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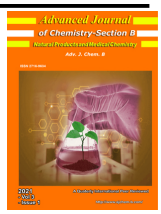

Original Research Article

Comparison of the phytochemical composition of *Euclea divinorum* Hern (Ebenaceae) leaves, tender stems and root bark

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ABSTRACT

Euclea divinorum Hern (Ebenaceae) has a long history of folkloric use in the treatment of different diseases. However, there are few reports on the responsible phytochemicals in its tender stems, leaves and root bark. The aim of this study was to compare the phytochemicals in *E. divinorum* leaves, tender stems and root bark. The plant materials were extracted serially by maceration with dichloromethane, hexane and ethanol. Alkaloids, phenols, saponins, flavonoids, steroids, cardiac glycosides, tannins, terpenes, and volatile oils were identified in qualitive Phytochemical screening of the extracts. UV Visible and Fourier Transform infrared spectroscopy indicated the presence of alcohols, phenols, alkanes, alkenes, alkynes, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in the extracts. Gas chromatography/mass spectrometry afforded identification and quantification of 30 compounds in the hexane and dichloromethane extracts. The major constituents were3,4-Methylenedioxybenzylacetone, Eicosane, Tetratriacontane, Hexatriacontane, 9-Hexadecen-1-ol, 2-Hydroxy-2-methyl-8,8-diphenyl-octa-5,7-dien-3-one, 1,4-Naphthoquinone and Octacosanal. The compounds identified in the different extracts of *Euclea divinorum* leaves, tender stems and root bark supports the use of this plant in traditional medicine.

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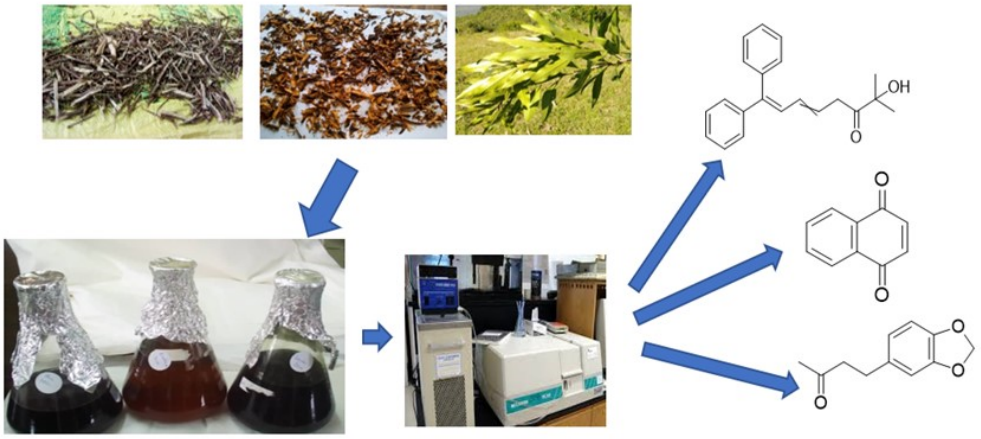
GRAPHICAL ABSTRACT



Introduction

Euclea divinorum Hern is a deciduous or evergreen medicinal shrub which grows at lower altitudes in various countries in Africa [1]. The plant is called magic guarri, diamond leaved Euclea, *Olkinyie* or *Uswet* (in Markweta dialect of Kenya). Its boiled stem bark is taken with meat soup for various ailments or for good health [2-4]. The branches (twigs) and root bark have been used by the local people of Kenya and Uganda; they are chewed as a disinfectant, used to impart red colour to the lips, clean and whiten the teeth(as toothbrushes) as well as treat gum bleeding and toothache [3, 5]. The plant root extract with those of *Carissa edulis* (Forsk.) Vahland *Carica papaya* L. are used for treatment of venereal diseases [6, 7]. The root and root bark are traditionally used to treat snakebites, headache, chest pain, ulcers[8, 9], gastrointestinal disturbances, arthritis, miscarriage, jaundice, diarrhoea, convulsions, cancers, skin diseases, malaria, tuberculosis, odontological diseases and gonorrhoea [2, 3, 10-17]. Outside East Africa, the roots of this plant is used for oral care, toothaches, fungal diseases, sores, wounds and abscesses[18, 19]. It is also used in South Western Ethiopia to purify drinking water in which the branches are added to the gourds or pots, and left to soak in the water for several hours[3].

Some phytochemical studies have been done on *E. divinorum.* Onyango et al. [4] reported the presence of tannins, saponins, alkaloids, steroids, terpenoids and reducing compounds in the methanol and ether extracts of roots and powdered root barks of *E. divinorum* while its water extract contained tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, reducing compounds and flavonoid aglycones. The ether extract had tannins, flavonoids, alkaloids, terpenoids, steroids and flavonoid aglycones. Another study [20] reported the presence of anthraquinones, saponins, tannins, polyphenols, and terpenoids in the dichloromethane : methanol extracts of leaves, roots and the stems of *E. divinorum*. However, alkaloids were present in only the root extract.

Earlier chemical studies of *E. divinorum* along with other species from the same genus yielded some naphthoquinones, triterpenes and flavonoids [21, 22]. Later, flavonoids in the ethanolic extracts of *E. divinorum* aerial parts

were characterized. The extract was partitioned between water and ethyl acetate, and the latter was subjected to column chromatography using methanol: chloroform mixtures of increasing polarities.(2R:3R)-aromadendrin-3-O-β-L-arabinopyranoside, catechin, quercitrin and myricitrin were identified [1]. Similarly, phytochemicals in the root bark of *E. Divinorum* were characterized. Naphthoquinones, triterpenoids and flavonoids were isolated. Specifically, 3-β-(5-hydroxyferuloly)lup-20-(30)-ene, isodiospryrin, shinalene, catechin, lupeol, lupine,7-methyljuglone and betulin(3-5) were reported in the chloroform extracts[10]. Further, the bioactive compounds in the leaves of *E. Divinorum* were characterized and two new compounds: Euclenal A (8-hydroxy-3-methoxy-1-naphthaldehyde) and Euclenal B (4-hydroxy-3,8-dimethoxy-1-naphthaldehyde) were identified [3]. Though different parts of this plant has proven bioactivities [10, 20, 23, 24], no study has compared the phytochemical composition of its leaves, tender stems and root bark. These parts of *E. divinorum* are commonly used [3] and in the present study, we compared the phytochemicals in the hexane, dichloromethane and ethanol extracts of *E. divinorum* leaves, tender stems (twigs) and root bark.

2. Materials and Methods

2.1 Ethical approval

This study was approved by the Department of Chemistry and Biochemistry, Moi University, Eldoret, Kenya for Immaculate Mbabazi (Approval No. MSC/ACH/9/18).

2.2 Collection of samples

Leaves, tender stems and root bark of *E. divinorum* were collected from Elgeyo Marakwet Rift Valley located in the North Rift region of Kenya (0°58'56.0"N and 35°35'16.5"E) near St. Joseph's Lawich Catholic. Purposive and random sampling methods were used during the collection of samples from the field where the first plant of *E. divinorum* was purposively selected and the rest were selected at random. The sampling method was also purposive because of the previous knowledge about the availability of *E. divinorum* plants in this location. The samples were obtained in clean sterilized manila bags and transported to the laboratory.

2.3 Sample preparation and extraction

The leaves, tender stems and root barks of *E. divinorum* were washed under tap water followed by distilled water and then shade-dried for 4 weeks. After, the plant materials were then warmed for 45 minutes in an oven at 30 °C before grinding separately into fine powder (**Figure 1**). Serial extraction method was used starting with hexane, dichloromethane (DCM) and finally ethanol.

For leaves, weighed aliquots (200 g) of the dry powder were placed in a 250 ml conical flask and then macerated at room temperature for 48 hours in 800 ml of 98.5% hexane with occasional shaking. The samples were filtered through Whatman No. 1 filter paper. The filtrates were concentrated on a rotary evaporator set at 45°C while the residues (labeled R1) were used in the subsequent extraction procedure. The concentrated extracts were collected in labeled 50ml sample vials, dried, weighed and masses of the extracts recorded[25]. The extracts were sealed and kept at 4°C. Residues (R1) were air dried, and then macerated with 800 ml of 99.5% DCM at room temperature for 48 hours with occasional shaking. These were then filtered through Whatman No. 1 filter paper, and the second sets of residues (labeled R2) were kept for further extraction.

Filtrates obtained were concentrated on a water bath set at 35°C.The concentrated extracts were collected in 50 ml sample vials, dried, weighed and the respective weights recorded. The extracts were sealed in labeled sample vial tubes and kept at 4 °C for further studies.

Figure 1: Dry ground powder of *E. divinorum* (a) leaves, (b) root barks and (c) tender stems.

Residues (R2) were air dried, then macerated in 800 ml of absolute ethanol at room temperature for 48 hours with occasional shaking. They were filtered and the filtrates were concentrated on a rotary evaporator at 65 °C. The concentrated extracts were collected in labeled sample vials, dried, weighed, sealed and kept at 4 °C.

The tender stem and root bark powders were subjected to the same treatment as described for the leaves to obtain the respective hexane, DCM and ethanol extracts.

2.4 Determination of total phenolic and total flavonoid contents

Total phenolic and total flavonoid contents were determined by Folin-Ciocalteau and aluminium chloride methods respectively.

