Vol. 17(3), pp. 100-105, March, 2023
DOI: 10.5897/JMPR2022.7287
Article Number: F6A5E1D70510
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Phytochemical screening and gas chromatography-mass spectrometry analysis of *Euphorbia ingens* organic root extract

Innocent Oluwaseun Okpako[1]*, Florence A. Ng'ong'a[2], Mutinda C. Kyama[3] and Sospeter N. Njeru[4]

[1]Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya.
[2]Department of Biochemistry, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[3]Department of Medical Laboratory Sciences, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[4]Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Nairobi, Kenya.

Received 12 December, 2022; Accepted 17 March, 2023

Euphorbia ingens **is a woody, succulent tree with ethnomedicinal applications; however, its chemical constituents are not well documented. This study determined the phytochemical profile of *Euphorbia ingens* dichloromethane-methanol root bark extract. Following sample collection and preparation, extraction was done using the cold percolation technique. This was followed by qualitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis. This study reports relative high abundance of phenols, tannins and saponins with moderate and low levels of flavonoids and terpenoids respectively in *E. ingens* dichloromethane-methanol root bark extract. However, alkaloids, quinones and sterols were not identified in this study. Additionally, 14 phytocompounds were identified through GC-MS analysis, including undecane; copaene; 3,4-altrosan; 9, 12, 15-octadecatrienoic acid; hexadecanoic acid; 1,2-benzenedicarboxylic acid; lanosterol; androst-2-en-17-one; lanosterol; 2-Bornanol; octadecanoic acid; squalene; 6-Pentylidene-4,5-secoandrostane-4,17.beta.-diol, and ethyl trans-4a, cis-4b, trans-8a, cis-10a-perhydro-trans-2,4a,8a-trimethyl-8-oxophenanthrene-2-carbothiolate. These phytocompounds have been documented to have diverse pharmacological activities from antimicrobial, antioxidant, anticancer, hypercholesterolemic and anti-inflammatory activities. Therefore, it was assumed in this study that these phytocompounds could be responsible for documented *E. ingens* pharmacological activities. Thus, the results of this study could support to some extent the ethnomedical uses of *E. ingens*. However, further biological investigations to validate the reported ethnobotanical uses of *E. ingens* are required.**

Key words: *Euphorbia ingens,* phytocompounds, ethnomedical, pharmacological potential, biological activity.

INTRODUCTION

In many regions, in traditional medicine, medicinal plants are often used for the treatment and management of various ailments. Therefore, they have long been under major consideration for treatment intervention in many parts of the world. Plants are repositories of various secondary metabolites which are the active players in the herbal plants' medicinal activity. The nature and the amount of secondary metabolites (commonly referred to as phytochemicals) vary from plant to plant. Phytochemicals are present in plant parts such as roots, leaves, fruits, stem, flowers and bark. Biological activities, including antiplasmodial, antimycobacterial, antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, anticancer activities among others, have been reported as being associated with plant-derived phytochemicals such as phenols, flavonoids, tannins, saponins, alkaloids, terpenoids and anthraquinnones (Starlin et al., 2019).

Euphorbia comprises one of the widespread genera of medicinal plants with therapeutic potential over a wide range of health issues. Taxonomically, they are Euphorbiaceae, some of them have latex, and the family as a whole includes monoecious and dioecious, herbaceous, shrubby, vining, and tree species (Simpson, 2010). Various Euphorbia species are reported as traditionally used to treat illnesses including, cancer, malaria, tuberculosis, digestive and respiratory disorders, skin and inflammatory conditions, migraine, intestinal parasites, warts and gonorrhea (Kemboi et al., 2020). Euphorbia ingens is a big, woody, succulent tree with a branching crown and shallow, extensive root system (Gildenhuys, 2006). The plant is native to southern Africa, especially within the savannah settings and it is commonly called candelabra tree (Palgrave et al., 2002). It is typically rooted on ledges or deeply in the sand, and so it can withstand protracted droughts, thus favoring warm climates such as those in Malawi, Mozambique, Zimbabwe, Zambia, Botswana, Tanzania, South Africa, Rwanda, Uganda, Swaziland, Kenya and Nigeria. Its latex is considered dangerous, since when it gets into contact with the skin and mucous membrane, it can cause extreme itchness, and can result in blindness when in contact with the eyes (Fred-Jaiyesimi and Ajibesin, 2012). When properly processed, the latex is used in traditional medicine for treatment of cancer and abnormalities such as swellings, fistula, lesions, wounds, abscesses and burns (Jain et al., 2021; Koduru et al., 2017). In some communities, it is used as purgative or for the treatment of ulcers and skin ailments (South African National Biodiversity Institute, 2004). In East Tropical Africa, E. ingens is used for the treatment of snakebites (Ernst et al., 2015). In addition to its traditional medicinal uses, there are reports on its ichthyocidal, antituberculous, antimicrobial and antifungal activities (Kena, 2016; Njeru and Meshack, 2016; Ross and Steyn, 2004).

