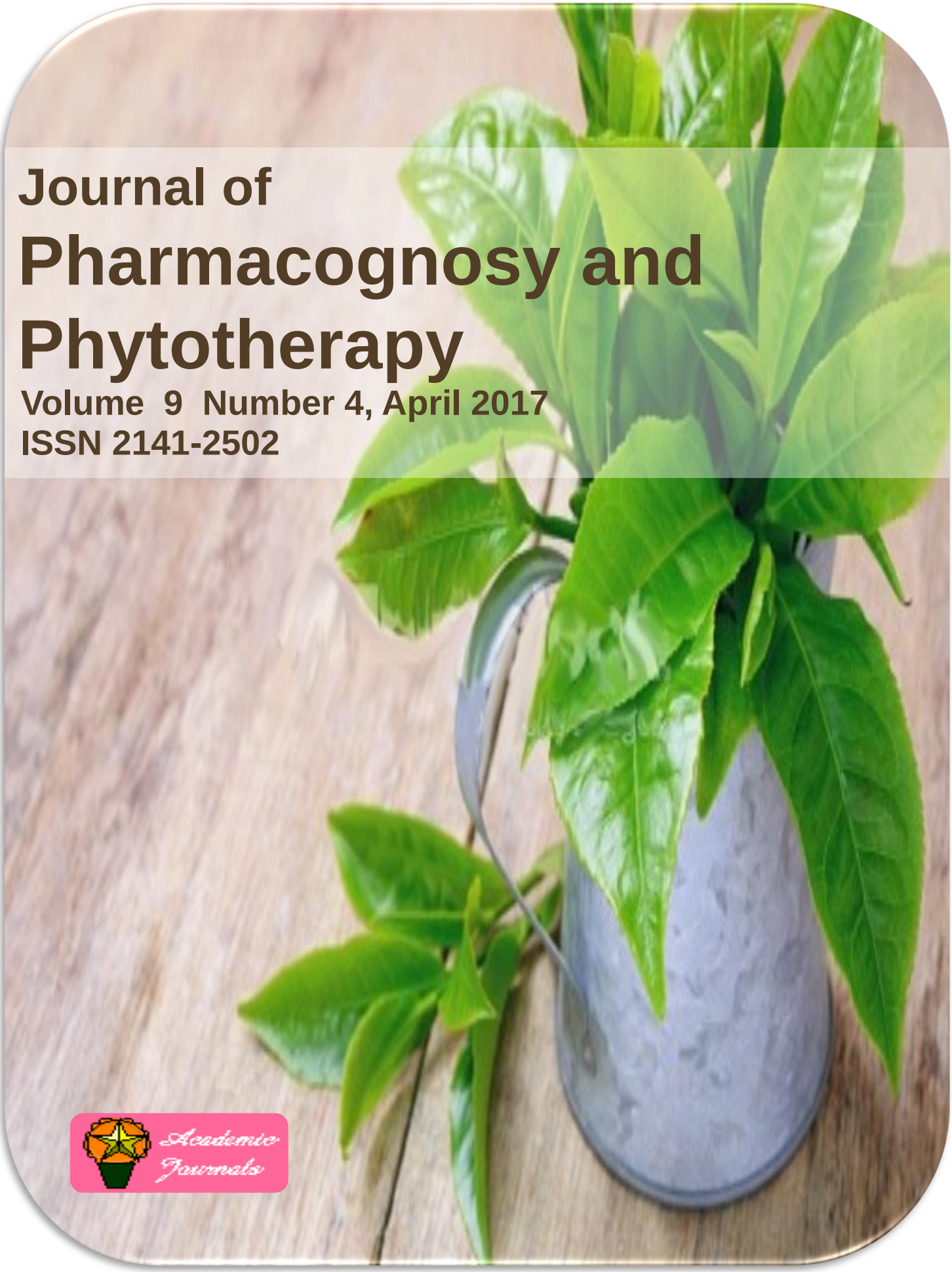

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Full Length Research Paper

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)

