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FTIR and GCMS analysis of *Euphorbia hirta* L. and its *In- vitro* Antibacterial and Antioxidant Activities

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ABSTRACT: **Introduction***: Euphorbia hirta* L. is a common weed plant belongs to family Euphorbiaceae. The present study brings out the importance of weed plants like *E. hirta* in medicine and health the care system. **Material and Methods:** Plant *E. hirta* has been collected from the garden of Department of Botany, University of Delhi and plant leaves were extracted in ethanol by the soxhlet method. Antibacterial screening has been done by disc-diffusion method. The antioxidant potential has been determined by TPC, TFC, FRAP, Total antioxidants through phosphomolybdate assay and by DPPH radical scavenging activity. FTIR and GCMS analysis have been done for characterization. **Result and Discussions:** Plant *E. hirta* showed significant antibacterial activity against *Escherichia coli, Staphylococcus aureus, Bacilus subtilis* and *Pseudomonas aeruginosa* with maximum ZOI diameter 19± 0.5 mm against *S. aureus* and *P. aeruginosa* both. Plant *E. hirta* L. showed significant antioxidant potential with 196.32 mg/g, 39.7133 mg/g, 595.99 mg/g, 525.84 mg/g values for TPC, TFC, FRAP activity and total antioxidants through phosphomolybdate assay respectively. Plant *E. hirta* also showed significant radical scavenging activity against DPPH free radical with a maximum % inhibition 68.505% at 1000µg/ml concentration. FTIR and GCMS of plant *E. hirta* have confirmed the presence of various active functional groups and compounds. **Conclusion:** The current study justifies the importance of *E. hirta* L. a common weed as a source of natural antioxidants having antibacterial potential and its role in herbal medicines.

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INTRODUCTION

Use of plants in health care system has been documented since ancient times in different traditional writings including Ayurveda, Unani and Sidhha. Many plants have been successfully used in treatment of various disorders like *Catharanthus roseus* (vincristine and vinblastine) for cancer, *Taxus brevifolia* (taxol) for cancer, *Cinchona* sp. (quinine) for malaria, *Digitalis lanata* (digoxin) for heart disease, *Withania somnifera* (withanolides) for cancer and parkinson's disease, *Berberis vulgaris* (berberine) for antidiabetic [1]. Hence, plants having medicinal activities are important part of today's health care system as it provides a natural way to get rid of disease with no or less side effects. Study and exploration of new plants is one of the most important parts of research as it will solve the issue of multidrug resistance against already existing plants.

Plant *Euphorbia hirta* L. is a common weed that belongs to family Euphorbiaceae and known by various vernacular names dhudi in Hindi, asthma weed in English and dugdhika in Sanskrit. *It* is widely distributed in India and easily found at roadsides, in parks and gardens. The plant is characterized by its creepy appearance almost flattened on the ground with the presence of milky latex and inflorescence as cyathium [2]. The plant has been reported to have different medicinal properties such as worm infestations, antibacterial, antifungal, antiurolithiatic, analgesic, antimalarial and antiviral [3-4]. The current study reveals the medicinal worth of *E. hirta* and its role in the health care system.

MATERIAL AND METHODS

Collection of plant and preparation of extract

Plant *E. hirta* has been collected from the garden of Department of Botany, University of Delhi in the month of July-August and identified using "Flora of Delhi". Leaves of the plant were shade dried and powdered using a mixer grinder and extracted in ethanol by the soxhlet method.

Antibacterial screening

For antibacterial Screening Disc Diffusion method has been used against *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* (MTCC 11949), *Bacilus subtilis* (MTCC 441) and *Pseudomonas aeruginosa* (MTCC 1688) procured from MTCC Chandigarh. Standard gentamicin has been procured from sigma (aldrich). Absorption of Bacterial cultures was adjusted to 0.1±0.02 at 600nm using spectrophotometer SP UV 1000 to reach 10^8 CFU/ml concentrations for final use.

For Disc Diffusion method LB Agar plates were taken and 20µl of bacterial culture was poured and spread on it. Sterilized discs were then dipped in the test samples and placed in the center of the agar plates. Agar plates were then sealed with parafilm and were incubated 12-16 h at 28ºC in the oven. Observations were taken after incubation and ZOI of plant samples and standards were compared [5].

