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Phytochemical screening, FT-IR and GC-MS analysis of *Euphorbia hirta*

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Abstract
The aim of present study is to identify the phytochemicals present in the plant *Euphorbia hirta* and to subject the plant extracts for FTIR and GCMS analysis. Preliminary phytochemical screening of the methanol extract of the plant showed the presence of flavonoids, alkaloids, saponins, tannins, proteins, carbohydrates, Quinone's, fats and oils. The FTIR spectroscopy of the chloroform, butanol and ethyl acetate extracts indicated the presence of OH, CH stretching saturated, C=O, C=C, NO2, C-N, Ar-O, C-O, R-O- C-Cl stretching respectively. Phytoconstituents in the methanol extract of *E. hirta* was studied using GC-MS analysis. Fifteen compounds were identified from the methanol extract. The main chemical constituent is glycol aldehyde dimer [Peak area: 41.22%; RT: 3.035; Mol. formula: C4H8O4].

Keywords: *Euphorbia hirta*, Methanol extract, GC-MS analysis, phytochemical screening

1. Introduction
The plant *Euphorbia hirta*, a small annual hairy plant of family Euphorbiaceae is popularly known by the name Dudhe Jhar in Nepal. The plant is erect or ascending herb growing up to the height of 50 cm, stem is slender and often reddish or purplish in color, covered with yellowish bristly hairs especially in younger parts. The plant bears small numerous clustered flowers on leaf nodes. From each leaf node there protrudes out a pair of opposite, elliptical, oblong or oblong-lanceolate leaf having serrated edge. Fruits are yellow, three celled, hairy and keeled capsules having the diameter of 1-2 mm that contain three brown, four sided, angular wrinkled seed.
The plant is distributed throughout the hotter part of Nepal, India and most of the tropical and sub-tropical countries mostly grow in open grasslands.
Euphorbia hirta is a well-known herb amongst the users of conventional medicines. It is used as the folk medicine against several skin disease, wounds, warts, gonorrhea, migraines and intestinal parasites throughout the world. The plant is popularly known as asthma plant because it provides a good cure for asthma disease and other respiratory problems [1]. The plant is also widely used against diarrhea, and dysentery. There are many testimonies that prove the effectiveness of *E. hirta* against dengue too [2]. Traditionally it is also used in the treatment of kidney stone, diabetes [3], and in conjunctivitis. It also exhibit anxiolytic and sedative [4], analgesic, antipyretic, anti-inflammatory [5], antimicrobial [6], anti-allergy [7], anti-oxidant [8], anti-tumor [9], anthelminthic [10], anti-cancer [11] and diuretic activities [12]. The current study was purposed to determine the bioactive compounds from the methanol extract of *E. hirta* plant, evaluate the pharmalogical potential and characterize them by GC-MS chromatographic technique.

2. Material and methods
2.1 Glassware and chemicals
All the glassware and chemicals are used during the test are of analytical grades. They were washed with good detergent, ringed in tap water and soaked in chromic acid clearing solution.

2.2 Collection of plant materials
The whole plant of *E. hirta* was collected from the field of chautara sangachokgadhi municipality-12 sindhupalchok Nepal in the month of June, 2016. The plant was washed with pure water and was completely dried under the shade for 15 days. Then the plant was crushed into fine powder and stored in sealed container in cold and dry place.

2.3 Preparation of plant extract
The extraction of whole plant was performed by cold percolation method with two different solvents viz. hexane and ethanol. During the process 200 g of plant powder was weighed and kept into two separating funnel 100g in each and 400 mL hexane poured into each of the funnel and soaked for 72 hours and filtered. The filtrate thus obtain was subjected to distillation. Now in each funnel containing residue, 400 ml of methanol was poured and soaked for 1 week and filtered. The solvents thus obtained were distilled off by distillation process at 60 ºC temperature. Two samples of semisolid mass was obtained by this process, hexane extract and methanol extract from solvent hexane and methanol respectively. Again chloroform, ethyl acetate and 2-butanol fraction were obtained by applying methanol extract with respective organic solvents followed by distillation process. The methanolic and hexane extracts thus obtained was subjected to phytochemical screening. Chloroform, n-butanol, ethyl acetate fraction of methanol extract were subjected to FTIR analysis and for GC-MS analysis methanol extract was used.

