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The Isolation, Characterization and Pharmacological Acitvities of Various Chemical Compounds of Euphorbia Hirta

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Abstract

Euphorbia hirta L. is an annual medicinal herb throughout many tropical continents used to cure various diseases. Several studies have isolated many bioactive compounds from *E. hirta*. This study aimed at providing a collection of bioactive constituents in *E. hirta*. This review summarizes the extraction solvent, the structures and the properties of 38 bioactive phytochemicals isolated from *E. hirta*. It could help to understand the relationship existing between phytochemicals and their activities.

Keywords: *Euphorbia hirta* , Medicinal herb, Phytochemicals

Introduction

The largest genus of family Euphorbiaceae is Euphorbia with about 1600 species. It is characterized by the presence of white milky latex which is more or less toxic. Latices of *E. ingens, E. mey, E. tirucalli*, and *E. triangularis* are possible sources of rubber. This group of plants has been a subject of intense phytochemical examination and isolated compounds which include:- flavanoids, triterpenoids, alkanes, amino acids, and alkaloids. *E. ipecacuanha* is known as wild ipecac; *E. antiquorum* is known as *Tridhara; E. lathyrus* is known as caper spurge; and *E. thymifolia* is known as *Laghududhika*.

There are many other species of Euphorbia which are used in traditional medicines. All species of Euphorbia exudes a milky juice when broken, which is more or less poisonous and used as an ingredient in arrow poisons. *E. hirta* possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties.

E. hirta belongs to the plant family *Euphorbiaceae* and genus Euphorbia. It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to oblong- lanceolate, acute or subacute, dark green above, pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds.

Material and methods

Qualitative phytochemical analysis

Collection of plant

The leaves of Euphorbia hirta were collected in September 2022 from Churu, Rajasthan, 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. 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.