Total Phenolic content and Total Flavonoid content

Total phenolic content (TPC) of plant extract was determined according to the method of Slinkard et al. [6]. Test tubes were covered with aluminum foil and 300 µl of plant extract (1mg/ml), 1 ml ethanol, 3.16 ml distilled water and 200 µl Folin-Ciocalteu reagent were added and incubated for 8 min at room temperature, then 600 µl sodium carbonate solution (10%) was added and incubated at 40 °C for 30 min in hot water bath. The absorbance was recorded at 765 nm using UV visible spectrophotometer. Ethanol and Gallic acid has been used as blank and standard respectively. Total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of extract.

The total flavonoid content (TFC) was determined according to the reported protocol [7]. 300 µl extract (1mg/ml), 3.4 ml aqueous ethanol (30%), 150 µl aqueous sodium nitrite solution (0.5 M), 150 µl aluminum chloride solution (0.3 M) was added to test tube, incubated for 5 min and then 1 ml sodium hydroxide solution (1 M) was then added, the content was mixed well and absorbance was recorded at 506 nm using UV visible spectrophotometer. Ethanol and Rutin has been used as blank and standard respectively. The total flavonoid content of each extract was expressed as mg of rutin equivalents (RE) per g of extract.

FRAP and Phosphomolybdate assay for total antioxidants

Ferric reducing antioxidant potential (FRAP) of the extracts was done according to the method by Benzie and Strain [8]. FRAP reagent was prepared by mixing in 25 ml acetate buffer (30 mM; pH 3.6), 2.5 ml TPTZ solution (10 mM in 40 mM HCl) and 2.5 ml ferric chloride solution (20 mM). The mixture was incubated for 15 min at 37 °C before use. 2.85 ml FRAP reagent, 150 µl plant samples (1mg/ml) was added to the test tube, incubated for 30 min in the dark, and its absorbance was recorded at 593 nm. Ethanol and Ascorbic acid have has been used as blank and standard, respectively. The results were expressed as mg of ascorbic acid equivalents (AAE) per g of extract.

Total antioxidant capacity (TAC) of extracts was done by phosphomolybdate assay proposed by Prieto et al. [9], the procedure described by Jan et al. [10] and Mashwami et al. [11] with slight modification. Test tubes were covered with aluminium foil and 300 µl plant extract (1mg/ml), 3 ml phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to it, incubated at 95 °C for 90 min. The mixture was then allowed to reach at room temperature and absorbance was recorded at 765 nm. Ethanol and Ascorbic acid has been used as blank and standard respectively. The antioxidant capacity was reported as mg of ascorbic acid equivalents (AAE) per g of extract.

DPPH radical scavenging activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of plant was done according to the method reported by Brand-Williams et al. [12] with slight modification [13]. The stock solution was prepared by dissolving 2.4 mg DPPH in 10 ml ethanol, was kept in a refrigerator until further use. The working solution of the radical was prepared by diluting the DPPH stock solution with ethanol to obtain an absorbance of about 0.98 (±0.02) at 517 nm [14]. To test tube, 3 ml DPPH working solution and 100 µl of plant extract (100µg/ml to 1mg/ml) was added, Incubated for 30 minutes at RT in dark and absorbance was measured at 517 nm. The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

Where, Ac and As are the absorbance of control and sample, respectively. The control has 100µl ethanol in place of the plant sample.

FTIR and GCMS analysis

Fourier transform infrared (FT-IR) spectrum of leaves of plant *Euphorbia hirta* L. was obtained using spectrometer (Shimadzu) in the range of 400–4,000 cm^{-1}. Gas Chromatoraphy- Mass Specrrometry of leaves of plant *Euphorbia hirta* L. was performed by using GCMS program GCMS_QP2010 Ultra with following working conditions: column oven temperature 60℃, injection temperature 260℃, injection mode split, flow control mode linear velocity, pressure 73.3kPa, flame thermionic detector, ion sources temperature 230℃ and carrier gas saver off.

RESULTS AND DISCUSSION

Antibacterial screening

Plant *E. hirta* has shown significant antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* with a

zone of inhibition diameter 15, 19, 18 and 19mm (± 0.5mm) respectively which are comparable with the standard Gentamicin with ZOI 20, 20, 20 and 21mm (± 0.5mm) respectively (Figure IA). Plant *E. hirta* showed excellent activity against both gram- negative (*E. coli* and *P. aeruginosa*) as well as gram- positive bacteria (*S. aureus and B. subtilis*) and hence, it can be used as effective alternative against bacterial human pathogens.

