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Phytochemical Screening, Antibacterial and Antioxidant Activities of *Euphorbia hirta* Crude Extract

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Abstract: The present study aims to analyse the antibacterial and antioxidant activity of the crude extract of *Euphorbia hirta*. The antibacterial activity was tested against *Escherichia coli*, *Proteus vulgaris*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using different solvent extracts of *Euphorbia hirta* with disc diffusion method and the DPPH scavenging activity was performed to analyze the free radical scavenging efficacy of the extracts. GC-MS analysis was performed to identify the biologically active compounds in ethyl acetate extract. The result reveals the antibacterial efficacy of the ethyl acetate mediated crude extract of *Euphorbia hirta* against all the tested organisms. The free radical scavenging activity of the ethyl acetate and methanol extracts were recorded with the IC₅₀ value of 47.8 µg/ml and 63.7 µg/ml, respectively, compared with aqueous extract with IC₅₀ value of 181.86 µg/ml. GC-MS analysis of ethyl acetate revealed the presence of potential antibacterial and antioxidant components suggesting the isolation and evaluation of the specific agent which can be exploited as a potential antimicrobial drug and antioxidant agent.

Keywords: *Euphorbia hirta*, Phytochemical, Antibacterial, Antioxidant, GCMS analysis

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Introduction

The use of natural products such as plants and its derivatives as medicine could be traced for back as the emergence of human civilization. The earliest mention of use of plants as medicine is found in Rigveda (4500-1600 B.C). The eight divisions of Ayurveda deals with the properties of drugs and science of life and healing process (Rastogi and Mehrotra, 2002). Nature has bestowed with rich source of medicinal plants, with range of biological activities. More than 80% of the world population primarily depends on herbal medicine to treat

various ailments (WHO) especially traditional medicines such as plant derivatives which plays a major role in substituting basic health needs of the people of developing countries (Shihabudeen and Priscilla, 2010).

More than 7000 plant species were used for the traditional medicinal care in India. Medicinal plants have chemotherapeutic action (Kusuma *et al.*, 2014). The biologically active ingredients such as alkaloids, flavonoids, phenols, tannins, glycosides, steroids and terpenoids are the

secondary metabolites derived from the plants (Hill, 1952; Cowan, 1999; Rufus *et al.*, 2013). Hundreds of plants were tested for their bactericidal efficacy; however, most of them were not adequately evaluated (Balandrin *et al.*, 1985). Indiscriminate use of synthetic antimicrobial agents results in the development of multidrug resistant microorganisms (Su *et al.*, 2015). The nature and mechanism of action of medicinal plants as biological agents were not established (Rafieian-Kopaei, 2013). In recent years the application of medicinal plants gains attention due to their therapeutic properties, cost effective and with no or less toxicity when compared with synthetic antibiotics (Nengroo and Rauf, 2019). *Euphorbia hirta* (family Euphorbiaceae), is an annual herb commonly found in tropical countries (Lind *et al.*, 1971). Numerous small green flowers crowd together to form a chime, characteristic feature of Euphorbiaceae. *Euphorbia hirta* have potential biological activities (Shanmugapriya *et al.*, 2012). The aim of the present study was to evaluate the antibacterial and antioxidant potential of the crude extracts of *Euphorbia hirta* and to explore the presence of new compounds with specific activities.

Materials and Methods

Collection of Plant Material:

Fresh plants of *Euphorbia hirta* were collected from the Medicinal Plant Garden, Presidency College, Chennai, India and identified with the Voucher specimen available with the Plant Biology and Biotechnology Department, Presidency College, Chennai, India. The leaves were collected, and shade dried for a week period and grinded into fine powder and stored in the airtight container for further use.

Extraction:

About 200 g of grinded powder of the leaf of *Euphorbia hirta* was packed in the filter paper and loaded in the thimble of the Soxhlet apparatus for the extraction using different solvents such as water, methanol and ethyl acetate. The extraction

was done by simply heating the solvent in the bottom of the flask and the warm solvent slowly fills the thimble loaded with the plant material and when it reaches the margin the contents will be emptied into the bottom flask. This cycle is continuously run for a period of 8 h till the complete extraction of the metabolites from the plant material *Euphorbia hirta*. The solvent extracts were further subjected to vacuum evaporation to separate the solvent from the extract and the concentrated extracts were refrigerated and re-suspended with respective solvent before the experiment.