Total phenolic content (TPC) of the crude extracts was determined as reported by Johari and Khong[26] with some modifications as follows. Crude hexane extract (0.1 g) of *E. divinorum* leaves was dissolved in 25 ml of hexane. The resultant solution of this extract (0.5 ml) was transferred into vials in triplicate, mixed with 2.5 ml of Folin-Ciocalteau reagent followed by 2.5 ml of 6% sodium carbonate solution. The solutions were incubated in the dark at 25°C for 30 minutes after which their absorbances were measured at 760 nm using a Beckman Coulter DU 720 General Purpose UV/Vis spectrophotometer.

To determine the TPC of hexane, DCM and ethanol extracts from leaves, tender stems and root barks, the respective dry extracts were dissolved in the respective solvents and the above procedure was followed.

A calibration curve of a standard reference was established using gallic acid (concentrations of 1, 10, 25, 75, 100 and 200 ppm). Thus, the TPC was reported as gallic acid equivalents in ppm of the extract [26].

Total flavonoid content (TFC) of the crude extracts were determined as reported by Pękal and Pyrzynska [27] with slight modifications. Briefly, crude hexane leaf extract (0.1g) was dissolved in 25 ml of hexane and the resultant solution (1 ml) was transferred into sample vials in triplicate and mixed with 5% (w/v) of sodium nitrite solution (0.3 ml). After 5 minutes, 2% (w/v) of aluminium chloride solution (0.5 ml), 1 M sodium hydroxide solution (2 ml) and finally distilled water (3 ml) were added. The mixtures were incubated at 25 °C for 20 minutes after which the absorbance of each was read at 510 nm.

To determine the TFC in the remaining extracts from leaves, tender stems and root barks, the respective dry extracts were dissolved in their respective solvent of extraction and the above procedure was repeated. Prepared 500 ppm solution of quercetin was used to prepare standard quercetin solutions of 5, 10, 50, 75 and 100 ppm. Aliquots (0.5 ml) of each solution was taken into a vial and subjected to the same treatment as the samples. A calibration curve was obtained from which the TFC was estimated as quercetin equivalent in ppm [27].

2.5 Preliminary phytochemical screening

Standard phytochemical screening procedures were followed to test for the presence of alkaloids, flavonoids, cardiac glycosides, phenols, saponins, quinones, steroids, tannins, terpenes and volatile oils in all the extracts of leaves, tender stems and root bark of *E. divinorum*.

2.5.1 Alkaloids

To the extract (2 ml), 1% hydrochloric acid was added and steamed. Drops of Wagner's reagent (1 ml) were then added to the resultant solution. A brown or reddish brown precipitate indicated the presence of alkaloids [28].

2.5.2 Cardiac glycosides

Glacial acetic acid (1 ml) was added to the extract (2 ml) in a test tube followed by ferric chloride (3 drops) and then concentrated sulphuric acid (1 ml). Presence of a brown ring at the interface was used to indicate the presence of cardiac glycosides [29].

2.5.3 Flavonoids

Four drops of concentrated hydrochloric acid followed by magnesium turnings (0.5 g) were added to the extract (2 ml) in a test tube. A pink colour formation after 3 minutes was used to indicate the presence of flavonoids [29].

2.5.4 Phenols

Measured 1% iron (III) chloride solution (1 ml) was added to the extract (2 ml) in a test tube. A blue or green colour formation was an indication of the presence of phenols [29].

2.5.5 Quinones

Concentrated sulphuric acid (1 ml) was added to the extract (1 ml) in a test tube. Formation of a red colour showed the presence of quinones [29].

2.5.6 Saponins

Distilled water (5 ml) was added to the extract (1 ml) in a test tube. The mixture was then shaken vigorously for two minutes. Appearance of foam lasting for 5 minutes confirmed the presence of saponins [29].

2.5.7 Steroids

Acetic anhydride (2 ml) was added to the extract (1 ml) in a test tube followed by 2 ml of concentrated sulphuric acid. A blue or green coloration indicated the presence of alkaloids [29].

2.5.8 Tannins

In a test tube containing 5 ml of the extract, a few drops of 1% solution of lead (II) acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins [30].

2.5.9 Terpenes

To the extract (2 ml), chloroform (5 ml), acetic anhydride (2 ml), and drops of concentrated hydrochloric acid were added. A reddish-brown colour formed at the interface was an indication of terpenes.

2.5.10 Volatile oils

To the extract (1 ml), 90% ethanol was added, followed by few drops of ferric chloride. Formation of a green colour indicated their presence [29].

2.6 Characterization of phytochemicals in leaves, tender stems and root bark extracts of *E. divinorum*

2.6.1. Ultraviolet visible spectroscopy

Dry powder of *E. divinorum* leaves (1 g) was placed in a 250 ml conical flask and macerated at room temperature for 48 hours in 50 ml of 98.5% hexane with occasional shaking. The sample was filtered through Whatman No.1 filter paper under gravity. The extract was centrifuged at 12,000 rpm for 10 minutes and again filtered. The sample was then diluted with hexane in a ratio of 1:10 and then scanned at wavelength ranging from 210nm to 800nm using a Beckman Coulter DU 720 General Purpose UV/Vis spectrophotometer[31]. The wavelength scans for all the samples (leaves, tender stems and root barks) extracted by maceration with hexane, DCM and ethanol were obtained as described above.

2.6.2. Fourier Transform Infrared spectroscopy

Potassium bromide pellets of the samples were prepared by grinding 10mg of hexane extract of the leaves with 250 mg of potassium bromide (FTIR grade). The 13mm potassium bromide pellets were prepared in a standard device under a pressure of 75 kN cm^{-2} for 3 minutes. The functional groups present in all the extracts were analyzed using a FTS- 8000Shimadzu Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu Corporation, Japan). The spectral resolution was set at 4 cm^{-1} and the scanning ranged from 400 to 4000 cm^{-1}. The FTIR spectra of all the extracts were obtained as described above. Every analysis was repeated twice for spectra confirmation [31].

2.6.3 Gas chromatography/mass spectrometry analysis of plant extracts

Hexane and DCM extracts of the leaves, tender stems, and root barks were considered for gas chromatography/mass spectrometry (GC/MS) analysis. Samples were diluted in their respective solvents of extraction (1:10 v/v), ultrasonicated for 15 minutes, filtered separately through Whatman No. 1 filter papers followed by 0.45µm syringe filters and transferred into sample vials for GC/MS analysis.

A Shimadzu QP 2010-SE GC/MS coupled to an auto sampler was used for the analysis. Ultrapure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1ml/minute. A BPX5 non-polar column (30 m × 0.25 mm × 0.25 µm) was used for separation. The GC was programmed as follows: the oven temperature was programmed from 50 °C (1 minute) to 250 °C (9 minutes) at a rate of 5 °C /min. Total run-time was 50 minutes. Measured 1 µL of the sample was injected. Injection was done at 200 °C in split mode, with split ratio set to 10:1. The interface temperature was set at 250°C. The EI ion source was set at 200 °C. Mass analysis was done in full scan mode within a range of 50-600 amu. Detected peaks were matched against the NIST 2014 MS library for identification.

2.7 Statistical analysis

All quantitative data were reported as means ± standard deviations of triplicates. The data obtained were analyzed using both descriptive and inferential statistics. Quantitative data (percentage yield, TPC and TFC) were subjected to Analysis of Variance test at $p < 0.05$ using SPSS for Windows (Version 20).

3. Results and Discussion

3.1 Percentage yield

The extraction yields shown in **Table1** were expressed as the percentages of initial mass of the sample macerated. As shown, extraction using ethanol gave the highest yields in comparison to hexane and DCM with the root barks having the highest yield of 7.60% followed by tender stems (3.30%). For the leaf extracts, a high yield was obtained using ethanol (2.51%) than using DCM (1.92 %) and hexane (1.13 %). This trend was also observed for the extracts of tender stems and root bark. However, these differences were not statistically different ($p = 0.07$). This result indicates that ethanol is a good solvent for extraction as compared to hexane and DCM probably because most compounds in the parts of *E. divinorum* extracted are polar, thus were able to dissolve in the more polar ethanol than other non-polar solvents used. Differences in solvent polarities used for extraction is known to play a key role in increasing the solubility of phytochemical compounds [25, 32, 33]. Further, differences in the structure of phytochemical compounds also determine their solubility in solvents of different polarities [34]. Indeed, the three solvents used had different polarities arranged as hexane < DCM < ethanol. This change appears to be related not only to the differences in the polarity of extracts of the components but also to the solvents used, which also plays a vital role in increasing the solubility of phytochemical compounds.

Table1: Organic extract yield of different parts of *E. divinorum*

Part used	Extract yield (%)		
	Hexane	Dichloromethane	Ethanol
Leaves	1.13	1.92	2.51
Tender stems	0.57	1.74	3.30
Root bark	0.51	2.35	7.60

Therefore, the results of the current study confirmed the effect of solvent extraction and the plant organ on the yield and consequently confirm the richness of this plant in polar substances.

