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Full Length Research Paper

Identification of compounds in hexane extracts of *Elephantorrhiza elephantina* and their comparison with selected over the counter products

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The purpose of this study was to analyze hexane extracts of *Elephantorrhiza elephantina* (Ee) roots and to compare their chemical composition with some selected over the counter (OTC) products. Selection of the OTC products was based on their usage in treating stomach ailments, similar to the usage of *E. elephantina* in some regions of Southern Africa. A gas chromatography/mass spectrometer (GC/MS) and a Fourier transform infrared/attenuated total reflection (FTIR/ATR) spectrometer were used for data acquisition. OTC products included dulcolax, fish oil, imodium, senokot, stool softener, castor oil, and triphala. GC/MS results indicated that *E. elephantina* roots contained hexadecanoic, 9,12-octadecadienoic, 9-octadecenoic, and octadecanoic acids as major components, making up about 3 mg fatty acids/g *E. elephantina* roots. The OTC products contained at least two fatty acids in common with *E. elephantina*, namely, hexadecanoic and octadecanoic acids. Principal component analysis of the FTIR/ATR data indicated high similarities between *E. elephantina* roots and senokot or triphala, and the least similarities between *E. elephantina* and fish oil, castor oil, and stool softener.

Key words: Fourier transform infrared/attenuated total reflection (FTIR/ATR) spectrometry, *Elephantorrhiza elephantina*, gas chromatography/mass spectrometry (GC/MS), hexane extracts, fatty acids, over the counter (OTC) products.

INTRODUCTION

Elephantorrhiza elephantina (Burch.) Skeels. species belongs to *Elephantorrhiza*, a genus of legume in the Fabaceae family that is widespread in Southern African regions including Botswana, Mozambique, South Africa, Swaziland, and Zimbabwe (Plowes and Drummond, 1990). *E. elephantina* (Ee) grows naturally in open grassy slopes and hillsides, and produces red roots that look like sweet potatoes. Its offshoots look like the twigs of a Jacaranda tree. It is called “Ntolwane” in some Nguni languages and “Mupangara” in Shona. Its roots are used as a traditional remedy for various ailments. Among Zulus, parts of the plant are added to food as an enema for dysentery and diarrhoea, while it is added in drinking water and administered to livestock to treat mange (Dold and Cocks, 2001). Indigenous people of Zimbabwe use *E. elephantina* to assist bowel movement or to regain their energy levels when they feel their bodies run down. They also use it to relieve constipation or as a colon cleanser by adding the ground *E. elephantina* powder as a food supplement to soft porridge, or boiling the powder in water and drinking it as tea.

Use of traditional plant-based medicines has been practiced for thousands of years worldwide, and it is claimed that about 80% of the populations in developing countries use plant-based medicines for their health care (Palombo, 2006; Appidi et al., 2008). Part of the attraction to traditional medicines is the belief in their multifunctional

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action, availability, and affordability as compared to typical doctor's prescription medicines. Plant medicines also provide abundant source of biologically active compounds needed for developing new chemicals for pharmaceuticals (Palombo, 2006; Tsedu and Maharaj, 2011) In South Africa, scientists along with traditional health practitioners are currently making intentional efforts to evaluate biological activities of herbal extracts that are currently used by indigenous people and significant progress is being made in this area, based on the current literature.

Studies on *E. elephantina* in different regions of South Africa have focused on testing the biological activities of *E. elephantina* extracts from polar solvents. In a few cases, investigators have isolated compounds from polar extracts and tested their activities. Aaku et al. (1998) tested 70% aqueous ethanol extracts of *E. elephantina* for their antibacterial and antifungal activity. They further purified some compounds in the extracts and found that only gallic acid and ethyl gallate showed activity against fungicide bacteria. Aqueous and methanol extracts of selected plants, including *E. elephantina*, were used to evaluate anti-diarrhoea activity of some medicinal plants used by Zulu traditional healers (Lin et al., 2002). While the extracts effectively controlled castor oil induced diarrhoea in rats, no reference was made to the chemical composition of these extracts. Similar studies on the anti-bacterial activities of medicinal plants in the treatment of diarrhoea using a host of plant species have been reported (Mathabe et al., 2006). Out of eight diarrhoea-causing strains evaluated, polar solvent extracts of *E. elephantina* demonstrated antibacterial activity in five of the strains.

In another study, not related to diarrhoea, acetone extracts of *E. elephantina* roots demonstrated significant activity against a tick-borne disease that is problematic to the livestock of South African farmers (Naidoo et al., 2005). *E. elephantina* roots were suspected to contain tannins, bitter polyphenolic compounds that are found in plants and are known to have beneficial effects on human and animal health (Germ et al., 2010). An in-depth study of the chemical composition of *E. elephantina* using polar extracts was reported (Mthembu, 2007). In that study, extracts of *E. elephantina* in 1:1 methanol/dichloromethane were analyzed by thin layer chromatography (TLC) using a mixture of toluene/methanol/acetone/pyridine (35:10:50:5) as the solvent. Compounds isolated were further identified by proton nuclear magnetic resonance spectroscopy and *E. elephantina* roots were found to contain β-sitosterol, gallic acid, methyl gallate, catechin, and pentahydroxyflavan, among other compounds. Identification of catechin in *E. elephantina* roots indicated the presence of tannins and phenolic substances. With some knowledge of the chemistry of *E. elephantina* roots, a toxicity study of aqueous extracts of *E. elephantina* roots on rats was conducted recently (Maphosa et al., 2010), where it was concluded that doses of up to 1600 mg/kg body weight did not cause acute toxicity in rats. According to the authors, these dosages are far in excess of the amounts of *E. elephantina* commonly used for various treatments. Additional studies have been reported on the potential of *E. elephantina* in treating goats that were infected with parasitic worms (Maphosa and Masika, 2012). In that study, one group of goats was treated with different doses of Prodose orange®, a commercial product used regularly by farmers, and the other group was treated with different doses of aqueous *E. elephantina* extracts. Results showed that *E. elephantina* extracts caused significant reduction of the total faecal egg count of worms, comparable to that of the commercial product. It is worth noting that the body-weight of goats on *E. elephantina* treatment increased, implying some nutritional value of *E. elephantina*.

