EVALUATION OF BIOACTIVE COMPOUNDS IN Euphorbia hirta Linn. LEAVES EXTRACT USING GAS CHROMATOGRAPHIC AND MASS SPECTROSCOPIC TECHNIQUES

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Abstract

GC-MS is the best sensitive technique used for separation and identification of the many structurally complex components that are present in plant extracts. The present study was aimed to find the bioactive compounds present in the ethanol, aqueous, methanol and hexane extracts of leaf of Euphorbia hirta Linn. by qualitative, quantitative photochemical screening, Gas chromatography and mass spectrometry analysis. Aqueous, ethanol and methanol extracts of Euphorbia hirta leaves showed the presence of tannin, saponin, steroids, terpenoids, flavonoids, triterpenoids, polyphenol, glycoside, anthocyanins and coumarins). Hexane extract of Euphorbia hirta leaves showed the presence of steroids, terpenoids, polyphenol and anthroquinone were present.On the basis of qualitative analysis, the rich content of phytochemicals present in ethanol extract as compared to other extract and used for subsequent studies. Significant amount of flavonoids, total phenol, terpenoids and saponin present in Euphorbia hirta leaves. The prevailing compounds are Diethyl Phthalate, Phthalic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, Gamma.-Sitosterol, Cholest-5-en-3-ol (3.beta.), beta.-Sitosterol, 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6 and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15-hexadecamethyl were found to be in this extract. Based on the results obtained in the present investigation, it may be concluded that the biological activities of the identified phytocomponents used for anti-microbial, anti-inflammatory, antioxidant, anti-diabetic, anti-ulcer, hepatoprotective, antiarthritic hyporcholesterolemic, and anti-cancer activities. Therefore, Euphorbia hirta leaves is recommended as a source of phytopharmaceutical value.

Keywords: Euphorbia hirta Linn, Qualitative and Quantitative Phytochemicals, GC-MS analysis, Bioactive compounds.

INTRODUCTION

Medicinal plants are known to be the main source of drug therapy in traditional medicine. It is an alternative to Western medicine and is strongly linked to religious beliefs and practices of indigenous cultures (Junaid and Nasreen, 2012). India is blessed with thousands of Ayurvedic and household formulations to treat various disorders, including anxiety, depression, arthritis, high blood pressure, hormonal imbalances, insomnia, migraines, skin problems, and other disorders. The medicinal property of a plant depends upon the physiologically active biochemical compounds called secondary metabolites. Plants have an almost limitless ability to synthesize secondary metabolites which present in the plant parts like leaves, fruits, buds, stem, flowers, bark, roots, etc. (Hussein and El-Anssary, 2018).

Many phytochemical compounds have been utilized by thousands of physicians in their practices and are consumed under medical management by tens of millions of people (Yuan et al., 2016). Crude plant extracts and medicines manufactured on the values of natural compounds even by pharmaceuticals companies may lead to large-scale exposure of humans to natural products (Ghosh et al., 2016). The major reason for continued use of herbal remedies is their usefulness, easy availability, low price, and moderately less or no toxic property (Alexandra et al., 2018. It can act on the body as powerful as pharmaceutical drugs, and it can start healing itself. As various phytochemicals with pharmacological activity have been isolated from several traditional Indian medicinal plants, it is pertinent to investigate the therapeutic effects of the traditional Indian medicinal plants (Kshetrimayum, 2017).

GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. It is the key tool used in sports anti-doping laboratories to test athlete's urine samples for prohibited performance enhancing drugs like anabolic steroids. Several GC-MS have left earth for the astro chemistry studies. As a unique and powerful technology the GC-MS provides a rare opportunity to perform the analysis of new compounds for characterization and identification of synthesized or derivatized compound (Mohammed et al., 2016). In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids (Sosa et al., 2016). Euphorbia hirta (Tamil : belonging to family Euphorbiaceae is a medicinal, rhizomatous herb distributed in southern Western Ghats of India and northern east coast of Tamil Nadu (Rahuman et al., 2008), Hence, the present study was aimed to find the phytochemicals / bioactive compounds present in the ethanol, aqueous, methanol and hexane extracts of leaf of Euphorbia hirta Linn. by qualitative, quantitative photochemical screening, Gas chromatography and mass spectrometry analysis.

MATERIALS AND METHODS

Qualitative phytochemical analysis

Collection of plant

The leaves of Euphorbia hirta were collected in September 2021 from Thanjavur, Tamil Nadu, India from a herb. The collected Euphorbia hirta leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered using mixer grinder.

