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*Original Article*

# Ethnomedicinal survey, phytochemical, isolation and identification of bioactive compounds from *Elephantorrhiza elephantina*, *Pentanisia prunelloides* and *Dioscorea sylvatica* used in the treatment of elephantiasis

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## ABSTRACT

**Introduction:** More than 200 diseases can be transmitted to people through ingesting food contaminated with microorganisms (bacteria, viruses and parasites) or chemicals. Other pathogens for example those causing malaria, tuberculosis and leprosy, as well as parasitic worms can be as chronic infections and impaired nutrition, growth, cognitive development and fertility. **Objective:** The aim of this research was to screen extracts from the three plants for phytochemicals. This includesthe isolation and identification of bioactive compounds of *Elephantorrhiza elephantina*. **Methods:**In this study, an ethnomedicinal survey, phytochemical analysis, isolation, and identification of bioactive compounds were conducted in *Elephantorrhiza elephantina, Pentanisia prunelloides* and *Dioscorea sylvatica* plant species used in the treatment of elephantiasis in most parts of the eastern Free State using standard methods. **Results:**The ethnobotanical survey documented 12 medicinal plants that are used to treat lymphatic filariasis. *Elephantorrhiza elephantina, Pentanisia prunelloides* and *Dioscorea sylvatica* were the three most used plant species. All three plants tested positive for the presence of tannins, saponins, flavonoids, steroids, terpenoids, glycosides, anthraquinones and alkaloids. Four compounds: acetyl salicylic acid, benzoic acid, resorcinol and nonanedioic acid were identified from *E. elephantina* rhizome. **Discussion:**Amongst 12 documented plant species, *E. elephantina, P. prunelloides* and *D. sylvatica* were the most frequently used plants and were selected for isolation and characterisation of bioactive compounds. Acetyl salicylic acid, benzoic acid, resorcinol and nonanediotic acid were isolated and identified from the methanol extract from *E. elephantina* rhizome. **Conclusion:**The presence or existence of isolated phenolic-flavonoids in *E. elephantin* demonstrated the basis for utilising it based on the isolated compounds.

**Key words:** Bioactive compounds, ethnomedicinal survey, *Elephantorrhiza elephantina*, elephantiasis, phytochemicals, traditional medicinal plants

## INTRODUCTION

Plants have been used to treat and manage a variety of illnessesfor centuries. Elephantiasis is one of them. Elephantiasis is categorised into filarial and non-filarial elephantiasis. The filarial elephantiasis is caused by infection with parasitic nematodes such as *Wuchereria bancrofti* (responsible for 90% of the cases), *Brugia malayi* and *Brugia timori*[1] whereas non-filarial elephantiasis occurs in the absence of parasitic infections[2], but in the presence of secondary infectious diseases. *Candida albicans* and *Escherichia coli* are two examples of pathogenic microorganisms that may cause secondary infections that harm the lymphatic system leading to the development of elephantiasis[3].*Candida albicans* is the principal pathogenic species that infects patients with immune dysfunction due to HIV infection, malignancy, immunosuppressive therapy and organ transplantation[4].

Elephantiasis, regarded as the most chronic manifestation of lymphatic filariasis, is believed to be aggravated by microorganism such as bacteria and fungus[5]. Elephantiasis is primarily characterized by skin cracks, folds, and ulcer that are more vulnerable to secondary infections, particularly those caused by *Candida* isolates[6]. The skin lesions and moisture in the lymphedema region due to elephantiasis contributes to secondary infections[7], which act as secondary infections to elephantiasis. Moreover, skin lesions such as wounds facilitate invasion of bacteria and fungi into the underlying tissue[8], hence housing micro-organisms like *E. coli* and *C. albicans*. These micro-organisms may not be the direct cause of elephantiasis, however they can further aggravate this disease leading to more damage on the patients if not treated.

Elephantiasis receives less attention than diseases such as acquired immune deficiency syndrome (AIDS) and tuberculosis (TB) since it is not fatal. However, it's the most obvious manifestations, is the swelling of limbs, and disfigured and socially isolated people[9]. According to the World Health Organization[10] and[11] 59.0% of the 68 million people who are infected by lymphatic filariasis, have been disfigured or incapacitated by it. The disease occurs in more than 80 countries throughout the global tropics[12] and is one of the 13 neglected tropical diseases (NTDs). According to Acharya et al.[13], NTDs are endemic in all the founding BRICS countries (Brazil, the Russian Federation, India, China and South Africa).

In an ethnobotanical survey, herbalists mentioned that several plants, including the ones studied

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during the current project, are used for the traditional treatment of elephantiasis. *Elephantorrhiza elephantina* (Burch.) Skeels, commonly known as '*Mositsane*' in Sesotho and Setswana[14], is a succulent belonging to the Fabaceae which is widespread in Southern African regions[15]. In South Africa, the root of this plant is used traditionally for the treatment of hypertension, syphilis and infertility in women[14]. In Basotho culture, these roots are deemed fundamental because of their red colour, is associated with blood and good health[14]. *Pentanisia prunelloides* (Klotzsch.) Walp., also known as '*Setima mollo*' in Sesotho meaning 'fire extinguisher', is a perennial herb with a large, thick tuberous root and leafy branches holding dense groups of small purple flowers. The plant belongs to the Rubiaceae and is widely used by different cultures for various medicinal purposes. The roots of this plant have been used to treat a swollen stomach, sore joints, rheumatism, dysentery, vomiting and indigestion[14,16]. The plant is used by the Zulu people to relieve inflammation, bacterial and viral infections, and to stimulate uterine concentrations[16].

*Dioscorea sylvatica* Eckl. (Dioscoreaceae) is commonly known as '*Marakalla*' in Sesotho. In some tropical region, it serves as a major source of carbohydrates in some regions of the tropics. The *D. sylvatica* is traditionally used for skin problems and rheumatism, by rubbing the freshly peeled rhizomes on the skin[17]. It is also used in the treatment of various conditions including allergy, dermopathy, cancer, renal infections and most commonly inflammation[17]. The researchers intended to screen extracts from the three plants for phytochemicals. Furthermore, the bioactive compounds of *Elephantorrhiza elephantina* were isolated and identified.

# MATERIALS AND METHODS

## Study area

This study was conducted in the eastern part of the Free State Province(Figure 1 and 2 ).The Free State Province is centrally situated between latitudes 26.6° and 30.7° S and longitudes 24.3° and 29.8° E [18]. The altitude is approximately 1300 m above sea level. The Free State is the third biggest Province with an area of around 129 825 km2, covering about 10.6% of the country's total area[18]. The Free State climate is typical of the interior plateau with rain falling in summer and lots of sunshine, and cold winter.

## Plant collection and identification

Plants were collected with the assistance of traditional healers and herbalists in four towns of the eastern Free State Province, namely, Bethlehem, Senekal, Lindley and Phuthaditjhaba. *Elephantorrhiza elephantina* (Burch.) Skeels, *Dioscorea sylvatica* Eckl, and *Pentanisia prunelloides* (Klotzsch) Walp. The traditional healers and herbalists initially identified the plants using their common names; but Dr Erwin Sieben (Plant ecologist andSenior Lecturer of Environmental Sciences, University of KwaZulu-Natal, South Africa) identified the plants scientifically. Voucher specimens were deposited at the herbarium of the University of the Free State, Qwaqwa Campus with the ethics approval number: UFS-HSD2015/0021.