Throughout this brief literature review, polar solvents (water, ethanol, methanol, and acetone) were used to prepare the extracts for their evaluations. There is a possibility that *E. elephantina* contains less polar compounds that may be extracted using non-polar solvents. Our preliminary studies on *E. elephantina* by gas chromatography/mass spectrometry (GC/MS) revealed that hexane extracts contained mainly fatty acids, among other compounds. Existence of fatty acids in *E. elephantina* has not been reported so far in the literature. The purpose of this study was thus to identify the major volatile compounds in *E. elephantina* roots using a non-polar solvent such as hexane. *E. elephantina* chemistry was also compared, under similar experimental conditions, with some over the counter (OTC) products commonly used for treating stomach ailments to see if they had anything in common with *E. elephantina*. Castor oil, dulcolax, fish oil, senokot, triphala, imodium, and stool softener were selected for this purpose. Based on the label information, castor oil is a stimulant laxative used for relieving occasional constipation. Dulcolax, containing bisacodyl, acacia, dibutyl phthalate, docusate sodium, and magnesium stearate, is used for relieving temporary constipation and irregularity. Fish oil, containing omega-3 fatty acids, including eicosapentaenoic (EPA) and docosahexaenoic acids, helps good blood circulation and it supports heart health. Imodium, with loperamide and magnesium stearate, controls symptoms of diarrhoea. Senokot, mainly senna concentrate, serves as a natural vegetable laxative. Stool softener, which contains docusate sodium, relieves occasional constipation. Triphala, a mixture of Indian gooseberry fruits and myrobalan, corrects bowel irregularities. A commonly used method for extracting volatiles is steam-distillation (Pirbalouti et al., 2012; Ze-kun and Chen-Haixia, 2012). However, for good yields, steam distillation requires large samples (fifty or more grams), and the process generally takes several hours for extraction. In this study, *E. elephantina* powder was dissolved in a methanol/chloroform mixture followed by extraction with

hexane. Samples as small as 0.2 to 0.4 g were extracted and analyzed by GC/MS. For spectral comparison, a Fourier transform infrared spectrometer equipped with an attenuated total reflection (FTIR/ATR) accessory was used to acquire data.

MATERIALS AND METHODS

Main instrumentations used

GC/MS analysis was performed via a quadrupole GCMS-QP2010 (Shimadzu Scientific, Inc., USA) instrument using 70 eV electron impact ionization. The instrument was equipped with an auto injector for better precision. Hexane extracts were separated using a SHRXI-5MS capillary column, 30 m long, 0.25 mm ID, and 0.25 µm df. Helium was used as carrier gas at a 1.0 ml/min flow rate. The oven temperature was held at 60°C for 1 min, then ramped at 15°C min^{-1} to 280°C and held for 10 min, giving a total run time of about 25 min. A split ratio of 1:20 was used. The mass range was set at 40 to 500 m/z. Chromatograms for *E. elephantina* and OTC products were obtained under identical experimental conditions. A batch mode was used for injection and this allowed us to automatically load up all the samples in 2 ml vials, with acetone rinses between the samples to minimize any carry-overs.

A Perkin Elmer Fourier transform-infrared (FT-IR) spectrum 100 (USA, CT) instrument equipped with a universal ATR accessory that uses a diamond crystal in contact with ZnSe was used to record sample spectra for comparisons. Either the solid powder of the sample or its gel was loaded on the ATR accessory. Uniform powder was obtained by grinding samples using a pestle and mortar, and finally sieving the powder through a120 mesh sieve size opening.

Samples and chemicals

A batch sample of sun-dried and ground *E. elephantina* roots was collected from the countryside of Nswazi and Nkwidze areas in the Southwestern region of Zimbabwe, about 50 and 75 miles from Bulawayo, respectively. Before shipping, the *E. elephantina* powder was vacuum packaged in plastic bags to remove atmospheric oxygen, thus to limit the growth of aerobic bacteria or fungi on the powder. Dr. Runner R. T. Majinda in the Department of Chemistry at the University of Botswana identified the plant species. Senokot, stool softener, dulcolax, imodium, castor oil, and fish oil were obtained from drug stores, and triphala was obtained from an Indian grocery store. Analytical grade methanol, chloroform, hexane, acetone, and BF_3/CH_3OH reagent for derivatization were purchased from Sigma Chemical Co. (MO, USA). For standards, 99% 9, 12-octadecadienoic (linoleic) acid, 90% 9-octadecenoic (oleic) acid, 99% decanoic acid, 99% hexadecanoic (palmitic) acid, and 97% octadecanoic (stearic) acid were purchased from Sigma-Aldrich (USA).

Procedures

Sample preparation for GC/MS analysis

In the initial studies, about 2.0 to 3.0 g of finely ground *E. elephantina* roots were mixed with 10 ml of 1:1 methanol/chloroform overnight. The mixture was refluxed for approximately 20 min. The solution was filtered using a Buchner funnel followed by a 0.45 µm filter to remove particulates. The solvent from the filtrate was removed by rotor vaporization at 40 to 50°C under vacuum. The residue was removed from the flask into a 10 ml test tube by re-dissolving in 3 to 4 ml of 1:1 methanol/chloroform. Volatiles from the thick brown solution were extracted three times with 2 ml portions of hexane to a total volume of 6 ml colorless solution. Excess hexane was removed by deaeration with nitrogen gas under the hood to about 2 ml. This solution was ready for injection and analysis by GC/MS.