Total Phenolic content and Total Flavonoid content

The total phenolic content assay measures the reduction of Folic- Ciocalteu reagent in the presence of phenolic compounds and Total flavonoid content is based on the ability of flavonoids to form a complex with Al^{3+}. TPC of Plant *E. hirta* was determined in terms of mg of Gallic acid equivalent per gram of extract and TFC was determined in terms of mg of Rutin equivalent per gram of extract with values 196.32 mg of GAE/g of extract and 39.7133 mg of RE/g of extract respectively (Figure IB). The above results confirmed that plant *E. hirta* is an excellent source of natural phenolics and flavonoids.

FRAP and Phosphomolybdate assay for total antioxidants

FRAP activity indicates the plant's ability to reduces (Fe3+) ions to Ferrous (Fe2+) and phosphomolybdate assay is based on the reduction of molybdenum (VI) is to molybdenum (V) in the presence of antioxidants. FRAP and total antioxidants through phosphomolybdate assay of Plant *E. hirta* were determined in terms of mg of Ascorbic acid equivalent per gram of extract and showed significant activity with values 595.99 and 525.84 AAE of mg/g for FRAP and Total antioxidants content by phosphomolybdate assay respectively (Figure IC).

DPPH radical scavenging activity

DPPH is a free radical molecule that absorbs maximum at 517 nm but on its aborbance decreases on reduction in the presence of antioxidants. Plant *E. hirta* has shown significant radical scavenging activity against free radical DPPH with % activity 25.615%, 56.32%, 60.49%, 62.97% and 68.505 % at 100, 250, 500, 750, 1000 µg/ml concentration respectively and are comparable with the standard Ascorbic acid (Figure ID).

Table 1: Represents FTIR analysis of leaves of plant *Euphorbia hirta* L. [15].

S. No.	Peak value (cm^{-1})	Stretching type	Functional Groups
1.	530.42	C-I stretching	Iodo compound
2.	1217.08	C-O stretching	Alkyl aryl ether, vinyl ether
3.	1367.53	O-H bending; S=O stretching	Phenol; sulfonate, sulfonamide
4.	1436.97	C-H bending	Alkane, Alkyl
5.	1739.79	C-H bending; C=O stretching	Aromatic compound; Esters, δ-lactone
6.	3014.74	O-H stretching (broad)	Carboxylic acid

Table 2: Represents GCMS analysis of leaves of *Euphorbia hirta* L.

Peak no.	% Amt.	Compound Name	Activity
16	5.37	Neophytadiene	Antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant [16]
21	10.47	n-Hexadecanoic acid	Antimicrobial antioxidant, Antioxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Flavor, Hemolytic [17]
29	28.02	(Z,Z)-6,9-CIS-3,4-Epoxy-nonadecadiene	Sexpheromone [18]
30	6.18	Octadecanoic acid	Larvicidal activities, antioxidant, anti-inflammatory, analgesic, antidiabetic, hypolipidemic, anthelmintic, wound healing, and antipyretic properties [19]
44	1.54	Pentacosane	Antioxidant activity [20]
48	5.48	Octacosanol	Antinociceptive and Anti-Inflammatory [21]
50	2.49	2H-1-benzopyran-6-ol	Antimicrobial [22]
51	1.09	Cholest-5-en-3-ol (3.Beta.)	Antibacterial [23]
53	9.13	Ergost-5-en-3-ol, (3 beta)	Anticancer, antimicrobial activity Antioxidant, Hypocholesterolemic [23]
55	1.20	Beta –amyrin	Antibacterial Antihyperglycemic and hypolipidemic [24]
57	2.69	Methyl Commate D	antimicrobial and anti-inflammatory [16]