## Intellectual property agreement statement

All the traditional healers and herbalists who participated in this project gave informed consent, and agreement was obtained that the plants would not be sampled for commercial purposes and that study's findings would be recorded to educate the people of the Free State Province and the rest of the world regarding their effectiveness in treating elephantiasis and related illnesses.

## Plant preparation and extractions

The collected plant materials were oven-dried at 50°C and processed into a fine powder using an industrial electric grinder (MRC Laboratory Equipment, Durban). Powered plant samples were stored in an air-tight container, and 15 g each were extracted with 150 mL of acetone, ethanol, and water, respectively. The plant extracts were filtered through Whatman No.1 filter paper discs, and the filtrates were dried using a rotary evaporator. The residues were stored at 10 °C until used.

## Ethnomedicinal survey

An ethnobotanical survey was conducted through structured questionnaires administered to two traditional healers and three herbalists from February 2015 to March 2016. The survey obtained information such as the identity of the plants used in the treatment of lymphatic filariasis, modes of preparation, plant parts used, and modes of administration. All five mentioned that they prepare their medicinal plants by first drying the plants. They administer medicinal plants orally after infusion and decoction of the dried plant material. Some plants were collected fresh with the assistance of elderly traditional healers and others were purchased from the herbalists.

## Phytochemical analysis

Phytochemical constituents such as tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, anthraquinones and alkaloids of *Elephantorrhiza elephantina, Pentanisia prunelloides* and *Dioscorea sylvatica* were determined using the standard procedures described

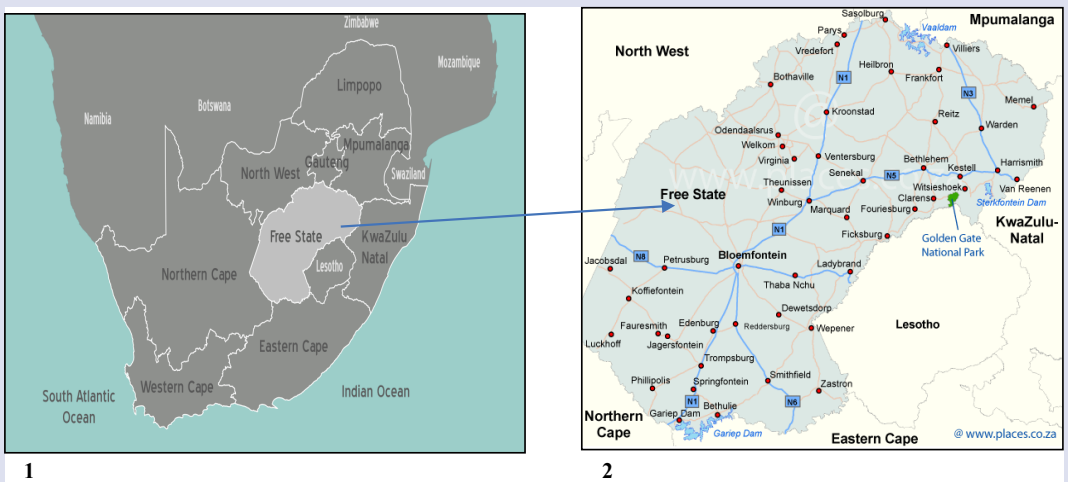


**Figure 1 and 2.** Map showing the Free State Province. The Arrow indicates the study area. Source: SA; Map of the Free State[19]

by Harbone[20],Trease and Evans[21],and Sofowora[22].The *Elephantorrhiza elephantina* (Burch.) Skeels species was chosen for further phytochemical analysis.

## Isolation and identification of bioactive compounds from Elephantorrhiza elephantina

### *Solvent-solvent extraction*

Rhizomes of *E. elephantina* were freshly extracted with 90% methanol and dried. The extract was re-suspended in methanol: $H_2O$ and sequential liquid-liquid extraction of the methanol: H2O phase with *n*-hexane: ethyl acetate (8:2); chloroform, ethyl acetate, ethyl acetate: *n*-hexane (9:1); and *n*-hexane: chloroform (7:3). The ethyl acetate phase was further extracted with a toluene: ethyl acetate: methanol (22:8:8) mixture. This mixture and the *n*-hexane: chloroform (7:3) phase, chloroform and ethyl acetate were used to partition[23].The obtained fractions from the separation funnel were transferred into pre-weighed beakers and then air-dried under a fan until constant weights were achieved.

Silica gel (200g, particle size 0.063–0.200mm, mesh ASTM) was suspended in a minimal volume of *n*-hexane, toluene and methanol, respectively, and then mixed thoroughly to remove air bubbles and swiftly put into the column (3.5 cm internal diameter) for packing[24]. The extract was re-dissolved in 15 mL *n*-hexane, chloroform, ethyl acetate and methanol, respectively, and slowly run through the different columns. The solvent was allowed to flow through the column until the surface of the silica gel was almost exposed. Individually, 5 mL of each solvent (*n*-hexane, chloroform, ethyl acetate and methanol) was gradually added and allowed to run through various numbers of columns, respectively until the solvent above the silica was clear. The column was carefully eluted with an eluent by placing a filled reservoir at the top of the column. The extracts formed bands along the column, and fractions were collected[24]. These fractions were spotted onto a thin-layer chromatography TLC plate and subjected to a suitable solvent system [*n*-hexane: ethyl acetate (8:2), ethyl acetate: *n*-hexane (9:1), and toluene: ethyl acetate: methanol (22:8:8), and n- hexane: chloroform (7:3)]. Fractions that showed similar spots were mixed, and their quantity was reduced in front of a fan and evaluated in the bioautography assay.

### *Preparative thin-layer chromatography (TLC)*

The preparative TLC of different fractions were prepared by loading 0.5 mg of each fraction in a 1 cm band onto 10X10 cm plastic-backed TLC plates (Silica gel 60 F254, 0.25 mm, Merck) over 7.5 cm[24]. The plates were developed simultaneously in some solvent systems [e.g., *n*-hexane: ethyl acetate (8:2), ethyl acetate: *n*-hexane (9:1) and toluene: ethyl acetate: methanol (22:8:8) and *n- hexane*: chloroform (7:3)]. The solvent front was marked, the plates were air-dried and the separated components were visualised under visible and ultraviolet light ($UV_{254\ nm}$ and $UV_{366\ nm}$). The plates were then stained with anisaldehyde (AS) spray reagent and heated at 110°C for 10-15 min, allowing for the colour development of various components previously not visible[24]. The active fractions were scraped off the TLC plates using a sterile blade and eluted from the silica with a suitable solvent. The scrapped fractions from the TLC plate were filtered through Whatman No.1 filter paper to get rid of excess silica.

Data was recorded by taking photographs of different chemical profiles of various fractions under UV-light.

### *Bioautography*

The test microorganism for the bioautographic bioassay was *S. aureus* (ATCC 12600) [25]. An overnight culture was prepared in MH broth medium (3 X 20 mL). The cultures were centrifuged at 3000 g for 10 min and the supernatant medium was discarded. The bacterial cells pellets were mixed and re-suspended in 10 mL of fresh broth. The broth culture was then sprayed onto a TLC plate and incubated overnight at 37°C with 100 % humidity. After incubation the plates were then allowed to dry slightly before spraying with 2 mg/mL solution of INT. The plates were then re-incubated for an additional 30-60 min to allow for colour development. Zones of inhibition appeared as white spots against a pink-coloured background[24]. A retention factor ($R_f$) was calculated using the clear spots observed the on TLC plate.