In the final studies, smaller amounts of *E. elephantina* samples were used to convert acids into methyl esters. A mass of 0.2 to 0.4 g fine powder of *E. elephantina* was ground in 4 ml 1:1 mixture of methanol/chloroform using a mortar and pestle. The mixture was centrifuged for 10 minutes at 2000 rpm using a VWR Clinical centrifuge. The supernatant was filtered into a 10 ml test tube through a 0.45 µm disc. The test tube contents were deaerated with nitrogen as before to remove the solvent. The residue was transferred to a 4 ml vial and methylation was completed according to Morrison's method (Morrison and Smith, 1964), with some modifications. To the *E. elephantina* residue in the vial, 750 µl 1:1 methanol/chloroform and 300 µl of 14% BF_3/CH_3OH were added, and the vial was heated in boiling water for 10 to 15 min after capping the vial to keep out moisture. To the cool contents, 1 ml of water and 2 ml hexane were added to the contents, vortexed, and the hexane layer containing fatty acid methyl esters was finally transferred to a clean 2 ml vial for analysis with GC/MS. If the hexane layer was not clear, further filtration through a 0.45 µm disc was performed. For comparative studies, dulcolax (1 tablet), fish oil (0.1902 g), castor oil (200 µl), imodium (1 tablet), senokot (1 tablet), stool softener (1 tablet), and triphala (0.4005 g) were treated as for *E. elephantina*. In some preparations, concentration adjustment had to be made before satisfactory chromatogram peaks were obtained.

Quantitative analysis of major compounds

A mixture of stock standard solution was prepared by dissolving 44.9 mg octadecanoic acid, 45.8 mg hexadecanoic acid, 54.0 mg 9-octadecenoic acid, 52.5 mg 9, 12-octadecadienoic acid in 1:1 methanol/chloroform and making them to volume in a 100 ml volumetric flask. A separate internal standard stock solution was prepared by dissolving 30.2 mg of decanoic acid in a 100 ml flask using the same solvent. Actual concentrations, ranging from 39.86 to 69.30 ng/µl were calculated based on the percent purities stated on the containers. To minimize loss of volume through evaporation, the stock solutions were kept in the refrigerator until used. *E. elephantina* samples around 0.3 g (Table 3) were weighed out and converted to methyl esters as described earlier. Exactly 200 µl internal standard were added to each sample before derivatization. For the working standards solution, 200 µl each of the stock standard and internal standard solutions were micro-pipetted into a 10 ml test tube, followed by removal of the bulk of the solvent using nitrogen gas. The residue was derivatized as the aforementioned. The final solutions, after extracting the methyl esters with hexane, were made up to 1500 µl before analysis with GC/MS. The original amount of each compound was calculated via Equation 1,

$$\frac{mg}{g} = \left(\frac{A_{comp} C_{\text{int}}}{A_{\text{int}} F}\right)\frac{1.5}{m_{spl}} \quad (1)$$

where A_{comp} and A_{int} are area counts of the compound and internal standard peaks, respectively, C_{int} is the concentration of the internal standard in ng/µl, F is the response factor, and m_{spl} is the mass in grams of the *E. elephantina* sample used. The numerator in the last term (1.5 ml) converts mg/L to mg in the final volume of each

sample before injection, given that 1 ng/µl is numerical equal to 1 mg/L.

FTIR/ATR spectrometry studies and statistical analysis

Using a Perkin Elmer spectrometer, each spectrum was recorded from 650 to 4000 cm^{-1}, giving 3351 data points per spectrum. Five spectra were recorded for each sample, resulting in a 40 × 3351 matrix for *E. elephantina* and the seven OTC products used for the comparative study. Correlation coefficients of the infrared spectra were calculated using the data analysis feature of Microsoft Excel and the coefficient values were compared. Correlation coefficient values close to unity indicate a high degree of spectral similarities. A principal component analysis (PCA) computer program was used to analyze the 40 × 3351 matrix. Background theory of PCA has been discussed in the literature (Andre, 2003; Msimanga, 2010). PCA uses spectral variables (wave numbers) to model the samples by reducing the number of variables to a few meaningful ones. Briefly, the covariance of raw data matrix is decomposed into scores and loadings. The scores are used to generate score plots that group together only those traces (spectra) with very similar features. Closeness of the groups signifies similarities. In this way, *E. elephantina* features can be compared with those of the OTC samples.

RESULTS AND DISCUSSION

Identified compounds in *E. elephantina* roots by GC/MS

Figure 1a is a chromatogram obtained by injecting 1 µl of the hexane extract during our preliminary studies. Majority of these peaks showed a very similar pattern of mass spectrum fragmentation (Figure 1b) with repeating intensities at 43, 55, 60, 73, 85, 87, and 129 m/z and no discernible molecular ion intensities. The NIST08 library search indicated that most of the peaks were of fatty acids origin as displayed in Table 1. To date, occurrence of fatty acids in *E. elephantina* roots has not been reported in the literature. The aforementioned results prompted us to do more investigation. Further, the 2 to 3 g sample preparations in our preliminary study were too concentrated to be resolved satisfactorily and multiple cleaning of the column with acetone after each injection became necessary. Sample amounts were thus scaled down to 0.2 to 0.4 g in the final analysis and a derivatization technique was used to identify the fatty acids. Converting the long-chain fatty acids to their methyl esters (FAMES) not only improved peak shapes but it also made the compounds to be more thermally stable and some esters could be identified by their molecular ions. Figure 2 shows a chromatogram of *E. elephantina* extract after converting fatty acids to their corresponding methyl esters. Table 2 lists the identity of the peaks including their percent similarities (%Sim) to reference library spectra, retention times (RT (min)), relative areas (Rel. %), and CAS numbers (CAS#). The NIST08 library software was used to identify peaks. Methyl esters of 9, 12-octadecadienoic, 9-octadecenoic, hexadecanoic, and octadecanoic acids were also confirmed by using reference standards. Other than FAMES, methyl benzoate (Sim = 98%), methyl -3-Phenylprop-2-enoate (Sim = 96%), and methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (Sim = 82%) were also found. Saturated FAMES are evidenced by distinct fragments appearing at 41, 43, 55, 74, 87, 129, and 143 m/z (Figure 3a). The molecular ion (heaviest m/z) is easily identified by intensities at (M-31) and (M-43) m/z. Thus methyl hexadecanoate (Figure 3a) is identified by its molecular ion at 270 m/z followed by 239 and 227 m/z. A base peak of 74 m/z is typical for saturated FAMES. It is a result of McLafferty re-arrangement of the methyl ester end of the molecule forming a more stable $[CH_3OCOH(CH_2)]^+$. The pattern for unsaturated FAMES varies with the number of C=C bonds. Thus, FAMES with one C=C bond show 41, 43, 55, 69, 74, 83, and 97 m/z intensities, with a base peak of 55 m/z (Figure 3b), while FAMES with two C=C bonds show 41, 55, 67, 81, 95, and 109 m/z intensities, with abase peak of 67 m/z (Figure 3c). As with saturated FAMES, loss of 31 m/z ($-OCH_3$) from the molecular ion is also visible in one and two C=C containing FAMES as indicated in the mass spectra of 9-octadecenoic acid methyl ester and 9, 12-octadecadienoic acid methyl ester (Figure 3). Another noteworthy FAME found in *E. elephantina* was cis-5, 8, 11, 14, 17-Eicosapentaenoic acid methyl ester, with 96% Sim. EPA, a member of the omega-3 fatty acid family, is found in fish oil and it is believed to lower serum cholesterol and triglyceride levels (Moghadasian, 2008). Based on the earlier mentioned analysis, *E. elephantina* roots contain a large diversity of fatty acids, with hexadecanoic acid, 9-Octadecenoic acid, 9,12-octadecadienoic acid and octadecanoic acid as the major components. Octadecanoic acid is added in most medicinal tablets as magnesium stearate. Figure 4 gives structures of the aforementioned fatty acids and other compounds found in *E. elephantina*. They are reported here as methyl esters for consistence, since they were analyzed and identified as such.

