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Mystroxylon aethiopicum chloroform root bark extracts phytochemical analysis using gas chromatography mass spectrometry

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Mystroxylon aethiopicum **has been used by many ethnic groups in Africa for the management of hemorrhagic diarrhea, stomachache, respiratory tract infections, urinary tract infections coughs, hypertension and gonorrhea. This study was carried out to identify low molecular weight phytochemicals present in the root bark extract of *M. aethiopicum* with the aid of gas chromatography-mass spectrometry (GC-MS) technique. The GC-MS analysis revealed the presence of various low molecular weight phytochemicals which belongs to four groups of secondary metabolites namely sesquiterpenes, dieterpenes, monoterpenes and fatty acids. The presence of these phytochemicals in the plant extract may be positively associated with pharmacological properties of *M. aethiopicum* and therefore justifying the ethnomedical usage of the plant.**

Key words: Gas chromatography-mass spectrometry (GC-MS) analysis, pharmacological properties, phytochemicals.

INTRODUCTION

The importance of medicinal plants and traditional health systems in solving health care problems of the world is gaining attention (Gadir, 2012). Medicinal plants have been of great value to human healthcare in most parts of the world for thousands of years (Pokhare et al., 2011). The medicinal value of plants is due to presence of bioactive compounds with interesting pharmacological activities such as anticancer, anti-inflammatory, antibacterial, antifungal and antioxidant (Ammal and Bai, 2013). Screening for bioactive compounds in medicinal plants is an important pre-requisite in investigations aiming at establishing lead compounds which can be further developed into potential herbal products for treatment of several ailments (Bohlin and Bruhn, 1999). Gas chromatography coupled to mass spectrometry (GC-MS) has commonly been used for analysis of relatively low molecular weight compounds (Eisenhauer et al., 2009; Prabhadevi et al., 2012). Taking into consideration the medicinal importance of bioactive compounds, it is essential to thoroughly investigate their composition and

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hence promote the use of such compounds as potential sources of drug templates (Bohlin and Bruhn, 1999). In recent years, there has been a growing interest in researching and developing new compounds from different sources including medicinal plants such as *Mystroxylon aethiopicum* to combat infectious diseases pathogenic microbes (Balouiri et al., 2016). This plant species was earlier reportedly used by many ethnic groups in Africa for the management of infectious diseases (Boer et al., 2005). Despite the wide use of such plant in the management of infectious diseases, there is lack of scientific studies regarding phytochemicals responsible for therapeutic effects. In this regard, the plant was chosen for determination of its bioactive compounds. This study therefore reports the phyto-chemical investigations of *M*. *aethiopicum* chloroform root bark extract using GC-MS technique.

MATERIALS AND METHODS

Studied taxon

Mystroxylon aethiopicum is a member of family Celastraceae and is a perennial evergreen phanerophyte (tree) that may grow up to 12 m high (Irish, 2012). This plant is found in a wide range of habitats including forest margins, evergreen forests, open woodland, riverine fringes, on termite mounds and rocky ridges (Burrows and Willis, 2005). The plant is mostly abundant in Ethiopia, Sudan, South Africa, Namibia, Angola, Cameroon, Madagascar, Seychelles and Comoro (Curtis and Mannheimer, 2005). In Tanzania, the species grows in highlands of Arusha and Kilimanjaro regions where it is locally known as “Oldonyanangui” in Maasai language (Kokwaro, 1993). Traditionally, the plant is currently knowledgably by many communities for the management of hemorrhagic diarrhea, stomachache, respiratory tract infections, urinary tract infections, coughs, hypertension and gonorrhea (Boer et al., 2005; Iwu, 2014). In Kenya, fine powder prepared from root barks of this plant is reportedly used in making tea that is considered to be a good medicine for stomachache (Burkil, 2004).

Plant materials and preparation of extracts

The plant materials were collected from Imbibya village in Arusha rural district, Tanzania. Plant species were identified by Mr. Gabriel Laizer, a botanist from Tropical Pesticide Research Institute (TPRI) and voucher specimen coded MA-0001 is kept at the Nelson Mandela African Institution of Science and Technology (NM-AIST). Root bark was harvested without affecting the plant, air dried under the shade and pulverized into fine particles using electric blender. Pulverized materials (250 g) were macerated in chloroform for 48 h. The respective extracts were filtered through Whatman No. 1 filter paper on a plug of glass wool in a glass column and solvents were evaporated through the vacuum using a rotary evaporator and the final residue obtained was subjected to GC-MS analysis.