### *High-Performance Liquid Chromatography analysis*

High-performance liquid chromatography-diode array detection (HPLC-DAD) analysis was carried out using an Agilent HPLC 1200 infinity series system, equipped with a photodiode array detector (Agilent Technologies, Waldbronn Germany). The chromatograms were recorded at 205 nm and 260 nm. An Agilent Zorbax Eclipse Plus C18 column (3.5 µm X 150 mm X 4.6 nm) (Agilent, Newport, CA, USA) was operated at an oven temperature of 25°C. The mobile phase was a mixture of 30% water (mobile phase A) and 70% methanol (mobile phase C). A flow rate of 1 mL/min was used throughout the analysis. The eluate was injected into HPLC-DAD system for quantitative and qualitative analysis[26].The results were obtained in chromatograms showing the peaks of identified compounds.

# RESULTS

### *Ethnomedicinal survey*

The ethnobotanical list with detailed information is presented in Table 1. A total of five informants, comprising two traditional healers and three herbal sellers were interviewed. Four of the interviewees were females ranging between the ages of 35 to 60 years and one male was between the age of 60 and 65 years. The study revealed that 12 medicinal plants belonging to 12 plant families were used to treat lymphatic filariasis and related ailments in the eastern Free State (Table 1). These plants belonged to the families Apiaceae, Apocynaceae, Asphodelaceae, Asteraceae, Crassulaceae, Dioscoreaceae, Equisetaceae, Fabaceae, Hyacinthaceae, Lamiaceae, Poaceae and Rubiaceae. Most frequently, plant parts used by the traditional healers and herbalists were roots and leaves, followed by rhizomes, stems and bark. Grinding plant material into fine powder was the preparatory method that was often used for plants in the treatment of lymphatic filariasis.

## Phytochemical analysis

Tables 2 and 3 show the phytochemical analysis and chemical composition of *E. elephantina, P. prunelloides* and D. *sylvatica* acetone, ethanol and water extracts for the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids and anthraquinones. All three plants tested positive for the presence of most of the screened phytochemicals except for steroids. Extracts from *P. prunelloides* and *E. elephantina* tested positive for the presence of saponins and glycosides. Phytochemical findings on the roots of both *E. elephantina* and *P. prunelloides* are reported by Zaheen et al.[27], validating the range of phytochemicals compounds including tannins, flavonoids and terpenes. Phytochemicals of *E. elephantina* is also reported[28].

## Solvent-solvent extraction

The bulk extraction of *E. elephantina* rhizome with methanol yielded a total mass of 471.62 g of thick red residue. In a solvent-solvent fractionation, 4 fractions were obtained. These were *n*-hexane (115.82 g), chloroform (113.80 g), ethyl acetate (122.5 g) and hydromethanol (119.48 g).

**Table 1. The list of the medicinal plants used in the treatment of lymphatic filariasis in the eastern Free State.**

| Family | Scientific name | Common name(Sotho) | Plant parts used | Other medicinal use | Application for lymphatic filariasis and other diseases |
|---|---|---|---|---|---|
| Apiaceae | ***Alepideapilifera* Weim.** | *Lesokwana* | Rhizomes and roots | For pains | Raw rhizomes and roots are chewed |
| Apocynaceae | ***Xysmalobium undulatum*(L.) W.T. Aiton** | *Pohotshehla* | Bark | Pains, sore wounds and headache | Powdered roots used for sores and wounds, used for heartburn and as a vermifuge in children; also as a decongestant and for headaches |
| Asphodelaceae | ***Aloe ecklonis* Salm-Dyck** | *Lekgala* | Leaves | Tuberculosis and phthisis | Decoction are used as a cure for TB and phthisis, extract of leaves used as a purgative |
| Asteraceae | ***Cineraria aspera*Thunb.** | *Mohoduwa pela* | Leaves | Relieve asthma and tuberculosis | Leaves are smoked to relieve tuberculosis |
| Crassulaceae | ***Cotyledon orbiculata* L.** | *Sereledi* | Leaves/ leaf sap/roots | Inflammation, sore throat and skin ailments | Leaves and leaf sap used for various ailments and as vermifuge, juice has been used to treat epilepsy |
| Dioscoreaceae | ***Dioscorea sylvatica* Eckl.** | *Marakalla/ leeto la tlou* | Roots | Menstrual cramps and inflammation | Infusion and decoction of roots used for easing menstrual cramps treat muscular spasm, colic and inflammation |
| Equisetaceae | ***Equisetum arvense* L.** | *Horsetail* | All aerial parts | Inflammation of vagina | Used as a decoction and helps get rid of excess fluid in the body by increasing urine output |
| Fabaceae | ***Elephantorrhiza elephantina* (Burch.) Skeels.** | *Mositsane* | Roots and rhizomes | Heart condition, fever, syphilis, and hypertension | The infusion of the inner parts of the roots is administered for enema and diarrhoea |
| Hyacinthaceae | ***Eucomis autumnalis*(Mill.) Chitt.** | *Kgampumpu/ mathethebane* | Roots, bark and stems | Stomach pains, low back-ache, coughs and syphilis | Infusion of swollen roots drunk for stomach pains |
| Lamiaceae | ***Salvia repens* Burch. Ex Benth.** | *Mosisidi* | Roots | Stomach pains and controls blood pressure | Infusion of ground roots given to children for pains and decoction of root taken in large doses before meal |
| Poaceae | ***Themeda triandra* Forssk.** | *Seboku* | Leaves | Stomach pains | Infusion of ground leaves and water is drunk for stomach pains |
| Rubiaceae | ***Pentanisia prunelloides* (Klotzsch) Walp.** | *Setimamollo* | Roots | Sores, boils, blood deficiency, dysentery, diarrhoea and internal tumours | The infusion of its roots is drunk to induce vomiting or herbal stomach pains |

**Table 2. Phytochemical screening of *E. elephantina*, *P. prunelloides* and *D.sylvatica*.**

| Plant name | Plant extracts | | |
|---|---|---|---|
| *E. elephantina* | Water | Ethanol | Acetone |
| **1.Tannins** | + | + | - |
| **2. Saponins** | + | + | + |
| **3. Flavonoids** | - | + | + |
| **4. Steroids** | - | - | - |
| **5. Terpenoids** | - | + | + |
| **6. Glycosides** | + | + | + |
| **7. Anthroquinones** | - | + | - |
| **8. Alkaloids** | - | + | - |
| *P. prunelloides* | **Water** | **Ethanol** | **Acetone** |
| **1.Tannins** | + | + | - |
| **2. Saponins** | + | + | + |
| **3. Flavonoids** | - | + | + |
| **4. Steroids** | - | - | - |
| **5. Terpenoids** | - | + | + |
| **6. Glycosides** | + | + | + |
| **7. Anthroquinones** | - | + | - |
| **8. Alkaloids** | - | + | - |
| *D. sylvatica* | **Water** | **Ethanol** | **Acetone** |
| **1. Tannins** | - | + | + |
| **2. Saponins** | - | + | + |
| **3. Flavonoids** | + | - | + |
| **4. Steroids** | - | - | - |
| **5. Terpenoids** | - | + | + |
| **6. Glycosides** | + | - | + |
| **7. Anthroquinones** | + | + | - |
| **8. Alkaloids** | + | - | - |