2.4 Phytochemical screening
Phytochemical analysis of the methanol and hexane extracts were undertaken using standard methods as described by Edeoga [13], Trease and Evans [14], Harborne [15], Daniel [16] and Prasthith [17].

2.4.1 Test for alkaloids (Dragendroff's test)
In 1 ml of extracts solution, few drops of Dragendroff's reagent was added and the color developed was noticed. Appearance of orange color indicates the presence of Alkaloids.

2.4.2 Test for terpenoids
In a test tube containing 1 ml of extracts, a few drops of thionyl chloride were added. Appearance of pink color indicates the presence of terpenoids.

2.4.3 Test for Coumarins
1 ml extract and 1 ml 10% sodium hydroxide was added. Formation of yellow color indicates the presence of Coumarins.

2.4.4 Test for tannins
To the few mg of powder, 10% alcoholic ferric chloride was added; formation of dark blue or greenish black color shows the presence of Tannins.

2.4.5 Test for flavonoids
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid indicates the presence of flavonoids.

2.4.6 Test for phenols
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

2.4.7 Test for volatile oils
To 2 ml of extracts, 0.5 ml of dilute NaOH and small amount of dilute HCl acid were added and the formation of white precipitates indicates volatile oils.

2.4.8 Test for Quinones
To 1ml of extract 2 drops of concentrated hydrochloric acid was added. Formation of red color indicates the presence of Quinones.

2.4.9 Test for sugars
To 1 ml of extract, Fehling's solution was added. Appearance of red color indicates the presence of sugar.

2.4.10 Test for carbohydrates
a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α–naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's Test: filtrates were treated with Benedict's reagent and heated gently. Orange red colored precipitate indicates the presence of reducing sugars.

c) Fehling's test: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitates indicates the presence of reducing sugar.

2.4.11 Detection of glucosides
Extracts were hydrolyzed with dil. HCl, and then treated with Ferric chloride solution and immersed in a boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose- pink color in the ammonical layer indicates the presence of anthranol glycosides.

2.4.12 Detection of saponins
a) Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minute. Formation of 1 cm of foam indicates the presence of saponins.

b) Foam Test: 0.5 g of extracts was shaken with 2 ml of water. If foam persists for ten minutes it indicates the presence of saponins.

2.4.13 Test for fixed oil (spot test)
A small quantity of extracts was pressed between two filter papers. Formation of grease spot indicates the presence of fixed oils and fats.

2.5 Fourier Transform Infrared Spectrophotometer (FTIR) analysis
The powdered sample of chloroform fraction, butanol fraction and ethyl acetate fraction of methanol extract of *E. hirta* were loaded in FTIR spectroscope (SHIMAZDU, FTIR spectrometer, model: IR Prestige-21), with a scan range 5,000-400 cm-1.

2.6 GC-MS analysis
GC-MS analysis of methanol extract of *Euphorbia hirta* was carried out on instrument GCMS-QP 2010 Ultra, equipped with a capillary column Rtx-5MS (30m×0.25mm×0.25µm). The instrument was operated in the EI mode (70 eV). Helium was used as the carrier gas. 1 µl of the methanol extract of whole plant was injected into GC with split less injection mode. The column head pressure was programmed to 68.3kPa. Column temperature maintained at 250,280 and 300 ºC with a hold time 1.00, 2.00 and 10.00 min. respectively.

The GC-Ms interface was programmed at 280 ºC. In the full scan mode, electron ionization mass spectra in the range 30-600 (m/z) were recorded. The start –end time was 3.00-25.00 minute. The identification of the compounds were done by comparing mass spectra with NIST library, USA/Wiley.

3. Results and Discussion

The extractive values for methanol and hexane extract of *E. hirta* are 8.7% and 2.0% respectively. The percentage yield of chloroform, ethyl acetate and butanol extracts obtained from 5.434 g of methanol extract are 7.45%, 3.95%, and 7.64% respectively.

The result of phytochemical screening of crude hexane and methanol extracts are listed in Table 1.