GC-MS analysis

GC-MS analysis was carried out using Agilent 6890N GC connected to the Agilent 5975 MS (Agilent technologies, USA) with capillary column (HP-5) of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. Helium gas (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 1 µL was employed. The injector temperature was maintained at 250°C, the ion-source temperature was 280°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The mass spectrometer operated in electron ionization mode with an ionizing energy of 70 eV and the ion source temperature was 230°C. The inlet line temperature was 200°C and the total GC-MS running time was 36 min. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectra of the detected compounds from the *M*. *aethiopicum* chloroform root bark extract were compared with the spectra of the known compounds stored in the NIST library. In this way, the name, molecular weight and structure of the compounds contained in the *M*. *aethiopicum* chloroform root bark extract were determined.

RESULTS

The GC-MS technique was used to identify thirty four volatile phytochemical compounds present in the *M*. *aethiopicum* chloroform root bark extract. The retention time, peak areas, molecular formulas, molecular weights and biological activities of these compounds are presented in Table 1. These phytochemicals belongs to four groups of secondary metabolites namely sesquiterpenes, dieterpenes, monoterpenes and fatty acids.

Sesquiterpenes seemed to be in high proportions than the rest of low molecular weight secondary metabolites identified in *M*. *aethiopicum* chloroform root bark extract. These sesquiterpenes are δ-cadinol, copaene, α-muurolene, caryophyllene, α-calacorene, α-humulene, cubenol, β-eudesmol, γ-cadinene, elixene, isolongifolene, aromadendrene, isoledene, thujopsene, α-cubebene, epizonarene, α-gurjenene, α-farnesene, cyperene, (*Z,Z*)-α-farnesene, α-curcumene, caryophyllene oxide, norelidol and farnesol (Figure 1).

Diertepenes identified in this study were geranyl linalool, totarol and geranylgeraniol, while monoterpenes were borneol and santolina epoxide (Figure 2). Additionally, fatty acids identified are 9,12,15-octadecatrienoic acid, (*Z,Z,Z*), 9,12-octadecadienoic acid (*Z,Z*), 9-octadecenoic acid, (*E*), tetradecanoic acid and *n*-hexadecanoic acid (Figure 2).

DISCUSSION

The gas chromatography coupled to mass spectrometer was used to analyze *M. aethiopicum* chloroform root bark extract. Secondary metabolites belonging to sesquiterpenes, diterpenes monoterpenes and fatty acids were identified. Most of these phytochemicals have been reported to possess interesting biological activities against human infectious diseases and non-communicable diseases as shown in Table 1. Compounds that have been reported to exhibit antitumor activities are

Table 1. Reported biological activities of volatile phytochemical compounds detected in *M. aethiopicum* chloroform root bark extract.

