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Geographical Comparison of Phytoconstituents in *Euphorbia hirta*: A Pilot Study in Ethiopia and India

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Abstract

The present study is a comparison between two *Euphorbia hirta* specimens collected in Addis Ababa, Ethiopia, and Avadi, Chennai, India to analyze the geographical differences seen in the phytoconstituents. This comparative study deals with both plants' phytochemical screening, FTIR, and GC-MS analysis of Euphorbia hirta. Phytochemical screening of the aqueous, petroleum ether, acetone, methanolic, and ethanolic extracts of both the *Euphorbia hirta* was done and the results revealed the presence of terpenoids, phlorotannins, tannins, flavonoids, cardiac glycosides, saponins, steroids, alkaloids, quinones, and coumarins. On comparing the results of Eh-E and Eh-I, it is apparent that the ethanol extract of both samples showed the existence of maximum phytochemical compounds. The GC-MS analysis of an aqueous extract of *Euphorbia hirta* from both continents revealed the presence of 12 and 15 phytoconstituents respectively. The similar chemical components from both regions are phytol and decanoic acid, which were found responsible for the major bioactivity. The study suggests that *Euphorbia hirta* can be utilized as a potential source of pharmaceuticals and can be further studied in the extraction of particular bioactive compounds.

Keywords: Euphorbia hirta, Bioactivity, Phytochemicals, Medicinal plants, Phlorotannins

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Introduction

Plants have always been man's ally in life, providing him with food, fuel, and medicine since the dawn of civilization. As they have been throughout human history, plants continue to be a significant source of medicine [1, 2]. *Euphorbia hirta* commonly called an asthma plant is an annual hairy erect herb that grows in arable lands, waste areas, roadsides, pathways, and gardens. *Euphorbia hirta* belongs to the family *Euphorbiaceae* [3]. Though the plant is a native of India, *Euphorbia hirta* is widely cultivated throughout the tropics and subtropics [4]. *Euphorbia hirta* can be either red or purple depending upon the sunshine period. It has oppositely occurred simple leaves that are asymmetric, lanceolate, and acute at the apex. It has semi-erect stems, covered with purplish-brown hairs, and

discharges white latex when cut. The fruit is found in capsules that contain small, elongated, four-sided red seeds [5]. Plants synthesize diverse chemical substances, which are further classified based on their functional group, chemical class, and biosynthetic origin. These plant derivatives have been employed for disease prevention and detection [6]. Various organs of the plant, mainly the leaves and the roots of *Euphorbia hirta* havetraditionally been used asfolk medicine as concoctions, to treat a variety of skin problems, treat tooth pain, wounds, headache, rheumatism, improve breastfeeding, diarrhea, warts, dysentery colic, gonorrhea, migraines and helps treat pain during pregnancy [7]. *Euphorbia hirta* has potent biological activities including analgesic, anti-arthritic, anti-coronary, antipyretic, anti-inflammatory [8], antimicrobial [9], anti-allergy [10], antioxidant [11], anti-tumor [12], anthelminthic [13], anti-cancer [14], antiseptic, diuretic [15], sedative [16] and anxiolytic. Due to these therapeutic potentials, *Euphorbia hirta* gained attention and is widely used in the treatment of asthma, cancer, asthmatic bronchitis, kidney stone, diabetes [17], dengue [18], intestinal disorders [19], conjunctivitis-ulcerated cornea [20]. Earlier ethnopharmacology studies on *Euphorbia* report that Topical dosage forms like paste, poultice, cataplasm, rubefacient, or emollient are applied to the skin and the forehead for headache, fever, joint pain, muscle pain, stomach-ache, skin rashes, and itching while preparations for internal use such as infusion and decoction are indicated for symptoms related to bleeding of the skin, nose, oral cavities, and the gastrointestinal tract. Several ethnomedicinal studies have also reported that leaves are the most frequently used part because of their remarkable identity and accessibility in addition to the fact that most biosynthesis of therapeutically active constituents occurs in leaves [21]. Theprimary objective of this collaborative research is intended to compare the bioactive properties of *Euphorbia hirta* growing in two regions - Ethiopia and India, and explore the characteristic variance due to varied geographical locations. Since in both countries, awareness ofthis plant regarding their potent pharmaceutical properties are less known and under-utilized, this initiative was taken by the researchers of both countries to qualitatively study the presence of phytochemicals in all of the sample extracts of the collected specimen. Further investigation of the extracts with the highest phytochemical occurrence was done using FTIR analysis and GC-MS.