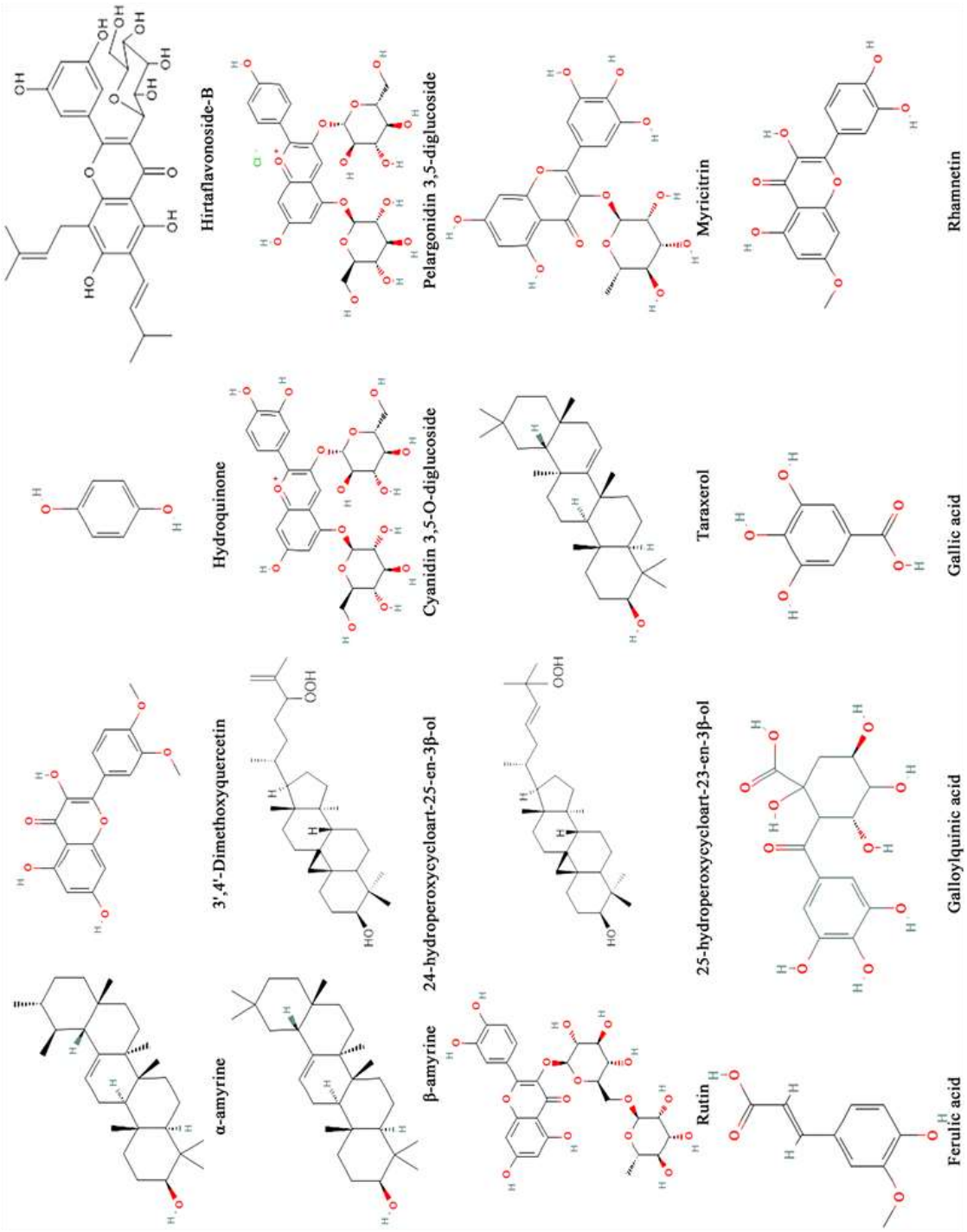
Biological Potential of Phytochemical

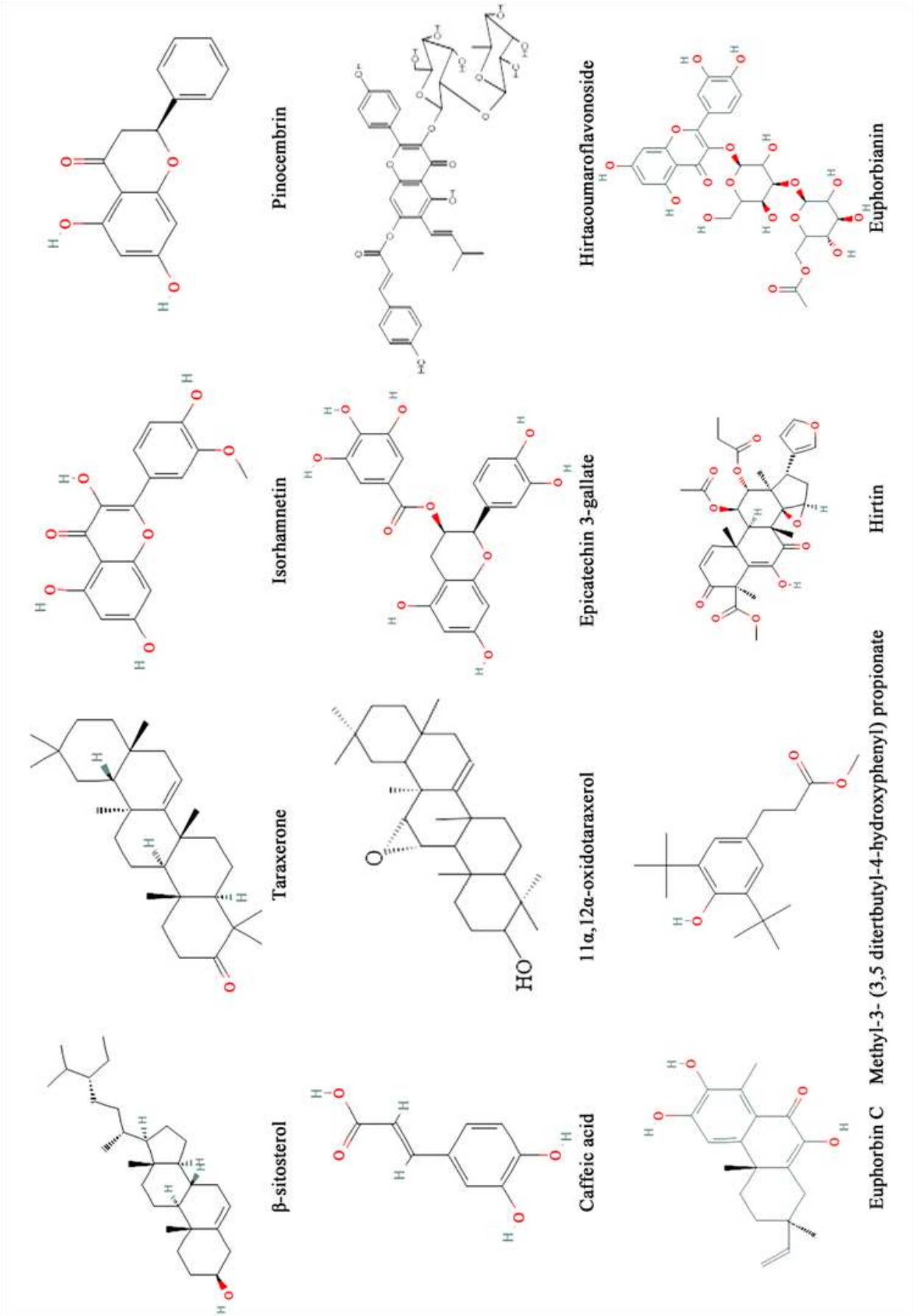
The bioactive compounds isolated from *E. hirta*, have demonstrated fourteen biological activities recorded.