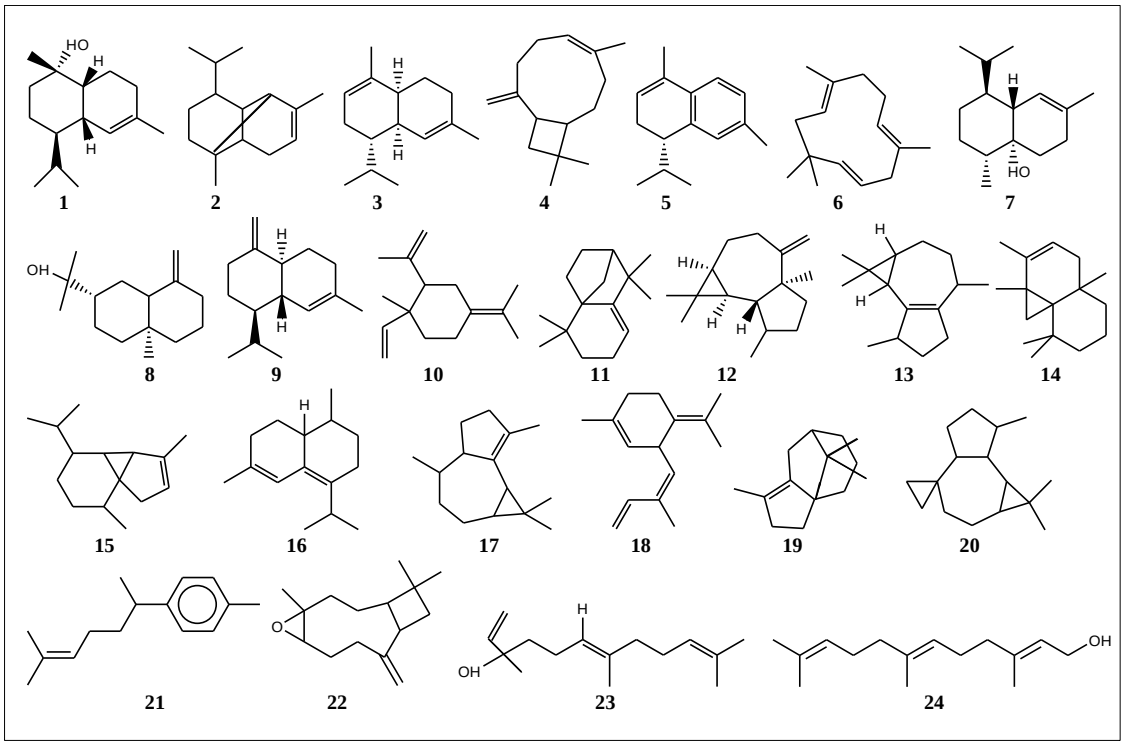

Figure 1. Structures of δ-cadinol (1), copaene (2), α-muurolene (3), caryophyllene (4), α-calacorene (5), α-humulene (6), cubenol (7), β-eudesmol (8), γ-cadinene (9), elixene (10), isolongifolene (11), aromadendrene (12), isoledene (13), thujopsene (14), α-cubebene (15), epizonarene (16), α-gurjenene (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.

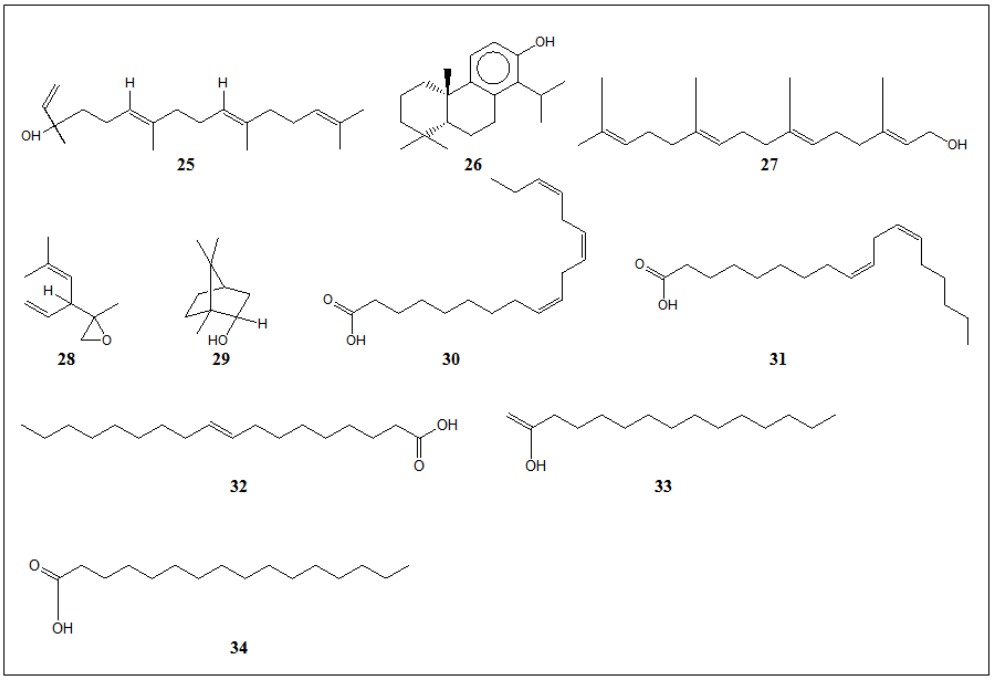

Figure 2. Structures of geranyl linalool (25), totarol (26), geranylgeraniol (27), borneol (28), santolina epoxide (29), 9,12,15-octadecatrienoic acid, (*Z,Z,Z*) (30), 9,12-octadecadienoic acid (*Z,Z*) (31), 9-octadecenoic acid, (*E*) (32), tetradecanoic acid (33) and *n*-hexadecanoic acid (34) from *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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The authors are thankful to the Commission for Science and Technology (COSTECH) through the Nelson Mandela African Institution of Sciences and Technology (NM-AIST) for financial support of this study. Herbalists from Imbibya village are appreciated for availing the authors, ethnomedical information on medicinal plants. Last but not the least, authors wish to acknowledge Mr. Joseph Malulu of the Tropical Pesticides Research Institute (TPRI) for his technical support.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al-Farhan KA, Warad I, Al-Resayes SI, Fouda MM, Ghazzali M (2010). Synthesis, structural chemistry and antimicrobial activity of borneol derivative. Central Eur. J. Chem. 8(5):1127-1133.

Ammal RM, Bai GV (2013). GC-MS Determination of bioactive constituents of *Heliotropium indicum* leaf. J. Med. Plants 1(6):30-33.

Asif M, Shafaei A, Jafari SF, Ezzat MO, Majid AS, Oon CE, Petersen SH, Kono K, Majid AM (2016). Isoledene from *Mesua ferrea* Oleo-gum resin induces apoptosis in HCT 116 cells through ROS-mediated modulation of multiple proteins in the apoptotic pathways: A mechanistic study. Toxicol. Letters 257:84-96.

Balouiri M, Sadiki M, Ibnsoud SK (2016). Methods for in *vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal. 6(2):71-79.

Barrero A, Quilez MJ, Lara A, Herrador M (2005). Antimicrobial activity of sesquiterpenes from the essential oil of *Juniperus thurifera* wood. Plant Med. 71(1):67–71.

Baskaran A, Karthikeyan V, Rajasekaran CS (2016). Gas chromatography-mass spectrometry (GC-MS) analysis of ethanolic extracts of *Barleria longiflora* Lf. World J. Pharm. Pharm. Sci. 5(4):1233-1246.

Boer HJ, Koola A, Broberg A, William R, Mziray WR, Levenfors JJ (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96(3):461-469.

Bohlin L, Bruhn JG (1999). Bioassay methods in natural product research and drug development. Springer Science & Business Media. Second edition: Kluwer Academic Publishers, Netherland. pp. 245-248.