Given the wide use in popular ethno-medicinal applications, Euphorbia species are currently top of interest in the search for potential drugs derived from plant-based natural products (Ernst et al., 2015). Studies have reported chemical constituents and pharmacological properties of a few Euphorbia species (Jain et al., 2021; Vasas and Hohmann, 2014), and mainly terpenoids, flavonoids and polyphenol classes have been found to exhibit a great variety of biological effects such as cytotoxicity, antiviral activity, antimicrobial and anticancer properties. In spite of this, specific information on the phytochemical constituents of *E. ingens* is still limited. Given the importance of this plant in African traditional medicine, this study determined the phytochemical profile and chemical compounds present in *E. ingens* organic root bark extract. It is expected that the findings of this study will pave way for further biological investigations, as well as other scientific justification for its ethno-medicinal uses.

MATERIALS AND METHODS

Plant collection and identification

Euphorbia ingens is not an endangered plant, and therefore it was collected from open field with no need of seeking prior authorization. The collection site was Embu, Kenya, located at around 0°46'27.0"S 37°40'54.9"E or at -0.774156, 37.681908 of GPS co-ordinates. Plant identification and authentication was done by a botanist at Egerton University, Kenya where voucher specimen number NSN9 was deposited and the name checked as acceptable (http://www.worldfloraonline.org/).

Preparation of plant material

The collected root barks were properly cleaned, shade dried at ambient temperature for 21 days with repeatedly turning the samples upside down to prevent fungal growth. The dried root barks were later milled into fine powder using an electric grinder (Christy 8 MILL, serial No; 51474). The powder (411 g) was soaked in a volume of 1 L of organic solvent (dichloromethane-methanol, 1:1), using the cold percolation technique. Following percolation and intermittent agitation, the resulting liquid was filtered with Whatman No. 1 filter paper. This was done repeatedly until the initial yellow colour of the filtrate became faded to indicate a consistent solvent extraction. After filtration, the filtrate was successively evaporated to dryness at 57°C, using a rotary vacuum evaporator (Rotavapor R-300; Buchi, Switzerland). The obtained extracts of the *E. ingens* was 149 g, and the yield percentage was calculated using the following equation (Evans, 2009):

$$\text{Percentage yield of extracts} = \frac{\text{Weight of the obtained extract material}}{\text{Weight of original fine plant powder used}} \times 100$$

Phytochemical screening of the plant extract

Euphorbia ingens crude root bark extract was prepared for analysis by dissolving 100 mg in 50 ml of dichloromethane-methanol (1:1). Qualitative tests for preliminary phytochemical screening was to determine the presence of alkaloids, terpenoids, flavonoids, sterols,

*Corresponding author. E-mail: innocentokpako9@gmail.com.

Table 1. Phytochemical profile of *E. ingens* dichloromethane-methanol crude root bark extract and their relative abundance.