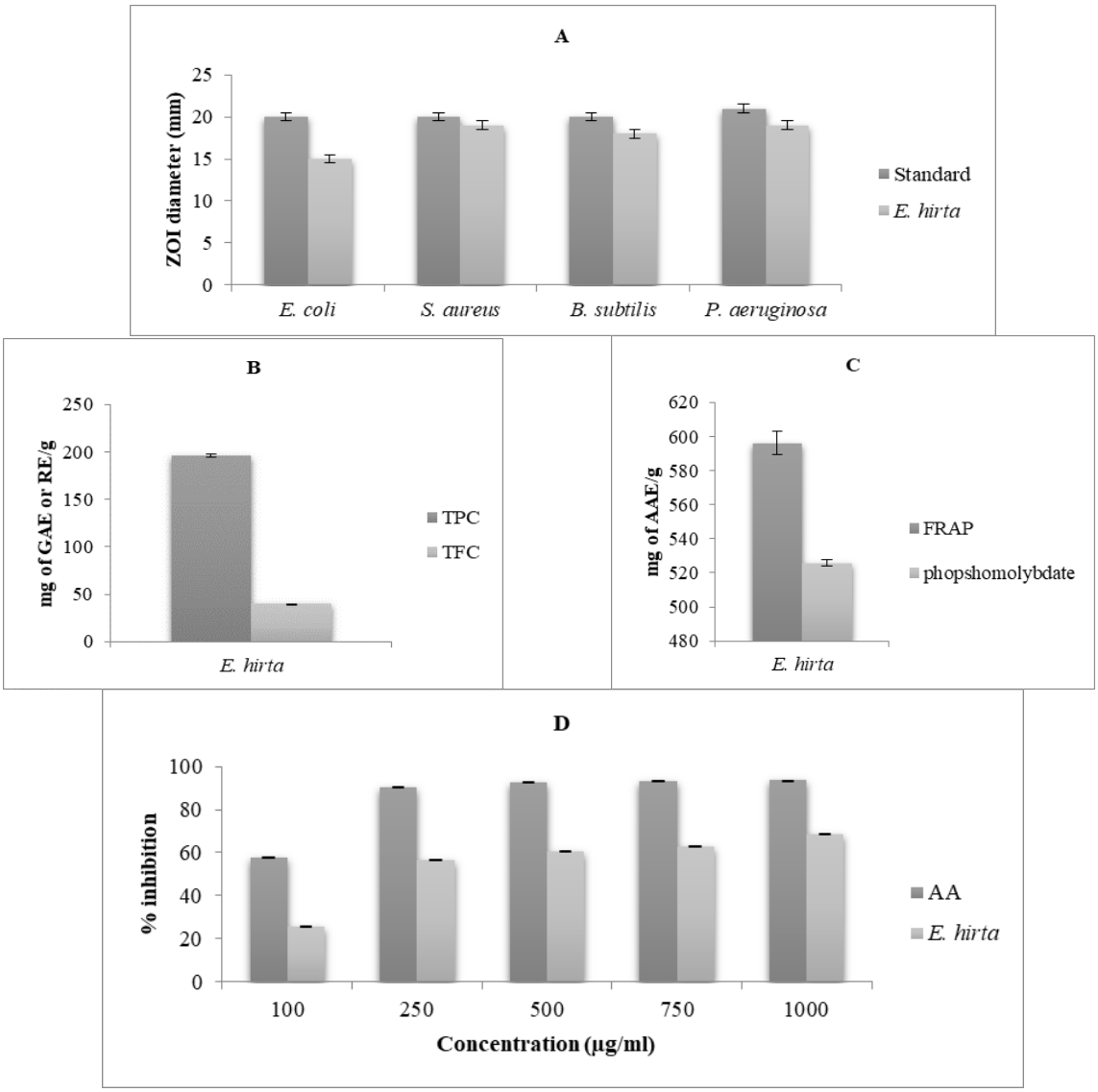

Figure 1. Represents antibacterial activity against *E. coli, S. aureus, B. subtilis* and *P. aeruginosa* (A), TPC and TFC (B), FRAP and total antioxidants through phosphomolybdate assay (C) and DPPH radical scavenging activity (D) of leaves of *Euphorbia hirta* L. AA= Ascorbic Acid, GAE= Gallic Acid equivalent, RE= Rutin Equivalent and AAE= Ascorbic Acid Equivalent.