Brilhante RS, Lima RA, Caetano EP, Leite JJ, Castelo DD, Ribeiro JF, Bandeira, TD, Aguiar CR, Monteiro AJ, Sidrim JJ, Rocha MF (2013). Effect of farnesol on growth, ergosterol biosynthesis, and cell permeability in *Coccidioides posadasii*. Antimicrob. Agents Chemother. 57(5):2167-2170.

Burkil HM (2004). The useful plants of west tropical Africa. First edition. Publisher Royal Botanic Gardens: Kew, Richmond, United Kingdom. pp. 25-28.

Burrows JE, Willis CK (2005). Plants of the Nyika Plateau: An account of the Nyika National Parks of Malawi and Zambia. Southern African Botanical Diversity Network. Report No. 31. SABONET, Pretoria.

Çelik K, Toğar B, Türkez H, Taşpinar N (2014). *In vitro* cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. Turk. J. Biol. 38(2):253-259.

Curtis BA, Mannheimer CA (2005). Tree Atlas of Namibia National Botanic Research Institute, Windhoek. pp. 406-410.

Delaquis PJ, Stanich K, Girard B, Mazza G (2002). Antimicrobial activity of individual and mixed fractions of *Dill*, *Cilantro*, *Coriander* and *Eucalyptus* essential oils. Int. J. Food Microb. 74(1):101-109.

Devi J, Muthu AK (2014). Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* Linn. Int. J. Pharm. Pharm. Sci. 6(2):755-

759.
Eisenhauer N, Klier M, Partsch S, Sabais AC, Scherber C, Weisser WW, Scheu S (2009). No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration. J. Appl. Soil Ecol. 42(1):31-36.
Gadir SA (2012). Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay. J. Chem. Pharm. Res. 4(12):5145-5148.
Ghannadi A, Rabbani M, Ghaemmaghami L, Malekian N (2012). Phytochemical screening and essential oil analysis of one of the Persian sedges; *Cyperus rotundus*. Int. J. Pharm. Sci. Res. 3(2):424-427.
González A, Gutiérrez CM, Moenne A (2014). Oligo-carrageenan kappa-induced reducing redox status and increase in TRR/TRX activities promote activation and reprogramming of terpenoid metabolism in *Eucalyptus* trees. Molecules 19(6):7356-7367.
Gurbuz I, Yesilada E, Demirci B, Sezik E, Demirci F, Baser KH (2013). Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (*Styrax liquidus*). J. Ethnopharmacol. 148(1):332-336.
Hadri AE, Gó mez del Río MA, Sanz JS, Gonzá lez CA, Idaomar M, Ribas OB, Benedí GJ, Sánchez MA (2010). Cytotoxic activity of á-humulene and transcaryophyllene from Salvia officinalis in animal and human tumor cells. Real Acad. Nac. Farm. 76 (3):343-356.
Ho PL, Chiu SS, Chan MY, Ang I, Chow KH, Lau YL (2011). Changes in nasopharyngeal carriage and serotype distribution of antibiotic-resistant *Streptococcus pneumoniae* before and after the introduction of 7-valent pneumococcal conjugate vaccine in Hong Kong. J. Diagnostic Micro. Infect. Dis. 71(4):327-334.
Irish J (2012). Namibia biodiversity database: *Mystroxylon aethiopicum* in Namibia. http://www.biodiversity.org.na/taxondisplay.php?nr=4902.
Iwu MM (2014). Handbook of African medicinal plants. Second edition. CRC Press, Taylor & Francis Group, Florida, United States. pp. 28-32.
Kajalakshmi K, Mohan VR (2016). Determination of bioactive components of *Myxsopyrum seratullum* (oleaceae) stemm by GC-MS analysis. Int. Res. J. Pharm. 7:36-42.
Keskes H, Belhadj S, Jlail L, El Feki A, Damak M, Sayadi S, Allouche N (2016). LC-MS–MS and GC-MS analyses of biologically active extracts and fractions from Tunisian *Juniperus phoenice* leaves. J. Pharm. Biol. 55(1):1-8.
Kokwaro JO (1993). Medicinal plants of East Africa. Second edition: Published and printed by Kenya literature bureau, Nairobi Kenya. pp. 176-177.
Krist S, Banovac D, Tabanca N, Wedge DE, Gochev VK, Wanner J, Schmidt E, Jirovetz L (2015). Antimicrobial activity of nerolidol and its derivatives against airborne microbes and further biological activities. Nat. Prod. Com. 10(1):143-148.
Kubo I, Muroi H, Himejima M (1992). Antibacterial activity of totarol and its potentiation. J. Nat. Prod. 55(10):1436-1440.
Kumar PP, Kumaravel S, Lalitha C (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr. J. Biochem. Res. 4(7):191-195.
Lavanya K, Prasada RA, Chakravarthy MB (2014). A review on biological and chemical properties of *Cyperus* species. Res. J. Pharm. Biol. Chem. Sci. 5(5):1142-55.
Lee YJ, Park SY, Kim SG, Kang JS, Lee SJ, Yoon S, Kim YH, Bae YS, Choi YW (2010). Identification of a novel compound that inhibits iNOS and COX-2 expression in LPS-stimulated macrophages from *Schisandra chinensis*. Biochem. Biophy. Res. Com. 391(4):1687-1692.
Legault J, Pichette A (2007). Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol. 59(12):1643-1647.
Li R, Yang JJ, Wang YF, Sun Q, Hu HB (2014). Chemical composition, antioxidant, antimicrobial and anti-inflammatory activities of the stem and leaf essential oils from *Piper flaviflorum* from Xishuangbanna, SW China. Nat. Prod. Com. 9(7):1011-1014.
Mancini E, Arnold NA, De Martino L, De Feo V, Formisano C, Rigano D, Senatore F (2009). Chemical composition and phytotoxic effects of essential oils of *Salvia hierosolymitana* Boiss and *Salvia multicaulis* Vahl simplicifolia Boiss growing wild in Lebanon. Molecules 14(11):4725-4736.
Manter DK, Kelsey RG (2007). Antimicrobial activity of extractable conifer heartwood compounds toward *Phytophthora ramorum*. J. Chem. Ecol. 33(11):2133-2147.
Merghache D, Boucherit OZ, Merghache S, Chikhi I, Selles C, Boucherit K (2014). Chemical composition, antibacterial, antifungal and antioxidant activities of Algerian *Eryngium tricuspidatum* essential oil. Nat. Prod. Res. 28(11):795-807.
Mickymaray S, Al Aboody MS, Rath PK, Annamalai P, Nooruddin T (2016). Screening and antibacterial efficacy of selected Indian medicinal plants. Asian Pacif. J. Trop. Biomed. 6(3):185-191.
Mossa JS, El-Feraly FS, Muhammad I (2004). Antimycobacterial constituents from *Juniperus procera*, *Ferula communis* and *Plumbago zeylanica* and their in vitro synergistic activity with isonicotinic acid hydrazide. Phytother. Res. 18(11):934-937.
Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M (2010). Synergistic properties of the terpenoids aromadendrene and 1, 8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. J. Phytomed. 17(13):1061-1066.
Naidoo N, Thangaraj K, Odhav B, Baijnath H (2009). Chemical composition and biological activity of the essential oil from *Cymbopogon nardus*. Afr. J. Trad. Complimentary Altern. Med. 6:395.
Pattnaik S, Subramanyam VR, Bapaji M, Kole CR (1996). Antibacterial and antifungal activity of aromatic constituents of essential oils.
Microbiology 89(358):39-46.
Pérez A, Cirio AT, Rivas VM Aranda RS, Torres NW (2011). Activity against *Streptococcus pneumoniae* of the essential oil and d-cadinene isolated from *Schinus molle* fruit. J. Essential Oil Res. 23(11):25-28.
Pokharen N, Dahal S, Anuradha M (2011). Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*. J. Med. Plants Res. 5(24):5785-5788.
Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N (2012). Phytochemical studies on *Allamanda cathartica* using GC–MS. Asian Pacific J. Trop. Biom. 2(2):550-554.
Rajeswari N, RamaLakshmi S, Muthuchelian K (2011). GC-MS analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum*. J. Chem. Pharm. Res. 3(3):792-798.
Rangasamy K, Namasivayam E (2014). *In vitro* antioxidant and free radical scavenging activity of isolongifolene. Asian J. Biol. Sci. 7(1):13-23.
Rasheed HM, Khan T, Wahid F, Khan R, Shah AJ (2015). Chemical composition and vasorelaxant and antispasmodic effects of essential oil from *Rosa indica* petals. Evidence-Based Comp. Altern. Med. 9:1-6.
Roukia H, Mahfoud HM, Ould MD (2013). Chemical composition and antioxidant and antimicrobial activities of the essential oil from *Teucrium polium* geyrii (Labiatae). J. Med. Plants Res. 7(20):1506-1510.
Sadashiva CT, Sharanappa P, Remashree AB, Raghu AV, Udayan PS, Balachandran I (2010). Chemical composition and antimicrobial activity of the essential oil from bark of *Pittosporum dasycaulon*. Adv. J. Biol. Res. 4(6):301-304.
Sagwan S, Rao DV, Sharma RA (2013). GC/MS spectroscopic analysis of some different *in vivo* methanolic plant extracts of *Maytenus emarginata* (willd.): an important medicinal plants. Int. J. Instit. Pharm. Life Sci. 3(5):76-84.
Sarada K, Margret RJ, Mohan VR (2011). GC–MS determination of bioactive components of *Naringi crenulata* (roxb) *nicolson*. Int. J. Chem. Tech. Res. 3(3):1548-1555.
Selvamangai G, Bhaskar A (2012). GC–MS analysis of phytocomponents in the methanolic extract of *Eupatorium triplinerve*. Asian Pacif. J. Trop. Biomed. 2(3):1329-1332.
Sermakkani M, Thangapandian V (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J. Pharm. Clin. Res. 5(2):90-94.
Shaik G, Sujatha N, Mehar SK (2014). Medicinal plants as source of antibacterial agents to counter *Klebsiella pneumoniae*. J. Appl. Pharm. Sci. 4(1):135-147.
Soares BV, Morais SM, Dos Santos Fontenelle RO, Queiroz VA, Vila-Nova NS, Pereira C, Brito ES, Neto MA, Brito EH, Cavalcante CS, Castelo-Branco DS (2012). Antifungal activity, toxicity and chemical