+: Present; -: Absent

**Table 3. Chemical composition of the plants used against elephantiasis in the Eastern Free State**

| Plant Species | Family | Common Name | Plant part used | Chemical Composition |
|---|---|---|---|---|
| *Elephantorrhiza elephantina* (Burch.) Skeels | Fabaceae | eland's bean, eland's wattle, elephant's root (Eng.); baswortel, mositsane (Sotho, Tswana); intolwane (Xhosa, Zulu). | Whole plant | TLC analysis of saponin fraction against Digitonin afforded sub-fraction 61 of *E. elephantina* which exhibited the presence of saponins and triterpenoids [29] Methyl esters of 9, 12-octadecadienoic, 9-octadecenoic,hexadecanoic, and octadecanoic acids (FAMES); methyl benzoate (Sim = 98%), methyl -3-Phenylprop-2-enoate (Sim = 96%), and methyl 3-(3,5-ditert-butylhydroxyphenyl) propionate(Sim = 82%) were also found[29], Flavonoids [29] |
| *Dioscorea sylvatica* Eckl. | Dioscoreaceae | Wild Yam (Eng.); Ingefu/ Uskolpati (Zulu); Marakalla (Sotho) | Whole plant | Previous phytochemical investigations on the genus: Alkaloid dioscorin Sapogenins, sterodial saponins, and related, steroids, glycosides of diosgenin, dioscin, Alkaloid (dioscorine, and dihydrodioscorine) – TLC, HPLC [30,31] Other compounds- Oxalate salts, Calcium Oxalate Raphide (light microscopic observation in the tuber/bulb[3130] |
| *Pentanisia prunelloides* (Klotzsch) Walp | Rubiaceae | Wild verbena, broad-leaved Pentanisia (Eng.); icimamlilo (Zulu); Setima mollo (Sotho) | Whole Plant | palmitic acid, diosgenin; (steroidal saponin) and oleanolic acid [32] Roots were reported to contain high levels of alcohol precipitate solids (0.7-7.0%), and these include polysaccharides and glycoproteins, amino acids (α-aminobutyric acid, valine, allo-isoleucine, serine, aspartic acid, asparagine and alanine. Several unidentified terpenes of medium and low polarity together with surcose and (-)-epicatechin were also isolated using TLC,MS, MS/MS, GC/MS, HPLC, CC [33] |

UHPLC/MS/MS, ultra-high-performance liquid chromatography–tandem mass spectrometry method; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography; Column Chromatography (CC); UPLC-QTOF/MS, ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry; MS, MS/MS, tandem mass spectrometry, long-chain fatty acids methyl esters (FAMES)

**Table 4. Masses of compounds extracted from the column chromatography fractions through preparative TLC.**

| Compounds extracted | Extracted mass (g) |
|---|---|
| E.Hex | 0.0362 |
| E.Ch | 0.0198 |
| $E.EA_1$ | 0.0185 |
| $E.EA_2$ | 0.01 |
| E.MeOH | 0.0176 |

## Column chromatography

From the *n*-hexane fraction, a total number of 120 fractions were collected from the column chromatography and they were spotted on a TLC plate (Figure 3). Fractions 16-31, 32-40 and 41-44 were combined based on the similarity of their TLC profile and spotted onto TLC plates (Figure 4A). Figure 5 shows a TLC chromatogram of combined fractions 16-31 under ($UV_{254\ nm}$ and $UV_{366\ nm}$) and after spraying with anisaldehyde spray reagent. The combined fractions (16-31) which were spotted as a long band are presented in (Figure 6). A bright blue fluorescing colour was observed under $UV_{366\ nm.}$

## Bioautography

With regards to the bioautography test, fractions 16-31 and 32-40 showed bacterial inhibition at Rf value of 0.74, whereas fractions 41- 44 displayed clear spots at Rf value of 0.81 (Figure 4B).

Concerning the chloroform fraction, 80 fractions were collected from column chromatography and they were spotted onto a TLC plate. Fractions 9-16 were combined based on the similarity of their TLC profile and spotted onto TLC plate. Figures 7 and 8 show a TLC chromatogram of combined fractions 9-16 viewed under UV light (UV366 nm) and after spraying with an anisaldehyde spray agent. Regarding ethyl acetate fraction, a total number of 80 fractions were collected and grouped according to their TLC profile (Figure 8). Figure 9 shows a TLC chromatogram of combined fractions (19-27 and 28-40). An olive-yellow fluorescing colour was observed under UV-light (UV366 nm).

Concerning hydromethanol fraction, 56 fractions were collected from the column chromatography and were spotted on the TLC plate according to their TLC profile. Figure 11A- shows a TLC chromatogram of combined fractions 3-7. An orange-yellow fluorescing colour was observed under UV-light (UV366 nm). The bioautography test of the hydromethanol fractions showed bacterial inhibition at Rf value of 0.59 (Figure 11B).

## Preparative (TLC)

The results for preparative TLC plates are presented in Figures 12 to 15. The preparative TLC on *n*-hexane resulted in compound E.Hex (observed as a bright blue fluorescing spot under $UV_{366\ nm}$), with a mass of 0.0362 g (Table 4 and Figure 12). Following preparative TLC on chloroform fraction, compound E.Ch was isolated with a mass of 0.0198 g. The preparative TLC on ethyl acetate resulted in the isolation of compounds $E.EA_1$ and $E.EA_2$ with masses of 0.0185 g and 0.01 g, respectively. Lastly, the preparative TLC on hydromethanol fraction resulted in the isolation of compound E.MeOH with a mass of 0.0176 g.

## High Performance Liquid Chromatography analysis

The results for the HPLC analysis are presented in Figures 16 to 20. Benzoic acid, nonanedioic acid, acetyl salicylic acid and resorcinol were identified following the HPLC analysis of compounds E.Ch, $E.EA_1$, $E.EA_2$, E.Hex and E.MeOH. Benzoic acid was identified from E.Ch and $E.EA_2$ with Rt of 6.332 and 6.397 minutes (Figure 16 & Figure 18). Nonanedioic acid was identified from $E.EA_1$ and E.Hex with retention times (Rt) of 9.216 and 9.349 minutes, respectively (Figure 17 & Figure

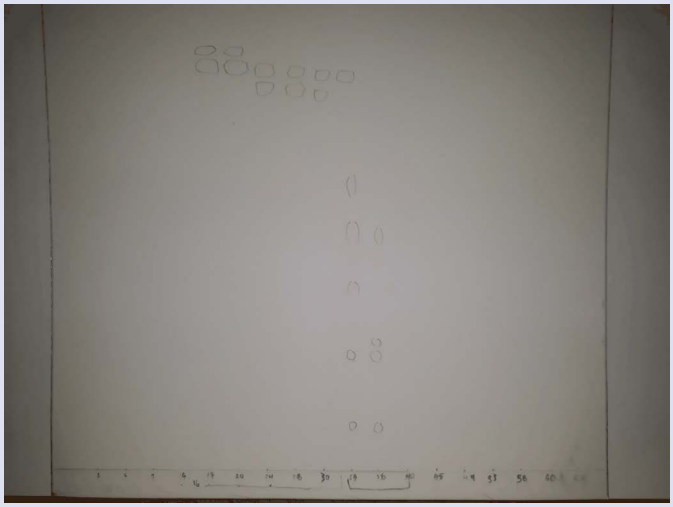

**Figure 3.** TLC chromatogram of *E. elephantina* methanol extract from rhizome. The *n*-hexane fractions were collected from the column chromatography and spotted on a TLC plate and developed with *n*-hexane: ethyl acetate (8:2) solvent system.