S/N	RT (min)	Peak area (%)	Name of compound	Molecular formula	Molecular weight (g/mol)	Reported bioactivity	References
1	13.89	8.29	δ-cadinol	$C_{15}H_{26}O$	222.37	Antifungal	Ho et al. (2011)
2	11.43	5.73	γ-cadinene	$C_{15}H_{24}$	204.35	Antibacterial	Kubo et al. (1992); Pérez et al. (2011); Vukovic et al. (2008)
3	31.03	5.00	Borneol	$C_{10}H_{18}O$	154.25	Antimicrobial	Al-Farhan et al. (2010); Tabanca et al., (2011)
4	10.17	3.19	Caryophyllene	$C_{15}H_{24}$	204.35	Antibacterial, antifungal	Baskaran et al. (2016); Sarada et al. (2011)
5	24.07	1.88	α-farnesene	$C_{15}H_{24}$	204.35	Insecticidal	Yang et al. (2014)
6	16.89	1.78	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antitumor	Kumar et al. (2010)
7	18.88	1.65	9,12-octadecadienoic acid (*Z,Z*)	$C_{18}H_{32}O_2$	280.45	Antitumor	Prabhadevi et al. (2012); Sermakkani and Thangapandian (2012)
8	13.06	1.58	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	Anti-inflammatory, antitumor, antibacterial, analgesic, anesthetic	Rajeswari et al. (2011)
9	14.01	1.49	β-eudesmol	$C_{15}H_{26}O$	222.37	Antifungal	Su and Ho (2013)
10	23.29	1.23	Santolina epoxide	$C_{10}H_{16}O$	152.23	Cardiovascular disorder	Rasheed et al. (2015)
11	16.82	1.20	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antitumor	Devi and Muthu (2014); Selvamangai and Bhaskar (2012)
12	9.30	1.15	Copaene	$C_{15}H_{24}$	204.35	Antibacterial	Solis et al. (2004)
13	15.68	0.99	Norelidol	$C_{15}H_{26}O$	222.37	Antifugal	Krist et al. (2015)
14	11.04	0.93	α-muurolene	$C_{15}H_{24}$	204.35	Antioxidant	Gurbuz et al. (2013)
15	13.61	0.91	Cubenol	$C_{15}H_{26}O$	222.37	Anti-inflammatory	Lee et al. (2010)
16	11.25	0.89	α-curcumene	$C_{15}H_{24}$	204.35	Antibacterial	Sadashiva et al. (2010); Merghache et al. (2014)
17	13.25	0.83	Thujopsene	$C_{15}H_{24}$	204.35	Antifungal	Manter and Kelsey (2007); Barrero et al. (2005)
18	8.84	0.61	α-cubebene	$C_{15}H_{24}$	204.35	Antibacterial, antioxidant	Naidoo et al. (2009)
19	9.16	0.60	Epizonarene	$C_{15}H_{24}$	204.35	Antidiabetic	Keskes et al. (2016)
20	8.78	0.59	α-gurjenene	$C_{15}H_{24}$	204.35	Insecticidal	González et al. (2014); Lavanya et al. (2014)
21	19.06	0.58	9,12,15-octadecatrienoic acid, (*Z,Z,Z*)	$C_{18}H_{30}O_2$	278.43	Antitumor	Prabhadevi et al. (2012); Mickymaray et al. (2015)
22	14.45	0.56	Farnesol	$C_{15}H_{26}O$	222.37	Antifungal	Brilhante et al. (2013)
23	21.44	0.52	Totarol	$C_{20}H_{30}O$	286.45	Antimicrobial	Mossa et al. (2004); Kubo et al. (1992)
24	12.78	0.40	Elixene	$C_{15}H_{24}$	204.35	Anti-inflammatory	Li et al. (2014)
25	9.89	0.32	(*Z,Z*)-α-farnesene	$C_{15}H_{24}$	204.35	Antioxidant	Çelik (2014)
26	10.74	0.26	α-humulene	$C_{15}H_{24}$	204.35	Antitumor	Hadri et al. (2010); Legault and Pichette (2007)
27	12.47	0.23	α-calacorene	$C_{15}H_{24}$	204.35	Antibacterial	Shaik et al. (2014)
28	18.73	0.19	9-octadecenoic acid, (*E*)	$C_{18}H_{34}O$	282.46	Antitumor	Sagwan et al. (2013); Kajalakshmi and Mohan (2016)
29	8.52	0.15	Isolongifolene	$C_{15}H_{24}$	204.35	Antioxidant	Rangasamy and Namasivayam (2014)
30	10.83	0.15	Aromadendrene	$C_{15}H_{24}$	204.35	Antibacterial	Mulyaningsih et al. (2010)
31	10.67	0.12	Isoledene	$C_{15}H_{24}$	204.35	Antitumor	Asif et al. (2016)
32	25.54	0.12	Geranylgeraniol	$C_{20}H_{34}O$	290.48	Antibacterial	Vik et al., (2007); Togashi et al. (2008)
33	7.80	0.07	Cyperene	$C_{15}H_{24}$	204.35	Antifungal	Ghannadi et al. (2012)
34	17.48	0.06	Geranyl linalool	$C_{20}H_{34}O$	290.48	Antibacterial, antifungal	Soares et al. (2012); Delaquis et al. (2002); Pattnaik et al. (1996)