Preparation of extract

10grams of Euphorbia hirta leaves powder were used for extraction. Extraction was performed with cold extraction using the maceration method into ethanol, aqueous, methanol and hexane solvent for 24 hours using the "intermittent shaking" method to obtain an extracts. The extracts were filtered using Whatman filter No 1 paper and filtrate was used for phytochemical analysis.

Qualitative Preliminary phytochemical analysis

Phytochemical tests were carried out different extracts (ethanol, aqueous, methanol and hexane) of Euphorbia hirta leaves using standard procedures to identify secondary metabolites in the following methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973). Total phenols estimated by the method of Edeoga et al., (2005). Saponin determined by the method of Obdoni and Ochuko (2001). Flavonoid determined by the method of Boham and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan et al., 2013). The mass spectrum was interpreted with the aid of the database and the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Results of the present study to examine the phytochemical analysis of aqueous, ethanol, methanol and hexane extract of Euphorbia hirta leaves. Aqueous, ethanol and methanol extracts of Euphorbia hirta leaves showed the presence of tannin, saponin, steroids, terpenoids, flavonoids, triterpenoids, polyphenol, glycoside, Anthocyanins and coumarins (Table 1). Hexane extract of Euphorbia hirta leaves showed the presence of steroids, terpenoids, polyphenol and anthroquinone were present.

Emodin was absent in all the extracts. On the basis of qualitative analysis, the rich content of phytochemicals present in ethanol extract as compared to other extract and used for subsequent studies.

Table 1: Phytochemicals analysis of different extracts of Euphorbia hirta leaves

S. No	Phytochemicals	Extracts			
		Aqueous	Ethanol	Hexane	Methanol
1	Tannin	+	++	-	++
2	Saponin	++	+	-	+
3	Flavonoids	++	++	-	++
4	Steroids	+	++	+	++
5	Terpenoids	+	++	+	+
6	Triterpenoids	+	++	+	+
7	Alkaloids	-	+	-	+
8	Anthroquinone	+	++	+	+
9	Polyphenol	++	++	+	++
10	Glycoside	+	++	-	+
11	Coumarins	++	+	-	++
12	Emodins	-	-	-	-
13	Anthocyanins	+	++	-	+

(-) Absent, (+) Present and (++) high concentrations

Table 2 and Figure 1 represent the quantitative analysis of phytochemicals in ethanolic extract of Euphorbia hirta leaves. Significant amount of flavonoids (73.41±5.13mg/gm), total phenol (198.65±13.90mg/gm), terpenoids (38.59±2.70mg/gm) and saponin (54.85±3.83mg/gm) present in Euphorbia hirta leaves.

Table 2: Quantitative analysis of phytochemicals in ethanolic extract of Euphorbia hirta leaves

S. No	Phytochemicals	Results (mg/gm)
1	**Flavonoids**	73.41±5.13
2	**Total phenol**	198.65±13.90
3	**Terpenoids**	38.59±2.70
4	**Saponin**	54.85±3.83