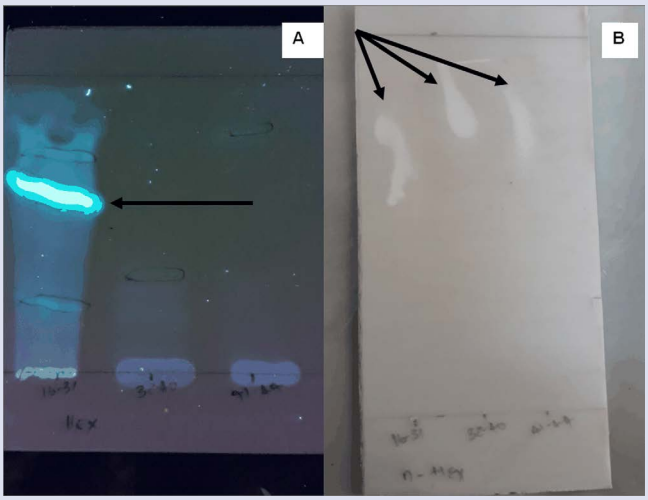


**Figure 4.** TLC plates of *E. elephantina* rhizome *n*- hexane fraction. Fractions 16-31, 32-40 and 41-44 collected from the silica column chromatography were spotted the on-TLC plate and developed with an *n*-hexane: ethyl acetate (8:2) solvent system. (**A**) A reference TLC plate viewed under UV366 nm. The arrow indicates the area of interest. (**B**) The TLC plate was sprayed with *S. aureus*. Arrows indicate clear zones or inhibition zones.

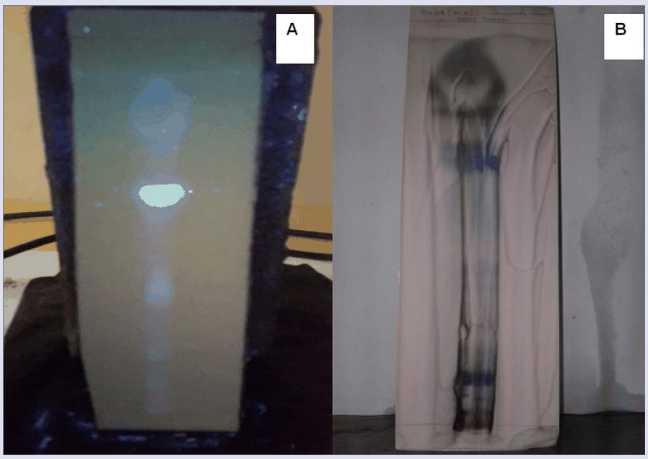


**Figure 5.** The TLC chromatogram of *E. elephantina* rhizome. The *n*-hexane combined fractions (16-31) collected from gravity-assisted column chromatography was spotted on the TLC plate and developed with an *n*-hexane: ethyl acetate (8:2) solvent system. (**A**) Reference TLC plate under UV light and (**B**) TLC plate sprayed with AS reagent.

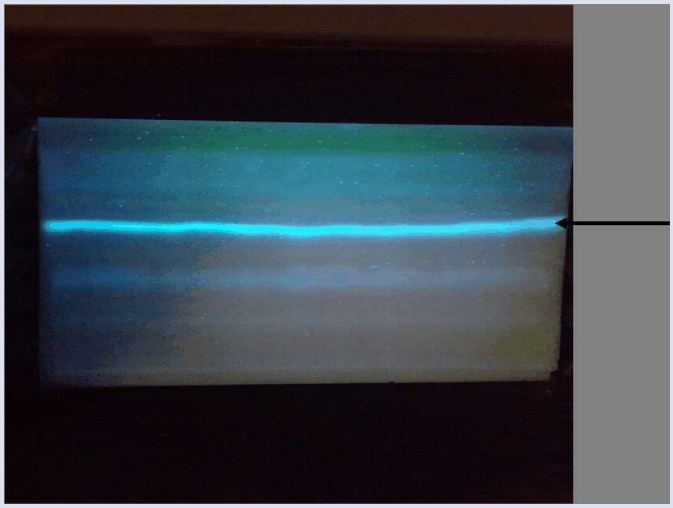

**Figure 6.** The preparative TLC chromatogram from the combined fractions (16-31) spotted as a long band onto a TLC plate. Arrow pointing to the scraped zone. The plate was developed with n-hexane: ethyl acetate (8:2) solvent system.

**Figure 7.** The TLC chromatogram of *E. elephantina* rhizome chloroform fraction. Fractions 3-6 and 9-16 were collected from the column chromatography and spotted on the TLC plate and developed with ethyl acetate: *n*-hexane (9:1) solvent system. Reference plate under UV-light.

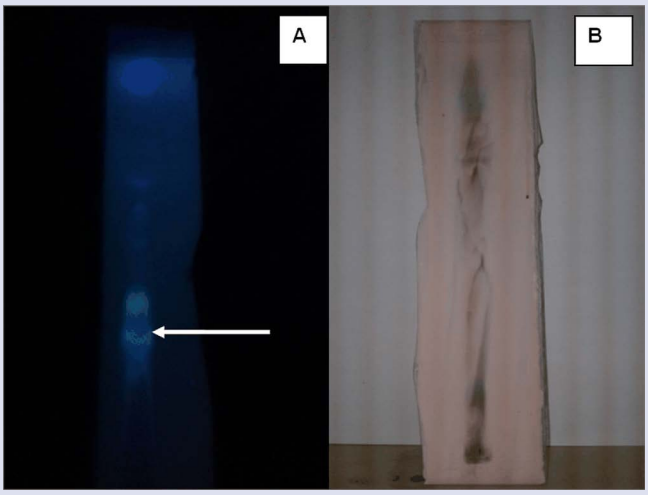


**Figure 8.** The TLC chromatogram of *E. elephantina* rhizome. The chloroform fraction collected from gravity-assisted column chromatography was spotted on a TLC plate and developed with ethyl acetate: *n*-hexane (9:1) solvent system. (**A**) reference TLC plate under UV light and (**B**) TLC plate sprayed AS with reagent