Quantitative analysis of major fatty acids by GC/MS

For quantitative analysis, it was necessary to optimize peak resolution, especially the region from 12.0 to 14.4 min (Figure 2) using a different temperature gradient program. Thus, the oven temperature was held at 150°C for 5 min, ramped to 200°C at 15°C min^{-1} and held for 10 min, and finally ramped to 280°C at 15°C min^{-1} and held for 5 min, giving a total run time of about 29 min. Since several preparative steps were involved, an internal standard method for calibration was adopted to minimize uncontrollable sample losses and any fluctuations in the experimental parameters. Table 3 summarizes the results for the four major components in *E. elephantina* as calculated according to Equation 1. Hexadecanoic acid

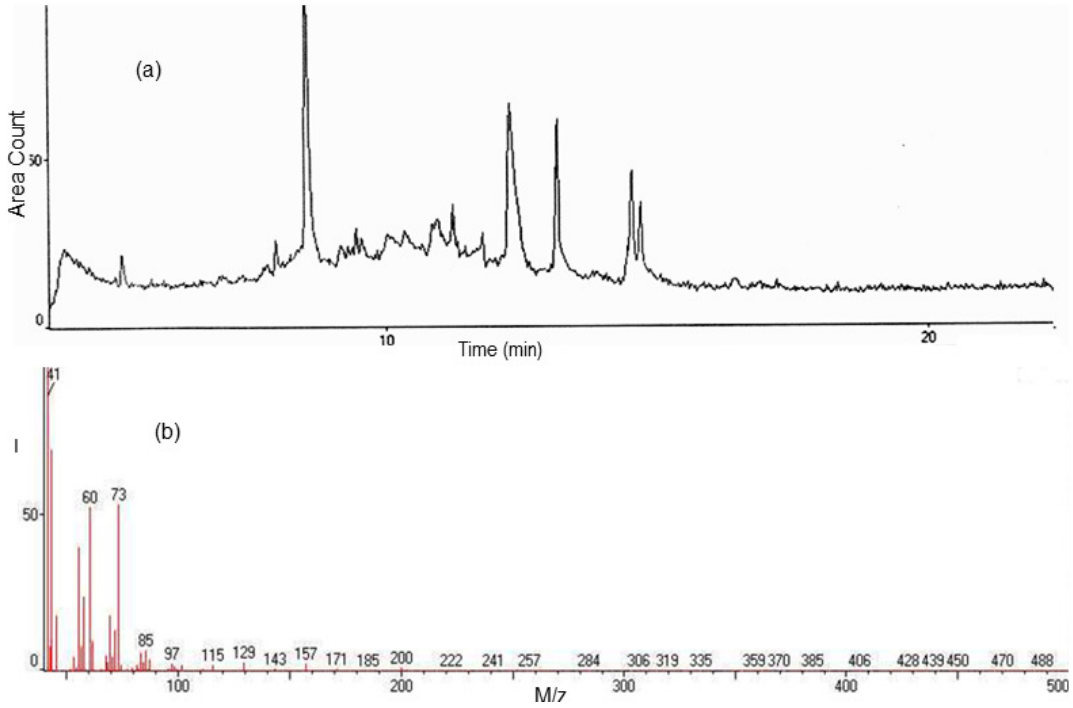

Figure 1. (a) Chromatogram of a 1 µl injection of *E. elephantina* hexane extract. (b) Mass spectrum of one of the major peaks showing a general pattern of the majority of the peaks in (a).

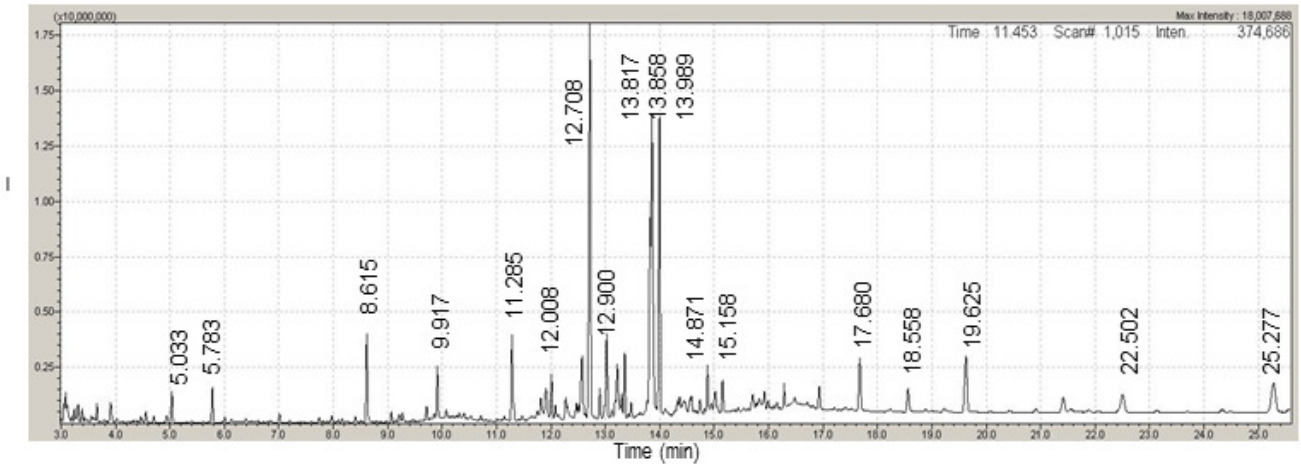

Figure 2. A chromatogram of hexane extract of *E. elephantina,* after derivatization showing all peaks with more than 1% relative area count under the experimental conditions specified in the text.