Phytoconstituent	Relative abundance
Alkaloids	-
Phenols	+++
Tannins	+++
Terpenoids	+
Flavonoids	++
Saponins	+++
Quinones	-
Sterol	-

+++= highly abundant, ++= moderately abundant, += less abundant, -= absent.
Source: Authors 2022

tannins, saponins, quinones and phenols according to Udu et al. (2021) and Njeru et al. (2015).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of *E. ingens* dichloromethane-methanol root bark extract was determined using gas chromatography mass spectrometer system (Model; Shimadzu, GC-MS QP-2010SE) and a low polarity BPX5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The oven temperature was programmed to begin at 55°C, constant for 1 min, then gradually increased by 10°C per minutes, until it reached isothermal temperature of 280°C with a final hold time of 15 min 30 s. The injector temperature was kept at 200°C. Helium was used as a carrier gas at a constant flow rate of 1.08 ml / min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using an AS3000 autosampler coupled with GC in the split mode, split ratio (10:1). The ion source and interface temperature were set at 200 and 250°C respectively. The EI mass spectra were collected at 70 eV in full scan mode over the range of m/z 35 to 550. The NIST mass spectra database was used for the qualitative identification of compounds detected in the extract.

RESULTS AND DISCUSSION

Plant extraction and phytochemical screening

The percentage yield of the *E. ingens* dichloromethane-methanol root bark extract was 36.25% w/w dry matter and yellowish in color. Preliminary phytochemical screening revealed relative high abundance of phenols, tannins and saponins with moderate and low levels of flavonoids and terpenoids respectively in *E. ingens* dichloromethane-methanol root bark extract. However, alkaloids, quinones and sterols were not identified in this study (Table 1).

The findings of this study are similar to those reported by Njeru and Meshack (2016), apart from the fact that they reported absence of flavonoids. These differences may be attributed to seasonal variations and environmental factors such as the light intensity, water, and temperature which are known to affect the phytochemical composition of plants (Bazargani et al., 2021). Notably, the plant materials for this study were picked during the dry season, whereas in the cited study, the plants were picked during the rainy season. The compounds reported in this study on E. ingens have also been reported in other Euphorbia species (Kemboi et al., 2020; Salehi et al., 2019).

Phenols are secondary metabolites synthesized by plants as a natural defense mechanism against pathogens. They also play a significant role in plants growth and signaling (Pratyusha, 2022). A striking feature of phenols is the presence of a benzene ring containing at least one hydroxyl group. The differences in phenols arise depending on the nature of compounds that are additionally attached to the benzene ring (Aldred, 2009). Phenols are important in the prevention and treatment of cancer, cardiovascular diseases, diabetes, obesity and infectious diseases caused by viruses, bacteria and protozoans (Pinto et al., 2021). Flavonoids are modulators of cellular activities as they have capacity to affect many enzymatic activities. Given their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties, flavonoids are important nutraceuticals and pharmaceuticals (Panche et al., 2016). Structurally, tannins can either be hydrolysable (tannic acid) or condensed tannins (pro-anthocyanidins); they have shown activities against bacteria, parasites, viruses and they also have antioxidant, anti-inflammatory, and immunomodulation activities (Smeriglio et al., 2017). Saponins are distinctively known for their stable foaming property in aqueous solutions. They are membrane-permeabilizing compounds with antimicrobial activities. They protect vital organs as they are antioxidants and possess anti-inflammatory activities that mechanistically act by down-regulating the expression of TNF-α (Hu et al., 2009). They also have a role in regulating cell cycle, apoptosis, autophagy, angiogenesis, and they are reported to show anti-cancer activity (Zhong et al., 2022). The most abundant compounds in the natural products in