Materials and Methods

Collection of samples

The whole plant of *Euphorbia hirta* was collected from two locations on different continents. **Figure 1** shows the pictorial representation of plant species. One from the outer skirts of the villages around 50 km from Addis Ababa (8.9806° N, 38.7578° E, 2,355 m a.s.l), the capital city of Ethiopia, and meanwhile other from the region of Avadi (13.1067° N, 80.0970° E, 10 m a.s.l), Chennai, Tamil Nadu, India. The samples from Ethiopia and India were designated as Eh-E and Eh-I respectively. The samples were identified using earlier reports [22, 23].

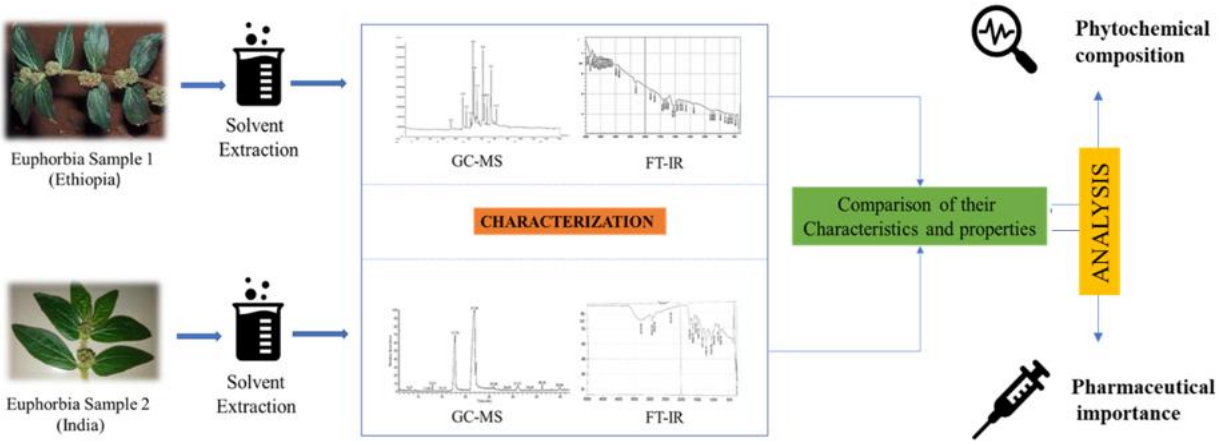


Figure 1. Comparative study on phytochemical, FT-IR, and GC-MS analysis of *Euphorbia hirta* growing in two different regions.

Preparation of samples

The collected plant samples (2 kg) were washed in running water to eliminate the dirt and air-dried for 15-20 days. A hundred grams of whole plant powder was extracted by maceration in 400 ml of methanol for 4 days with frequent agitation. Further crushed into a fine powder using a blender and used for further analysis.

Preparation of the extract

The air-dried powder of both samples was subjected to successive extractions by the addition of 10g of the powdered samples into five different conical flasks containing 100 ml of petroleum ether, acetone, methanol, ethanol, and distilled water respectively. This concoction

was plugged using a cotton thread and put on a rotary shaker at 190-220 rpm for 24 hours. The supernatant was then extracted, and the solvent was evaporated to produce the 400 mg concentrated extract. The crude samples are preserved for later usage at ambient temperature [24].

Phytochemical screening

Qualitative analytics were done for the crude samples of both plants by using standard approaches. The following tests were performed using standard protocols on 50 mg of *Euphorbia hirta* solvent extracts (petroleum ether, acetone, methanol, ethanol, and aqueous) [25].