Values expressed as Mean ± SD for triplicate

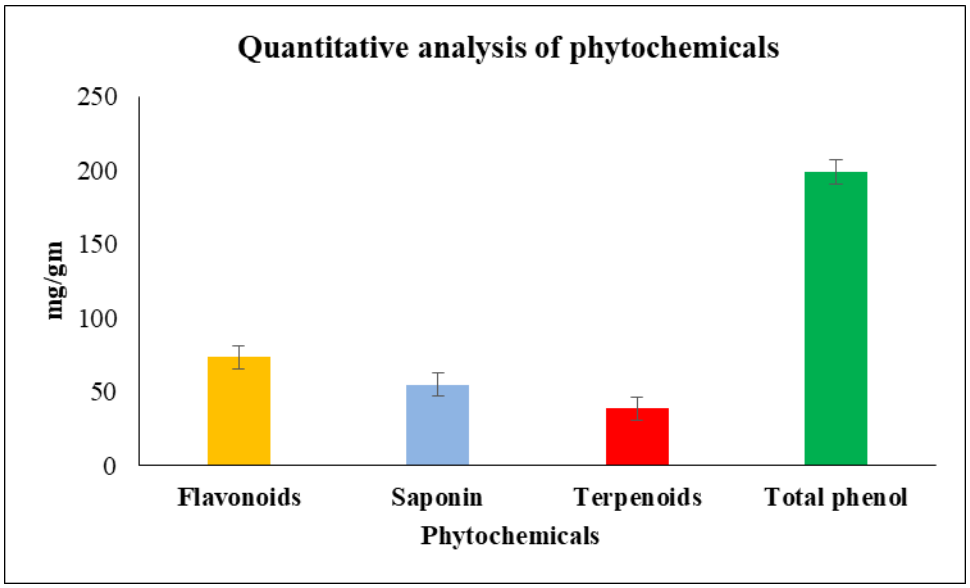

Figure 1: Quantitative analysis of phytochemicals

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid reduces blood cholesterol, increases bile secretion and lipid levels and antimicrobial activity against some strains of bacteria such as Staphylococcus aureus (Gryglewski et al., 1987). Phenolic acid possesses diverse biological activities, for instance, anti-inflammatory, antiulcer, antioxidant (Silva et al., 2007) cytotoxic, anti-spasmodic and anti-depressant activities (Ghasemzadeh et al., 2010).

Most recent researches have focused on the health aspects of flavonoids for humans. Flavonoids have gained recent attention because of their broad pharmacological and biological activities. Flavonoids have been reported to exert various biological property including cytotoxicity, coronary heart disease prevention, hepatoprotective, antimicrobial, antitumor as well as anti-inflammatory activities (Al-Huqail et al., 2019). The best-described property of flavonoids is in their capability to act as powerful antioxidants which might shield the form from free radicals and reactive element species. Flavonoids have been reported as enzyme inhibition, anti-inflammatory, oestrogenic, antimicrobial, anti-allergic, vascular activity, antioxidant and cytotoxic antitumor activity (Havsteen, 2002).

Tannin containing plant extracts are used as astringents, diuretics, against diarrhoea, duodenal and stomach tumours (De Bruyne et al., 1999) and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara et al., 2005). Recently, tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers (Blytt et al., 1988).

Alkaloids are significant in protecting and the survival of plant because they ensure their survival against insects, micro-organisms (antibacterial and antifungal activities) and herbivores (feeding deterrens) and also against other plants by means of allelopathically active chemicals (Molyneux et al., 1996). Alkaloids have many pharmacological activities including antimalarial activity (quinine), antiarrhythmic effect (quinidine, spareien), antihypertensive effects (many indole alkaloids) and anticancer actions (dimeric indoles, vincristine and vinblastine) (Wink et al., 1998). Some alkaloids have stimulant property as caffeine and morphine, nicotine used as the analgesic and quinine as the antimalarial drug (Rao et al., 1978).

Saponins may be considered as part of plants defence systems and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Lacaille-Dubois and Wagner, 2000). Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immune stimulant, hypo cholesterolaemic, anticarcinogenic hypoglycaemia and to act as antifungal and antiviral properties of saponins (Morrissey and Osbourn, 1999: Traore et al., 2000).

Among plant secondary metabolites, terpenoids are the structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses which involves herbivores and their natural enemies (McCaskill and Croteau, 1998). Basically, the terpenoids are known to greatly contribute to the therapeutic values such as: anti-

hyperglycemic activity, anti-inflammatory activity, anti-parasitic activity, enhancer of skin permeation for many drugs across cell membrane, anti-viral activity, anticancer activity and antimicrobial activities (Degenhardt et al., 2003). Terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. In addition, terpenoids can have medicinal properties such as anti-carcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (Dudareva et al., 2004).

Identification of bioactive compounds in ethanol extract of Euphorbia hirta leaves by GC MS analysis

GC-MS analysis is one of the first steps towards understanding the nature of active principles in medicinal plants and to decide whether the plant species has any individual compound or group of compounds. The spectrum profile of GC-MS confirmed the presence of main components with their retention time. The heights of the peak show the relative concentrations of the components present in the extracts. In comparison of the mass spectra of the constituent with the NIST library, the phytoconstituents were characterized and identified.

In the present study, thirty compounds were identified in extract of Euphorbia hirta leaves by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 3 and fig 2. The prevailing compounds are Diethyl Phthalate, Phthalic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, Gamma.-Sitosterol, Cholest-5-en-3-ol (3.beta.), beta.-Sitosterol, 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6 and Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15-hexadecamethyl were found to be in this extract. The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results.