3.2 Total phenolic and total flavonoid contents

3.2.1 Total phenolic content

The TPC of the extracts were determined using the Folin-Ciocalteau method. Folin-Ciocalteau reagent consists of a mixture of sodium molybdate, sodium tungstate and other reagents which when added to plant extracts react with phenolic compounds to produce a solution of a blue complex which absorbs at 760 nm. The assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes [35, 36]. A calibration curve (**Figure 2**) was prepared for the quantitative analysis and the linearity for gallic acid standard was established from the range of 1 ppm to 100 ppm which was fitted on the line $y = 0.0022x + 0.0012$. As shown in **Table 2**, the TPC was highest for ethanol than all the extracts. This could be because ethanol just like methanol is a polar protic solvent [25, 37]. Thus, it extracted more polyphenols which are inherently polar, and their solubility is through hydrogen bond formation [38]. The root bark extract recorded the highest TPC followed by tender stems and then lastly the leaf extracts. Hexane being the least polar, extracted the least quantity of phenolic compounds compared to ethanol and DCM. The DCM tender stem extract contained the least quantity of phenolic compounds and the root bark contained the highest. Overall, the root bark extracts had the highest quantity of phenolic compounds ($p < 0.05$) compared to the rest of the parts of the plant extracted. Further, ANOVA test showed that there were significant differences ($p = 0.008$) among the mean TPC of hexane, DCM and ethanol extracts.

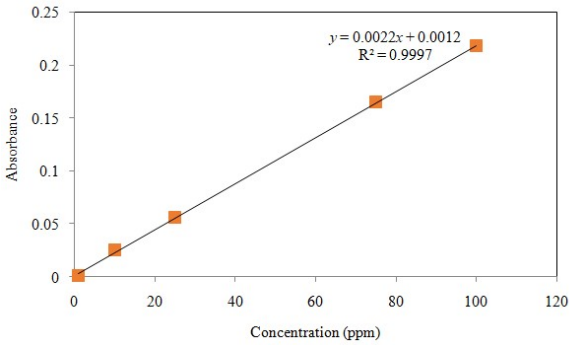


Figure 2: Calibration curve for TPC using Gallic acid standard

Table 2: Total phenolic content of the different solvent extracts of *E. divinorum*

Part used	Hexane	Dichloromethane	Ethanol
Leaves	299.0 ± 2.6	1190.0 ± 12.0	1516.0 ± 17.1
Tender stems	231.0 ± 16.6	828.0 ± 11.4	2800.0 ± 5.7
Root barks	472.0 ± 6.3	1569.0 ± 5.3	3105.0 ± 3.3

Values are means ± standard deviations of triplicates, expressed as in ppm gallic acid equivalent.

Table 3: Total flavonoid content of the different extracts of *E. divinorum*

Part used	Hexane	Dichloromethane	Ethanol
Leaves	84.3 ± 1.41	23.4 ± 0.40	63.10 ± 1.97
Tender stems	55.6 ± 1.33	27.6 ± 1.24	81.60 ± 2.88
Root bark	193.3 ± 1.09	96.1 ± 0.61	309.70 ± 11.90

All values are means ± standard deviations of triplicates, expressed as quercetin equivalent in ppm.

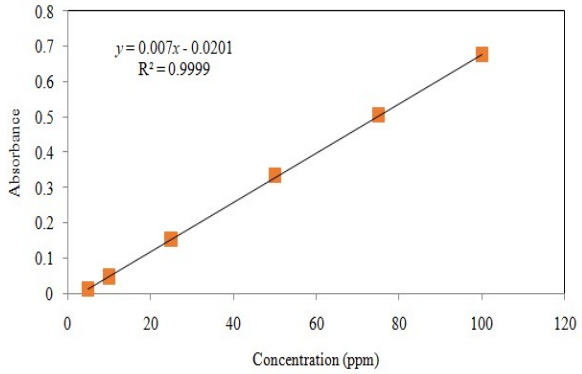


Figure 3: Calibration curve for TFC using Quercetin standard

3.2.2 Total flavonoid content

The aluminium chloride method was used for TFC determination. The method is based on the nitration of the aromatic ring of quercetin with its position 3 or 4 non-sterically hindered, giving a yellow complex of aluminium that turns red after addition of sodium hydroxide [27].The calibration curve (prepared using quercetin as a standard) is shown in **Figure 3**. Linearity for the standard was established from the range of 5 ppm to 100 ppm which was fitted on the straight line that gave the equation $y = 0.007x - 0.00201$.

It was found that TFC were highest for ethanolic extracts compared to DCM and hexane extracts. In the root barks for instance, TFC were found to be 193.30 ± 1.09, 96.10 ± 0.61, and 309.70 ± 11.90ppmfor the hexane, DCM and ethanolic extracts respectively. This could have been because ethanol with the highest degree of polarity among the solvents, was able to extract much of the flavonoids which are relatively polar[25, 39]. Low TPC in plant extracts indicates that the extract contains more flavonoid heterosides than aglicones [25]. Unlike for the TPC, ANOVA test for TFC indicated that there were no significant differences between hexane, DCM and ethanol extracts ($p = 0.45$). Overall, it should be emphasized that the recovery of phytochemicals from plants are influenced by dielectric constant, chemical structure of organic solvents, as well as the chemical properties of plant phytochemicals [25], explaining the variations observed in the TPC and TFC obtained from the different parts of *E. divinorum* using the three solvents.

3.3 Phytochemical analysis of *E. divinorum* extracts

3.3.1 Phytochemical screening

Qualitative phytochemical screening of medicinal plants is an essential step to their detailed phytochemical and pharmacological investigation [40]. Phytochemical screening tests using the different extracts of *E. divinorum* in this study revealed the presence of several secondary metabolites including alkaloids, flavonoids, cardiac glycosides, phenols, saponins, quinones, steroids, tannins, terpenes and volatile oils (**Table 4**). Alkaloids were detected in only hexane extract of the root barks. Cardiac glycosides and steroids were detected in hexane extracts of the leaves, tender stems, and root barks and in dichloromethane extract of the tender stems. Flavonoids were present in ethanolic extract of the root barks only whereas phenols were detected in ethanol extracts of the leaves, tender stems and root barks only. Quinones were confirmed present in the hexane extracts of tender stems and root barks, DCM extracts of leaves and tender stems, and ethanol extract of the root barks. Phenols, saponins, tannins and terpenes were present only in the ethanol extracts of leaves, tender stems and root barks.

Table 4: Secondary metabolites identified in different extracts of *E. divinorum* parts.

Phytochemical	Solvent	Leaves	Tender stems	Root barks
Alkaloids	Hexane	-	-	+++
	Dichloromethane	-	-	-
	Ethanol	-	-	-
Cardiac glycosides	Hexane	++	+	+++
	Dichloromethane	-	+++	-
	Ethanol	-	-	-
Flavonoids	Hexane	-	-	-
	Dichloromethane	-	-	-
	Ethanol	-	-	+++
Phenols	Hexane	-	-	-
	Dichloromethane	-	-	-
	Ethanol	+	++	+++
Quinones	Hexane	-	++	+++
	Dichloromethane	-	++	++
	Ethanol	+	-	-
Saponins	Hexane	-	-	-
	Dichloromethane	-	-	-
	Ethanol	+++	+++	+++
Steroids	Hexane	+++	+	+++
	Dichloromethane	-	-	-
	Ethanol	-	-	-
Tannins	Hexane	-	-	++
	Dichloromethane	-	-	-
	Ethanol	++	++	+++
Terpenes	Hexane	-	-	-
	Dichloromethane	-	-	-
	Ethanol	+++	+++	+++
Volatile oils	Hexane	+	++	+++
	Dichloromethane	+	+	-
	Ethanol	+++	+++	+++

+++ represents very high, ++ indicates moderate, + indicates little/traces, and –indicates absent.

However, tannins were also detected in the hexane extract of the root barks. Volatile oils were detected in all the extracts with the exception of DCM extract of the root bark. Saponins and phenols were only detected in ethanol extracts of the leaves, tender stems and root bark of *E. divinorum.*

The results of the present study agreed well with previous studies. Onyango et al. [4] reported the presence of tannins, saponins, alkaloids, steroids, terpenoids and reducing compounds in the methanol and ether extracts of roots and powdered root barks of *E. divinorum* while its water extract contained tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, reducing compounds and flavonoid aglycones. The ether extract showed the presence of tannins, flavonoids, alkaloids, terpenoids, steroids and flavonoid aglycones. Another study [20] reported the presence of anthraquinones, saponins, tannins, polyphenols, and terpenoids in the DCM : methanol extracts of leaves, roots and the stems of *E. divinorum*. However, alkaloids were present in only the root extract. Ngari et al.[23] reported the presence of triterpenoids and amino acids, resins and tannins in the DCM extract of *E. divinorum* roots. The presence of alkaloids only in the hexane root barks extracts in the current study corroborate observations made by previous authors. The identified secondary metabolites could be responsible for the medicinal properties of *E. divinorum*. For example, alkaloids are adenosine receptor antagonists, have analgesic, antitussive, stimulant, antispasmodic, antiprotozoal, antiarrhythmic, antipyretic, antimalarial, antitumor, vasodilating, aphrodisiac, antihypertensive and antibacterial activities[41]. Tannins have reported antifungal, anti-inflammatory, antidiabetic, wound healing and antibacterial activities [42] while saponins possess hypocholesterolemic, antibacterial and anticancer agents.