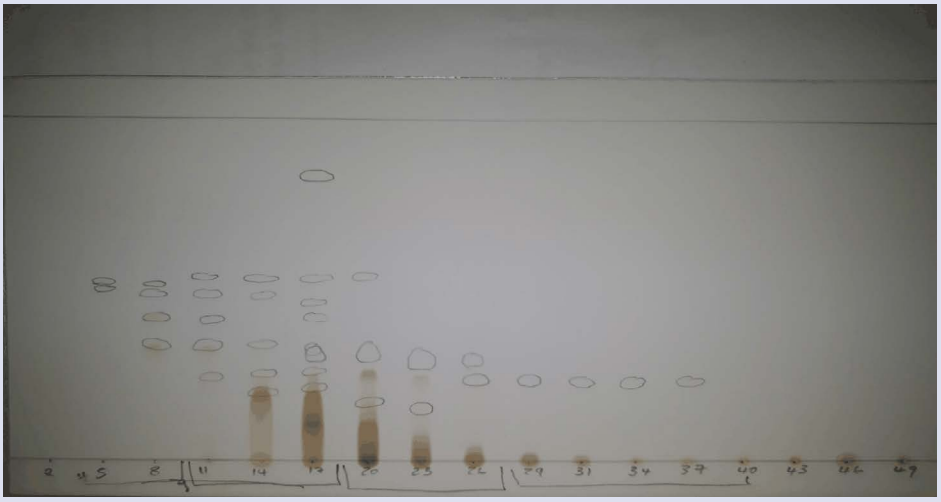

**Figure 9.** The ethyl acetate fractions collected from the column chromatography and spotted onto a TLC plate and developed with toluene: ethyl acetate: methanol (22:8:8) solvent system.

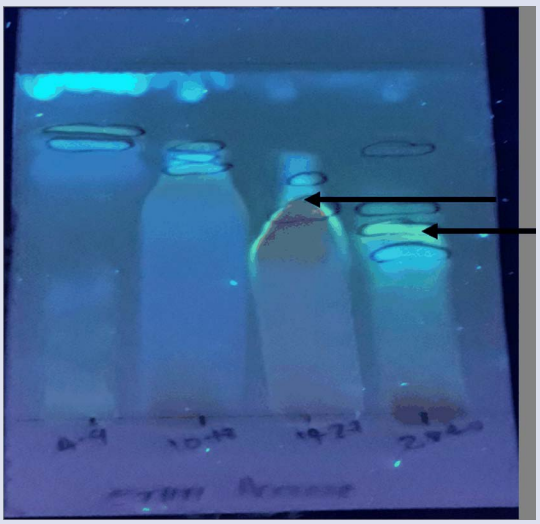

**Figure 10.** TLC plates of *E. elephantina* rhizome ethyl acetate fraction. Fractions 4-9, 10-18, 19-27 and 28-40 collected from the column chromatography were spotted on the TLC plate and developed with a toluene: ethyl acetate: methanol (22:8:8) solvent system. Reference plate under UV-light.

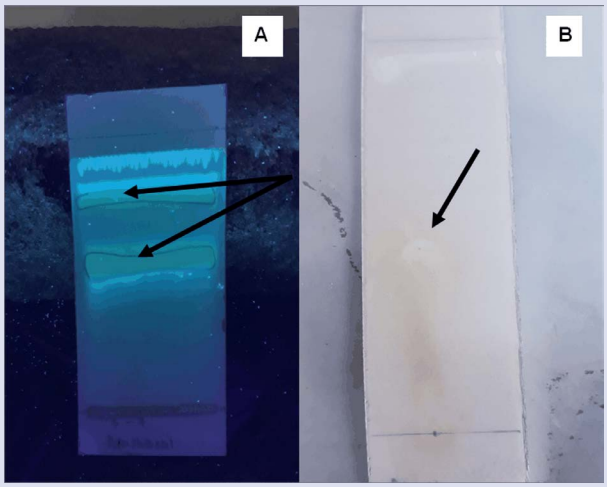


**Figure 11.** TLC plates of *E. elephantina* rhizome methanol fraction. Fractions 3-7 collected from the silica column chromatography were spotted on TLC plate and developed with *n*-hexane: chloroform (7:3) solvent system. (**A**) reference plate under UV-light and (**B**) TLC plate with *S. aureus* bacterial overlay. The arrow shows a clear zone or spot.

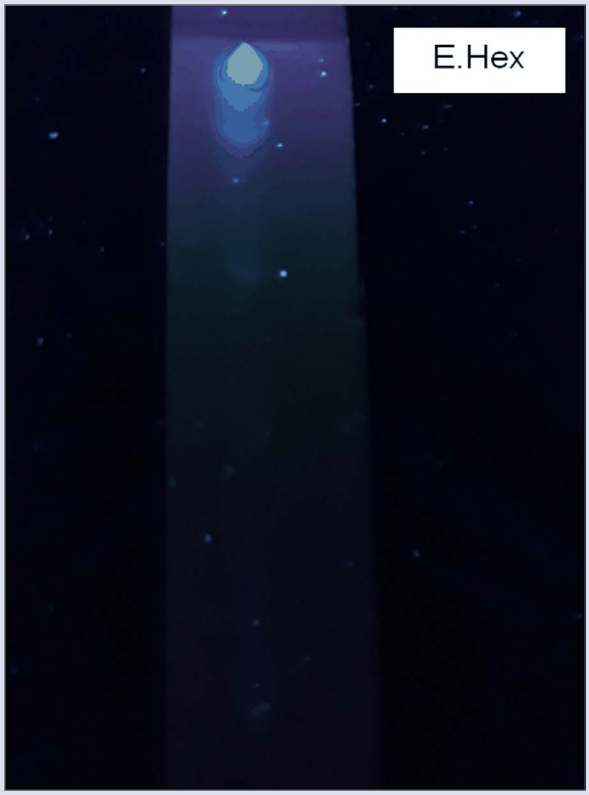


**Figure 12. E.Hex** obtained from *n*- hexane fraction was spotted on TLC plates and developed with *n*-hexane: ethyl acetate (8:2) solvent system. TLC plate viewed under UV light.

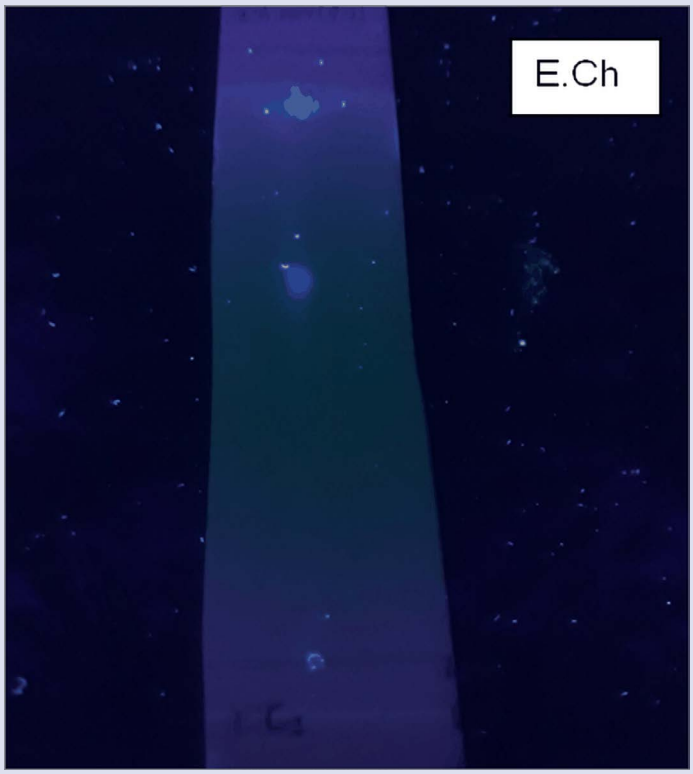


**Figure 13. E.Ch** obtained from chloroform fraction was spotted on TLC plate and developed with ethyl acetate: *n*- hexane (9:1) solvent system. TLC plate viewed under UV light.