composition of the essential oil of *Coriandrum sativum* L. fruits. Molecules 17(7):8439-8448.
Solis C, Becerra J, Flores C, Robledo J, Silva M (2004). Antibacterial and antifungal terpenes from *Pilgerodendron uviferum* (D. Don) Florin. J. Chilean Chem. Soc. 49(2):157-161.
Su YC, Ho CL (2013). Composition and two activities of the leaf essential oil of *Litsea acuminata* (Blume) kurata from Taiwan. Rec. Nat. Prod. 7(1):27-34.
Tabanca N, Kirimer N, Demirci B, Demirci F, Baser KH (2001). Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. phrygia and the enantiomeric distribution of borneol. J. Agric. Food Chem. 49(9):4300-4303.
Togashi N, Inoue Y, Hamashima H, Takano A (2008). Effects of two terpene alcohols on the antibacterial activity and the mode of action of farnesol against *Staphylococcus aureus*. Molecules 13(12):3069-3076.
Vik A, James A, Gundersen LL (2007). Screening of terpenes and derivatives for antimycobacterial activity; identification of geranylgeraniol and geranylgeranyl acetate as potent inhibitors of *Mycobacterium tuberculosis in vitro*. Plant Med. 73(13):1410-1412.
Vukovic N, Milosevic T, Sukdolak S, Solujic S (2008). The chemical composition of the essential oil and the antibacterial activities of the essential oil and methanol extract of *Teucrium montanum*. J. Serb. Chem. Soc. 73(3):299-305.
Yang W, Zhao A, Congai Z, Qizhi L, Wangpeng S (2014). Composition of the essential oil of *Cynanchum mongolicum* (Asclepiadaceae) and insecticidal activities against *Aphis glycines* (Hemiptera: Aphidiae). Pharmacog. Magazine 10(1):130-134.