Table 1: phytochemical screening of methanol and hexane extract of *E. hirta*

Phytochemical constituents	Methanol extract	Hexane extract
Alkaloids	+	-
Flavonoids	+	-
Carbohydrates	+	-
Steroids	+	-
Tannins	+	+
Saponins	+	-
Proteins and amino acids	-	-
Caumarin	-	-
Quinone	+	+
Glycosides	-	-
Phenols	+	-
Fats and oils	+	-

(+) present and (-) absent

The present investigation of phytochemical analysis of methanol and hexane extract of powdered *Euphorbia hirta* indicated the presence of lot of phytochemicals as mentioned in the table 1. The literature confirmed the therapeutic application of this plant is due to these compounds.

Flavonoids found in plants are for large number of biological actions and pharmacological effect such as anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, immune stimulating effects. Some reports have also mentioned that the anti-malarial activity of *E. hirta* is due to the presence of flavonoids [18]. Saponins detected during analysis are reported to have cytotoxic [19], anti-ulser activity [20] as well as possess sweetness and bitterness, foaming and emulsifying properties suggesting the action of saponins as a chemical barrier against potential pathogens in plants. Alkaloids are responsible for anti-microbial [21] and anti-tumor activity [22].

Tannins in plants are found to possess spasmolytic activity, free radical scavenger and anti-oxidant properties. Plants rich on phenolic content might be used as a good anti-oxidant [23], anti-tumor [24] reagent.