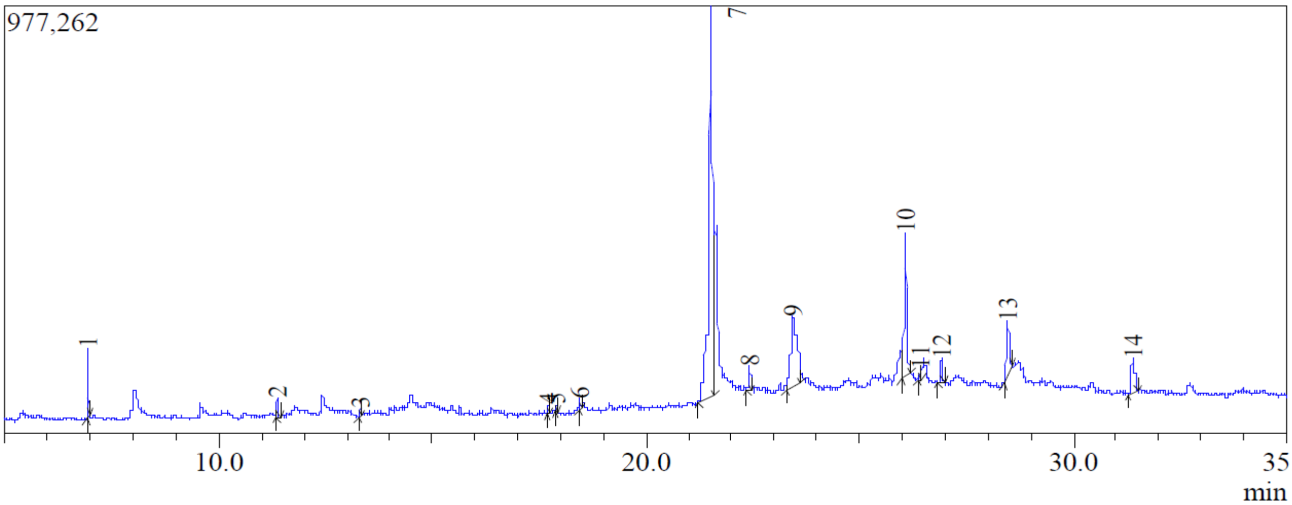

Figure 1. GC-MS chromatogram of the chemical constituents present in *E. ingens* dichloromethane-methanol crude root bark extract.
Source: Authors 2022

general are terpenoids (Yang et al., 2020), however, the study findings reported low levels in terpenoids while a moderate level was reported by Njeru and Meshack (2016). Previous studies on differential gene expression analysis revealed that the expression level differed among gene paralogs involved in terpenoid biosynthesis in the leaf, stem, and root of other related plants (Jeon et al., 2022). Plants' terpenoids are anti-hyperglycemic, anti-inflammatory, anti-parasitic, anti-viral, anti-cancer, anti-microbial and they render skin permissible to many drugs across cell membrane (Ramawat and Mérillon, 2013).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chromatogram of GC-MS spectra of compounds from the *E. ingens* root bark extracts is shown in Figure 1, while Table 2 contains information on the identity of the compounds. The individual compounds identification was established on the basis of the peak area, and retention time. The structures of these compounds are additionally presented in Figure 2.

Among the identified bioactive compounds, tetracyclic triterpenoid lanosterol had the highest percent peak area (54.96%). All steroids are derived from lanosterol. An important steroid in humans is cholesterol, which is central to lipid physiology and is a precursor of various steroid hormones. Lanosterol has been demonstrated to be effective in combating cataracts, the leading cause of blindness in the world (Nagai et al., 2020). Undecane was shown to inhibit the immune cells degranulation and the secretion of histamine and TNF-α (Choi et al., 2020). Copaene is a tricyclic sesquiterpene with antioxidant and antigenotoxic properties (Türkez et al., 2014). 3,4-Altrosan is a bacteriostatic compound and also effective against fungi (Upgade and Bhaskar, 2013). 9, 12, 15-octadecatrienoic acid and hexadecanoic acid (palmitic acid) have anti-inflammatory, cancer preventive, hepatoprotective, antioxidant, and hypocholesterolemic properties (Starlin et al., 2019). 1,2-Benzenedicarboxylic acid (also known as phthalic acid) has antimicrobial activity (Osuntokun and Cristina, 2019). Squalene plays an antioxidant role, prevents cardiovascular diseases, and has antiproliferative activity against ovarian, breast, lung and colon cancer (Lozano-Grande et al., 2018).

Conclusion

In the present study, five phytochemical classes with known diverse pharmacological properties were identified in the *E. ingens* root bark dichloromethane-methanol extract. The GC-MS results revealed the presence of 14 secondary metabolites with previously reported biological activities that include antimicrobial, antioxidant, anticancer, hypercholesterolemic and anti-inflammatory effects. The results of this study support the therapeutic uses of *E. ingens* in traditional medicine that to a larger extent, are likely to be due to the presence of the bioactive compounds identified. Based on this, further biological investigations could also be useful towards further exploitation of *E. ingens* in the development of novel therapeutic compounds for management of various human diseases.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

Table 2. The GC-MS identified compounds from *E. ingens* dichloromethane-methanol crude root bark extracts.