ester, the most abundant, was found to be about 1 mg/g *E. elephantina* with a relative standard deviation less than 5%, while the least abundant octadecanoic acid ester was about 0.5 mg/g *E. elephantina* with 11% RSD. Our attempts to quantify tetradecanoic (myristic) acid ester gave over 20% RSD. Myristic acid ester had a relative peak area of 4.4% (Table 2) under the experimental conditions used. These four major components contribute about 3 mg/g *E. elephantina* fatty acids content.

Comparison of the chromatograms of *E. elephantina* and OTC products

Table 4 lists by retention times the chromatographic peaks found in OTC samples that were in common with those of *E. elephantina*. All chromatograms were obtained under the same experimental conditions. The 9th column of Table 4 shows that all %RSD of the retention times were less than 1%, a good precision of

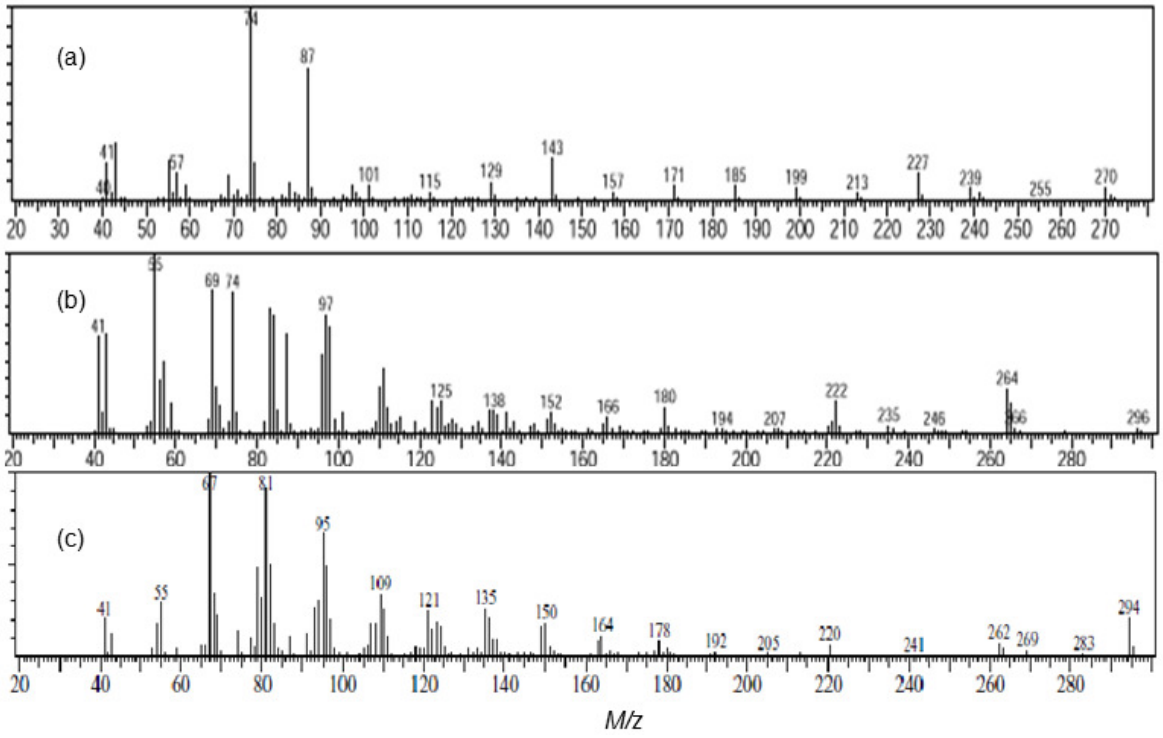

Figure 3. Mass spectrum features of (a) a saturated, (b) one C=C bonded, and (c) two C=C bonded fatty acid methyl esters. The saturated one C=C and two C=C fatty acid methyl esters show base peaks of 74, 55 and 67 m/z, respectively

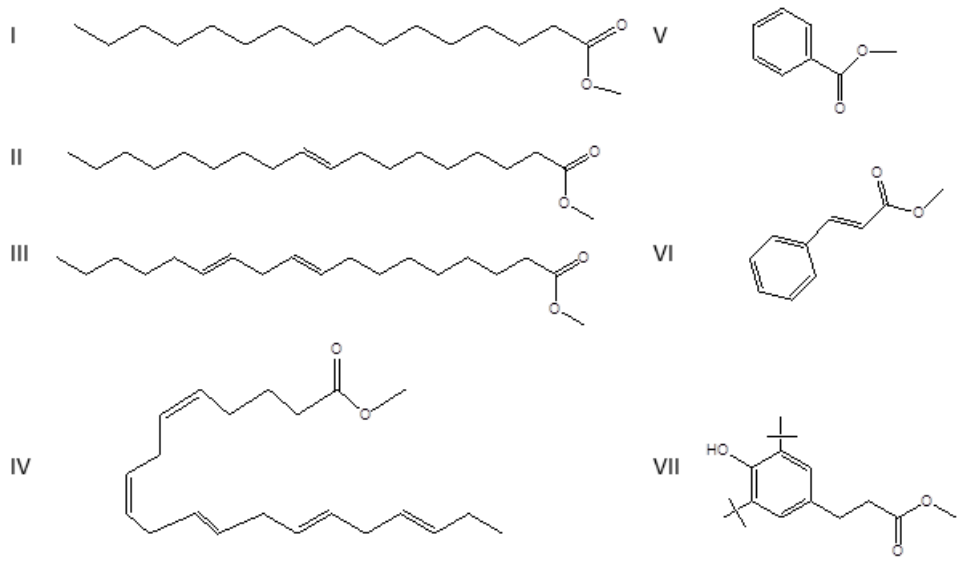

Figure 4. Representative structures of compounds identified as methyl esters: I. Hexadecanoic acid methyl ester, II. 9-Octadecenoic acid, methyl ester, III. 9,12-octadecadienoic acid methyl ester, IV. cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (EPA), V. Methyl benzoate, VI. Methyl -3-Phenylprop-2-enoate, VII. Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate.

