.13	2.99	Antioxidant, Anticancer
23	21.205	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32	1.41	Antifungal, Antibacterial, Antiviral, Soap and cosmetics production
24	21.385	Vanillin/ Propyl ester	$C_8H_8O_3$	152.15	1.47	Antifungal, antimicrobial, flavour (Fitzgerald et al., 2005)
25	21.692	3-Cyclohexen-1-carboxaldehyde	$C_7H_{10}O$	110.15	0.73	Allergenic
26	21.712	Butyrophenone	$C_{10}H_{12}O$	148.20	1.06	Antiemetics
27	22.151	1,9 octadecadiene	$C_{18}H_{34}$	250.46	0.78	Not stated

Table 1. Contd.

28	22.276	Citronellol	$C_{10}H_{20}O$	156.27	0.60	antibacterial, antidepressant, antiseptic, antispasmodic, anti-inflammatory, deodorant, diaphoretic, diuretic, febrifuge, fungicidal, insect repellant, anthelmintic
29	22.501	Aromadendrene oxide Longipinocarveol	$C_{15}H_{24}O$	220.35	1.89	Antioxidant activity
30	22.626	N-acetyl-d-Serine	$C_5H_9NO_4$	147.13	0.70	Antifungal, nutrient additive
31	22.851	2-Aminoresorcinol	$C_6H_5NO_4$	125.13	2.28	Intestinal-alpha-glucosidase inhibitor.
32	22.958	Alpha-Guaiene	$C_{15}H_{24}$	204.35	0.92	Antimicrobial, insecticidal
33	23.086	Caryophyllene	$C_{15}H_{24}$	204.36	0.68	Aldose-reductase inhibitor, Allergenic, Analgesic, Anti-asthmatic.
34	23.178	3-Buten-2-one, 4-hydroxy trimethyl-7-oxabicyclo-heptyl-	$C1_3H_{20}O_3$	224.30	1.84	Antioxidant
35	23.427	1-Acetyl-3methylurea	$C_4H_8N_2O_2$	116.12	0.64	Antioxidant and antimicrobial
36	23.718	Ledol/Cedvanoxide	$C_{15}H_{26}O$	222.36	0.71	anti-inflammatory and analgesic activities
37	23.961	2-Benzothiozolamine	$C_8H_9N_3S$	224.30	1.86	Antitumor
38	24.145	2,5-octadiene-tetramethyl-	C_8H_{14}	166.30	0.99	Natural antioxidant, antihyperuricemic and anti-inflammatory
39	24.329	Spiro[2.3]hexan-4-one, 5,5-dichloro	$C_6H_6Cl_2O$	193.07	0.48	Antimicrobial
40	24.531	Cyclodecanone	$C_{10}H_{18}O$	154.25	0.98	Antifungal
41	24.644	Semicarbazone	$C_6H_{11}N_3O$	141.17	0.58	Antiviral, anticancer
42	24.816	Orcinol	$C_7H_8O_2$	124.13	3.14	antifungal, antimicrobial, and keratolytic (Vanderpas, 2003).
43	25.154	2-(1-Hydroxycyclohexyl)-furan	$C_{10}H_{14}O_2$	166.22	0.90	Antimicrobial
44	25.386	Paradrine	$C_9H_{13}NO$	151.21	0.58	Analgesic Stimulates the sympathetic nervous system
45	25.540	Alloaromadendrene oxide	$C_{15}H_{24}O$	220.35	0.49	Antioxidant, anticancer
46	25.677	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	$C_{15}H_{26}O$	238.37	2.42	Antioxidant, cosmetic fragrance
47	25.884	2-Acetylbenzoic acid	$C_9H_8O_3$	164.16	0.74	Hair dye, Pharmaceutical intermediate
48	26.241	Thumbergol	$C_{20}H_{34}O$	290.48	0.41	Antifungal
49	26.704	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	1.48	Antioxidant, Hypocholesterolemic, Nematicide,Pesticide, Antiandrogenic, Flavor, Hemolytic, 5-alpha reductaseinhibitor
50	27.250	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	1.19	Antioxidant, anti-inflammation Hypocholesterolemic, Nematicide Pesticide, Lubricant,Antiandrogenic, Flavor,Hemolytic, 5-alphareductase inhibitor.
51	27.635	Metanephrine	$C_{10}H_{15}NO_3$	197.23	0.72	Metabolite
52	27.997	Methoxamine	$C_{11}H_{17}NO_3$	211.26	0.63	α1-adrenergic receptor agonist, Sympathomimetic agent
53	28.816	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{20}H_{36}O_3$	264.40	5.74	Antibacterial, antifungal
54	28.965	2-furanmethanol, tetrahydro-acetate	$C_5H_{10}O_2$	144.17	2.91	Anticancer
55	29.404	9,12,15-Octadecatrienoic acid (α-linolenic acid)	$C_{18}H_{30}O_2$	278.40	4.52	Anti-Inflammatory, Hypolipidemic, Antiaggregant, Anti-leukotriene, Antiprostatic, Immunostimulant, Vasodilator, 5-alpha reductase inhibitor
56	29.511	Ethyl 9,12-hexadecadinoate	$C_{18}H_{32}O_2$	280.40	0.56	Antibacterial
57	30.449	Bicyclo heptane, 7,7-dimethyl 1-2-methylene	$C_{10}H_{16}$	136.23	0.47	Cellulose Biosynthesis Inhibitors
58	31.286	Doconexent/ Methyl parinarate	$C_{22}H_{32}O_2$	328.49	0.81	anti-inflammatory
59	31.648	9-Octadecenamide	$C_{18}H_{35}NO$	281.48	0.90	Antioxidants; food preservatives; food coloring agents; flavoring agents; anti-infective agents; excipients
60	33.031	Tocainide	$C_{11}H_{16}N_2O$	192.26	0.13	Antiarrhythmic agent

Table 1. Contd.