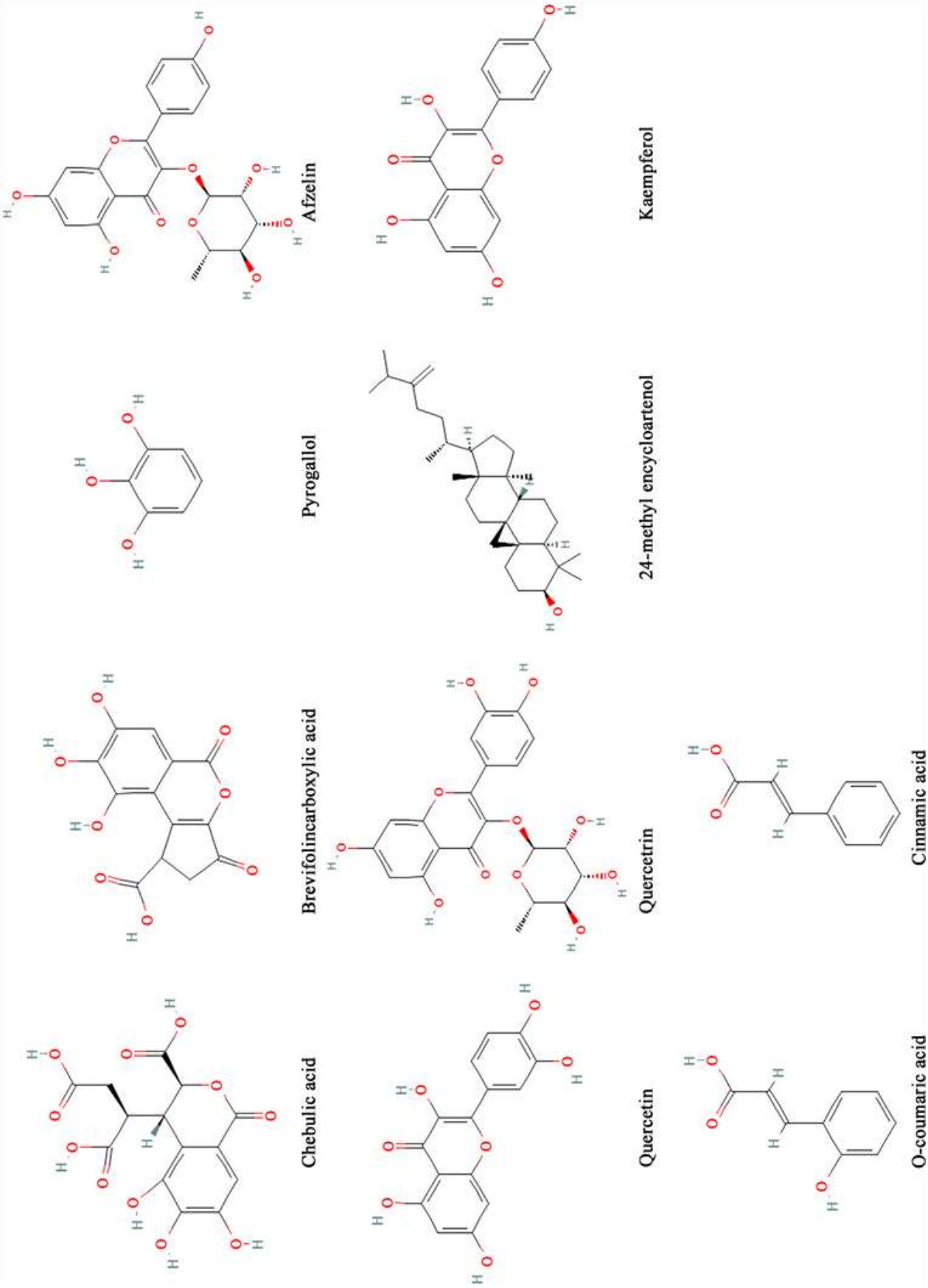
Class	Compounds	Parts used	Extraction solvents
Diphenol	Hydroquinone	Leaves	Ethyl acetate
Triphenol	Pyrogallol	Whole plant	Methanol
Phenolic acids	Galloylquinic acid	Leaves	
	Ferulic acid and Gallic acid	Aeral part	Methanol
	Caffeic acid	Aerial parts	Methanol
	O-coumaric acid	Leaves	Ethyl acetate
	Hydroxyl cinnamic acid	Leaves	Water
	Chebulic acids and Brevifolincarboxylic acid	Aerial parts	Ethanol extract
Flavonoids	Quercetin	Stems	80% hot methanol