Test for alkaloids: 50 mg of solvent-free extract, mixed and washed with a few drops of HCl. Added 2 drops of Mayer's Reagent. The appearance of a creamy white precipitate indicates the presence of alkaloids. *Test for sterols:* The array of colors shows that sterols are present when 50 mg of chloroform dissolved extract is mixed with 2 mL of acetic anhydride and 2 drops of concentrated Sulfuric acid. *Test for tannins:* A white color precipitate shows that Tannins are present when the 50 mg of extract was dissolved in water and 10% Lead acetate. Test for flavonoids: Brick red or magenta color appearance indicates the presence of flavonoids when 50 mg of the extract is heated after the addition of 5 mL alcohol followed by magnesium ribbon and HCL. *Test for terpenoids:* The appearance of a reddish-brown color precipitate on the addition of 2 ml of chloroform and a few drops of concentrated sulphuric acid to the extract shows the presence of Terpenoids. *Tri-terpenoids:* The appearance of purple color on exposing the extract to tin and thionyl chloride, indicates the existence of Tri-terpenoids.

Test for saponins: A froth layer of up to 2 cm is observed when 50 mg of extract and 20 ml of distilled water were dissolved and mixed vigorously. This indicates the presence of saponin. *Test for cardiac glycosides:* The appearance of the blue-green color on the addition of 1mL of glacial acetic acid, ferric chloride, and sulphuric acid to the 2ml of extract indicates the presence of cardiac glycosides. *Test for coumarin:* The yellow color indicates the presence of coumarin when the 50 mg of extract was mixed with 10% ferric chloride. *Test for quinones:* The appearance of red color on the addition of a few drops of concentrated sulfuric acid to the 50 mg of extract indicates the presence of quinones. *Test for phlorotannin:* Boiled 1ml of 1% aqueous hydrochloric acid was mixed with 2ml of extract. Red color deposition indicates that phlorotannins are present.

GC-MS analysis

The ethanol extract of *Euphorbia hirta.* obtained from two locations (India and Ethiopia) was characterized by GC-MS (TSQ QUANTUM XLS) which identifies the components present in the extract. GC-MS (TSQ QUANTUM XLS) is made of thermo-scientific, and the software required for the instrument is XCALIBUR (ver-2.2). The column's size is TG-5MS (30m0.25mm0.25um). The temperature (°C) was held constant at 280 °C for both the injector and the interface [26]. GC-MS mass spectrum analysis was performed using the National Institute Standard and Technology (NIST) database of over 62,000 patterns. The mass spectrum of the unidentified component was correlated with that of the identified components in the NIST-011 database [27]. The name and molecular structure of the compound have been determined.

FTIR-analysis

The illustration of the ethanol extract of *Euphorbia hirta* collected from India and Ethiopia are been established by the FTIR spectrum (VTHT, India, and AASTU, Ethiopia). The samples were determined using Shimadzu 8400s and the range of 4000-400 cm^{-1}, with 4 cm^{-1} resolution are used [28, 29].

Results and Discussion

Phytochemical screening

The phytochemical analysis of the *Euphorbia hirta* samples from both locations (Ethiopia and India) has confirmed the existence of medicinallyimportant compounds [30, 31] namely, tannins, phlorotannins, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, alkaloids, quinones, coumarins in different solvents extracts **(Table 1)**. The present study revealed that there are significant variations in the phytochemical composition of the sample extracts obtained from Ethiopia and India. On comparing the results of Eh-E and Eh-I, it is apparent that the ethanol extract of both samples showed the existence of maximum phytochemical compounds. In contrast to that, the petroleum ether extract of Eh-I, followed by the acetone extract of Eh-E displayed the presence of the least compounds. However, in the results of other extracts, both samples exhibited similar trends in component occurrence with trivial variations. Tannin, cardiac glycosides, alkaloids, and coumarins tend to be the most available compounds across all the extracts analyzed. Saponins and steroids have been the rarely available compound collectively. The differences in the phytochemicals between the two countries may be attributed to the different topography and climatic conditions. Earlier reports showed the presence of alkaloids, flavonoids, tannins, steroids, and phenolic compounds which correlate with the outcomes of the current study [32]. The former reports on the phytochemical analysis of *Euphorbia hirta* solvent extracts exhibited phenol, tannins, and quinone in aqueous, methanol, and ethanol extract which is parallel to the findings of the current study [33]. However, the

absence of tannins in aqueous extract and the presence of saponins, flavonoids, and terpenoids only in aqueous extract contradict the outcomes of the present study. Also, the absence of coumarins in all the solvent extracts in the previous study completely denies the facts of the current phytochemical study on *Euphorbia hirta*. The results clearly illustrate that the ethanol extract of *Euphorbia hirta* from both locations showed the presence of the maximum number of Phytocompounds compared to all the other extracts. Hence, ethanol extract was further analyzed by GC-MS and FT-IR to study its precise composition. Above all, this comparative phytochemical study between these two samples and the earlier reports depicts the influence of location in variations found in their phytochemical composition [34].