3.3.2 UV-Visible spectra of the extracts

The extracts of *E. divinorum* were scanned at wavelength between 210 to 800 nm to identify compounds containing σ-bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings. The spectra for the different extracts are shown in **Figure 4**. Leaf extracts recorded absorption at wavelengths of 410.0, 539.0, 699.0nm (for hexane extract), 414.0, 453.0, 669.0 nm (for DCM extract) and 370.0, 436.0, 655.0 nm for the ethanol extract. Tender stems recorded absorptions at 409.0, 674.0 nm (for hexane extract), 416.0, 452.0, 669.0 nm (for DCM extract) and 400.0, 665.0 for the ethanol extract. Root bark recorded absorption at 369.0, 395.0, 446.0 nm (for hexane extract), 342.0, 420.0, 665.0 nm and 352.0, 439.0, 699.0 nm for the ethanol extract. Overall, the analysis indicated that the absorption peaks occurred between 342.0 nm for DCM root bark extract and 699.0 nm for hexane and DCM leaf extracts, DCM tender stems extract and the ethanolic extract of the root bark. These absorption bands are due to the presence of flavonoids, phenols and their derivatives in the extracts [31].

3.3.3 FTIR spectra of *E. divinorum* extracts

Over the years, FTIR spectroscopy has proven to be a valuable tool in the identification of the functional groups of compounds in plant extracts [25, 31].

The results of FTIR spectroscopic analysis of different extracts of *E. divinorum* revealed the existence of various phytochemical constituents. The FTIR spectra were interpreted using the peak values as previously reported elsewhere [43-45]. The spectrum of the standard is given in **Figure 5**.

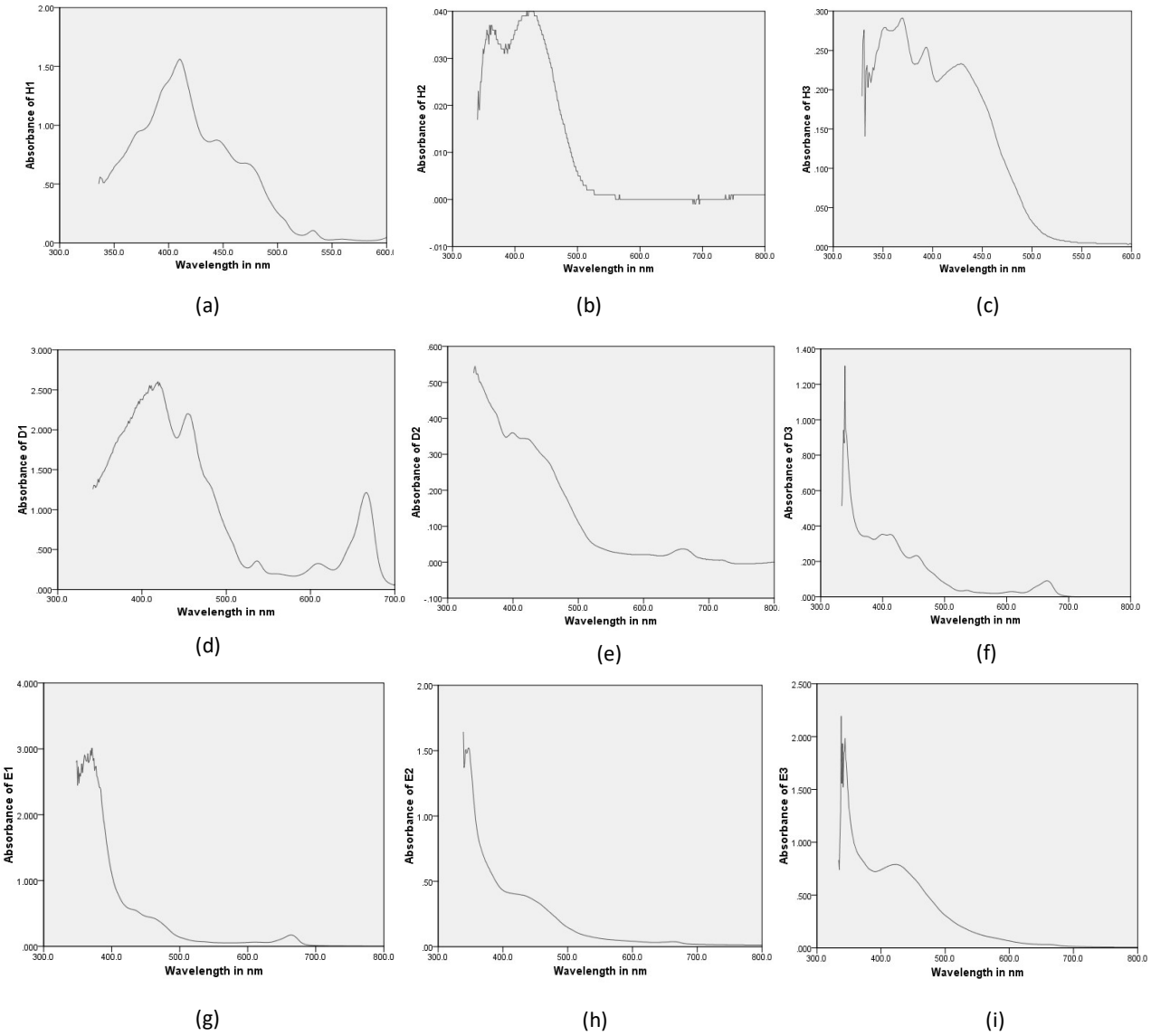


Figure 4: UV-Visible spectra of extracts of *E. divinorum*: (a) hexane leaf extract, (b)hexane tender stems extract, (c) hexane root bark extract, (d) DCM leaf extract, (e) DCM tender stems extract, (f) DCM root bark extract, (g)ethanolic leaf extract, (h) ethanolic tender stems extract, and (i) ethanolic root bark extract.

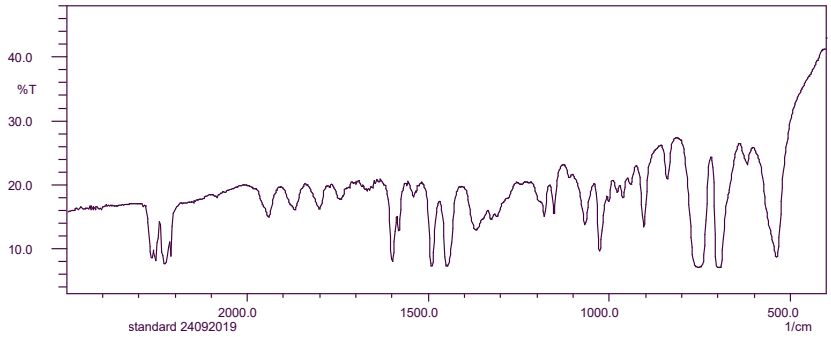


Figure 5: FTIR spectrum of the standard (KBr)

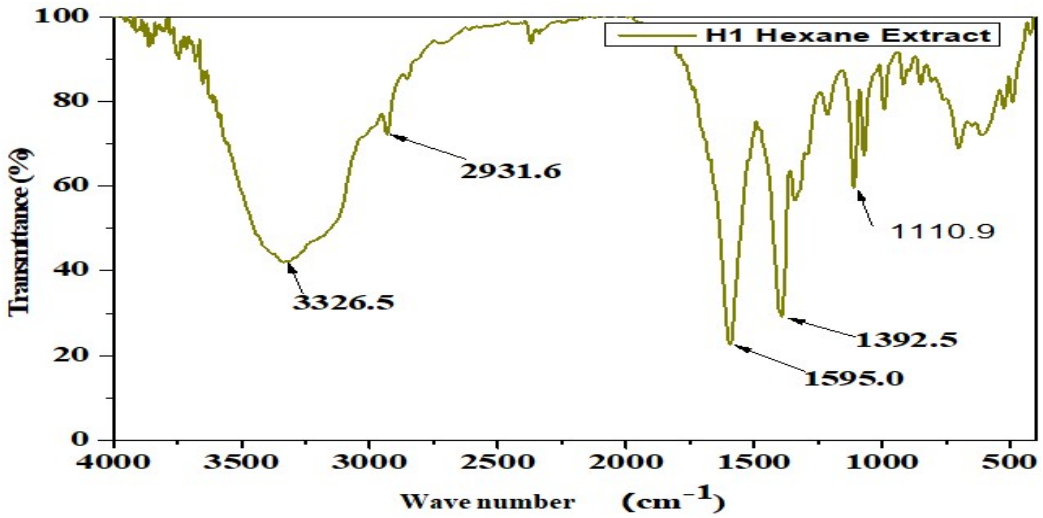


Figure 6: FTIR spectrum of hexane extract of *E. divinorum* leaves.

The intense absorption at 3326.5 cm^{-1} (**Figure 6**) is due to stretching of -OH groups from phenolic compounds present in the extract [44] or NH/OH stretching of amines and amides [30]. The band at 2931.6 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes. The sharp weak peak at 2353.6 cm^{-1} is due to absorption of the C≡N group of nitrile compounds. Absorption at 1595.0 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1392.5 cm^{-1} is due to Asymmetric in-plane bending of –CH_3. The absorption peaks at 1110.9 cm^{-1} and 1070.4 cm^{-1} are due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes [45].

In **Fig. 7**, the intense absorption at 3346.3 cm^{-1} is due to stretching of -OH groups from phenolic compounds present in the extract. The band at 2925.9 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes.