Phytochemical Analysis:

Qualitative phytochemical analysis of different extracts of *Euphorbia hirta* was analyzed for the presence of tannins, terpenoids, cardiac glycosides, flavonoids, phenols, carbohydrates, saponins, alkaloids, proteins, emodins, coumarins, steroids and anthocyanin following the methods of Harborne (1973) and Thooyavan and Karthikeyan (2017).

Antibacterial Assay:

Antibacterial efficacy of aqueous, methanol and ethyl acetate solvent mediated leaf extracts of *Euphorbia hirta* were tested against pure strains of *Escherichia coli*, *Proteus vulgaris*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the Department of Microbiology, Presidency College, Chennai, India.

Each of the bacterial strains was cultured in the Muller Hinton agar and the subcultures were maintained overnight at 37 C, the bacterial growth was harvested. To evaluate the antibacterial activity the disk diffusion method was followed. The concentrated plant extracts were re-suspended in their respective solvents and the samples were loaded on the sterile filter paper disc with the diameter of 6 mm. Different concentrations of solvent extracts (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml concentration) were loaded with the sterile disc along with 10 mg of standard antibiotic amoxicillin as positive

control. Muller Hilton agar was poured into the sterile Petri dishes and the plates were inoculated with the bacterial culture and the even spreading of the inoculated culture was confirmed. The plates were incubated overnight at 37 C, and the diameter of the zone of inhibition of bacterial growth around each disc was measured using Vernier caliper and recorded.

Antioxidant activity:

Antioxidant activity of different extracts were analyzed using DPPH scavenging activity followed by the method of Brand-William *et al.* (1995). Different concentrations (40 µg/ml, 80µg /ml, 120 µg /ml, 160 µg/ml and 200 µg/ml) of aqueous, methanol and ethyl acetate extracts of *Euphorbia hirta* were tested for its free radical scavenging activity using DPPH assay. 40 µg/ml to 200 µg/ml concentration of sample was dissolved in DMSO and made up to 40 µl and 2.96 ml of DPPH solution (0.1 mM) was added to each tube and the tubes were kept in dark for 20 min for incubation at room temperature and the optical density (OD) was read at 517 nm and to test the control 3 ml of DPPH was taken in a test tube. The scavenging efficacy of the plant extracts were calculated using the following equation:

$$\text{DPPH Scavenging Activity \%} = \frac{\text{Absorbance of Control} - \text{Absorbance of the extract}}{\text{Absorption of Control}} \times 100$$

Gas –Chromatography and Mass Spectrometry:

Ethyl acetate mediated leaf extract of *Euphorbia hirta* was subjected to GC-MS analysis followed by the method of Tyagi and Agarwal (2017). The analysis of ethyl acetate extract was subjected to GC-MS analysis using JOEL–GC mate equipped with Elite 1 capillary column coupled with turbo mass. Elucidated GC-MS spectrum was interpreted with the database at National Institute of Standard and Technology (NIST). The name of the compound, molecular weight and the structures were identified using NIST.

Results

Phytochemical analysis:

The preliminary phytochemical analysis showed the strong presence of tannin, alkaloids and flavonoids and moderate presence of steroids, terpenoids, coumarins, cardiac glycosides, carbohydrates, and phenol with reference to ethyl acetate extract. The observation revealed that the aqueous extract possess traces of steroids, terpenoids, tannins, cardiac glycosides and flavonoids with moderate presence of cardiac glycosides. The analysis of methanol extract revealed the moderate presence of steroids, terpenoids, alkaloids, carbohydrates, flavonoids with traces of tannin, coumarins, cardiac glycoside and phenols. Thus, the ethyl acetate mediated extract of *Euphorbia hirta* was found to have more bioactive compounds compared with aqueous and methanol extracts (Table 1).

Antibacterial activity:

Antibacterial efficacy of aqueous, methanol and ethyl acetate mediated crude extracts of *Euphorbia hirta* were tested against gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) using disc diffusion method. The observation with reference to antibacterial activity has been shown in Table 2. The results revealed the potential antibacterial activity of methanol and ethyl acetate mediated plant extracts against the growth of *Bacillus cereus*, a food poisoning bacteria and inhibiting the growth of tested pathogenic organisms. The aqueous extract showed mild to moderate inhibition of activity against tested organisms. However, the methanol extract of *Euphorbia hirta* showed a marked inhibition in the growth of *Pseudomonas aeruginosa* and the results obtained with reference to ethyl acetate extract showed moderate activity against all the tested organisms. A dose-dependant inhibition was recorded with ethyl acetate extract. Maximum activity was recorded with *Pseudomonas aeruginosa* with 18