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Full Length Research Paper

Anti-asthma potential of dried *Draco Spilopterus* Wieg. 1834 (Philippine Flying Dragon) using mesenteric mast cell count by atopic allergy method

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Decocted *Draco spilopterus* has been utilized in the Philippines for the treatment of asthma associated with its strong folkloric beliefs however no scientific evidence was available to prove such claims. Thus, the researchers ventured into this study to determine the anti - asthma potential of dried *D. spilopterus* on male albino rats using mesenteric mast cell count by atopic allergy method. Fifteen *D. spilopterus* were used in the study. Decocotion was the extraction method employed. Seven male albino rats were used in each trial and randomly divided into different test groups which were as follows: Treatment groups (600, 800 and 1000 mg/kgbw); negative control group (water); positive control group (Prednisolone 10 mg/kgbw); untreated group; and normal group. Mesentric mast cell count by atopic allergy method was employed in this study. Results revealed that the test solutions with a dose of 600, 800 and 1000 mg/kgbw elicited a mean anti-asthmatic activity of 42.25, 66.44 and 89.32%, respectively. The median effective dose was 663.90 mg/kgbw. The anti - asthma activity was dose - related; with increasing dose, the disrupted mast cell decreases. The test solution obtained from *D. spilopterus* is a potential alternative in the management of asthma but further studies have to be conducted.

Key words: Draco spilopterus, mesenteric mast cell count.

INTRODUCTION

Asthma is a chronic disease that affects the airways of the lungs characterized as airway hyper responsiveness and airflow obstruction at the bronchial level (Blitski et al., 2013; Nimgulkar et al., 2011; Varona et al., 2014). According to the World Health Organization (WHO) estimates, 235 million people suffer from asthma. It is a public health problem in all countries regardless of level of development. Over 80% of asthma deaths occur in low and lower - middle income countries, such as the Philippines. According to the WHO data in May 2014,

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asthma deaths in the Philippines reached 2.37% of total deaths. Asthma belongs to the top 10 causes of death in the Philippines. In a study by Lai et al. (2006) the estimated direct per - patient cost of asthma, including maintenance, urgent care, and drug as the basis, in the Philippines was $212±11. The health care expenditure for the treatment for asthma in the Philippines is costly. Furthermore, drugs used in the management of asthma are associated with several drawbacks. Inhaled corticosteroids work quickly and effectively in about 95% of patients with acute asthma. While it is challenging to beat the efficacy of inhaled steroids, new drugs should better them on their side effects profiles, especially when used frequently in a long - term fashion. There is still a concern about the use of inhaled corticosteroids as patients fear long - term side effects such as osteoporosis and stunting growth in children (Barnes, 2009). Thus, this necessitates the search for new therapies for asthma, driven by the prospect of a significant need for anti - asthma medications globally.

We ventured into this study to provide another management regimen for asthma which is native and rich in the Philippine forests to help especially those who are located in remote areas with difficult access to hospitals and medical care facilities. Influenced by folkloric belief originating from the ancient Chinese, the *D. spilopterus*, also known as the Philippine Flying Dragon, could effectively cure asthma. With this belief, some Filipinos have adapted this kind of regimen. The belief has been and is still practiced in the Philippines. However, no scientific evidence was available to prove such claims. Thus, this study was conducted, for the first time, in order to determine the number disrupted mesenteric mast cells of the rats (Figure 3), in relation to the treatment groups; determine which among the doses would produce the least disruption on the mesenteric mast cells; determine the percent activity of the test solutions; lastly, determine the median effective dose of the test solutions (Figure 2).

MATERIALS AND METHODS

Collection and preparation of the animal sample

The dried *D. spilopterus* were purchased in a store located along Osmeña Boulevard, Cebu City, Philippines. They were authenticated by the Department of Biology, University of San Carlos. They were washed to remove adhering materials and cut into small pieces.

Extraction of the animal sample

The extraction method used was decoction. Fifteen cut dried *D. spilopterus* were placed in a pot. Water was added and was brought to a boil for 15 to 20 min. Once it boiled, the pot was covered and removed from the heat. The stove was set to lowest heat and the liquid was allowed to simmer for about 45 min. Once the end of the simmer time was reached, the pot was removed from heat and was set aside for about two hours to cool down. The boiled flying lizards were strained out of the liquid and the remaining extract was squeezed out. The filtrate was evaporated to dryness using an evaporating dish using a water bath.

Preparation of the stock solution

About 9.6 g of dried powder extract from the boiled *D. spilopterus* was weighed and dissolved in sufficient amount of water to obtain a 100% w/v stock solution. From the stock solution, three different arbitrary doses of 600, 800 and 1000 mg/kgBW of rat, were administered to the treatment groups.

Preparation of the positive and negative controls

Prednisolone (20 mg/5 ml) syrup was used for the dose adjustments of the individual rats. Only one dose served as the positive control (10 mg/kg). The National Asthma Education and Prevention Program Expert Panel recommend 40 to 180 mg/day P.O. dosage of prednisolone. The dose was calculated by the average therapeutic dose of humans to rat on the basis of BSA (conversion factor - 0.018 for rats) by referring to the table of Paget and Barnes (1964). The negative control was plainly water with a dose of 0.2 ml/20 g.

Test animals

All test animals were housed in the University of San Carlos animal house and the research protocol was conducted under the supervision of a certified and trained animal technician ensuring proper animal handling was observed throughout the duration of the study. Male albino rats weighing about 100 to 200 g were selected. Prior to experimentation, the rats were divided into seven groups (Table 1) each containing three rats and then acclimatized for three days with a diet of standard rat pellets.

Mesenteric mast cell count by atopic allergy method

The procedure adopted for mesenteric mast cell count is by atopic allergy method in accordance with the study of Reddy et al. (2010) and Balaji et al. (2014).