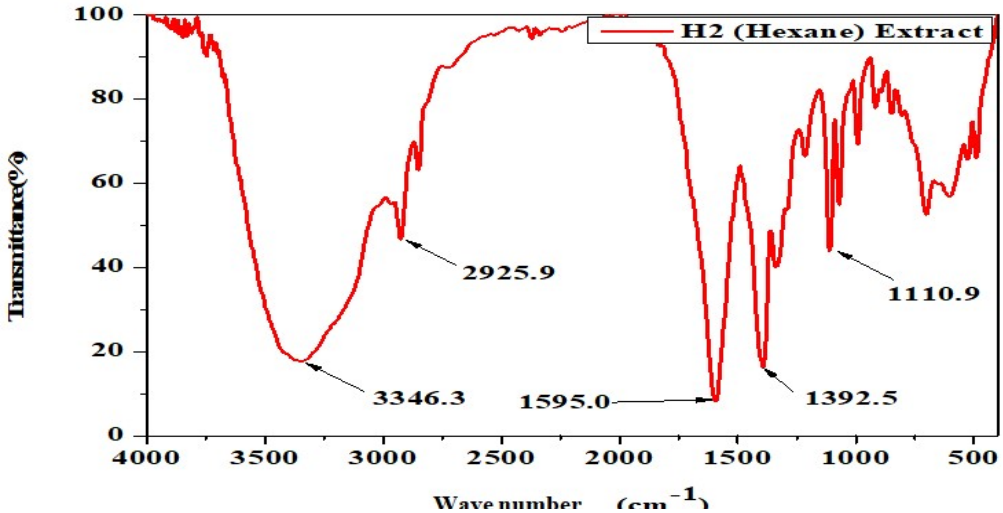


Figure 7: FTIR spectrum of hexane extract of the tender stems of *E. divinorum*

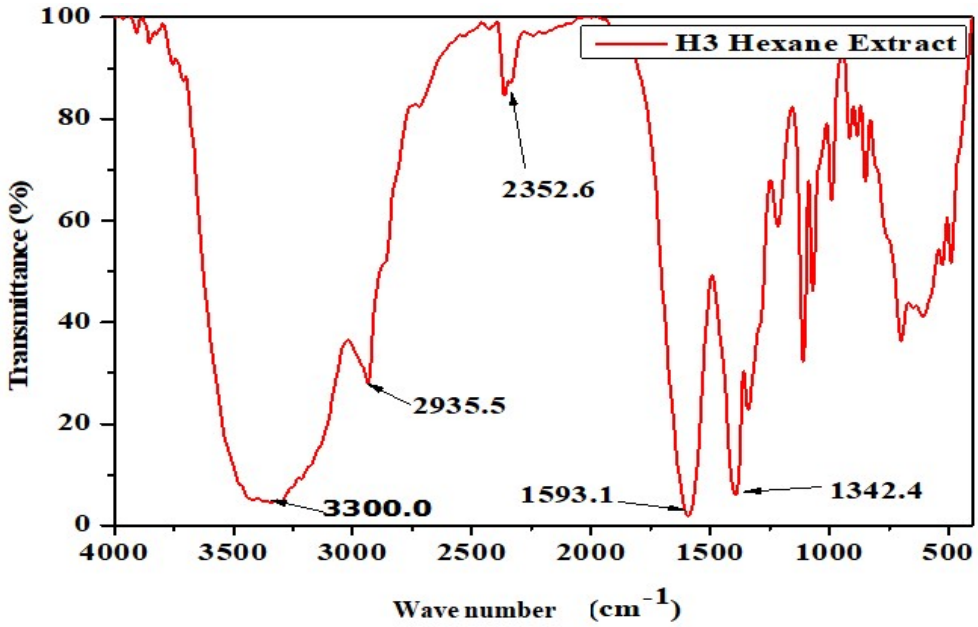


Figure 8: FTIR spectrum of hexane extract of the root bark of *E. divinorum*

Absorption at 1595.0 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1392.5 cm^{-1} is due to Asymmetric in-plane bending of $-CH_3$. The two absorption peaks at 1110.9 cm^{-1} and 1070.1 is due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 $cm^{-1}$and 910.2 cm^{-1} are due to =C-H bending vibrations of alkenes. The absorption band at 700 cm^{-1} is due to the stretching vibrations of -C-Br bond of aliphatic bromo-compounds [45]. The intense absorption at 3300 cm^{-1} in **Figure 8** is due to stretching of -OH groups from phenolic compounds present in the extract [44]. The band at 2935.5 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes. The sharp weak peak at 2353.6 cm^{-1} is due to absorption of the C≡N group. Absorption at 1593.1 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1394.1 cm^{-1} is due to asymmetric in-plane bending of $-CH_3$. Absorption at 1340.4 cm^{-1} is due to symmetric in-plane bending of $-CH_3$. The absorption at 1292 cm^{-1} is due to O=C-O-C-stretch from the aromatic esters. The absorption peaks at 1110.9 cm^{-1} and 1070.4 cm^{-1} are due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes while the absorption band at 702 cm^{-1} is due to the bending vibrations of ≡C-H bond of alkyne.

The intense absorption at 3339.3 cm^{-1} (**Figure 9**) is due to stretching of -OH groups from phenolic compounds present in the extract. The band at 2941.0 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes. The sharp weak peak at 2356.3 cm^{-1} is due to absorption of the C≡N group. Absorption at 1586.1 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1400.5 cm^{-1} is due to asymmetric in-plane bending of $-CH_3$. Absorption at 1340.4 cm^{-1} is due to symmetric in-plane bending of $-CH_3$. The absorption at 1292.7 cm^{-1} is due to O=C-O-C-stretch from the aromatic esters. The two absorption peaks at 1103.8 cm^{-1} and 1099.4 are due to C-O stretching vibrations from secondary and primary alcohols respectively.

The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes [46, 47]. In **Figure 10**, the intense absorption at 3336 cm^{-1} is due to stretching of -OH groups from phenolic compounds present in the extract. The band at 2929.8 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3hybridized carbon of alkanes. Absorption at 1586 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1397.3 cm^{-1} is due to Asymmetric in-plane bending of $-CH_3$. Absorption at 1339.4 cm^{-1} is due to Symmetric in-plane bending of $-CH_3$. The absorption at 1292 cm^{-1} is due to O=C-O-C- stretch from the aromatic esters. The two absorption peaks at 1110.9 cm^{-1} and 1070.4 cm^{-1} is due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes.