		Whole plant	Acetone-water (7:3)
		Leaves	Hydroalcoholic
	Quercetrin	Aeral part	Methanol
		Whole plant	Methanol, ethanol
		whole plant	50% ethanol/methanol
	Afzelin and Myricitrin	Aerial parts	Methanol and 50% ethanol/methanol
	3',4'-Dimethoxyquercetin, Hirtacoumaroflavonoside and Hirtaflavonoside-B	Whole plant	Methanol
	Rutin	Whole plant	Ethanol
	Cyanidin 3,5-O-diglucoside and Pelargonidin 3,5-diglucoside		
	Rhamnetin	Aeral part	Methanol
	Pinocembrin and Isorhamnetin	Aerial part	85% ethanol
	kaempferol	Stems	80% hot methanol
	Epicatechin 3-gallate	Aerial parts	Methanol
	Euphorbianin		
Tannins	Euphorbin C		
Terpenoids	α-amyrine	Stems	CH_2Cl_2
	β-amyrine	Whole plant	Ethanol
		Aerial parts	n-Hexane
	Taraxerol	Stems	CH_2Cl_2, ethanol
	Taraxerone and 11α, 12α-oxidotaraxerol	Whole plant	Petroleum ether
	24-methyl encycloartenol and β-sitosterol	Aerial parts	n-hexane