Table 1. Phytochemical Analysis of the Extracts of Euphorbia hirta

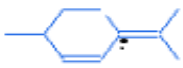
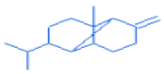
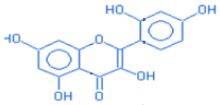
Phytochemical compound	Sample 1 – Ethiopia					Sample 2 - India				
	Aqueous	Petroleum Ether	Acetone	Methanol	Ethanol	Aqueous	Petroleum Ether	Acetone	Methanol	Ethanol
Tannin	+	-	+	+	+	+	-	-	+	+
Phlobatannin	+	+	-	-	+	-	-	+	-	-
Saponin	-	+	-	-	-	+	-	-	-	+
Flavonoids	-	+	+	-	+	+	-	+	+	-
Terpenoids	+	-	-	-	+	-	-	+	+	+
Cardiac Glycosides	-	-	-	+	+	+	+	+	+	+
Steroids	-	+	-	-	-	-	+	-	+	+
Alkaloids	+	+	+	+	+	+	-	+	-	-
Quinones	-	+	-	+	+	+	+	-	+	+
Coumarins	-	+	+	+	+	-	+	+	-	+

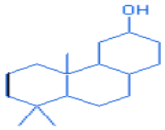
+: Present
-: Absent

GC-MS analysis

The components available in the ethanol extract of *Euphorbia hirta L.* of the samples obtained from both locations were determined by GC-MS **(Figures 2 and 3)**. The effectual data like molecular structure, molecular formula, molecular weight (MW), and retention time (RT) of the components in the ethanol extracts of *Euphorbia hirta* samples are presented in **Tables 2 and 3**. Twelve components were identified in each of the ethanol extracts of *E. hirta* collected from Ethiopiaand India [35, 36].

Table 2. GC-MS analysis of ethanol extract of Euphorbia hirta - (Ethiopia Sample)

S. No	Name of compound	Retention Time	Molecular weight	Molecular structure
1	Isoterpinolene	12.57	136.23	
2	Trans-á-copaene	14.88	204.3511	
3	9-Hexadecenoic acid, methyl ester[Z}	16.88	268.4348	
4	[E]-13-Docosenoic acid	21.43	338.5677	
5	Methyl eicosa-5,8,11,14,17-Pentaenoate	20.4	316.5	
6	Morin	19.63	302.23	
7	Heptadecanoic acid, 16-methyl-, methyl ester	19.05	298.5038	

8	Phytol	18.82	296.5	
9	3-Eicosene, [E]-	17.72	280.5	
10	Hexadecanoic acid, methyl ester	17.08	270.4507	
11	Dodecanoic acid, 10-oxo-	15.6	270.41	
12	3-Phenanthrenol, tetradecahydro-4b,8,8-trimethyl-[3S-(3à,4aá,8aà,10aá)]-	6.43	270.23	