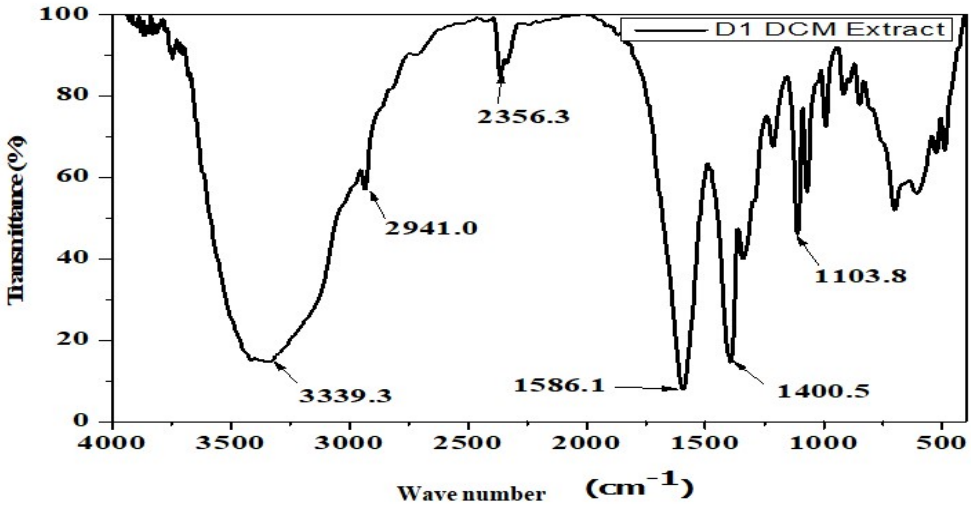


Figure 9: FTIR spectrum of DCM extract of the leaves of *E. divinorum*

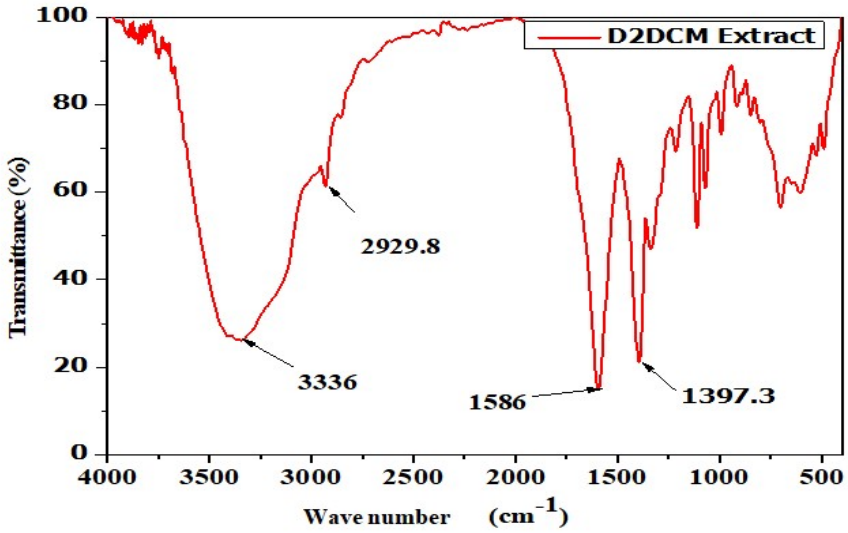


Figure 10: FTIR spectrum of DCM extract of the tender stems of *E. divinorum.*

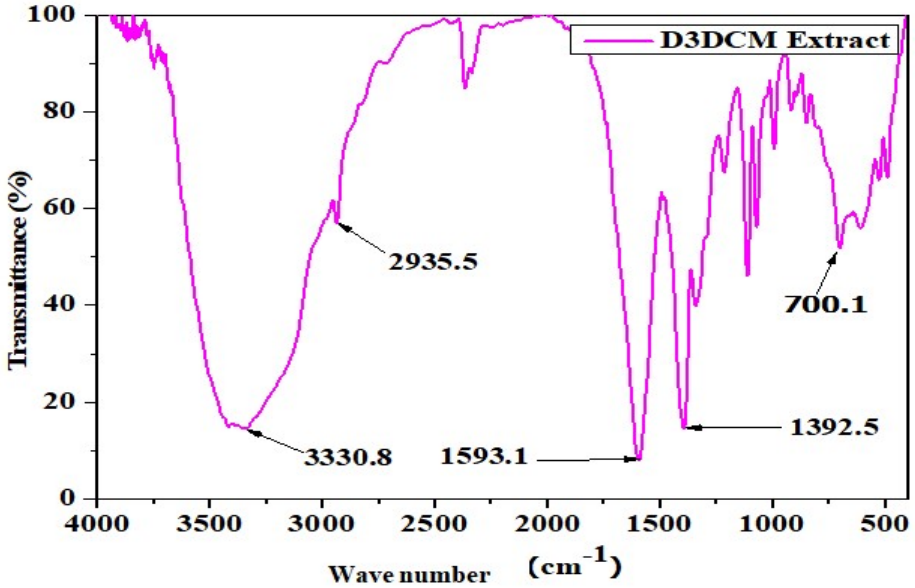


Figure 11: FTIR spectrum of DCM extract of the root barks of *E. divinorum.*

The intense absorption at 3330.8 cm^{-1} (**Figure 11**) is due to stretching of -OH groups of polyhydroxy or phenolic compounds present in the extract [45].

The band at 2935.5 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes. The sharp weak peak at 2353.6 cm^{-1} is due to absorption of the C≡N group. Absorption at 1593.1 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1392.5 cm^{-1} is due to asymmetric in-plane bending of $-CH_3$. Absorption at 1340.4 cm^{-1} is due to symmetric in-plane bending of $-CH_3$. The absorption at 1292 cm^{-1} is due to O=C-O-C- stretch from the aromatic esters or $-NO_2$ groups. The two absorption peaks at 1110.9 cm^{-1} and 1070.4 is due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 cm^{-1} and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes. The absorption band at 700.1 cm^{-1} is due to the bending vibrations of ≡C-H bond of alkyne.

For the ethanolic leaf extract (**Figure 12**), the intense absorption at 3269.1cm^{-1} is due to stretching of -OH groups from phenolic compounds present in the extract. The band at 2935.5 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3hybridized carbon of alkanes. Absorption at 1591.2 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1396.4 cm^{-1} is due to asymmetric in-plane bending of $-CH_3$. Absorption at 1342.4 cm^{-1} is due to symmetric in-plane bending of $-CH_3$.

The absorption at 1292.1 cm^{-1} is due to O=C-O-C- stretch from the aromatic esters. The two absorption peaks at 1110.9 cm^{-1} and 1070.4 cm^{-1} is due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibrations of alkenes. The absorption band at 702 cm^{-1} is due to the bending vibrations of≡C-H bond of alkyne.

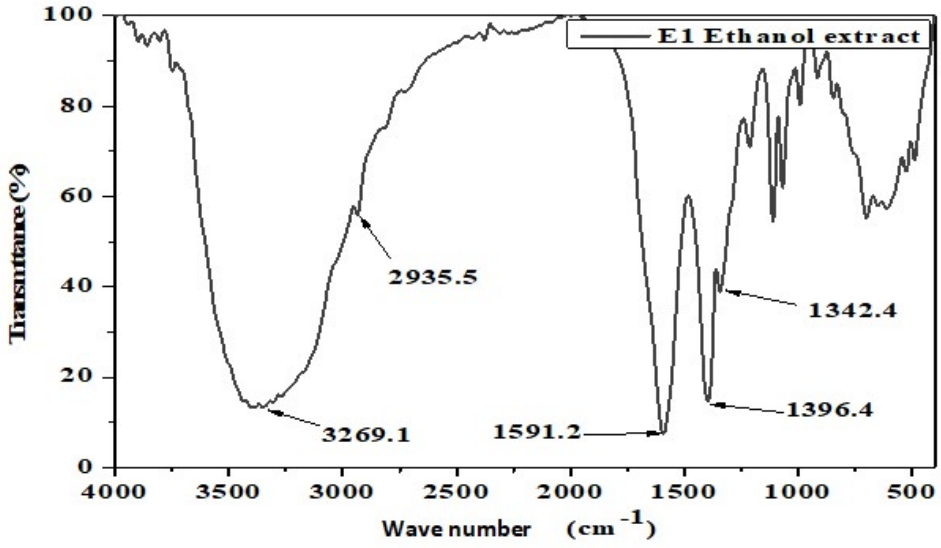


Figure 12: FTIR spectrum of ethanol extract of the leaves of *E. divinorum*

The intense absorption at 3319.3 cm^{-1} (**Figure 13**) is due to stretching of -OH groups from phenolic compounds present in the extract. The band at 2933.1 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3hybridized carbon of alkanes. The absorption at 1593.1 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorptions at 1396.4 $cm^{-1}$and 1340.4 cm^{-1} are due to asymmetric and symmetric in-plane bending of $-CH_3$respectively. The absorption at 1292 cm^{-1} is due to O=C-O-C- stretch from aromatic esters. The two absorption peaks at 1110.9 cm^{-1} and 1070.4 cm^{-1} are due to C-O stretching vibrations from secondary and primary alcohols respectively. The two weak bands at 991.3 and 914.2 cm^{-1} are due to =C-H bending vibration of alkenes. The absorption band at 700.1cm^{-1} is due to the bending vibrations of ≡C-H bond of alkynes[45]. For the ethanolic extract of the root bark (**Figure 14**), the intense broad absorption at 3209.3 cm^{-1} is due to stretching of -OH groups from alcohols present in the extract. The band at 2941.3 cm^{-1} is due to a C-H group asymmetric stretching which is sp^3 hybridized carbon of alkanes. Absorption at 1593.1 cm^{-1} is due to stretching of Ar-C=C groups of aromatic alkenes. Absorption at 1394.4 cm^{-1} is due to Asymmetric in-plane bending of $-CH_3$. Absorption at 1342.4 cm^{-1} is due to Symmetric in-plane bending of $-CH_3$. The two absorption peaks at 1100.1 cm^{-1} and 1059.4 is due to C-O stretching vibrations from secondary and primary alcohols respectively.

Thus, the FTIR spectra confirmed the presence of alcohols, phenols, alkanes, alkenes, alkynes, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in the different extracts of *E. divinorum.* The various functional groups observed in different extracts reflected the biochemical profile of *E. divinorum* which could be responsible for its various medicinal properties.

3.3.4 GC/MS results

GC/MS analysis is best suited for small molecular weight compounds (50-500 amu) that are thermally stable and volatile. It is a powerful tool that can be used to identifypossible compounds with high certainty, especially if a match exists in the NIST library [44]. Hexane leaf extract contained 4 compounds, with Tetratriacontane (47.43%) and 2-Hydroxy-2-methyl-8,8-diphenyl-octa-5,7-dien-3-one (13.53%)being the major constituents(**Table 5, Figure 15**).

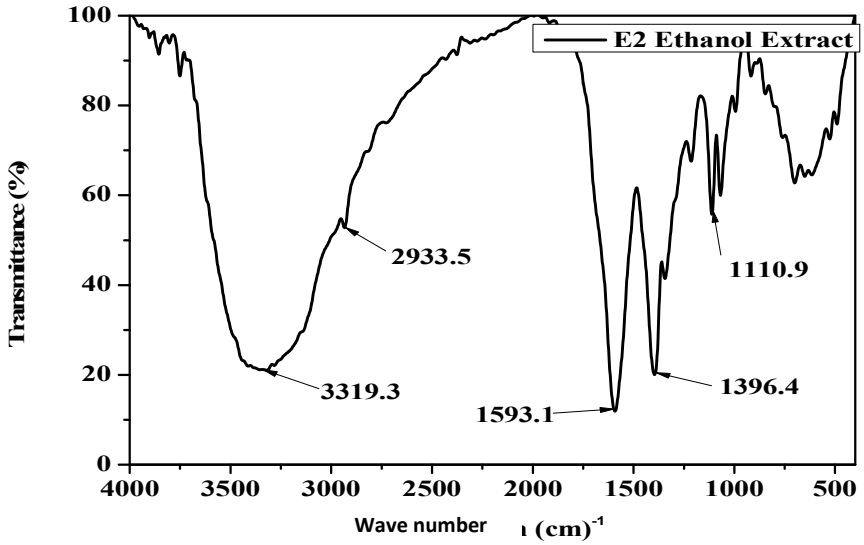


Figure 13: FTIR spectrum of ethanol extract of the tender stems of *E. divinorum*