	24-hydroperoxycycloart-25-en-3β-ol and 25-hydroperoxycycloart-23-en-3β-ol	Stems, roots and leaves	CH_2Cl_2
	Hirtin	Latex	
Hydroxyphenylcarboxylic acid esters	Methyl-3-(3,5ditertbutyl-4-hydroxyphenyl) propionate	Leaves	Methanol




Pinocembrin
Hirtacoumaroflavonoside
Euphorbianin
Isorhamnetin
Epicatechin 3-gallate
Hirtin
Taraxerone
11α,12α-oxidotaraxerol
Methyl-3- (3,5 ditertbutyl-4-hydroxyphenyl) propionate
β-sitosterol
Caffeic acid
Euphorbin C


Afzelin
Kaempferol
Pyrogallol
24-methyl encycloartenol
Brevifolincarboxylic acid
Quercetrin
Cinnamic acid
Chebulic acid
Quercetin
O-coumaric acid

Compounds	Activities	Mechanisms
Quercetrin, 3',4'-Dimethoxyquercetin, Hirtacoumaroflavonoside and Hirtaflavonoside-B	Anti-diabetes	Inhibition of α-glucosidase, regulation of postprandial hyperglycemia
Quercetrin	Anti-diabetes	Pancreatic β cells MIN6-protective effect
Quercetin, rutin, myricitrin, cyanidin 3,5-O-diglucoside, Pelargonidin 3,5-diglucoside, α-amyrine, β-amyrine, taraxerol	Anti-diabetes	High binding affinity to protein relating diabetes Type 2
Myricitrin	Anti-viral	Inhibition of Japanese encephalitis virus
Galloylquinic acid	Anti-viral	Effective against NS1, NS3 and envelope proteins domain III of ZIKA virus
Euphorbianin and rutin	Anti-viral	High binding affinity against protease M^{pro}, RNA-dependent RNA polymerase RdRp of SARS-CoV-2
β-amyrin	Anti-inflammatory	iNOS protein inhibition on the LPS-induced RAW 264.7 cells
Quercetrin, Ferulic acid, Gallic acid and Rhamnetin	Anti-inflammatory	Effective against turpentine-induced arthritis, formalin-induced experimental peritonitis and cotton pellet-induced granuloma models to the rats
β-amyrin and 24-methyl encycloartenol β-sitosterol	Anti-inflammatory	Inhibition effects on TPA-induced inflammation in ear to the mice
Afzelin, Quercetrin and Myricitrin	Anticancer	Cytotoxic against human epidermoid carcinoma KB 3-1 cells
25-hydroperoxycycloart-23-en-3β-ol and 24-hydroperoxycycloart-25-en-3β-ol	Anticancer	Cytotoxicity against a human cancer cell line, colon carcinoma (HCT 116) and non-small cell lung adenocarcinoma
Quercetin	Anticancer	Cytotoxicity against human breast adenocarcinoma MCF-7 cells
Afzelin, Quercetrin and Myricitrin	Antimalarial	Proliferation inhibition of *Plasmodium falciparum*
Isorhamnetin and Pinocembrin	Antimalarial	Multiple plasmepsin protease inhibition
Taraxerol	Antimicrobial	Against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