**Figure 14. E.EA$_1$** obtained from ethyl acetate fraction was spotted on TLC plate and developed with toluene: ethyl acetate: methanol (22:8:8) solvent system. TLC plate viewed under UV light.


**Figure 15. E.MeOH** obtained from hydromethanolic fraction was spotted on the TLC plate and developed with n-hexane: chloroform (7:3) solvent system. TLC plate viewed under UV light.

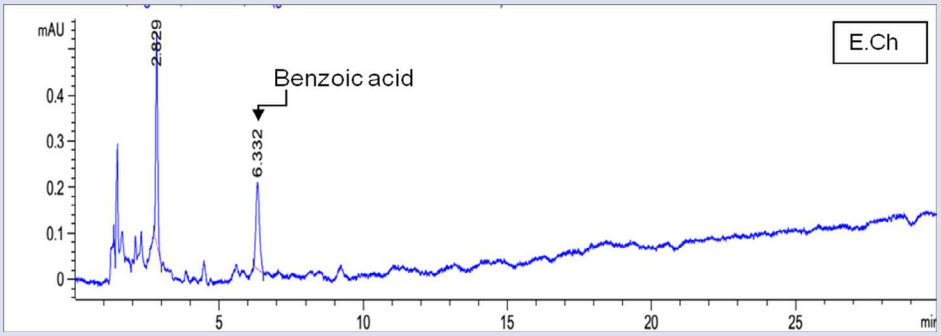


**Figure 16.** The HPLC chromatogram of benzoic acid isolated and identified from *E. elephantina* chloroform fraction. The peaks were detected in less than 30 minutes.

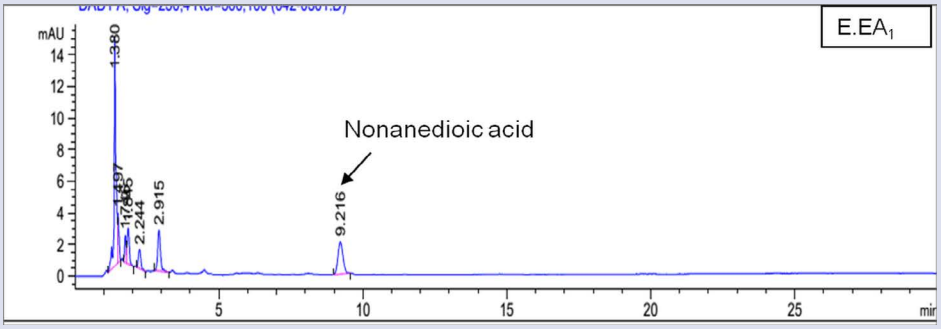


**Figure 17.** The HPLC profile of nonanedioic acid isolated and identified from *E. elephantina* ethyl acetate fraction; showing the peaks detected in less than 30 minutes.

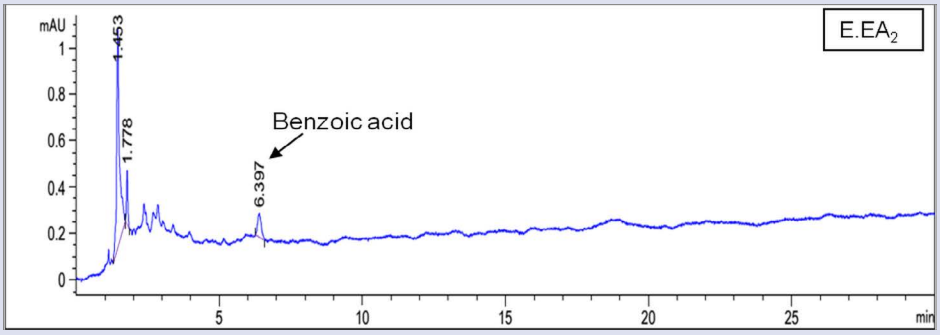


**Figure 18.** The HPLC profile of benzoic acid isolated and identified from *E. elephantina* ethyl acetate fraction, showing the peaks detected less than 30 minutes.

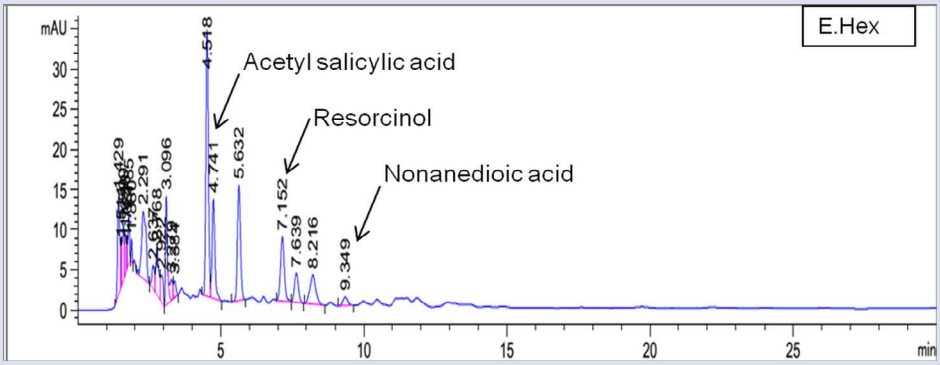


**Figure 19.** The HPLC profile of acetyl salicylic acid, resorcinol and nonanedioic acid isolated and identified from *E. elephantina n*-hexane fraction, showing the peaks detected less than 30 minutes.

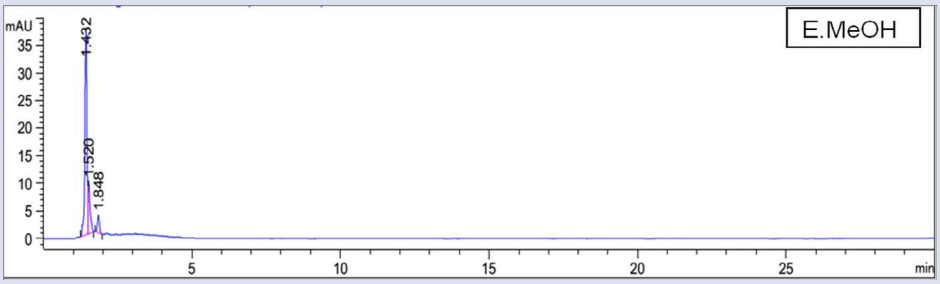


**Figure 20.** HPLC chromatogram of hydromethanol fraction of *E. elephantina* rhizome, showing the peaks detected in less than 30 minutes.

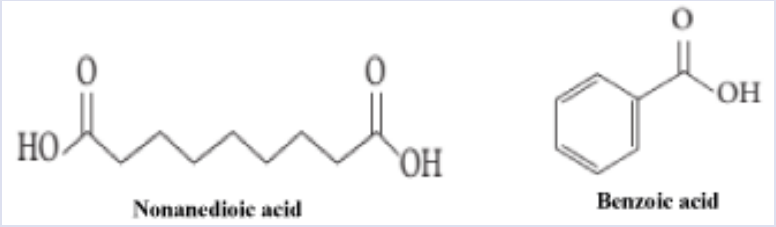


**Figure 21.** Chemical structures of compounds isolated from rhizome of *E. elephantina*[34].

18). Figure 20 shows resorcinol and acetyl-salicylic acid compounds isolated from E.Hex with Rt of 4.741 and 7.639 minutes. The chemical structure of the identified benzoic acid and nonanedioicacidis shown in figure 21.