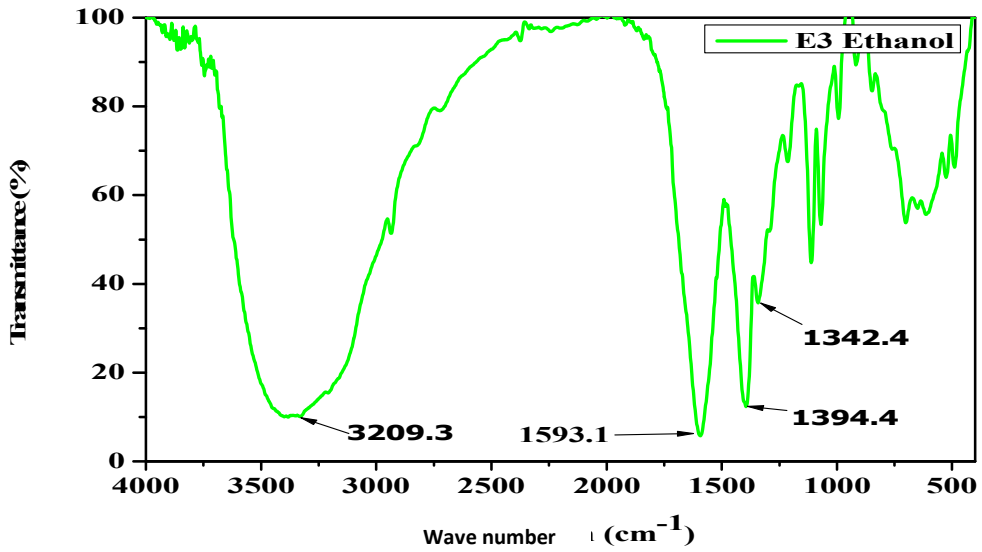


Figure 14: FTIR spectrum of ethanol extract of the root bark of *E. divinorum*

Hexane extract of the tender stems recorded 12 compounds, one of which (Eicosane) was also identified in the hexane extracts of leaves and root bark. However, an only trace of Tetratriacontane was found in this extract. The major constituents were Eicosane (56.26%) and Hexatriacontane (18.17%). In the root bark extract, 8 compounds were identified. Hexatriacontane (70.91%) and 1,4-Naphthoquinone (13.34%) dominated.

Table 5: Compounds identified in hexane extracts of *E. divinorum* by GC/MS.

Sample	Retention time (minutes)	Peak area %	Constituents
Leaf extract	10.866	2.53	1-Methyl-2-Pyrrolidinone
	40.517	2.11	Eicosane
	44.488	**47.43**	Tetratriacontane
	45.991	**13.53**	2-Hydroxy-2-methyl-8,8-diphenyl-octa-5,7-dien-3-one
Tender stems extract	34.489	0.39	Palmitic acid
	34.889	1.12	Ethyl palmitate
	38.181	0.89	Ethyl-9,12-octadecadienoate
	38.327	0.62	Ethyl 9α-linolenate
	40.516	**56.26**	Eicosane
	41.753	0.66	4,8,12,16-Tetramethylheptadecan-4-olide
	42.336	1.97	Tetracosane
	43.939	0.83	9-Tricosene
	43.941	0.56	Heptacosanol
	46.609	**18.17**	Hexatriacontane
	46.910	Traces	Tetratriacontane
	47.142	4.82	Heneicosane
Root bark extract	27.772	**13.34**	1,4-Naphthoquinone
	29.549	0.73	7-Ethoxycoumarin
	30.873	3.71	Eicosane
	31.367	**4.96**	Tetratetracontane
	35.395	0.78	4-Vinyl guaiacol
	40.765	**4.37**	Tetratriacontane
	41.428	0.94	Squalene
	46.920	**70.91**	Hexatriacontane

Peak areas in **bold** are for major constituents.

The DCM extract of the leaves showed presence of 9 compounds (**Table 6, Figure 16**) with Tetratriacontane (20.83%), Eicosane (15.89%) and Octacosanal (13.11%) being the dominant components. On the other hand, DCM extract of the tender stem*s* contained 5 phytoconstituents which included Eicosane (42.59%) and Hexatriacontane (28.70%) as the dominant components. The DCM extract of the root bark of *E. divinorum* produced a chromatogram with four prominent peaks which were identified to be 3,4-Methylenedioxybenzylacetone (76.01%), 9-Hexadecen-1-ol (13.65%), 2-Ethylhexyl acrylate (7.47%) and 2,6,11-Trimethyldodecane (2.87%).

Table 6: Compounds identified in dichloromethane extracts of *E. divinorum* by GC/MS

Sample	Retention time (minutes)	Peak area %	Constituents
Leaf extract	29.043	7.84	Octadecanal
	33.048	2.99	Cis, cis,cis-7,10,13-Hexadecatrienal
	34.895	1.38	Ethyl palmitate
	40.356	**20.83**	Tetratriacontane
	40.615	2.67	Tetradecyl acrylate
	41.415	5.86	Squalene
	44.488	**15.89**	Eicosane
	44.987	**13.11**	Octacosanal
	47.059	5.13	Heptacosanol
Tender stems extract	30.381	8.94	Tetratriacontane
	32.395	**28.70**	Hexatriacontane
	40.757	7.19	Tetratetracontane
	43.861	2.10	γ-Tocopherol
	46.618	**42.59**	Eicosane
Root bark extract	16.097	7.47	2-Ethylhexyl acrylate
	17.209	2.87	2,6,11-Trimethyldodecane
	31.286	**76.01**	3,4-Methylenedioxybenzylacetone
	40.608	**13.65**	9-Hexadecen-1-ol

Peak areas in **bold** are for major constituents.

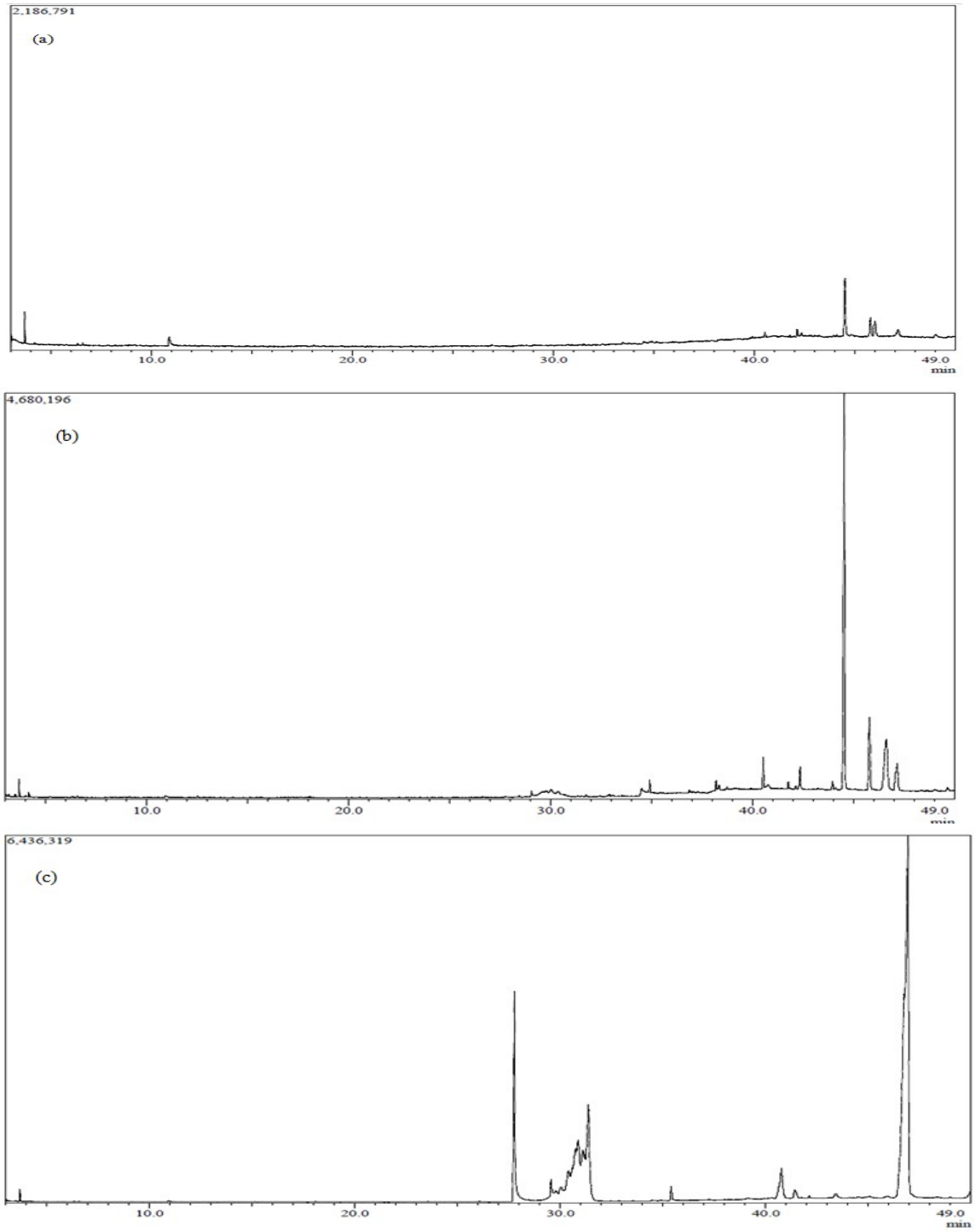


Figure 15: Gas chromatograph for hexane extracts of *E. divinorum*(a) leaves, (b) tender stems, and (c) root bark.