Sensitization of the test animals

After three days of acclimatization, the rats were sensitized only on the first day of experimentation by injecting 0.5 ml of horse serum and 0.5 ml of triple antigen, subcutaneously, specifically near the abdomen. The horse serum was for the induction the allergic reaction, which was supported by the administration of the antigen.

Administration of doses

Group 1 was used as negative control without any drug treatment and Group 2 was used as positive control and was administered with prednisolone, 10 mg/kg body weight, orally. Groups 3, 4 and 5 were treated with 600 mg/kg body weight, 800 mg/kg body weight and 1000 mg/kg body weight of the test solution prepared from *D. spilopterus* extract. Group 6 was used as the untreated group and therefore received no treatment. Group 7 was used as the normal group and was not sensitized by the allergen and no treatment was received. Complete allergic reaction of the horse serum occurs after 5 to 24 h after administration. After sensitization, the rats were treated with prednisolone and test solutions of different doses from the 5th to 12th day.

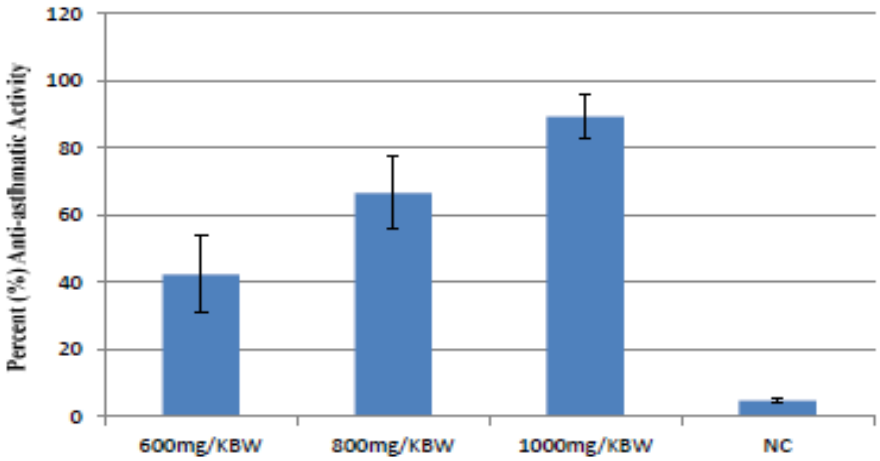


Figure 1. Percent anti-athma activity.

Staining of the mesenteric mast cells

On the 13th day, all rats from each group were sacrificed for microscopic examination on the mast cells. The mesenteries of the sacrificed animals along with pieces of intestine were suspended in Krebs-Ringer solution in order to obtain free, membrane-bound granules for rupturing cells. The mesentery pieces were exposed with 5% horse serum for 10 min. Pieces of mesentery were stained superficially with toluidine blue for mast cells to stain metachromatically. The cells stained a different color from the dye solution and the rest of the tissue. Mast cells stained red-purple (metachromatic staining) and the background stained blue (orthochromatic staining). Metachromasia, tissue elements staining a different color from the dye solution, is due to the pH, dye concentration and temperature of the basic dye. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements. The tissue was immersed in 0.1% toluidine blue (in 4% aqueous saline) for 10 min. The tissue was cleared in xylene for 5 to 10 min. It was rinsed with acetone thrice and was placed on a microscopic slide, stretched with the help of a needle. The intestinal pieces were cut and removed.

Counting of disrupted mast cells

The tissue was examined under the microscope in 100X magnification. Three slides per animal were used. Disrupted mast cells were stained red-purple and the normal mast cells were stained blue. Each slide was divided in three grids and the disrupted mast cells in four randomly selected fields for each grid were counted. With the numbers of disrupted mast cells, the percent anti-asthmatic activity was calculated using the formula shown below. Anti-asthmatic activity shown by the negative control is considered negligible:

$$Percent\ Activity\ (positive\ control) = \frac{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

$$Percent\ Activity\ (treatment\ groups) = \frac{Untreated_{disrupted\ mast\ cell} - Test\ solution_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

$$Percent\ Activity\ (negative\ control) = \frac{Untreated_{disrupted\ mast\ cell} - Negative\ control_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

Median effective dose (ED_{50})

A linear regression analysis was used to obtain a linear equation through Microsoft Excel 2007 that will be then be used to calculate ED_{50}.

Statistical analysis

Statistical analysis was performed using Microsoft Excel software and StatPlus 2009 program. To confirm the variability of the data and validity of results, the number of disrupted mast cells was compared using one-way analysis of variance (ANOVA). Differences between the untreated and the test solutions were considered statistically significant at standard *P* value ≤ 0.01.

RESULTS AND DISCUSSION

The test solution of *D. spilopterus*, based on the results, indicates an anti-asthma property. This was exhibited by the number of disrupted mast cells obtained and counted from the intestinal mesentery of the test animals. The anti-asthma activity exhibited by the *D. spilopterus* was dose-related; with increasing dose, the disrupted mast cell decreases.

The median effective dose (ED50) is 663.90 mg/KBW. Theoretically, the dose in which 50% anti-asthmatic activity will be exhibited by the test solutions is 663.90 mg/KBW. Linearity was verified by analysis of different concentrations. As a result, regression analysis showed good correlations with R^2 = 0.9997 in the doses 600, 800 and 1000 mg/KBW. This means that there is a direct relationship between the dose and the percent anti-asthmatic activity (Figure 1).

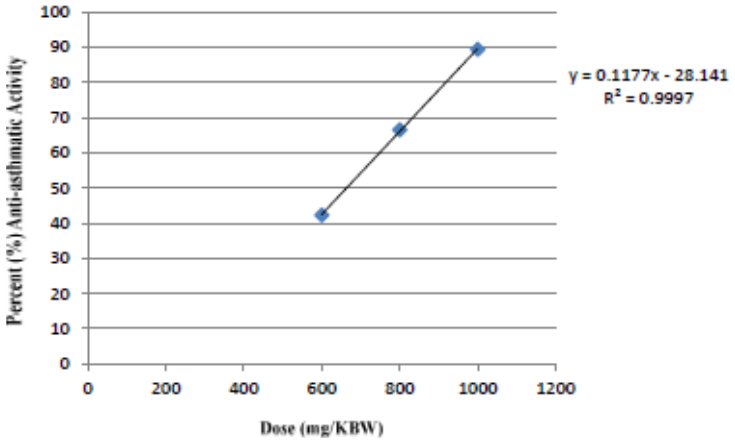


Figure 2. Median effective dose.