25-hydroperoxycycloart-23-en-3*β*-ol and 24-hydroperoxycycloart-25-en-3*β*-ol	Antimicrobial	Against *P. aeruginosa*, *S. aureus* and *Escherichia coli*, *Candida albicans* and *Trichophyton mentagrophytes*
quercetin and kaempferol	Antimicrobial	Against *E. coli*, *P. aeruginosa*, *Proteus mirabilis*, and *S. aureus Aspergillus flavus*, *Aspergillus niger*, *T. mentagrophytes*, and *C. albicans*
Taraxerone and 11*α*, 12*α*-oxidotaraxerol	Antimicrobial	Against *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *Sarcina lutea*, *S. aureus*, *E. coli*, *Shigella dysenteriae*, *S. sonnei*, *S. shiga*, *S. boydii*, *S. flexneriae*, *P. aeruginosa*, *Salmonella typhi*, *Klebsiella* sp. *Aspergillus flavus*, *A. niger*, *Penecillum* sp. *Trichoderma viride*, *C. albicans*, *Botryodiplodia theobromae*
Caffeic acid and Epicatechin 3-gallate	Antimicrobial	Cellular membrane destruction and ensuing membrane permeability perturbation of *P. aeruginosa*
Hydroquinone and O-coumaric acid	Antimicrobial	Against MRSA: *S. aureus* B39
Euphorbin C	Antimicrobial	Against *Helicobacter pylori*
β-Amyrin	Anti-atherosclerosis	Inhibition of atherosclerotic initiation induced by pro-inflammatory cytokines in SVEC4-10 endothelial cells
Quercetin	Antidiarrhoeic	Decrease both the total number of faeces and the number of diarrhoeic faeces induced in mice by castor oil
Quercetin	Anti-stress	Improvement in the swimming time, increases the time spent in open arm and decreases the time spent in the closed arm in mice
Taraxerol	Antiasthmatic	Inhibition of the contractile effect of histamine in guinea pigs
Hirtin	Anti-thrombotic disorders	Azocaseinolytic, gefibrinogenolytic, fibrinolytic and thrombin-like activities
Hydroxyl cinnamic acid derivatives	Antioxidant	Protection interaction with reference bovine serum albumin protein (BSA) against metal-catalyzed oxidation (MCO) system mediated oxidative damage
Methyl-3-(3,5- ditertbutyl-4-hydroxyphenyl) propionate	Antioxidant	DPPH radical scavenging activities *in vitro*

Twentynew chebulic acid and brevifolincarboxylic acid derivatives	Antioxidant	DPPH radical scavenging activities *in vitro*
Quercetrin	Anti-snake venom	Inhibition of protease, phospholipase-A2, hemolytic activity and hyaluronidase activities *in vitro*, inhibition *in vivo* of hemorrhage and edema induced in mice
Pyrogallol (1,2,3-Benzenetriol)	Anti-snake venom	Inhibition of protease activity *in vitro*
Rutin	Anti-hemorrhoid	Remarkable healing on croton oil-inducing hemorrhoid in Wistar Albino rats

Mechanisms of compounds isolated isolated from *E. hirta*. Quercetrin following by quercetin and *β*-amyrine were the most characterized biomolecules. They had properties such as anti-diabetes, anti-inflammatory, antimalarial, anticancer, anti-snake venom, antimicrobial, antidiarrhoeic and antistress. Otherwise, not identified lignans from ethanol extract of *E. hirta* has demonstrated an anticancer activity against cell lines Hep G2 with IC_{50} value of 7.2 ± 0.17 and 8.5 ± 0.36 µM. The peptide fractions from protein hydrolysate of *E. hirta* have Shown also a cytotoxicity against a gastric carcinoma cell line (KATO-III, ATCC No. HTB103) at 100 µg peptides ml^{-1}.

Pharmacological activities

Antibacterial activity

The ethanolic extract of *E. hirta* inhibited the growth of the *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtili* and aqueous and chloroform leaf extracts of *E. hirta* possess an antibacterial activity against *Klebsiella pneumonia*. The extract is noncytotoxic and antibacterial.

Antimalarial activity

The bioassay-guided fractionation of the methanolic extract of aerial parts of *E. hirta*, monitored against *P. falciparum parasites*, yielded a main active chromatographic fraction showing 90% growth inhibition of *P. falciparum* at a concentration of 5 µg/ml.

Anti-inflammatory activity

The *n*-hexane extract of aerial parts of *E. hirta* showed anti-inflammatory effects in the model of phorbol acetate-induced ear inflammation in mice. It exhibited a dose-dependent effect.

Galactogenic activity

The powdered *E. hirta* showed a galactogenic activity in guinea pigs before puberty by increasing the development of the mammary glands and induction of secretion.