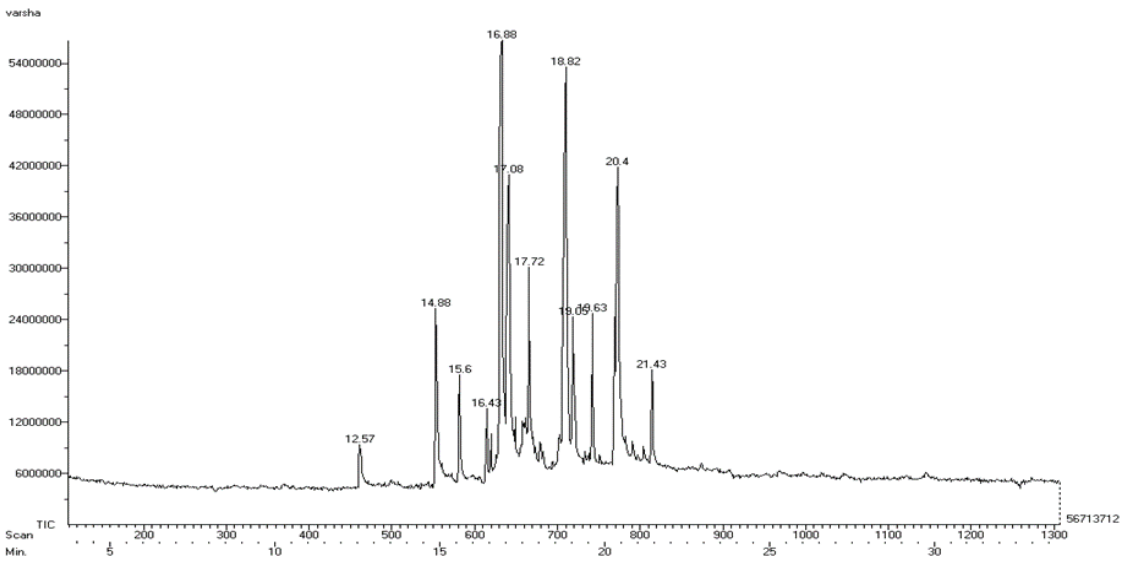
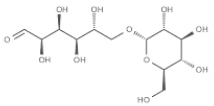
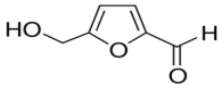
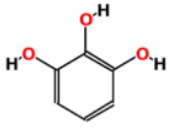
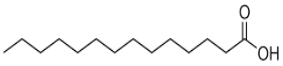
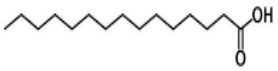
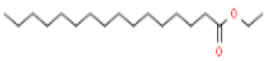
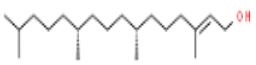
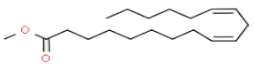
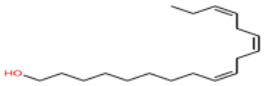
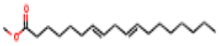
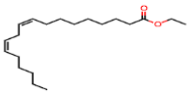
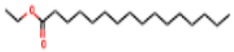
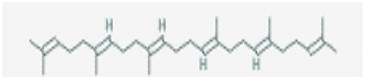
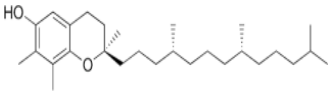


Figure 2. GC-MS chromatogram analysis of ethanol extract of Euphorbia hirta - (Ethiopia Sample)

Table 3. GC-MS analysis of ethanol extract of *Euphorbia hirta* - (India Sample)

S. No	Name of compound	Retention Time	Molecular weight	Molecular structure
1	2,3 – Dihydro -3,5- dihydroxy-6-methyl-4H-pyran-4-one	9.66	2.54	
2	5-Hydroxymethyl-2-furancarboxaldehyde	12.06	7.82	
3	1,2,3-Trihydroxybenzene	16.39	3.72	
4	Myristic acid	25.52	1.03	
5	Pentadecylic acid	30.48	13.27	
6	Ethyl palmitate	31.34	7.47	
7	Phytol	35.11	3.93	

8	Methyl linoleate	35.81	7.29	
9	9,12,15-Octadecatrien-1-ol	36.00	18.56	
10	7,10-Octadecadienoic acid methyl ester	36.47	6.22	
11	Ethyl linoleate	36.65	7.7	
12	Ethyl stearate	37.25	2.18	
13	Ethyl hexadecane	48.78	0.44	
14	Squalene	49.43	1.63	
15	gamma-Tocopherol	54.41	0.76	