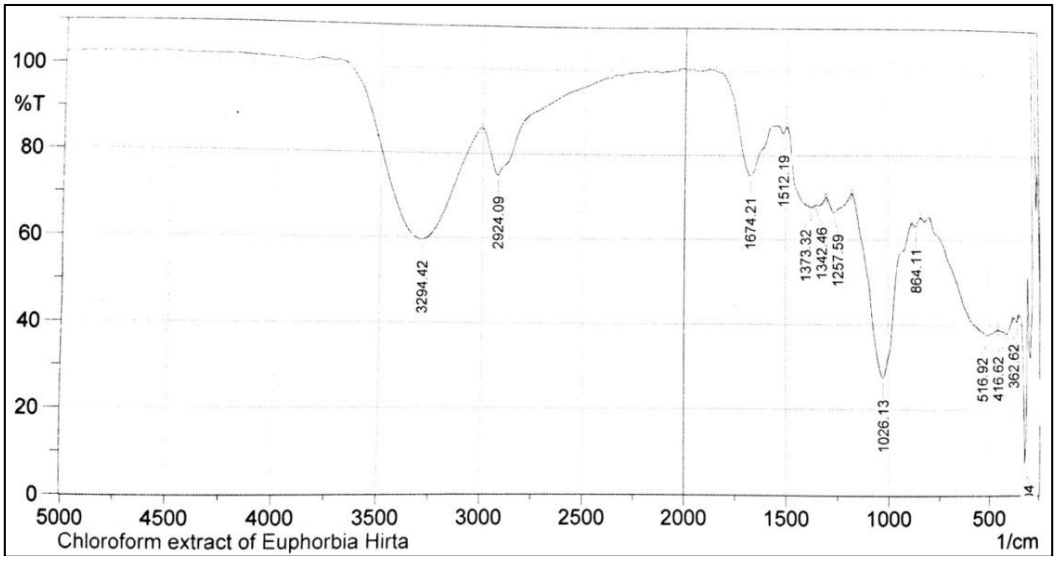

Fig 1: FTIR fingerprints of chloroform extract of *Euphorbia hirta*

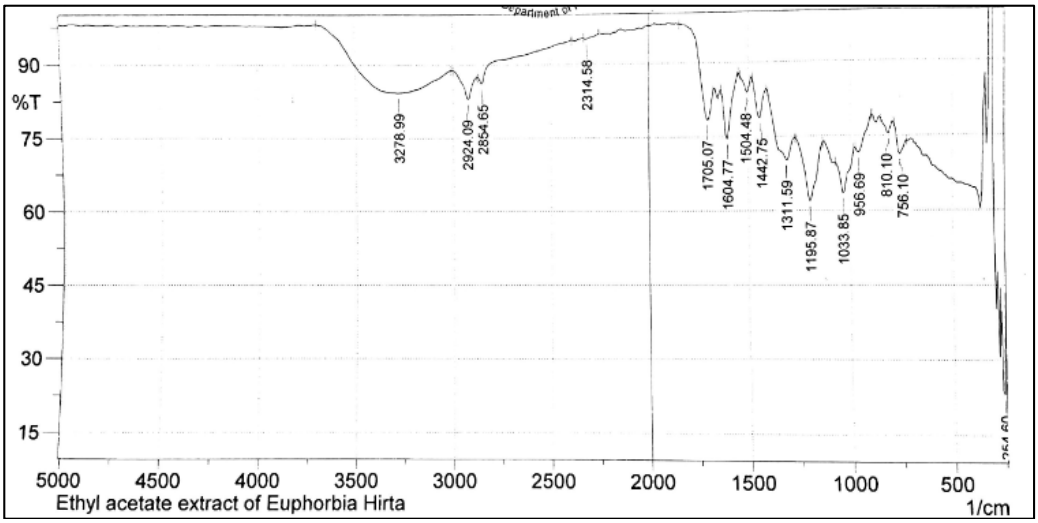

Fig 2: FTIR fingerprints of ethyl acetate extract of *Euphorbia hirta*

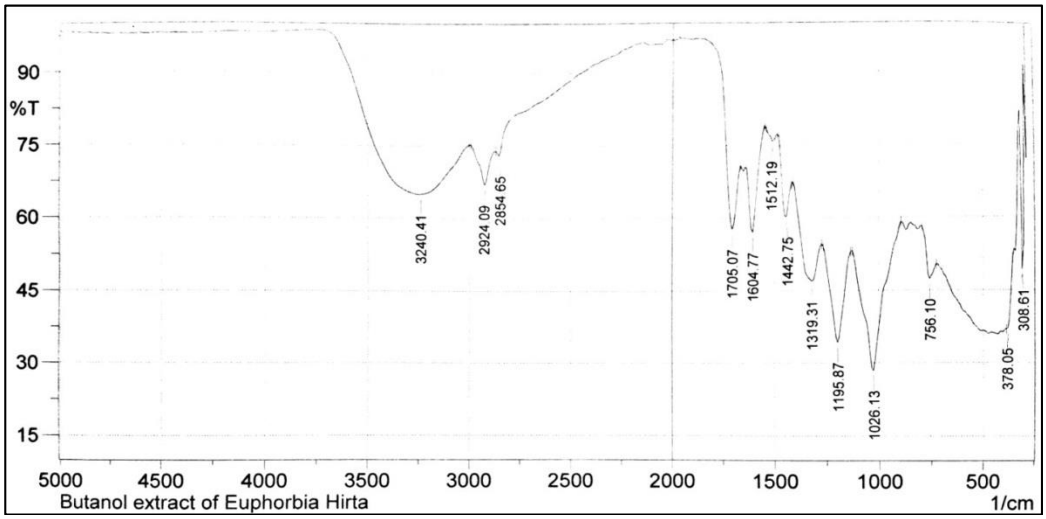

Fig 3: FTIR fingerprints of butanol extract of *Euphorbia hirta*

The results of fingerprint for the chloroform, ethyl acetate and n-butanol fraction of methanol extract confirmed the existence of various functional groups as listed in table 2.

Table 2: FTIR spectroscopic data of the chloroform, ethyl acetate and n-butanol fraction of methanol extract of *E. hirta*

S.N	Frequency range cm^{-1}			Type and groups
	Chloroform	Ethyl acetate	n-butanol	
1	3294.42	3278.99	3240.41	-OH
2	2924.04	2924.04	2924.04	C-H
3	-	2854.65	2854.65	C-H
4	-	1705.07	1705.07	C=O
5	1674.21	1604.77	1604.77	C=C
6	1512.19	1504.48	1512.19	NO_2
7	-	1442.75	1442.75	Aromatic multiple band
8	1373.32, 1342.46	1311.59	1319.39	C-N
9	1257.59	-	-	Ar-O
10	-	1195.87	1195.87	C-O-
11	1026.13	1033.85	1026.13	R-O-
12	-	956.69	-	C-O-
13	864.11	810.10	-	C-H
14	-	756.10	756.10	C-Cl
15	416.62	-	-	S-S