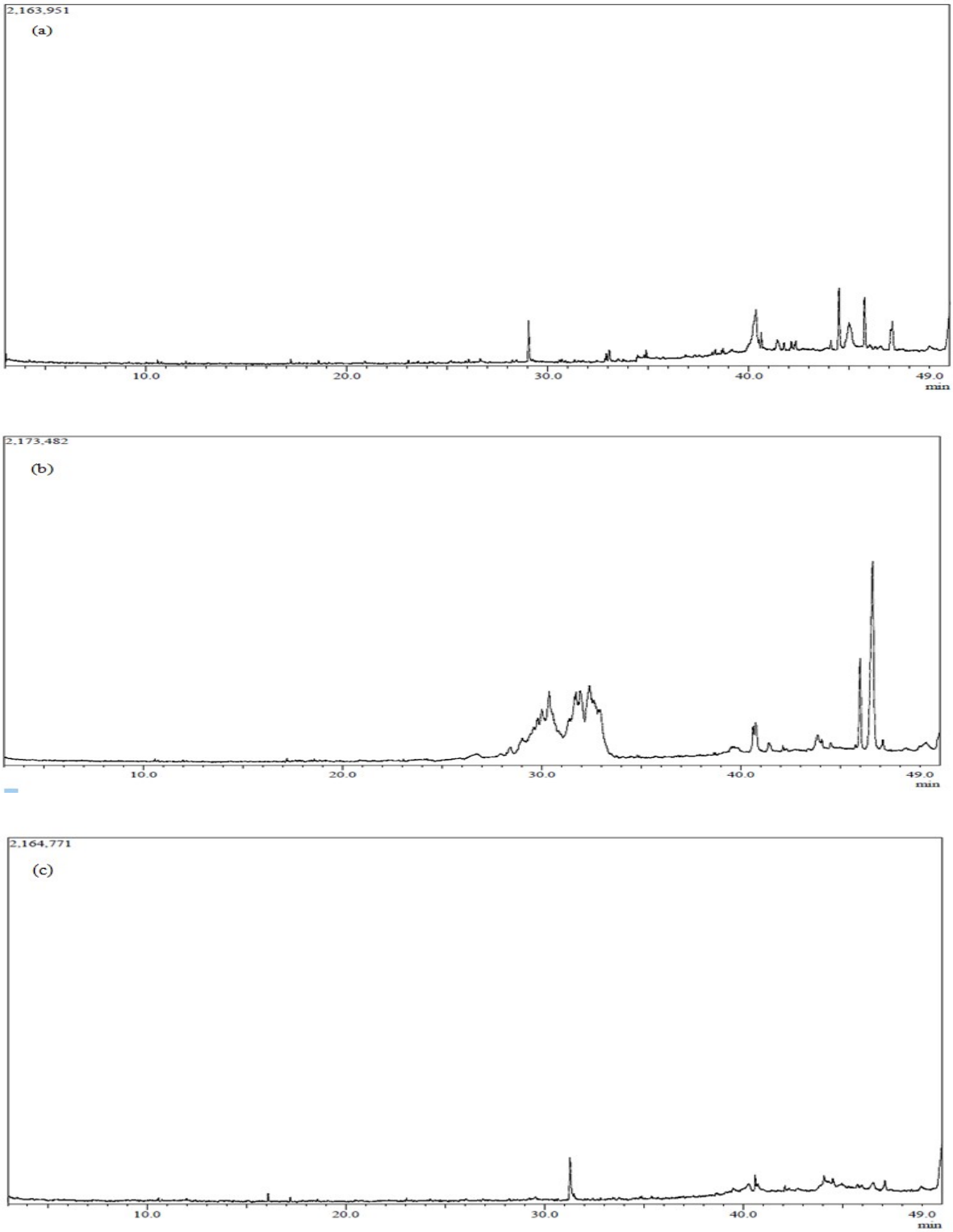


Figure 16: Gas chromatograph for DCM extracts of *E. divinorum* (a) leaves, (b) tender stems, and (c) root bark.

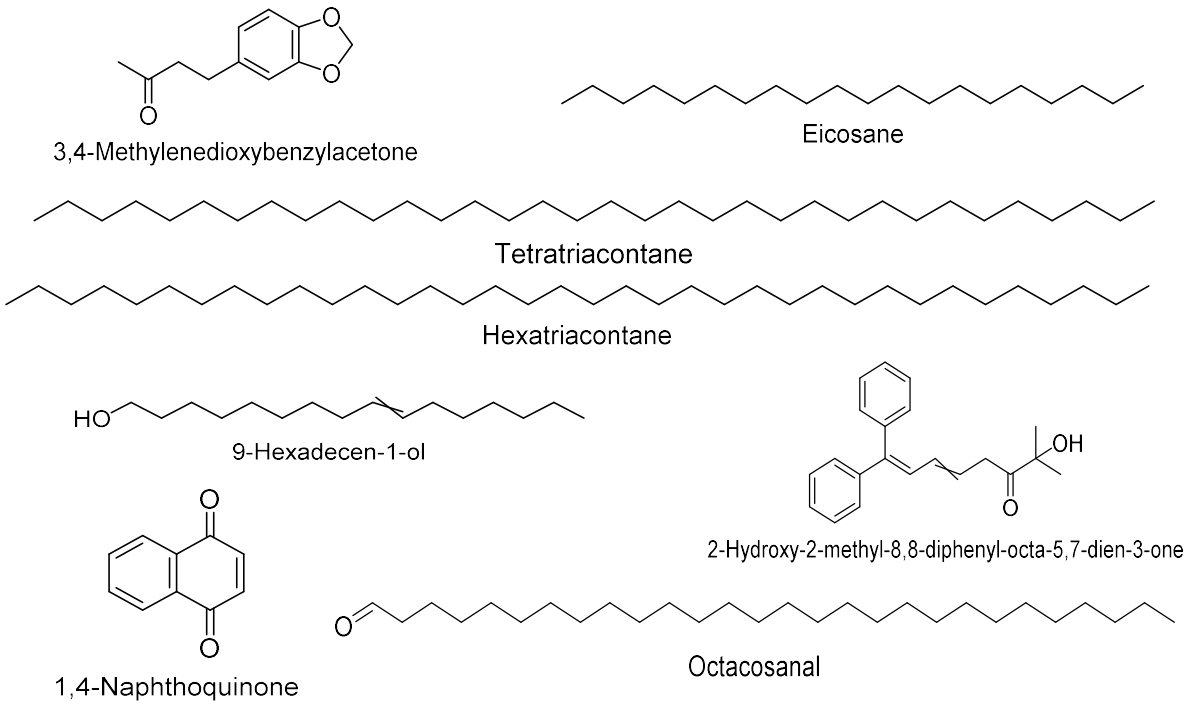


Figure 17: Structures of some of the major compounds identified in hexane and DCM extracts of *E. divinorum* leaves, tender stem and root bark.

A total of 30 compounds were identified in the hexane and DCM extracts of *E. divinorum*. The major compounds were 3,4-Methylenedioxybenzylacetone, Eicosane, Tetratriacontane, Hexatriacontane, 9-Hexadecen-1-ol, 2-Hydroxy-2-methyl-8,8-diphenyl-octa-5,7-dien-3-one, 1,4-Naphthoquinone and Octacosanal (**Figure 17**).Some of the compounds have reported medicinal properties. For example, naphthoquinones such as 1,4-naphthoquinonehave been reported to have cancer chemoprotective activity [48].Various natural and synthetic naphthoquinone analogues are precursors in the synthesis of natural products and pharmaceuticals, which exhibit antibacterial, antifungal, antiviral, antitumor, trypanocidal, antimalarial, antileismanicidal, molluscicidal and insecticidal activities[49]. Squalene is another bioactive compound with several pharmacological activities including anticancer, antibacterial, antifungal, antioxidant and cardioprotective properties[50, 51].Further, Palmitic acid and hydrocarbons such as Eicosane have been reported to down regulate pro-inflammatory cytokines which aid in wound healing [52]. Thus, the results of the current support the use of *E. divinorum* leaves, tender stems and root bark in the management of bacterial infections, cancer and venereal diseases.

Conclusion

Various secondary metabolites including flavonoids, alkaloids, tannins, phenols and saponins were found to be present during qualitative analysis of the extracts of *E. divinorum* leaves, tender stems and root bark. The presence of these phytochemicals was also supported by the spectroscopic studies showing characteristic peaks obtained in Infra-red, Ultraviolet and Visible regions. The presence of phenolics, alkenes, aldehydes, alcohols and aromatic compounds was also supported by the obtained FTIR spectra. The GC/MS results confirmed the

presence of compounds whose functional groups were detected during FTIR and UV-Visible spectrometry. The root bark extracts were found to contain the highest amounts of phenolics and flavonoids as compared to other parts of the plant extracted irrespective of the solvent used for extraction. The current study supports the use of the different parts of the plant studied in traditional herbal medicine. Further studies are required to isolate and test the bioactivity of pure compounds from this medicinal plant.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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