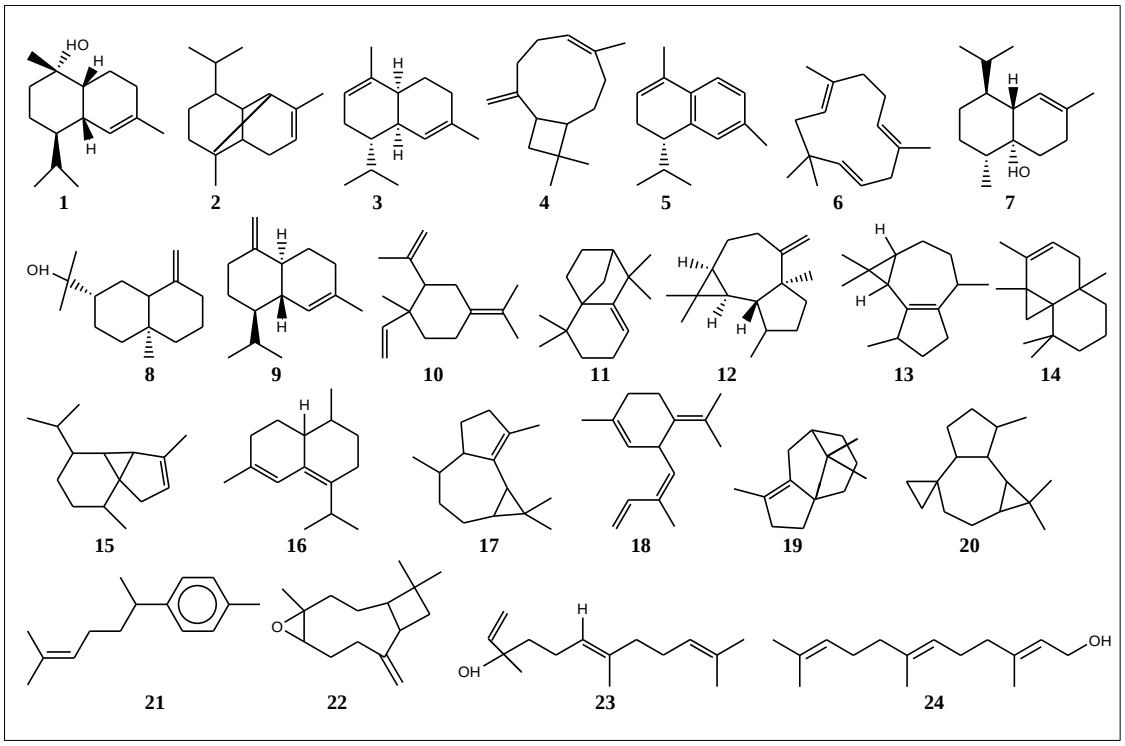

Figure 1. Structures of δ-cadinol (1), copaene (2), α-muurolene (3), caryophyllene (4), α-calacorene (5), α-humulene (6), cubenol (7), β-eudesmol (8), γ-cadinene (9), elixene (10), isolongifolene (11), aromadendrene (12), isoledene (13), thujopsene (14), α-cubebene (15), epizonarene (16), α-gurjenene (17), α-farnesene (18), cyperene (19), (*Z,Z*)-α-farnesene (20), α-curcumene (21), caryophyllene oxide (22), norelidol (23) and farnesol (24) from *M. aethiopicum* chloroform root bark extract.