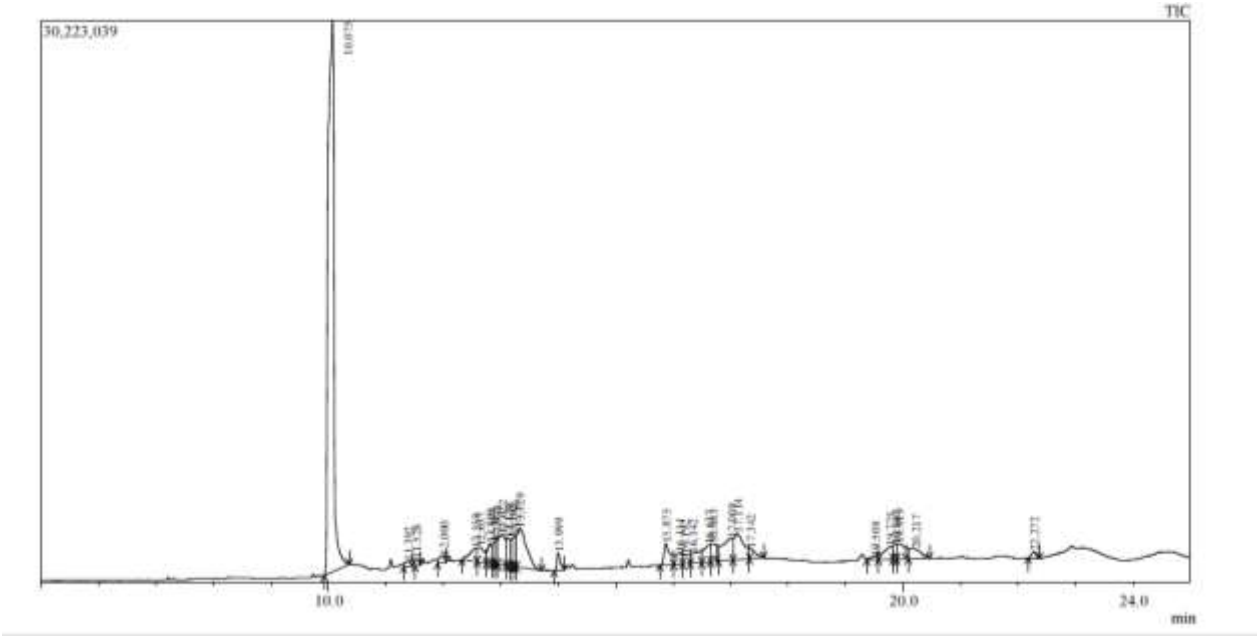

Fig. 2: Chromatogram of Euphorbia hirta leaves extract

Table 3: Identification of bioactive compounds in Euphorbia hirta leaves extract using GCMS

Peak	R. Time	Area %	Molecular Formula	Molecular Weight	Name of the compounds
1	10.075	52.33	$C_{12}H_{14}O_4$	222	Diethyl Phthalate
2	11.392	0.59	$C_{10}H_{10}O_4$	194	Phthalic acid
3	11.528	0.43	$C_{14}H_{16}O_6$	280	1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxo
4	12.000	0.77	$C_{21}H_{38}O_4Si$	382	Silane, diethyl(2,6-dimethoxyphenoxy) nonyloxy
5	12.558	1.60	$C_{21}H_{26}N_2O_3$	354	Yohimban-16-carboxylic acid, 17-hydroxy methyl ester
6	12.637	1.63	$C_{32}H_{62}O_4$	510	Hexanedioic acid, 2,3,4,5- tetrahexyl-, dimethyl ester
7	12.809	1.20	$C_{15}H_{20}O_4$	264	Phthalic acid, ethyl pentyl ester
8	12.883	0.96	$C_{10}H_{10}O_3$	178	Benzoic acid, 2-(1-oxopropyl)
9	12.933	0.96	$C_{25}H_{50}O_2Si$	410	cis-10-Nonadecenoic acid, tert-butyldimethyl
10	13.022	3.24	$C_{24}H_{35}NO$	353	5-Myristoyl-3-naphthamine
11	13.116	1.28	$C_{18}H_{36}$	252	5-Octadecene
12	13.192	1.47	$C_{13}H_9F_3N_2O_2$	282	Niflumic acid, trimethylsilyl ester
13	13.250	1.44	$C_{20}H_{13}F_3N_2O$	354	4,5-Dihydro-4-[4-[trifluoromethyl]phenyl]
14	13.329	5.93	$C_{20}H_{16}ClNO_3$	353	3-(p-Chlorophenyl)-5-(1-naphthyloxymethyl)
15	13.999	1.09	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid
16	15.875	2.04	$C_{16}H_{30}O$	238	9,12-Octadecadienoic acid
17	16.111	1.23	$C_{18}H_{34}O_2$	282	Ethyl 9-hexadecenoate
18	16.192	0.93	$C_{15}H_{24}O_2$	236	5,6-Azulenedimethanol, 1,2,3,3a,8,8a- hexahydro-2,2,8-trimethyl
19	16.342	1.63	$C_{21}H_{44}O$	312	1-Heneicosanol
20	16.617	1.76	$C_{29}H_{50}O$	414	Gamma.-Sitosterol
21	16.683	1.49	$C_{41}H_{72}O_2$	596	Cholest-5-en-3-ol (3.beta.)- tetradecanoate
22	17.008	3.70	$C_{29}H_{50}O$	414	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4 -vinylhexadecahydrocyclopenta[a]phenanthren-3-ol
23	17.114	4.27	$C_{29}H_{50}O$	414	Beta.-Sitosterol
24	17.342	0.86	$C_{20}H_{30}O_2$	302	Norethandrolone
25	19.508	0.47	$C_{12}H_{20}O_6$	260	Beta.-D-Allofuranose, 2,3:5,6-bis-O-(1-methy ethylidene)
26	19.775	1.87	$C_{30}H_{48}O$	424	4,4,6a,6b,8a,11,11,14b- l-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one
27	19.867	0.78	$C_{32}H_{52}O_2$	468	Urs-12-en-3-ol, acetate
28	19.919	1.74	$C_{18}H_{30}O_2$	278	5H-3,5a-Epoxynaphth[[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl
29	20.217	1.85	$C_{16}H_{26}$	218	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.
30	22.272	0.44	$C_{16}H_{50}O_7Si_8$	578	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15-hexadecamethyl