## DISCUSSION

The study revealed that 12 medicinal plants belonging to 12 plant families were used to treat lymphatic filariasis and related ailments in the eastern Free State (Table 1). These plants belonged to the families Apiaceae, Apocynaceae, Asphodelaceae, Asteraceae, Crassulaceae, Dioscoreaceae, Equisetaceae, Fabaceae, Hyacinthaceae, Lamiaceae, Poaceae and Rubiaceae. According to Maphosa et al. [35], the plant species used to treat inflammation, pains, TB and other pathogenic infections belong to the family Apocynaceae, Apiaceae, Poaceae, Asphodelaceae, Asteraceae, Crassulaceae, Equisetaceae, Hyacinthaceae, and Lamiaceae. Xaba et al.[36] reported on the phytochemical and pharmacological properties of *D. sylvatica*. Although few challenges such slower healing action of elephantiasis with medicinal plants may rise, the survey has established that all the informants often used medicinal plants to treat lymphatic filariasis and related diseases, and this study verified the suggestive usage of those medicinal plants as vital health care components. All the informants mentioned that they collect their plants from the veld and sometimes cultivate those that are difficult to access. Most frequently, plant parts used by the traditional healers and herbalists were roots and leaves, followed by rhizomes, stems and bark. Barks, stems, roots, rhizomes, corms and bulbs are used regularly because it is believed that they have compounds needed for the treatment and healing mechanisms of different ailments[37]. Grinding plant material into fine powder was the preparatory method that was often used for plants in the treatment of lymphatic filariasis. Grinding of plant material assists the solvent to penetrate the plant's cellular structure[38,39]. The most commonly used method of preparation was decoction and infusion mainly using water as the solvent of choice. The survey of the current study indicated that the most prominent method of herbal administration was orally based on the prescribed dosage, but few of them were applied on wounds and sores.

*Elephantorrhiza elephantina*, *Pentanisia prunelloides* and *Dioscorea sylvatica* were individually screened for the presence of phytochemical constituents, and the absence of steroids was observed in all three screened plants. The presence of saponins in tested plants indicates the importance of these medicinal plants, such as saponins are known to be rich in pharmaceutical properties and can increase immune responses[34,32,17]. In the undertaken by Mpofu et al.[14], the presence of saponins from *E. elephantina* and *P. prunelloides* extracts were confirmed after spraying with vanillin sulphuric acid and baking. Additionally, all three plant species indicated the presence of flavonoids which are reported to contain anti-inflammatory, anti-tumuor and anti-oxidant properties[40].

*n*-hexane, chloroform, ethyl acetate and hydromethanol fractions were analysed using the TLC were collected from the column chromatography and were spotted on the TLC plate according to their TLC profile. After spraying with anisaldehyde spray reagent, a bright blue fluorescing colour was observed under $UV_{366\ nm.}$ An olive-yellow fluorescing colour was observed under UV-light ($UV_{366\ nm}$) on ethyl acetate fraction. While an orange-yellow fluorescing colour was observed under UV-light ($UV_{366\ nm}$) on a hydromethanol fraction. The bioautography test of hydromethanol fractions showed bacterial inhibition at Rf value of 0.59 (Figure 11B). In this study, bioautography assay was used to determine and track the antibacterial activity of *E. elephantin* through the isolation process. The bioautography results showed many clear zones, which indicated the inhibition of bacterial species by the compounds presents in those areas. According to Shahverdi et al.[41], the bioautographic technique has used growth inhibition of microorganisms to detect anti-microbial components of extracts chromatogram on the TLC plate.

Following the HPLC analysis of compounds E.Ch, E.EA1, E.EA2, E.Hex and E.MeOH, the benzoic acid, nonanedioic acid, acetyl salicylic acid and resorcinol were identified. The identification of compounds was performed based on the retention time observed when compared with standards and/or matched with other studies. A study undertaken by Mradu et al.[42] , reports on the HPLC fingerprints of standard phenolic compounds that could be used as the benchmark for comparison during the qualitative and quantitative analyses of unknown compounds present in any plant sample or species. In this study, Nonanedioic acid was identified from $E.EA_1$ and E.Hex with retention times (Rt) of 9.216 and 9.349 minutes, respectively. These results are similar to findings obtained by Msimanga et al.[43], on the identification of compounds from *E. elephantina* hexane extract. Benzoic acid was also identified from

E.Ch and E.EA$_2$ with Rt of 6.332 and 6.397 minutes. Msimanga et al.[43] , identified benzoic acid from *E. elephantina* with an Rt of 5.783 minutes. While in a study undertaken by Mradu et al.[42] , benzoic acid displayed an Rt of 6.75 minutes.

Benzoic acid, acetyl-salicylic acid and resorcinol are phenolic compounds that are widespread in plants and synthesize thousands of various chemical structures[42] . The adaptation of plants to biotic and abiotic environmental conditions, presence of taste, colour and health benefits of medicinal plants is due to these phenolic compounds[42]. Flavonoids are a class of polyphenolic secondary metabolites that are associated with antioxidant, antiradical and anti-inflammatory properties[44]. According to Bharti et al.[45] and Al-Khayri et al.[44], numerous phenolic-flavonoids that are found in plants possess antipyretic, analgesic, anti-inflammatory and antioxidant properties. In a study undertaken by Maroyi[34], benzoic acid and nonanedioic acid were isolated from rhizomes of *E. elephantina* and the structures were characterised through the nuclear magnetic resonance (NMR) (Figure 20).According to Giraudeau[46], NMR is an essential technique in quantifying analysis of complex mixtures such as plants, herbal medicinal products and biofluids with no need for identical reference materials. In this study, NMR could not be conducted due to insufficient quantities of isolated compounds after HPLC analysis. This study has confirmed that *E. elephantina* is vital for the isolation of active compounds which could be beneficial in treating various ailments associated with inflammatory and helminths.

## CONCLUSION

The ethnobotanical survey showed that ailments such as TB, inflammation, sore wounds, skin problems, fever, heart condition, coughs, boils and headaches are mostly common in South Africa. This study documented 12 plant species commonly used by traditional healers and herbalists of the eastern Free State Province for treatment of lymphatic filariasis and water was preferred by traditional healers and herbalists in preparation of extracts. Amongst 12 documented plant species, *E. elephantina*, *P. prunelloides* and *D. sylvatica* were the most frequently used plants and were selected for isolation and characterisation of bioactive compounds. Acetyl salicylic acid, benzoic acid, resorcinol and nonanediotic acid were isolated and identified from the methanol extract from *E. elephantina* rhizome. Although the structural elucidation of compounds isolated from *E. elephantina* could not be achieved in this study, literature reported on a several compounds that have been previously isolated from *E. elephantina* rhizome extracts; compounds such as dihydrokaempferol, kaempferol, catechin, ethyl gallate and gallic acid[47].The presence or existence of isolated phenolic flavonoids in *E. elephantina* demonstrated the basis for utilising it based on the isolated compounds.

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