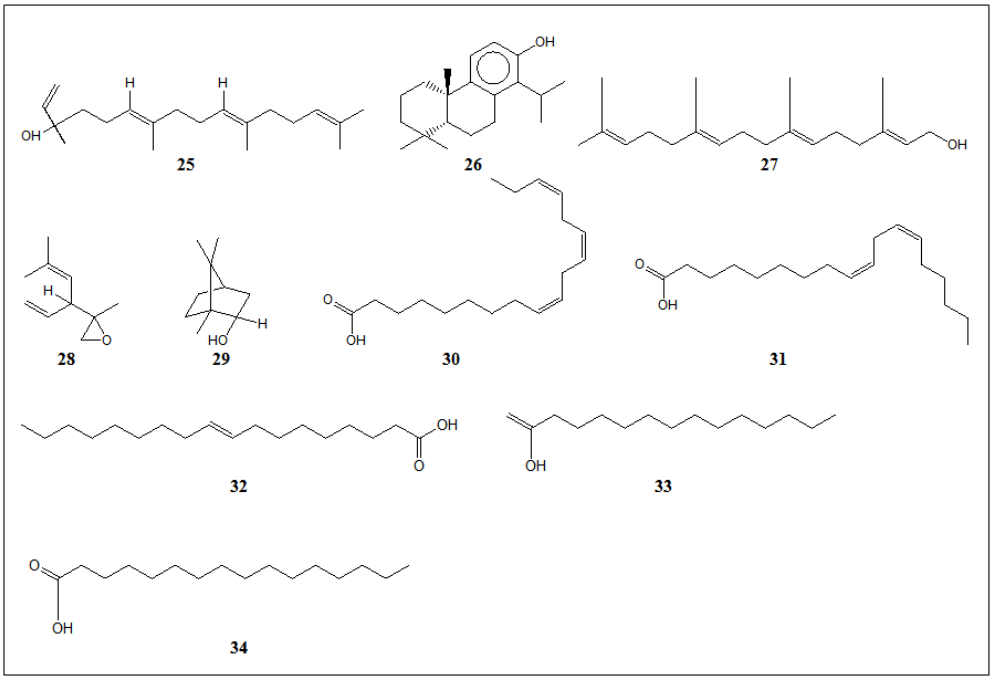

Figure 2. Structures of geranyl linalool (25), totarol (26), geranylgeraniol (27), borneol (28), santolina epoxide (29), 9,12,15-octadecatrienoic acid, (*Z,Z,Z*) (30), 9,12-octadecadienoic acid (*Z,Z*) (31), 9-octadecenoic acid, (*E*) (32), tetradecanoic acid (33) and *n*-hexadecanoic acid (34) from *M. aethiopicum* chloroform root bark extract.

α-humulene, isoledene, caryophyllene oxide, 9,12,15-octadecatrienoic acid (*Z,Z,Z*), 9,12-octadecadienoic acid (*Z,Z*), 9-octadecenoic acid (*E*), tetradecanoic acid and *n*-hexadecanoic acid (Kumar et al., 2010; Prabhadevi et al., 2012; Sermakkani and Thangapandian, 2012; Rajeswari et al., 2011; Devi and Muthu, 2014; Selvamangai and Bhaskar, 2012; Prabhadevi et al., 2012; Mickymaray et al., 2015; Hadri et al., 2010; Legault and Pichette, 2007; Sagwan et al., 2013; Kajalakshmi and Mohan, 2016; Asif et al., 2016).

Compounds that have been reported to exhibit antibacterial and antifungal activities are δ-cadinol, copaene, caryophyllene, α-calacorene, β-eudesmol, γ-cadinene, aromadendrene, thujopsene, α-cubebene, cyperene, α-curcumene, caryophyllene oxide, norelidol, farnesol, geranyl linalool, totarol, geranylgeraniol and borneol (Ho et al., 2011; Kubo et al., 1992; Pérez et al., 2011; Vukovic et al., 2008; Baskaran et al., 2016; Sarada et al., 2011; Rajeswari et al., 2011; Su and Ho, 2013; Solis et al., 2004; Krist et al., 2015; Sadashiva et al., 2010; Merghache et al., 2014; Manter and Kelsey, 2007; Barrero et al., 2005; Naidoo et al., 2009; Shaik et al., 2014; Mulyaningsih et al., 2010; Ghannadi et al., 2012). α-muurolene, isolongifolene, α-cubebene and (*Z,Z*)-α-farnesene have been reported to possess antioxidant activities (Gurbuz et al., 2013; Naidoo et al., 2009; Çelik, 2014; Rangasamy and Namasivayam, 2014).

Three of the identified compounds were reported to exhibit anti-inflammatory activities while two phytochemicals were reported as insecticidal. These include cubenol, elixene, caryophyllene oxide, α-gurjenene and α-farnesene, respectively (Lee et al., 2010; Li et al., 2014; Rajeswari et al., 2011; González et al., 2014; Lavanya et al., 2014; Yang et al., 2014). Epizonarene and santolina epoxide have been reported to exhibit antidiabetic and cardiovascular disorder, respectively (Keskes et al., 2016; Rasheed et al., 2015).

The reported biological activities of the identified compounds in this study validates the ethnomedical information on to the use of *M. aethiopicum* root bark for the management of hemorrhagic diarrhea, stomachache, respiratory tract infections, urinary tract infections, coughs, gonorrhea, cancer and hypertension (Boer et al., 2005; Iwu, 2014).

Conclusion

The GC-MS analysis of *M. aethiopicum* chloroform root bark extract led to identification of low molecular weight phytochemicals. These phytochemicals are grouped as sesquiterpenes, diterpenes, monoterpenes and fatty acids. The presence of vast number of phytochemicals in the root bark extracts of *M. aethiopicum* justifies its use for various ailments in Africa. Findings from this study have therefore validated the medicinal potential of *M. aethiopicum* root bark.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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