Antiasthmatic activity

E. hirta is reported to have an antiasthmatic activity due to the relaxation effect on the bronchial tubes and a depressant action on respiration.

Effect on urine output and electrolytes

Ethanolic and aqueous leaf extracts of *E. hirta* significantly induced diuresis in rats. The diuretic effect of the ethanol extract was significant at 6 h (for 100 mg/kg) and at 24 h (for 50 mg/kg). The water extract induced a significant increase in urine Na^+, K^+ and HCO^{3-} loss. The ethanol extract (100 mg/ml) caused a

significant decrease in the K^+ loss whereas the water extract increased its excretion. The HCO^{3-} urine output following the injection of both extracts was tremendously enhanced.

Antidiarrheal activity

The antidiarrheal effect of the herb decoction was studied in mice. It demonstrated an activity in models of diarrhea induced by castor oil, arachidonic acid, and prostaglandin E_2. Quercitrin, a flavanoid glycoside isolated from *E. hirta*, showed an antidiarrheal activity, at a dose of 50 mg/kg, against castor oil and prostaglandin E_2-induced diarrhea in mice.

Antioxidant activity

The aqueous extract of *E. hirta* L. showed an antioxidant effect and a free radical scavenging activity in various *in vitro* models like total antioxidant and total ferric reducing power determination, assay for free radical-scavenging activity using ABTS, DPPH, and hydroxyl radical scavenging assays. It showed maximum antioxidants and free radical scavenging activities, at 0.25 mg/ml. The free radical scavenging effect on DPPH and hydroxyl was found as 68.80 ± 5.21 and 73.36 ± 5.21%, respectively.

Antifertility activity

E. hirta at a dose of 50 mg/kg reduced the sperm motility and density of cauda epididymal and testis sperm suspension significantly, leading to 100% infertility.

Antiamoebic activity

The polyphenolic extract of *E. hirta* inhibited the growth of *Entamoeba histolytica* with a minimum active concentration of less than 10 μg/ml.

Antifungal activity

An ethanolic extract of E. hirta showed an antifungal activity against plant pathogens *Colletotrichum capsici*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Phomopsis caricae-papayae*, and *Aspergillus niger* using the paper disc diffusion technique.

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. 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.

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. Phenolic acid possesses diverse biological activities, for instance, antiinflammatory, antiulcer, antioxidant cytotoxic, anti-spasmodic and anti-depressant activities.

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 antiinflammatory activities. 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.

Tannin containing plant extracts are used as astringents, diuretics, against diarrhoea, duodenal and stomach tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals. Recently, tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers.

Alkaloids are significant in protecting and the survival of plant because they ensure their survival against insects, microorganisms (antibacterial and antifungal activities) and herbivores (feeding deterrens) and also against other plants by means of allelopathically active chemicals. 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). Some alkaloids have stimulant property as caffeine and morphine, nicotine used as the analgesic and quinine as the antimalarial drug.

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. 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.

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. 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. 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.

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. 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.

Conclusion

In the present review, we have made an attempt to provide the mophological, phytochemical, ethnopharmacological, and pharmacological information on *E. hirta*, a herb used traditionally for medicinal purposes. The literature survey revealed that *E. hirta* contains afzelin, quercitrin, myricitrin, rutin, gallic acid, quercitin, euphorbin-A and ephorbin-B, euphorbin-C, euphorbin-D, β-amyrin, 24-methylenecycloartenol, β-sitosterol, heptacosane, n-nonacosane, shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose, and chtolphenolic acid.

This herb shows antibacterial, anti-inflammatory, antimalarial, galactogenic, antiasthmatic, antidiarrheal, anticancer, antioxidant, antiferlity, antiamoebic, and antifungal activities. Further research is going on to find out more activities in constituents of *E. hirta*.

There are many other traditional uses of *E. hirta* in Ayurveda which serves as the basis for further studies. This review will definitely help the researchers to know its different properties.

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