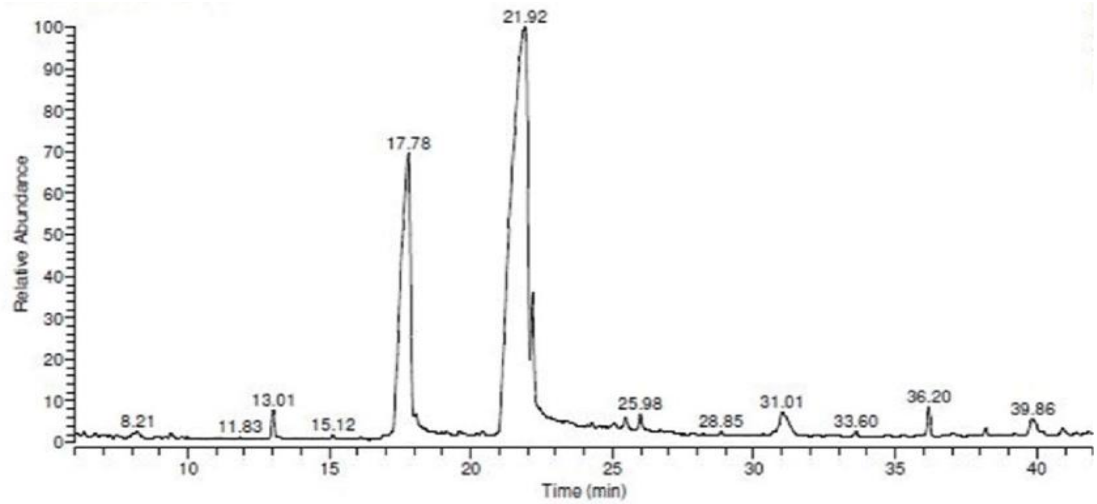


Figure 3. GC-MS chromatogram of ethanol extract of *Euphorbia hirta* - (India Sample)

Characterization study by FTIR analysis

The FTIR analysis of sample 1 (AASTU, Ethiopia) exhibited that the peak of 1035 cm^{-1} corresponds to the Carbonyl group, and the peak of 1215 cm^{-1} was specific to the Carboxyl group **(Table 4)**. Furthermore, the two peaks at 2924 cm^{-1} and 3354 cm^{-1}ascribed to the predominance of the alkene group and alcohol/phenols group. On the other hand, the FTIR analysis of sample 2 (VTHT, India) exhibited peaks at 1033.85 cm^{-1} and 1195.87 cm^{-1} which indicates the presence of aldehyde and carbonyl group respectively **(Table 5)**. Moreover, the peak of 2924.09 cm^{-1} shows the existence of the alkene group and the broad peak at 3278.99 cm^{-1} corresponds to the alcohol group Hence, the present study inferred that C-O to C-C, COOH, CH_2- and O-H functional groups are responsible for the catalytic reduction of *Euphorbia hirta*-sample 1. Alternatively, the catalytic reduction of *Euphorbia hirta*- sample 2 has been mediated by the R-O, C-O, CH_2-, and O-H functional groups. The above characterization peaks of sample-1 (Ethiopia) and sample-2 (India) obtained in FT-IR correspond to the earlier reports [26, 27, 30].

Table 4. FT-IR spectroscopic analysis of ethanol extract of *Euphorbia hirta* - (Ethiopia Sample)

Peak region (cm^{-1})	Compound	Nature	Discussion
1035	C-O to C-C	Anti-symmetric coupled stretch	Carbonyl
1215	COOH-	stretch and bend combination	Carboxyl

2924	CH_2-	Anti-symmetric stretch	Alkene
3354	O-H	broad stretch	Alcohol/Phenols

Table 5. FT-IR spectroscopic analysis of ethanol extract of *Euphorbia hirta* - (India Sample)

Peak region (cm^{-1})	Compound	Nature	Discussion
1033.85	R-O	stretch	Aldehyde
1195.87	C-O	stretch	Carbonyl
2924.09	CH_2-	stretch	Alkene
3278.99	O-H	stretch	Alcohol/Phenols

Conclusion

In Conclusion, *Euphorbia hirta* from Ethiopia and India possess significant bioactive properties and could potentially be used in the pharmaceutical industry. From the present pilot study, it can be suggested that when compared to all the other extracts, the ethanol extract of *Euphorbia hirta* from both countries showed a substantial presence of Phyto-compounds in the phytochemical screening and further detailed analysis could confirm their utilization as pharmaceutically important plants. Thereby the study suggests that *Euphorbia hirta* can be utilized as a potential source of pharmaceuticals and can be further studied in the extraction of bioactive compounds.