the retention times to use as the basis for identification. The seven OTC products and *E. elephantina* contained hexadecanoic acid ester (RT = 12.708 min) and octadecanoic acid ester (RT = 13.989 min). All contained

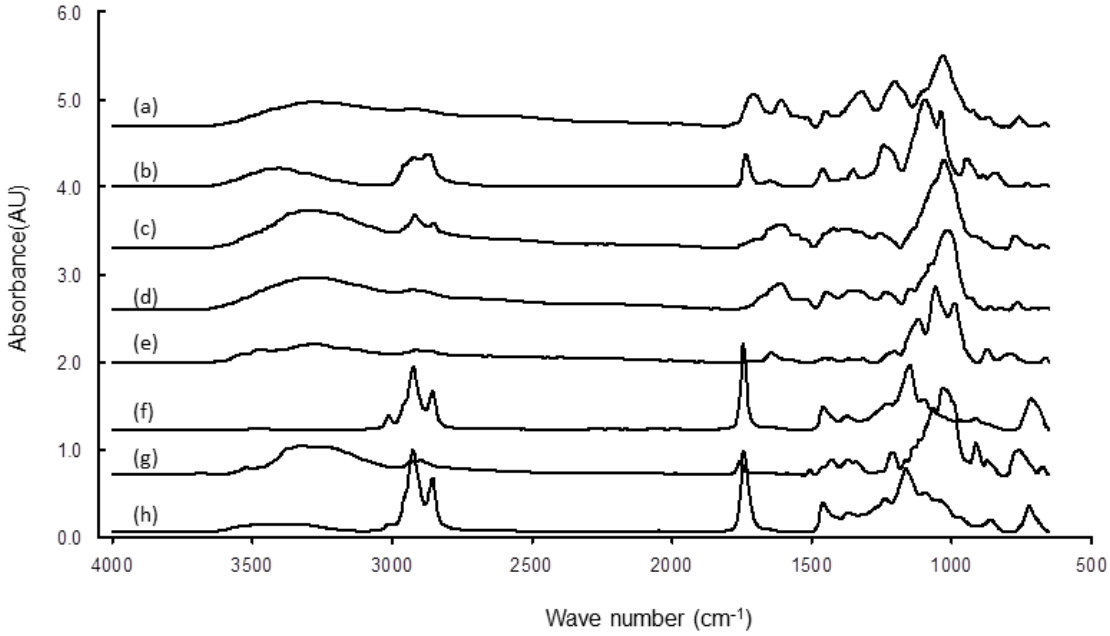

Figure 5. FTIR/ATR spectra of (a) triphala, (b) stool softener, (c) senokot, (d) *E. elephantina*, (e) imodium, (f) fish oil, (g) dulcolax, and (h) castor oil. Spectra were normalized to unity for visual comparison.

tetradecanoic acid (RT = 11.285 min), except stool softener and castor oil. Senokot and triphala chromatograms showed the most similarities to those of *E. elephantina*, although they had additional minor peaks at 4.561, 6.361, and 7.397 min, not present in *E. elephantina*. Castor oil, fish oil, stool softener, imodium, and dulcolax showed least similarities with *E. elephantina*. These dissimilarities can be attributed to extra peaks in these compounds, not present in *E. elephantina*. For example, castor oil showed a major peak at 15.226 min that was identified as 12-hydroxy-9-octadecenoic acid ester. Dulcolax contained significant amounts of dibutyl phthalate (RT = 13.02 min), while stool softener and imodium had 2-Butenedioic acid ester (RT = 14.597 min) in common, but not observed in *E. elephantina*. Based on Table 4, *E. elephantina* shows greater similarity with senokot and triphala which are used as laxatives. These similarities are also confirmed by the principal component analysis score plots in Figure 6 discussed subsequently. Next in similarities to *E. elephantina* are imodium and dulcolax. One would think that imodium, an anti-diarrheic OTC, should show very close similarities with *E. elephantina*, based on their uses. However, the differences may be attributed to the diversity of fatty acids found in *E. elephantina* as compared to imodium. Further, plant medicines are known for their multiple uses from region to region, as exemplified by *E. elephantina* under the introduction section. In Asian countries, *Sesbania grandiflora* (Fabaceae) is a folk remedy for dysentery, eyes, fevers, headaches, small pox, sores, sore throat and stomatitis (Saravanakumar et al., 2010). Doses become a factor as well. In small doses, the bark of *S. grandiflora* is used for dysentery, in large doses, it becomes a laxative, and in still larger doses, it is used as emetic. Clearly, the presence of fatty acids in *E. elephantina* and in some aforementioned OTC products is significant. Omega-3 fatty acids, including EPA, help good blood circulation and they support heart health (Cicero et al., 2009). In a recent study, lipid suppositories were prepared from fatty acids and tested as laxatives (Ormarsson et al., 2012). The fatty acids used included omega-3 fish oil, myristic acid, palmitic acid, stearic acid, EPA, and DHA among others. Majority of the named acids were found in *E. elephantina* as shown in Table 2. The clinically tested suppositories effectively stimulated bowel movement, although the exact mechanism behind the bowel stimulation is not clear. This work is one of the few studies that have linked fatty acids to laxatives and it backs up *E. elephantina* uses as a laxative.