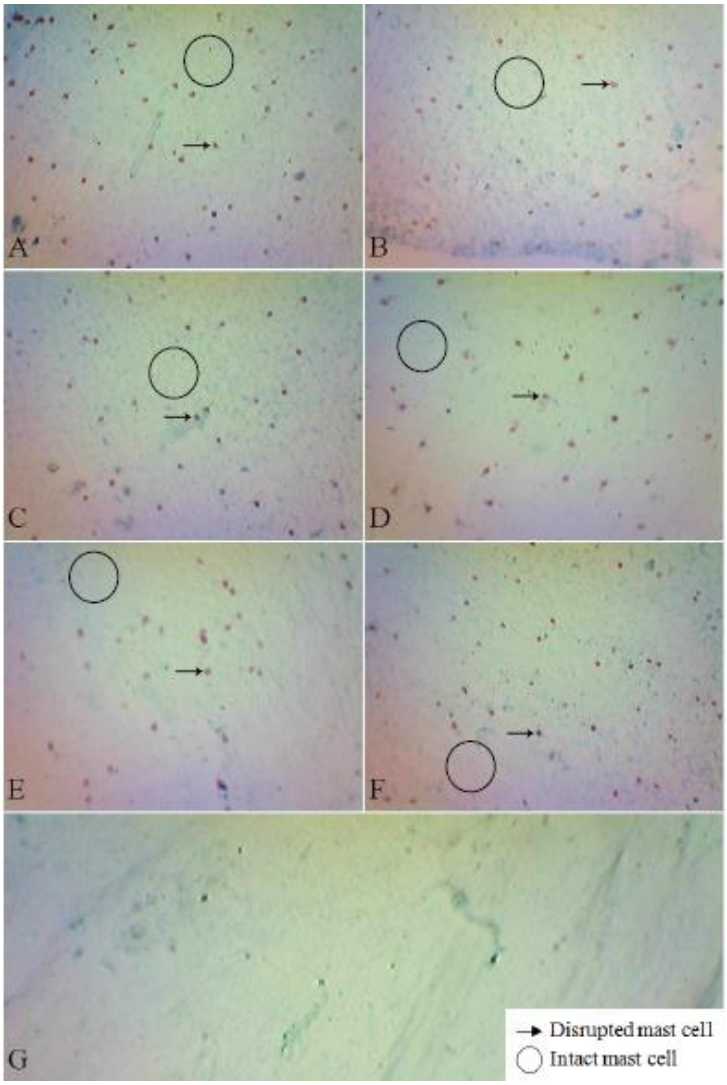


Figure 3. Comparison of disrupted mast cells among the positive control, negative control, test solutions and untreated; A: Negative Control; B: Positive Control; C: 600 mg/KBW; D: 800 mg/KBW; E: 1000 mg/KBW; F: Untreated; G: Normal.

Table 1. Treatment groups.

Treatment groups	Treatment
1	Negative control (Water - 0.2/20 g)
2	Positive control (Prednisolone - 10 mg/KBW)
3	Test solution (600 mg/KBW)
4	Test solution (800 mg/KBW)
5	Test solution (1000 mg/KBW)
6	Untreated (Sensitized without treatment)
7	Normal (No sensitization and treatment)

Table 2. One-way ANOVA for percent anti-asthma activity.

Source of variation	d.f.	SS	MS	F	p-value
Between Groups	3	11,760.8268	3,920.2756	53.4157	0.0000123
Within Groups	8	587.1346	73.3918		
Total	11	12,347.9614			

A probability (*p*) value of 0.0000123 was obtained. A *p*-value of < 0.01 indicates that a statistical significant difference exists between the different doses in relation to their anti-asthmatic activity. This indicates that the observed difference in outcomes is due to the observed effect. The treatment groups have a dose dependent relationship. As the dose increases, the percent anti-asthmatic activity also increases (Table 2).

The probable mechanism of *Draco spilopterus*'s anti-asthma activity is that it offers significant protection against degranulation by stabilizing the mast cells, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators. This may be due to the presence of alkaloids.

Chemical analysis on the secondary metabolites *D. spilopterus* has never been conducted however, based on literature, chemical tests was performed on another lizard species, *Tropidurus semitaeniatus*, utilized in traditional medicine in Northeast Brazil which revealed that it contains alkaloids (Santos et al., 2012). A study also revealed that an alkaloid produced statistically significant protection against Tween 80 - induced degranulation, as also to sensitized mast cells challenged with an antigen (horse serum) (Ghosal et al., 1986).

Based on the results, the proponents conclude that the extract of dried *D. spilopterus* in doses of 600, 800 and 1000 mg/KBW elicited an anti-asthmatic activity on male albino rats. The positive control group administered with 20 mg/5 ml prednisolone produced an average of 170 disrupted mast cells. The negative control group administered with plainly water produced an average of 343 disrupted mast cells. The treatment groups with doses 600, 800 and 1000 mg/KBW produced an average of 275, 232 and 190 disrupted mast cells, respectively. Based on the number of disrupted mast cells, the dose of 1000 mg/KBW showed the most significant relationship with the least number of disrupted mast cells. The average calculated percent anti-asthmatic activity of the test solutions were 42.25% for 600 mg/KBW, 66.44% for 800 mg/KBW, 89.32% for 1000 mg/KBW. Using the obtained percent activity, the median effective dose (ED50) is 663.90 mg/KBW.

These findings provide a preliminary data on the anti-asthma effect of decocted *D. spilopterus*. Further investigations on the isolation of specific compounds and toxicity studies may warrant the development of the animal extract into a drug product.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Balaji G, Chalamaiah M, Hanumanna P, Vamsikrishna D, Kumar J, Venu-babu V (2014). Mast cell stabilizing and anti – anaphylactic activity of aqueous extract of green tea (*Camellia sinensis*). Int. J. Vet. Sci. Med. 2:89-94.