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Conflict of interest: None

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Ethics statement: None

References

1. Sun Y, Chen Q, Liu P, Zhao Y, He Y, Zheng X, et al. Impact of Traditional Chinese Medicine Constitution on Breast Cancer Incidence: A Case-Control and Cross-Sectional Study. Pharmacophore. 2021;12(2):46-56. doi:10.51847/uqUyzrY0b0
2. Saravanan R, Dhachinamoorthi D, Senthilkumar K, Srilakshmi M, Divya Sri T. Antibacterial Activity of Euphorbia hirta L. Extracts. Int J Ayurveda Res. 2012;3(3):439-41.
3. Ghosh P, Ghosh C, Das S, Das C, Mandal S, Chatterjee S. Botanical Description, Phytochemical Constituents and Pharmacological Properties of Euphorbia hirta Linn: A Review. Int J Health Sci. 2019;(3):14.
4. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacogn Rev. 2010;4(7):58-61.
5. Sandeep BP, Nilofar SN, Chandrakanth SM. Review on Phyto-chemistry and Pharmacological aspects of Euphorbia hirta L. Linn. J Pharm Health Care Sci. 2009;1(1):113-33.
6. Basma AA, Zakaria Z, Latha LY, Sasidharan S. Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L. Asian Pac J Trop Med. 2011;4(5):386-90.
7. Rajesh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Assessment of Euphorbia hirta L. Leaf, Flower, Stem and Root Extracts for Their Antibacterial and Antifungal Activity and Brine Shrimp Lethality. Molecules. 2010;15(9):6008-18.
8. Tran N, Nguyen M, Le KP, Nguyen N, Tran Q, Le L. Screening of Antibacterial Activity, Antioxidant Activity, and Anticancer Activity of Euphorbia hirta Linn. Extracts. Appl Sci. 2020;10(23):8408.
9. Youssouf MS, Kaiser P, Tahir M. Anti-anaphylactic effect of Euphorbia hirta. Fitotherapia. 2007;78(8):535-9. doi:10.1016/j.fitote.2007.06.003
10. Asha S, Thirunavukkarasu P, Vinayagam MM, Sadiq A. Antioxidant Activity of Euphorbia hirta Linn Leaves Extracts. European J Med Plants. 2016;10;14:1-14.
11. Karki S, Shrestha K, Gautam R, Jha R. Phytochemical screening, FT-IR and GC-MS analysis of Euphorbia hirta. J Pharmacon Phytochem. 2020;9(1):1883-9.
12. Hore SK, Ahuja V, Mehta G, Kumar P, Pandey SK, Ahmad AH. Effect of aqueous Euphorbia hirta leaf extract on gastrointestinal motility. Fitoterapia. 2006;77(1):35-8.