The IR gives the broad peaks at 3294.42 cm^{-1}, 3278.99 cm^{-1} and 3240.41 cm^{-1} which indicates the presence of OH stretching. Peaks at 2924.09 cm^{-1}, and 2854.65 cm^{-1}corresponds to the saturated C-H stretching. The peak obtained at 1705.07 cm^{-1} indicated C=O functional group whereas peaks at 1674.21 cm^{-1}, 1604.77 $cm^{-1}$are due to C=C aromatic system. The presence of NO2 group was confirmed by 1512.21 cm^{-1}, 1504.48 cm^{-1} and peaks at 1442.75 cm^{-1} revealed the aromatic multiple band. Similarly the IR peaks at 1373.32 cm^{-1}, 1342.46 cm^{-1}, 1311.59 cm^{-1} and 1319.39 cm^{-1}

showed the C-N functional group. More ever peak at 125759 refers to Ar-O stretching whereas peaks at 1195.87 cm^{-1} and 956.69 cm^{-1} hinted the presence of C-O. The peaks at 1026.13 cm^{-1}, 1033.85 cm^{-1} clued the R-O functional group.
Furthermore Peaks at 864.11 cm^{-1} and 810.10 cm^{-1} clearly showed the C-H stretching and peaks of 756.10 cm^{-1} is for alkyl chloride functional group. The peaks at 416.62 cm^{-1} may be due to aryl disulphide stretching [25, 26].
The chromatogram of the GC-MS analysis of methanol extract of *E. hirta* is given in figure 4, which clearly showed the presence of fifteen major phytochemicals at different retention time. Table 3 shows phytochemical constituents of *E. hirta* in methanol extract.

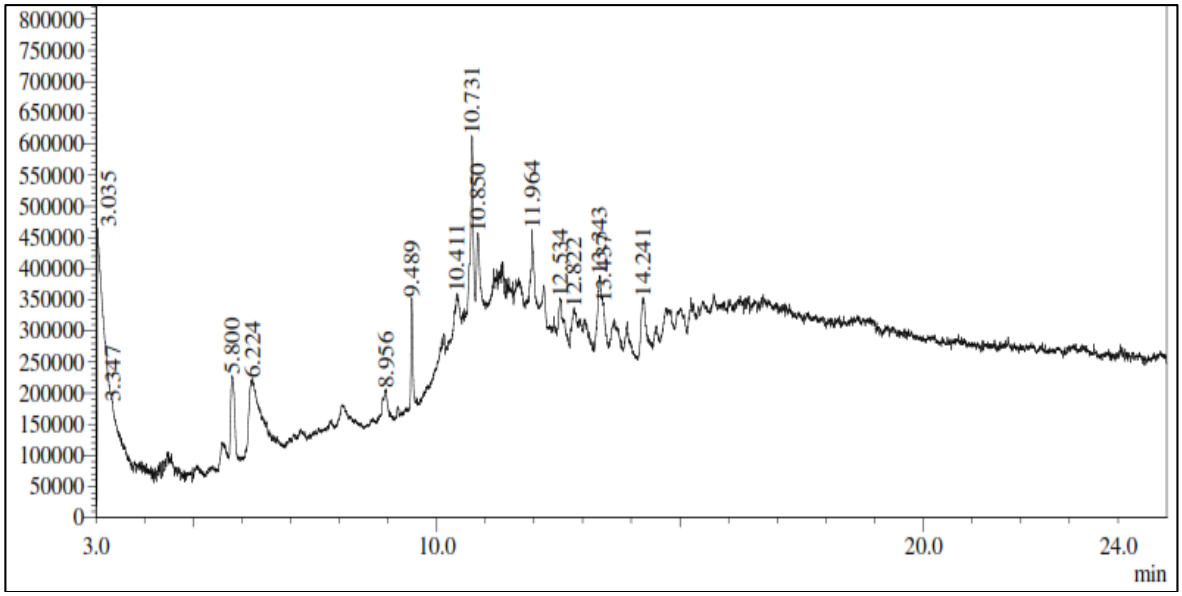

Fig. 4: GC-MS chromatogram of methanol extract of *Euphorbia hirta*

Identification of compounds is based on the retention time (RT), Molecular formula; molecular weight and peak area in percentage are present in table 3. The first compound with less retention time (3.035 min.) was Glucoldehyde dimer whereas 13-Octadecenal, (Z)- was the last compound which took longest retention time (14.241 min.) to identify. The result revealed that, Glucoldehyde dimer (41.22%) was found as major component followed by 11,14,17-Eichosatrionic acid, methyl ester (10.92%). The GC-MS analysis of methanol extract of E. hirta confirmed the presence of palmmitic acid, aldehyde compound, diterpenes, fatty acid ester compound and ester compound. These identified compounds may be responsible for the versatility of action against several infectious diseases (Table 4).