Comparison using FTIR/ATR spectra and statistics

The spectra of eight samples after probing the original samples with FTIR/ATR are displayed in Figure 5. For each sample, the average of five spectra was calculated and normalized to unity. Spectra of fish oil (Figure 5f) and castor oil (Figure 5h) show more clearly defined peak bands, since these two samples are more homogeneous than *E. elephantina*, senokot, and triphala, which contain plant tissues (particulates). C-H vibrations are seen around 2850 and 2920 cm^{-1} in fish oil and castor oil spectra. The C=O stretching are also noticeable around

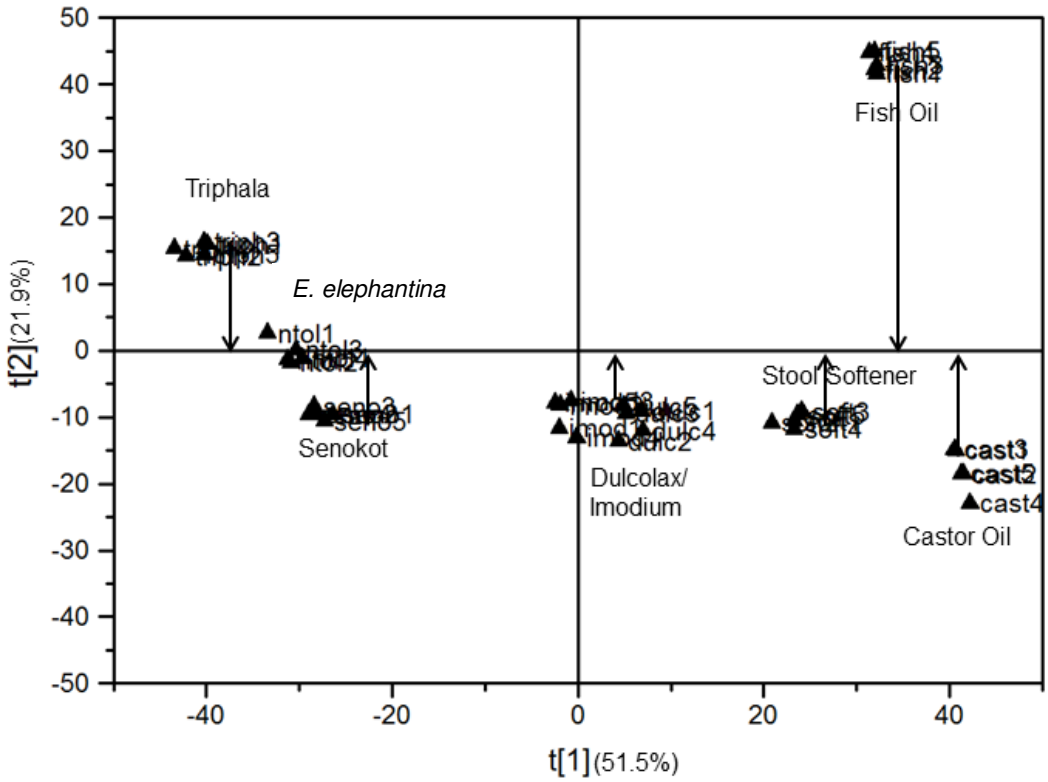

Figure 6. Score plot of the first and second principal components of *E. elephantina* and the OTC products. Proximity among groups indicates similarities between those groups.

Table 1. Mass spectrum identification of one of the major peaks from Figure 1 for the first 15 hits, based on the NIST08 Library search. The names indicate that the peak is likely to be a fatty acid.

Hit number	SI	Name	Molecular weight	Molecular form
1	85	Octanoic acid	144	$C_8H_{16}O_2$
2	85	Glycine, N-methyl-N-[1-oxododecyl]-SS Sarco	271	$C_{15}H_{29}NO_3$
3	84	Octanoic Acid SS n-Caprylic Acid SS n-Octano	144	$C_8H_{16}O_2$
4	83	Nonanoic Acid SS n- Nonanoic Acid SS n-Nono	158	$C_9H_{18}O_2$
5	83	Nitrous Acid, butyl ester SS n-Butyl nitrite SS	103	$C_4H_9NO_2$
6	83	Dodecanamide, N,N-bis[2-hydroxyethyl]-SS	287	$C_{16}H_{33}NO_3$
7	83	Undecanoic Acid SS-n-Undecanoic Acid SS	186	$C_{11}H_{22}O_2$
8	83	Decanoic Acid SS n-Capric Acid SS n-Decanoic Acid	172	$C_{10}H_{20}O_2$
9	82	Octanoic Acid	144	$C_8H_{16}O_2$
10	82	Decanoic Acid	172	$C_{10}H_{20}O_2$
11	82	Dodecanoic Acid SS n-Dodecanoic Acid SS	200	$C_{12}H_{24}O_2$
12	82	Nonanoic Acid	158	$C_9H_{18}O_2$
13	82	Tridecanoic Acid	214	$C_{13}H_{26}O_2$
14	82	Pentadecanoic Acid SS Pentadecylic Acid SS	242	$C_{15}H_{30}O_2$
15	82	Heptadecanoic Acid SS n-Heptadecanoic Acid	270	$C_{17}H_{34}O_2$

1700 to 1748 cm^{-1}. Otherwise, the rest of the spectra show similar features. Similarities between *E. elephantina* and OTC products by correlation coefficients are senokot (0.9785), dulcolax (0.8880), triphala (0.8629), imodium (0.8558), softener (0.6034), castor oil (0.3104), and fish oil (0.1652) in decreasing order. Dissimilarities between *E. elephantina* and fish oil or castor oil are also supported by the chromatogram results summarized in Table 4.

Table 2. List of all *E. elephantina* compounds identified by GC/MS and reference compounds. Only those compounds with relative peak areas ≥ 1 % are listed.