Peak No.	RT (min)	Compound identified	Peak area %	Molecular weight (amu)	Molecular formula
1	6.938	Undecane	2.09	156	$C_{11}H_{24}$
2	11.374	Copaene	0.76	204	$C_{15}H_{24}$
3	13.319	3,4-Altrosan	0.40	162	$C_6H_{10}O_5$
4	17.713	9,12,15-Octadecatrienoic acid	0.36	278	$C_{18}H_{30}O_2$
5	17.900	Hexadecanoic acid	0.26	270	$C_{17}H_{34}O_2$
6	18.447	1,2-Benzenedicarboxylic acid	0.45	278	$C_{16}H_{22}O_4$
7	21.520	Lanosterol	54.96	426	$C_{30}H_{50}O$
8	22.426	Androst-2-en-17-one	1.33	288	$C_{19}H_{28}O_2$
9	23.437	Lanosterol	10.75	426	$C_{30}H_{50}O$
10	26.073	2-Bornanol	13.41	348	$C_{16}H_{20}N_4O_5$
11	26.427	Octadecanoic acid	0.87	400	$C_{22}H_{44}O_4Si$
12	26.923	Squalene	1.33	410	$C_{30}H_{50}$
13	28.467	6-Pentylidene-4,5-secoandrostane-4,17.beta.-diol	5.04	362	$C_{24}H_{42}O_2$
14	31.403	Ethyl trans-4a,cis-4b,trans-8a,cis-10a-perhydro-trans-2,4a,8a-trimethyl-8-oxophenanthrene-2-carbothiolate	3.68	336	$C_{20}H_{32}O_2S$

Source: Authors 2022

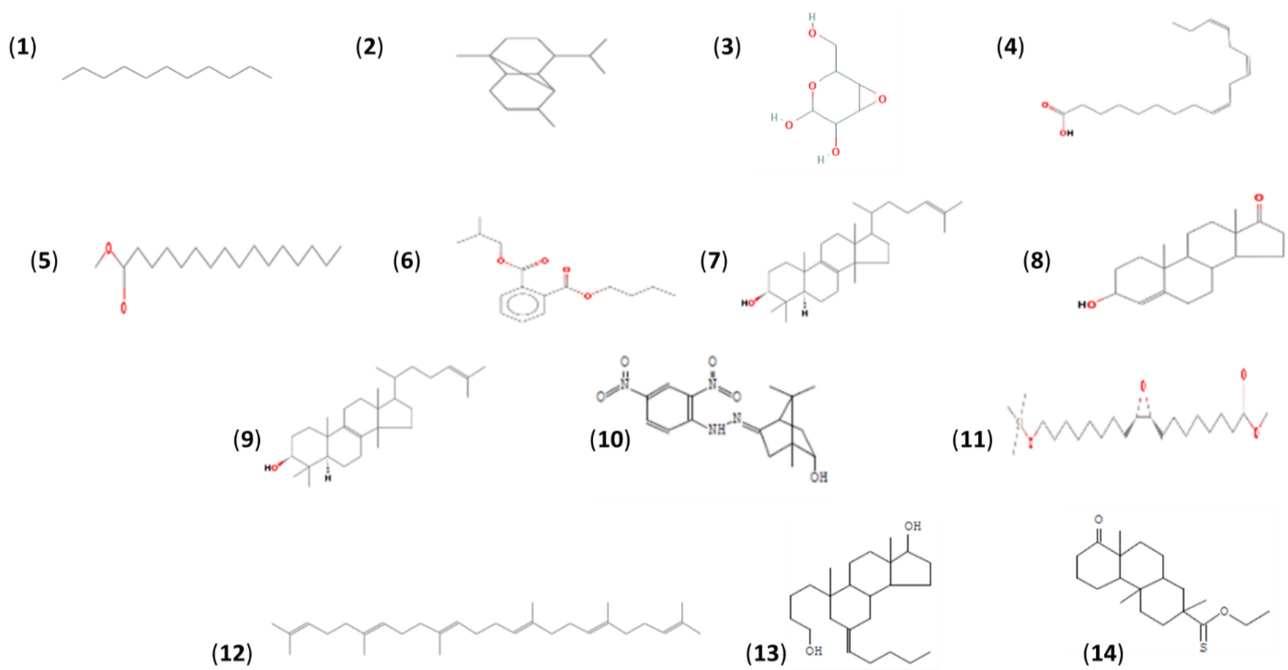

Figure 2. Structures of the compounds (**1 - 14**) presented in Table 2.
Source: Authors 2022