Table 3: GC-MS showed phytochemical compounds in methanol extract of *Euphorbia hirta*

P.N	RT	Area %	Name of compound	Base m/z	Fragment peaks (m/z) (% occurrence)	Matching peaks (m/z) with original chromatogram
1	3.035	41.22	Glycoldehyde dimer	31.05	13(5%), 31(100%), 42(7%), 60(8%), 73(2%), 85(3%), 103(1%)	73
2	3.347	3.27	1,3-Dibora-2,4,5-triselenole, 1,3-dipropyl-	302.00	27(70%), 41(97%), 53(25%), 106(23%), 166(20%), 170(70%), 186(20%), 209(25%), 214(65%), 262(40%), 302(100%), 308(30%), 346(60%)	166
3	5.800	6.97	Phosphoric acid, bis(trimethylsilyl)monomethyl ester	240.95	15(10%), 28(4%), 45(12%), 59(10%), 73(40%), 89(8%), 98(8%), 119(3%), 133(12%), 147(4%), 167(2%), 181(2%), 195(5%), 211(10%), 241(100%), 256(10%)	45, 167, 195, 241, 256
4	6.224	3.70	1,2,3- Benzenetriol	126.00	26(2%), 39(10%), 52(40%), 63(3%), 80(35%), 97(13%), 108(25%), 125(3%), 126(100%)	39, 52, 63, 80, 97, 108, 126
5	8.956	2.37	2,3-bis (1-methylallyl)pyrriolidine	124.05	39(15%), 55(15%), 67(4%), 82(3%), 97(15%), 124(100%), 150(2%), 162(3%)	124, 150
6	9.489	4.26	Hexadecanoic acid, Methylester	74.00	27(10%), 41(30%), 57(35%), 74(100%), 87(7%), 101(8%), 115(3%), 129(8%), 143(20%), 157(2%), 171(5%), 185(5%), 199(5%), 213(3%), 227(10%), 239(7%), 270(8%)	41, 57, 74, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239
7	10.411	2.05	3,4-Epoxycyclo hexylmethyl 3,4-epoxycyclohexane carboxylate	323.20	27(35%), 41(100%), 55(60%), 67(75%), 81(97%), 93(50%), 110(25%), 125(10%), 127(40%), 141(7%)	41, 55, 67, 81, 127
8	10.731	10.92	11,14,17-Eicosatrienoic acid, methyl ester	79.05	39(15%), 41(75%), 55(60%), 67(61%), 79(100%), 95(55%), 108(40%), 121(18%), 135(12%), 149(5%)	41, 55, 67, 79, 95, 108, 121, 135, 149
9	10.850	4.78	phytol	71.05	27(10%), 41(40%), 57(40%), 71(100%), 95(20%), 111(10%), 123(20%), 140(3%), 196(3%), 278(3%), 296(3%)	41, 57, 71, 111, 123
10	11.964	4.34	Oleic acid, 3-	323.25	27(22%), 41(53%), 43(92%), 57(100%), 71(76%),	41, 113, 137

			(octadecyloxy)propyl ester		97(40%), 98(30%), 113(20%), 137(10%), 151(10%), 264(20%), 281(12%), 322(20%)	
11	12.534	2.01	9- Tetradecenal, (Z)-	323.20	27(15%), 41(60%), 55(100%), 67(43%), 81(40%), 95(30%), 121(15%), 135(8%), 149(3%), 192(3%)	41, 55, 67, 81, 95, 135
12	12.822	1.89	13-Oxabicyclo[10,1,0]tridecane	323.20	27(30%), 41(80%), 55(100%), 67(67%), 82(52%), 96(30%), 111(21%), 135(5%)	41, 55, 67, 135
13	13.343	5.83	9-Octadecenoic acid, 1,2,3-propanetriyl ester	323.20	27(15%), 41(50%), 55(100%), 69(72%), 83(63%), 97(57%), 123(15%), 137(15%), 151(10%), 264(20%), 339(10%), 393(10%)	41, 55, 151
14	13.437	2.05	2-Pentyl-cyclohexane-1,4-diol	41.05	27(48%), 41(100%), 55(95%), 69(80%), 83(43%), 97(57%), 111(60%), 135(5%), 150(22%), 168(18%)	41, 83
15	14.241	4.35	13-Octadecenal,(Z)-	323.20	27(15%), 41(55%), 55(100%), 69(45%), 81(35%), 95(30%), 121(15%), 135(8%), 149(4%), 248(3%)	41, 55, 69, 81, 95, 135, 149
		100				

GC-MS identified compound 1, 2, 3-benzenetriol is an aromatic alcohol and reported to have anticancer, antiseptic, antioxidant, antidermattic, fungicide and insecticide activity. This compound was reported in methanolic extract of fruit of *Terminalia chebula* [26].