61	**13.224**	**Hydroquinone**	**$C_6H_4(OH)_2$**	**110.11**	**0.60**	**Antioxidant (Vanderpas, 2003)**
62	10.091	Catechol	$C_6H_6O_2$	110.11	0.24	Antibacterial, Antifungal
63	11.106	Glucopyranuronamide	$C_6H_{11}NO_6$	193.15	0.11	Antimicrobial,
64	16.38	Phenylephrine/Adrenalone	$C_9H_{13}NO_2$	167.21	0.06	Decongestant, hemorrhoidal, Vasoconstriction.

Main Activity Sources:* Duke (2013, 2016).

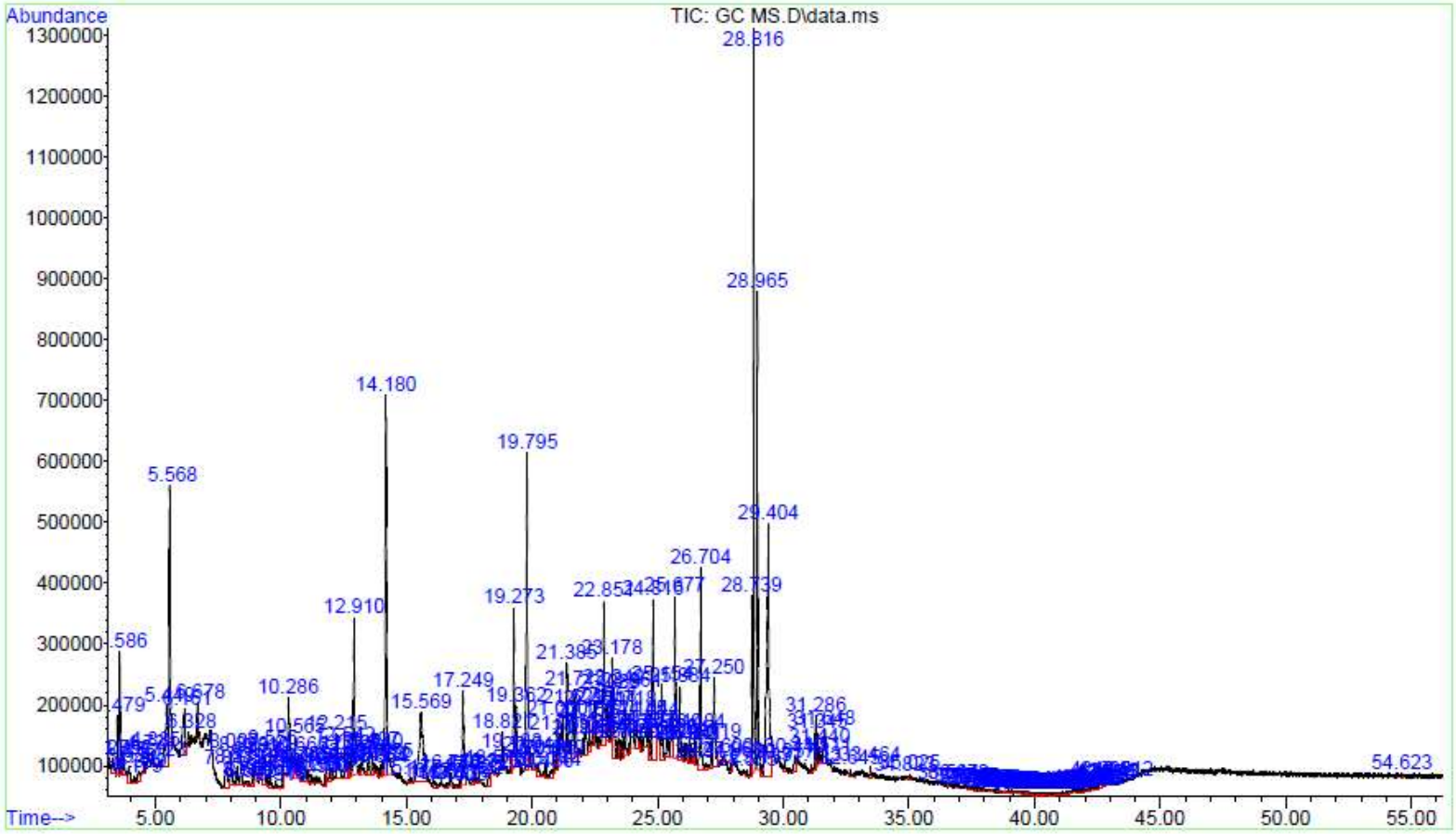

Figure 1. Chromatogram of GC-MS Phytochemical Characterization of *C. crepidioides* leaf Hexane fraction.

in vivo studies, ultimately leading to the discovery and development of new natural therapeutic agents and novel drugs.

Conclusion

This study revealed that *C. crepidioides* contains several bioactive compounds with various biological activities. The compounds identified from the GC-MS analysis of the hexane fraction of *C. crepidioides* provided clear justification for the plant medicinal use and ethnopharmacological activities. However, isolation of individual phytochemical constituents and further *in vivo* studies to validate their biological activities are required for novel drug development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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