ACKNOWLEDGEMENTS

The authors are grateful for the support and funding by Pan African University Institute for Basic Sciences, Technology, and Innovation. The authors appreciate the Egerton University Laboratory for plant identification and authentication. They also appreciate the Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute where the plant extraction and preliminary phytochemical screening was done and the Chemistry Department, College of Pure and Applied Sciences, Jomo Kenyatta University of Agriculture and

Technology for GC-MS analysis of the extract.

REFERENCES

Aldred E (2009). Phenols. In pharmacology: A handbook for complementary healthcare professionals. London, United Kingdom: Churchill Livingstone Pp 149-166.

Bazargani M, Falahati-Anbaran M, Rohloff J (2021). Comparative analyses of phytochemical variation within and between congeneric species of willow herb, *Epilobium hirsutum* and *E. parviflorum*: contribution of environmental factors. Frontiers in Plant Science 11:2289.

Choi D, Kang W, Park T (2020). Anti-allergic and anti-inflammatory effects of undecane on mast cells and keratinocytes. Molecules 25(1554):1-13.

Palgrave CK, Drummond RB, Moll EJ (2002). Trees of Southern Africa. Cape Town: Struik Publishers P 1212.

Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N (2015). Global medicinal uses of *Euphorbia* L.(*Euphorbiaceae*). Journal of Ethnopharmacology 176:90-101.

Evans WC (2009). Trease and Evans' pharmacognosy. Philadelphia, United States: Saunders Ltd. Pp. 1-603.

Fred-Jaiyesimi AA, Ajibesin KK (2012). Ethnobotanical survey of toxic plants and plant parts in Ogun State, Nigeria. International Journal of Green Pharmacy 6(3):174-179.

Gildenhuys S (2006). The three most abundant tree *Euphorbia* species of the Transvaal (South Africa). Euphorbia World 2(1):9-14.

Hu Y, Chen X, Duan H, Hu Y, Mu X (2009). Pulsatilla decoction and its active ingredients inhibit secretion of NO, ET-1, TNF-α, and IL-1α in LPS-induced rat intestinal microvascular endothelial cells. Cell Biochemistry and Function 27(5):284-288.

Jain V, Dudhal A, Chitale R, Bankar P (2021). A survey of some medicinally important plants of the family *euphorbiaceae* from Baramati area of Pune district of Maharashtra, India. Prasad Bankar 6(4):456-462.

Jeon MJ, Roy NS, Choi BS, Oh JY, Kim YI, Park HY, Um T, Kim NS, Kim S, Choi IY (2022). Identifying terpenoid biosynthesis genes in *Euphorbia maculata via* full-length cdna sequencing. Molecules (Basel, Switzerland) 27(14):4591.

Kemboi D, Peter X, Langat M, Tembu J (2020). A Review of the Ethnomedicinal Uses, Biological Activities, and Triterpenoids of *Euphorbia* Species. Molecules 25(17):4019.

Kena MA (2016). Antifungal activities of Monsonia burkeana and *Euphorbia ingens* extracts against *Penicillium digitatum*, the causal agent of citrus green mould. African Plant Protection 19:1-3.

Koduru S, Grierson DS, Afolayan AJ (2017). Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. Current Science 92(7):906-908.

Lozano-Grande MA, Gorinstein S, Espitia-Rangel E, Dávila-Ortiz G, Martínez-Ayala AL (2018). Plant sources, extraction methods, and uses of squalene. International Journal of Agronomy 2018:1-13.

Nagai N, Fukuoka Y, Sato K, Otake H, Taga A, Oka M, Hiramatsu N, Yamamoto N (2020). The intravitreal injection of lanosterol nanoparticles rescues lens structure collapse at an early stage in Shumiya cataract rats. International Journal of Molecular Sciences 21(3):1048.