Hexadecanoic acid, methyl ester may contribute as Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5- Alpha reductase inhibitor, Antialopecic agent. It has previously been reported in the leaves alcohol extract of *kigelia pinnata* [27], in the methanol extract of leaf of *Cassia italica* [28], P. Senthamil Selvan *et al*. [29] also found hexadecanoic acid, methyl ester as a key component of leaf of *Cissus vitiginea* along with 11 other compound by GC-MS analysis of methanol extract of the plant. Same compound has early been reported in the aqueous-methanolic extract of *Mundulea serica* [30], andin the ethanolic extract of the leaves of *Naravelia zeylanica* [31].

Phytol, a diterpene is one component of the plant, obserbed to have Antimicrobial, Anti-inflammatory, Anticancer, Diuretic and antiseptic activity [32]. The same compopund was previously reported in the ethanolic extract of the leaves of *Naravelia zeylanica* [33], in the ethanol extract of the whole plant of *Hedyotis leschenaultiana* [34]. Furthermore similar report was also observed in the leaves of Lantana camara [35] and in the leaves of *Mimosa pudica* [36].

Table 4: Biological activities of plant extract *E. hirta*

S. N	Name of compound	MW	Mol. Formula	Nature of compound	**Reported biological activity
1	Glycoldehyde dimer	120	C4H8O4	-	-
2	1,3-Dibora-2,4,5-triselenole, 1,3-dipropyl-	348	C6H14B2Se3	-	-
3	Phosphoric acid, bis(trimethylsilyl)monomethyl ester	256	C7H21O4PSi2	Ester	-
4	1,2,3- Benzenetriol	126	C6H6O3	Aromatic alcohol	Anticancer(Lung), Antiseptic, Antioxidant, Antidermattic, Fungicide, Insecticides
5	2,3-bis(1-methylallyl)pyrriolidine	179	C12H21N	-	-
6	Hexadecanoic acid, Methyl ester	270	C17H34O2	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5- Alpha reductase inhibitor, Antialopecic
7	3,4-Epoxycyclo hexylmethyl 3,4-epoxycyclohexane carboxylate	252	C14H20O4	-	-
8	11,14,17-Eicosatrienoic acid, methyl ester	320	C21H36O2	Unsaturated fatty acid ester	Antiarthritic, Anticoronary, Anti-inflammatory
9	phytol	296	C20H40O	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
10	Oleic acid, 3-(octadecyloxy)propyl ester	592	C39H76O3	Ester	-
11	9- Tetradecenal, (Z)-	210	C14H26O	Aldehyde compound	Sex pheromone
12	13-Oxabicyclo[10,1,0]tridecane	182	C12H22O	-	-
13	9-Octadecenoic acid, 1,2,3-propanetriyl ester	884	C57H104O6	Fatty acid	-
14	2-Pentyl-cyclohexane-1,4-diol	186	C11H22O2	Alcohol	-
15	13-Octadecenal,(Z)-	266	C18H34O	Aldehyde	Antimicrobial

**Source: Dr. Dukes Phytochemicals and Ethnobotanical Databases, Online source

11, 14, 17 -Eicosatrienoic acid, methyl ester the second major component of the plant may have Antimicrobial, Anti-inflammatory, Anticancer, Diuretic activity. Therefore the present study validates and strengthens the candidature of *Euphorbia hirta* plant as a curative of multiple diseases amidst the users of traditional medicine.

4. Conclusion

The result of the present study indicated the presence various secondary metabolites and further confirmed by FTIR and GC-MS spectrometry, which clearly depicted total 14 chemical constituents in methanolic extract of *Euphorbia hirta*. While studying the biological activity of GC-MS found compounds it can be concluded that the plant E. hirta may

serve as potent source of medicine due to the presence of these phytochemicals.

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