Barnes PJ (2009). New Drugs for Asthma. National Heart and Lung Institute, Imperial College of London. Discovery Medicine.

Blitski MS, Wenzel S, Vitari C (2013). What is Asthma? Am. J. Respir. Crit. Care Med. 188:7-8.

Ghosal S., Shanthy A., Mukhopadhyay M (1986). Effect of lycoriside, an acylglucosyloxy alkaloid, on mast cells. Pharm. Res. 3:240.

Lai CKW, Kuo SH, de Guia T, Lloyd A, Williams AE, Spencer MD (2006). Asthma control and its direct healthcare costs: findings using a derived Asthma Control Test score in eight Asia – Pacific areas. Eur. Respir. Rev. 15(98):24-29.

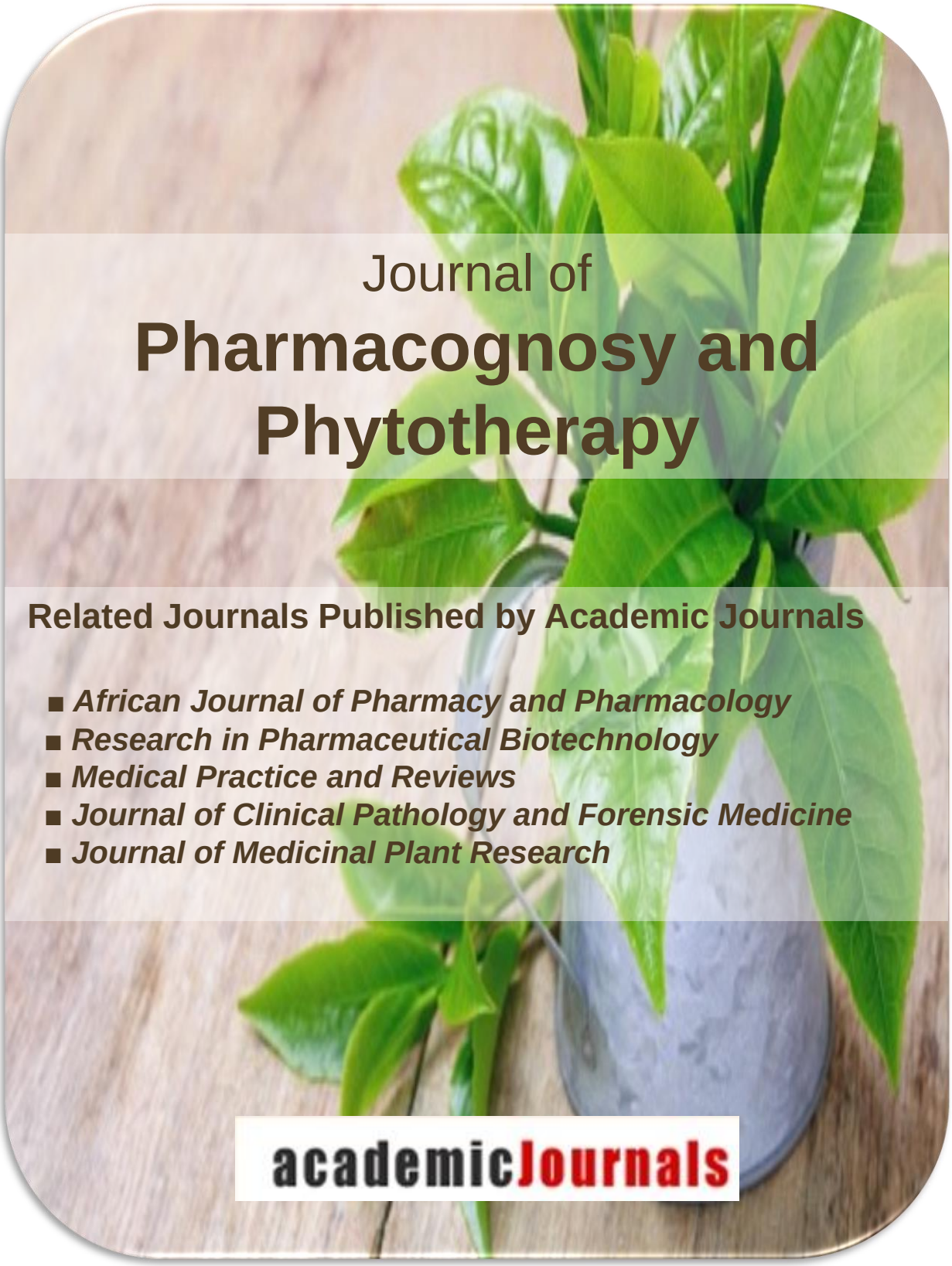
Nimgulkar CC, Patil SD, Kumar BD (2011). Anti-asthmatic and anti-anaphylactic activities of Blatta orientalis mother tincture. Homeopathy 100:138-143.

Reddy J, Gnanasekaran D, Vijay D, Ranganathan TV (2010). In vitro studies on anti asthmatic, analgesic and anti convulsant activities of the medicinal plant Bryonia laciniosa. Linn. Int. J. Drug Discov. 2(2):1-10.

Santos IJM, Matias EFF, Santos KKA (2012), "Evaluation of the Antimicrobial Activity of the Decoction of Tropidurus hispidus (Spix, 1825) and *Tropidurus semitaeniatus* (Spix, 1825) Used by the Traditional Medicine," Evid. Based Complementary Altern. Med. 6 p.

Varona LL, Alava HDA, Abong JM, Castor MAR, De Leon JC, Kwong SL (2014). Prevalence of Asthma Among Filipino Adults Based on the National Nutrition and Health Survey (NNHeS). Philipp J. Int. Med. 52(4):1-7.

World Health Organization (2013). Asthma. http://www.who.int/topics/asthma/en/



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