Table 4: Biological activity of compounds identified in Euphorbia hirta leaves extract using GCMS

Peak	R. Time	Name of the compounds	Biological activity**
1	10.075	Diethyl Phthalate	Antimicrobial and Antifouling activity Personal care products, plasticizers, cosmetics.
2	11.392	Phthalic acid	Arachidonic acid-Inhibitor
3	13.999	n-Hexadecanoic acid	**Antioxidant**, *Hypocholesterolemic* Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic,5-Alphareductase inhibitor
4	15.875	9,12-Octadecadienoic acid	Hypocholesterolemic,Nematicide Antiarthritic, **Hepatoprotective**, Antiandrogenic, Nematicide, 5-Alphareductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, **Anticancer**
5	16.617	Gamma.-Sitosterol	Growth hormone of animals and plants, anti-ulcer, antimicrobial, antipyretic and hypolipidemic agent
6	16.683	Cholest-5-en-3-ol (3.beta.)	Antimicrobial activity
7	17.114	Beta.-Sitosterol	A steroid precursor helps reducing LDL, help reduction of prostate hyperplasia, Used in Skin ointments
8	19.775	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6	Anti-bacterial, **antioxidant** and antitumor and **cancer preventive** activities
9	22.272	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15-hexadecamethyl	Antimicrobial activity

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

Table 4 revealed the biological activity of compounds identified in Euphorbia hirta leaves extract using GCMS. Diethyl phthalate act as antimicrobial and antifouling activity personal care products, plasticizers and cosmetics. Phthalic acid possesses Arachidonic acid-Inhibitor property. n-Hexadecanoic acid have antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic,5-alphareductase inhibitor. 9,12-Octadecadienoic acid act as hypocholesterolemic, nematicide antiarthritic, hepatoprotective, antiandrogenic, nematicide, 5-alphareductaseinhibitor, antihistaminic, anticoronary, insectifuge, antieczemic and anticancer activity. Gamma.-Sitosterol enchance the growth hormone of animals and plants, anti-ulcer, antimicrobial, antipyretic and hypolipidemic agent. Cholest-5-en-3-ol (3.beta.) has antimicrobial activity. Beta.-Sitosterol is a steroid precursor helps reducing LDL, help reduction of prostate hyperplasia, and also used in skin ointments. 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6 have anti-bacterial, antioxidant and antitumor and cancer preventive activities. Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15-hexadecamethyl act as antimicrobial activity (Velavan, 2015). The biological activity of the chemical compound collected from Dr. Duke's phytochemical and ethnobotanical online databases (https://phytochem.nal.usda.gov/phytochem/search).

Conclusion

Based on the results obtained in the present investigation, it may be concluded that the biological activities of the identified phytocomponents used for anti-microbial, anti-inflammatory, antioxidant, anti-diabetic, anti-ulcer, hepatoprotective, antiarthritic hypocholesterolemic, and anti-cancer activities. Therefore, Euphorbia hirta leaves is recommended as a source of phytopharmaceutical value

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