Njeru SN, Meshack AO, Samwel ON, Silas MN (2015). Antimicrobial and cytotoxicity properties of the crude extracts and fractions of *Premna resinosa* (Hochst.) Schauer (Compositae): Kenyan traditional medicinal plant. BMC Complementary and Alternative Medicine 15:295.

Njeru SN, Meshack AO (2016). Potency of extracts of selected plant species from Mbeere, Embu County-Kenya against *Mycobacterium tuberculosis*. Journal of Medicinal Plants Research 10(12):149-157.

Osuntokun OT, Cristina GM (2019). Bio isolation, chemical purification, identification, antimicrobial and synergistic efficacy of extracted essential oils from stem bark extract of *Spondias mombin*(Linn). International Journal of Molecular Biology 4(4):135-143.

Panche AN, Diwan AD, Chandra SR (2016). Flavonoids: an overview. *Journal of* Nutritional Science 5:1-15.

Pinto T, Aires A, Cosme F, Bacelar E, Morais MC, Oliveira I, Ferreira-Cardoso J, Anjos R, Vilela A, Gonçalves B (2021). Bioactive (poly)phenols, volatile compounds from vegetables, medicinal and aromatic plants. Foods 10(1):106.

Pratyusha S (2022). Phenolic compounds in the plant development and defense: an overview. In Plant stress physiology - perspectives in agriculture. London, United Kingdom: IntechOpen.

Ramawat KG, Mérillon JM (2013). Terpenes: chemistry, biological role and therapeutic applications. Natural Products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. New Delhi: Springer-Verlag, pp. 2665-2671.

Ross MJ, Steyn GJ (2004). Preliminary results on the ichthyocidal properties of *Euphorbia ingens* (*Euphorbiaceae*). African Journal of Aquatic Science 29(2):265-269.

Salehi B, Iriti M, Vitalini S, Antolak H, Pawlikowska E, Kręgiel D, Sharifi-Rad J, Oyeleye SI, Ademiluyi AO, Czopek K, Staniak M, Custódio L, Coy-Barrera E, Segura-Carretero A, de la Luz Cádiz-Gurrea M, Capasso R, Cho WC, Seca AML (2019). *Euphorbia*-derived natural products with potential for use in health maintenance. Biomolecules 9(8):1-20.

Simpson MG (2010). Diversity and classification of flowering plants: eudicots. Plant Systematics 2010:275-448.

Smeriglio A, Barreca D, Bellocco E, Trombetta D (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. British Journal of Pharmacology 174(11):1244-1262.

South African National Biodiversity Institute (2004). *Euphorbia ingens | PlantZAfrica*. http://pza.sanbi.org/euphorbia-ingens

Starlin T, Prabha P, Thayakumar B, Gopalakrishnan V (2019). Screening and GC-MS profiling of ethanolic extract of *Tylophora pauciflora*. Journal of Biomedical Informatics 15(6):425-429.

Türkez H, Çelik K, Toğar B (2014). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells *in vitro*. Cytotechnology 66(4):597.

Udu R, Oyweri J, Gathirwa J (2021). Antimalarial activity of *Nigella sativa* L. seed extracts and selection of resistance in *Plasmodium berghei* ANKA in a mouse model. Journal of Pathogens 2021:1-10.

Upgade A, Bhaskar A (2013). Characterization and medicinal importance of phytoconstituents of *C. Papaya* from down south Indian region using gas chromatography and mass spectroscopy. Asian Journal of Pharmaceutical and Clinical Research 6(4):101-106.

Vasas A, Hohmann J (2014). *Euphorbia* diterpenes: isolation, structure, biological activity, and synthesis (2008-2012). Chemical Reviews 114(17):8579-8612.

Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X (2020). Advances in Pharmacological Activities of Terpenoids. Natural Product Communications 15(3):1-13.

Zhong J, Tan L, Chen M, He C (2022). Pharmacological activities and molecular mechanisms of *Pulsatilla* saponins. Chinese Medicine (United Kingdom) 17(1):1-12.