Peak number	%Sim	RT (min)	Relative %	CAS#	Name
1	98	5.033	1.17	106-65-0	Butanedioic acid, methyl ester
2	98	5.783	1.46	93-58-3	Benzoic acid, methyl ester
3	96	8.615	3.91	103-26-4	3-phenyl-2-propenoic acid, methyl ester, (methyl cinnamate)
4	93	9.917	2.14	1732-10-1	Nonanedioic acid, dimethyl ester (dimethyl azelate)
5	95	11.285	4.41	124-10-7	T radecanoic acid, methyl ester (methyl myristate)
6	94	12.008	1.56	7132-64-1	Methyl pentadecanoate
7	92	12.474	3.07	10030-74-7	Methyl hexadec-9-enoate
8	96	12.708	24.05	112-39-0	Methyl hexadecanoate (methyl palmitate)
9	82	12.900	1.36	6386-38-5	Methyl 3-(3,5-di-tert-butyl-4-hydroxy-phenyl)propionate
10	86	13.217	2.00	77745-60-9	Cis-10-Heptadecenoic acid, methyl ester.
11	95	13.354	2.70	1731-92-6	Methyl heptadecanoate (methyl Margarate)
12	86	13.817	8.77	113-63-0	9, 12-octadecadienoic acid, methyl ester (linoleic acid ester)
13	96	13.858	13.55	1937-62-8	9-Octadecenoic acid, methyl ester (methyl oleate)
14	96	13.996	10.39	112-61-8	Methyl octadecanoate (methyl stearate)
15	96	14.871	1.99	10417-94-4	Cis-5, 8, 11, 14,17-Eicosapenta-enoic acid, methyl ester (EPA)
16	90	15.158	1.32	1120-28-1	Ecosanoic acid, methyl ester (methyl aracidate)
17	88	17.680	2.98	2442-49-1	Methyl tetracosanoate (lignoceric acid, methyl ester)
18	88	18.558	1.02	55373-89-2	Pentacosanoic acid, methyl ester
19	88	19.625	4.29	5802-82-4	Hexacosanoic acid, methyl ester, (methyl cerotate)
20	88	22.502	1.05	55682-92-3	Methy Octacosanoate
21	88	25.277	2.37	5024-21-5	Tetracontanedioic acid, dimethyl ester

%Sim: % similarities to reference library spectrum; RT (min): retention times in minutes; Rel. %: relative area counts; CAS#: CAS numbers (CAS#).

Table 3. Amounts of major fatty acid esters expressed in mg/g *E. elephantina* sample.

E. elephantina sample	Hexadec	9,12-Octa	9-Octadec	Octadeca
0.3023 g	0.9703	0.5721	0.8385	0.4571
0.3023 g	1.1054	0.7082	0.9927	0.5218
0.3112 g	1.0277	0.6255	0.8688	0.4746
0.3112 g	1.0683	0.6054	0.8671	0.5498
0.3058 g	1.0464	0.7352	0.9798	0.5820
0.3058 g	1.0154	0.7513	1.0354	0.4631
Mean	1.039	0.665	0.930	0.508
%RSD	4.46	11.2	8.81	10.1

%RSD: Percentage relative standard deviations.

Where spectral similarities are very close as indicated by the correlation coefficients of senokot, dulcolax, triphala, and imodium versus *E. elephantina*, PCA can provide more selectivity than correlation coefficients. PCA searches for latent variables that are unique to each of the eight products and uses those variables to classify the products in score plots. Thus, when the 40 × 3351 data matrix was analyzed by PCA, the first two principal components accounted for 73.4% information captured (data variability). The first and second principal components score plot (Figure 6) showed seven distinct groups of samples (Triphala, *E. elephantina*, Senokot, Fish Oil, Stool Softener, Castor Oil, and Dulcolax/Imodium). Dulcolax and imodium are grouped together, an indication of high similarities between these two products. By drawing a straight line through *E. elephantina* and the origin in Figure 6, and then dropping perpendicular lines from each group to this line, relative similarities of each group to *E. elephantina* can be determined. Senokot and Triphala are the closest to *E.*

Table 4. Comparison of *E. elephantina* compounds and selected over the counter products using retention times. Only peak areas ≥ 1 % are included.

E. elephantina RT(min)	Fish oil	Triphala	Senokot	Softener	Imodium	Dulcolax	Castor	% RSD in RT
5.033	-	-	-	5.080	-	-	-	-
5.783	-	-	-	-	-	-	-	-
8.615	-	8.616	-	-	-	-	-	-
9.917	-	-	-	-	-	-	-	-
11.285	11.289	11.283	11.283	-	11.283	11.316	-	0.12
12.008	-	-	-	-	-	-	-	-
12.573	-	-	-	-	-	-	-	-
12.708	12.710	12.697	12.702	12.693	12.696	12.694	12.699	0.05
12.900	-	-	-	-	13.012	13.024	-	0.53
13.217	-	-	-	-	-	-	-	---
13.354	-	-	13.351	-	-	-	-	0.02
13.817	-	13.808	13.814	-	-	-	-	0.15
13.858	13.883	13.842	13.857	-	-	13.836	13.849	0.13
13.989	13.982	13.979	13.990	13.980	13.981	13.973	13.984	0.04
14.871	-	-	-	-	-	-	-	0.08
15.158	-	-	15.154	-	-	-	-	0.02
17.680	-	17.672	17.676	-	-	-	-	0.02
18.558	-	-	-	-	-	-	-	-
19.625	-	-	-	-	-	-	-	-
22.502	-	-	-	22.594	-	-	-	0.29
25.277	-	-	-	-	-	-	-	-
21	4	7	8	4	4	5	3	-

elephantina, indicating similarity as determined by PCA on infrared data. Next in similarity to *E. elephantina* are imodium and dulcolax, whose togetherness implies that the two have very similar chemical composition. Stool softener, fish oil, and castor oil, are further away from *E. elephantina*. The PCA results are in part supported by the correlation coefficients and the chromatogram profiles observed earlier.

Conclusion

Hexane extracts of *E. elephantina* contain a large diversity of fatty acids, with hexadecanoic, 9-Octadecenoic, 9,12-octadecadienoic and octadecanoic acids as the major components. The major components account for about 3 mg fatty acids/g *E. elephantina* roots. Other minor compounds include cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (Omega 3), methyl benzoate, methyl -3-phenylprop-2-enoate, and methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate. The OTC products examined in this study contained at least two fatty acids in common with *E. elephantina*, namely, hexadecanoic and octadecanoic acids. Both chromatograms and principal component analysis of the FTIR/ATR data indicated high similarities between *E. elephantina* roots and senokot or triphala, followed by imodium and dulcolax. The least similarities were observed between *E. elephantina* and fish oil, castor oil, and stool softener. The role played by fatty acids from *E. elephantina* to relieve constipation, to ease bowel movement, and restore energy requires further studies.

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