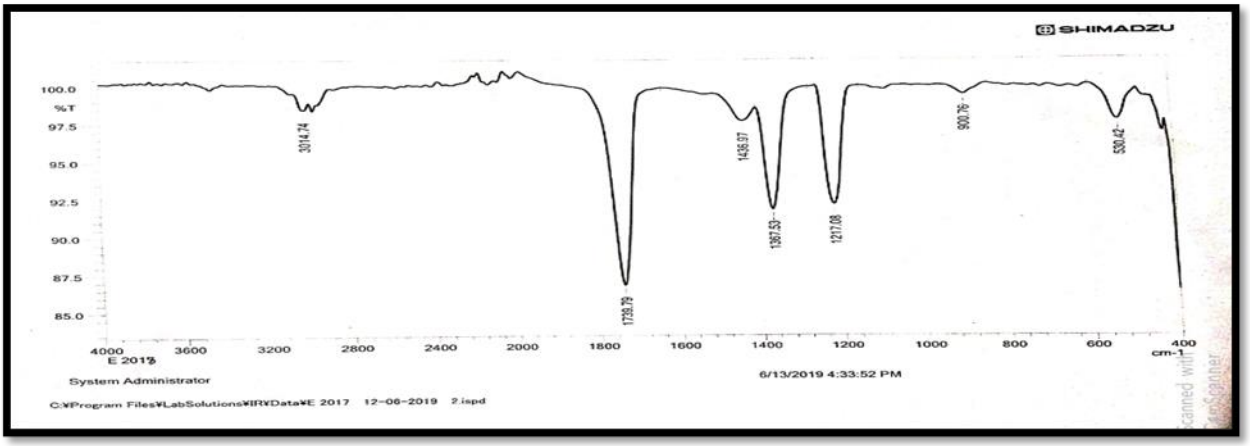

Figure 2. Represents FTIR graph of leaves of *Euphorbia hirta* L.

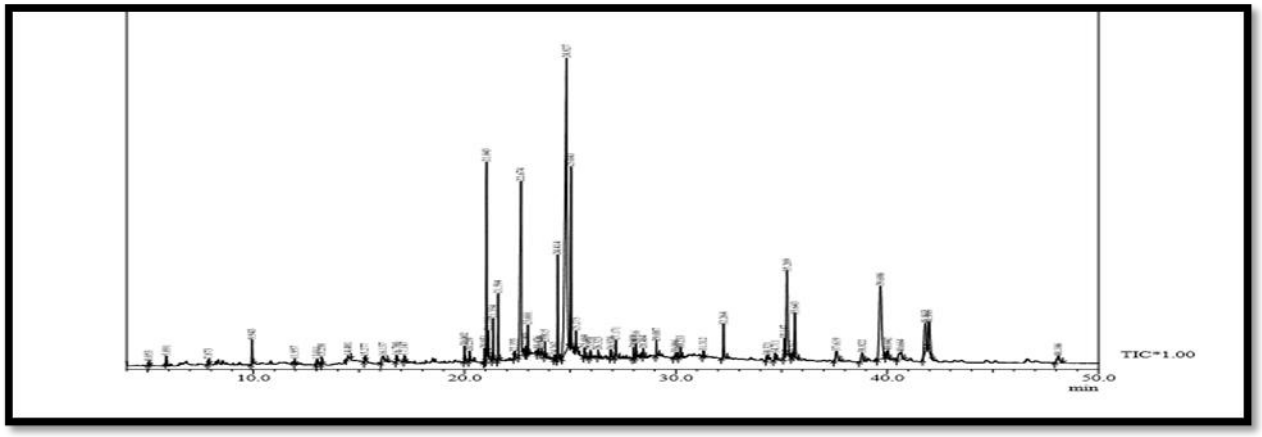

Figure 3. Represents graph of GCMS of leaves of *Euphorbia hirta* L.

CONCLUSION

Plant *Euphorbia hirta* L. showed significant antibacterial and antioxidant activity with 95% activity for *S. aureus* and *P. aeruginosa* and 90% activity for *B. subtilis* as compared to standard gentamicin (Figure I). Functional groups of a drug molecule play critical role in its overall activity including its interaction with the target and its mechanism of action. FTIR of Plant *E. hirta* has shown the presence of different functional groups responsible for drug- like properties of a compound (Table I and Figure II). GCMS has revealed the presence of various compounds reported to have different medicinal activity including antibacterial, antioxidant, anti-inflammatory, larvicidal, anticancer, antidiabetic etc (Table II and Figure III). The current study provides the basics to conduct further study on the plant to validate its other medicinal properties along with its bioactive compound(s) responsible for a specific activity. Hence, plant *Euphorbia hirta* L. may have a potential role in the field of herbal medicine.

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CONFLICT OF INTEREST

The authors confirm no conflicts of interest.

DATA AVAILABILITY STATEMENT

Not declared.

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript. Journal and Publisher will not be responsible for any copyright infringement and plagiarism issue.

AUTHOR CONTRIBUTIONS

The entire study was conceptualized, designed and conducted the study with the help of other authors, wrote the first draft of the manuscript, and other authors contributed significantly to the revision of the manuscript. All Authors read and approved the final manuscript.

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