13. Anitha P, Geegi PG, Yogeswari J, Anthoni SA. In Vitro Anticancer Activity of Ethanolic Extract of Euphorbia hirta (L.). J Sci Technol Arts. 2014;3(1):01-07.
14. Johnson BP, Abdurahman M, Tiam EA, Abdu-Aguye I, Hussaini IM. Euphorbia hirta L. leaf extracts increase urine output and electrolytes in rats. J Ethnopharmacol. 1999;65(1):63-9. doi:10.1016/s0378-8741(98)00143-3
15. Khan S, Ahmed B, Khalilullah H, Masoodi MH. Neuropharmacological activity of Euphorbia hirta L.and its isolated compound. J Pharmacogn Phytochem. 2014;3(2):138-46.
16. Widharna RM, Soemarrdji AA, Wirasutisna KR, Kardonol BS. Anti-diabetes Mellitus activity in vivo of ethanolic extract and ethyl acetate fraction of Euphorbia hirta L.L. Herb Int J Pharmacol. 2010;6(3):231-40. doi:10.3923/ijp.2010.231.240
17. Khurshid R, Saleen M, Karim S, Mir M. Anti-pyritic, anti-viral, anti-thrombotic properties of Euphorbia hirta L.against Dengue fever. Pharmacia. 2013;60(3):8. doi:10.1155%2F2018%2F2048530
18. Parekh J, Sumitra Chanda V. In vitro Anti-microbial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol. 2007;31(1):53-8.
19. Guzman G, Dacanay A, Andaya B, Alejandro G. Ethnopharmacological studies on the uses of Euphorbia hirta L.in the treatment of dengue in selected indigenous communities in Pangasinan (Philippines). J Intercult Ethnopharmacol. 2016;5(3):239. doi:10.5455/jice.20160330124637
20. Harbone JB. Phytochemical Methods. London: Chapman and Hill. 1973.
21. Trease GE, Evans WC. Pharmacognosy. 11th edn. London: Brailliar Tiridel and Macmillian Publishers; 1989.
22. Prajapati ND, Purohit SS, Sharma AK, Kumar T. Handbook of medicinal plants. India: Agarbios; 2013.
23. Perumal A, Krishna S, Madhusree. GC-MS Analysis, Antioxidant and Antibacterial Activities of Ethanol Extract of Leaves of Aegle Marmelos (L.) Correa. J Drug Deliv Ther. 2018;8(4):247-55. doi;10.22270/jddt.v8i4.1781
24. Sudha T, Chidambarapillai S, Mohan VR. GC-MS Analysis of Bioactive Components of Aerial Parts of Fluggealeucopyrus Wild (Euphorbiaceae). J Appl Pharm Sci. 2013;3(5):2231-3354. doi:10.7324/JAPS.2013.3524
25. Karki S, Shrestha K, Gautam R, Jha RN. Phytochemical screening, FT-IR, and GC-MS analysis of Euphorbia hirta. J Pharmacogn Phytochem. 2020;9(1):1883-9.
26. Noviyanti Utami W, Marpaung L, Lenny S. Isolation of Phenolic Compounds of Patikan Kebo Leaves (Euphorbia hirta. L). Int J Eng Sci Technol Stud. 2018;4(9):157-61.
27. Priyadarshini G, Elizabeth AA, Anthony J, Ram Krishna Rao M, Prabhu K, Ramesh A, et al. The GC MS Analysis of One Medicinal Plant, Premna tomentosa. Int J Pharm Sci. 2017;9(9):1595-7.
28. Gopinath SM, Rakesh CK, Ashwini Patil GM, Dayananda KS. Preliminary Phytochemical Evaluation of Leaf Extracts of Euphorbia hirta, SyzygiumCumini of Siddarabetta, Tumkur District, Karnataka. Int J Pharma Bio Sci. 2012;3(2):431-5.
29. Sandhu A, Bhardwaj N, Gupta R, Menon V. Antimicrobial Activity and Phytochemical Screening of Tinospora Cordifolia and Euphorbia hirta. Int J Appl Biol. 2013;4.
30. Das N, Kalita PP, Sarma MP, Bhattacharjee M. Molecular Modeling of HEV Core Protein and Active Compounds from Northeast Folk Medicine. J Biochem Technol. 2021;12(2):12-8. doi:10.51847/j3XkpgE1XE
31. Adjdir S, Benariba N, Adida H, Kamila G, El Haci IA, Terki M, et al. Phenolic Compou nds and Antimicrobial Activity of Ziziphus jujuba Mill. Fruit from Tlemcen (Algeria). J Biochem Technol. 2021;12(1):40-4. doi:10.51847/mAJJfuBvan
32. Nelly Nzuzi P, Clément Inkoto L, Koto-te-Nyiwa N, Florent Mukeba B, Jules Munganga K, Taba K, et al. Review on the phytochemistry, toxicology, and bioactivities of Euphorbia hirta L.: A potential antisickling medicinal plant species. J Med Plant Herbal Ther Res. 2020;7:8-18.
33. Al-Snafi AE. Pharmacology and therapeutic potential of Euphorbia hirta (Syn: Euphorbia pilulifera) - A review. IOSR J Pharm. 2017;7(3):7-20.
34. Mohammad Abu BN, Mohammad SH, Mohammad A, Rabeya S, Sanjeeda S, Rabiul Islam BM. Euphorbia hirta Linn. A wonderful miracle plant of Mediterranean region: A review. J Med Plants Stud. 2017;5(3):170-5.
35. Indra R, Prerna J, Priya T, Mohit P, Pallavi D, Shweta B, et al. Comparative GC-MS analysis of euphorbia hirta and euphorbia milli for therapeutic potential utilities. Plant Arch. 2020;20(2):3515-22.
36. Basyal D, Neupane A, Pandey DP, Pandeya S. Phytochemical Screening and In Vitro Antioxidant and Anti-inflammatory Activities of Aerial Parts of Euphorbia hirta L. J Nepal Chem Soc. 2021;42(1):115-24.