Table 1: Phytochemical analysis of various extracts of *Euphorbia hirta*

Bioactive compounds	Name of solvent		
	Aqueous	Methanol	Ethyl acetate
Steroids	+	++	++
Terpenoids	+	++	++
Tannins	+	+	+++
Saponins	-	-	-
Coumarins	-	+	++
Alkaloids	+	++	+++
Cardiac glycosides	+	+	++
Carbohydrates	++	++	++
Flavonoids	+	++	+++
Phenols	-	+	++
Anthocyanins	-	-	-
Protein	-	-	-

mm in diameter with inhibition at 100 µg/ml concentration followed by *Staphylococcus aureus* (14 mm)> *Escherichia coli* (14 mm)> *Bacillus cereus* (13 mm)> *Proteus vulgaris* (13 mm). The results of antibacterial activity of the plant extract showed variable antibacterial activity of the plant extract with reference to aqueous and methanol extracts when compared with ethyl acetate extract, suggesting that the ethyl acetate mediated *Euphorbia hirta* plant extract is a better candidate for the identification and isolation of pharmacologically active compound. The ethyl acetate extract showed potential antibacterial activity against both gram-positive and gram-negative bacteria tested in the present study.

Antioxidant activity:

The antioxidant activity of different extracts of *Euphorbia hirta* (Table 3; Fig. 1) was evaluated using DPPH free radical scavenging activity. A dose-dependant free radical scavenging activity was observed with methanol and ethyl acetate extracts with the IC₅₀ value of 63.7 µg/ml and 47.8 µg/ml, respectively. The ethyl acetate mediated extract showed higher radical scavenging activity with the IC₅₀ value of 47.8 µg/ml. The results were compared with the standard ascorbic acid. However, with the aqueous plant extract the IC₅₀

value recorded was 181.86 µg/ml.

GC-MS analysis:

The phytochemical investigation of ethyl acetate was referenced with GC-MS and mass spectrum of unknown compounds were compared with the NIST library and the results have been presented in Table 4 and Figure 2. Eight major compounds were identified. The mass spectrometry analysis of the compounds eluted with time intervals was done to identify the structure of the eluted compound. In the present study GC-MS spectrum of ethyl acetate extract of *Euphorbia hirta* showed the presence of bioactive compounds such as Desulphosinigrin (RT 12.17), Ethyl iso - allocholate (RT 14.27), 9, 12, 15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy) methyl]ethyl ester, (ZZZ) (RT 16.00), Pentadecanoic acid, 14 - methyl, methyl ester (RT 17.23), 1 - Monolinoleoyl-glycerol trimethylsilyl ether (RT 17.62), 16-octadecenoic acid, methyl ester (RT 19.07), Ethyl iso-allocholate (RT 25.35) and 21- Acetoxyl-6a, 11a-dihydroxy-16a, 17a-Propylmethylenedioxy-pregna-1, 4-diene-3, 20-dione (RT 28.27).

Discussion

The major chemical compound identification from

Table 2: Antibacterial activity of various extracts of *Euphorbia hirta*

Extracts	Bacterial strains	Zone of Inhibition (mm)				
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Amoxicillin
Aqueous	<i>Bacillus cereus</i>	7	8	9	9	13
	<i>Staphylococcus aureus</i>	7	7	8	8	13
	<i>Escherichia coli</i>	7	8	8	9	14
	<i>Proteus vulgaris</i>	-	-	7	7	11
	<i>Pseudomonas aeruginosa</i>	8	8	9	9	16
Methanol	<i>Bacillus cereus</i>	9	9	10	10	15
	<i>Staphylococcus aureus</i>	8	8	9	10	14
	<i>Escherichia coli</i>	7	8	9	9	15
	<i>Proteus vulgaris</i>	8	8	9	10	13
	<i>Pseudomonas aeruginosa</i>	11	11	12	13	15
Ethyl acetate	<i>Bacillus cereus</i>	11	11	12	13	14
	<i>Staphylococcus aureus</i>	11	11	12	14	14
	<i>Escherichia coli</i>	10	10	11	14	15
	<i>Proteus vulgaris</i>	9	10	11	13	12
	<i>Pseudomonas aeruginosa</i>	16	16	17	18	16

Table 3: Antioxidant activity of different extracts of *Euphorbia hirta*

Concentration µg/ml	Aqueous	Methanol	Ethyl acetate
40	16.858 ± 0.2238	42.852 ± 0.2479	47.062 ± 0.2760
80	22.868 ± 0.1575	54.870 ± 0.3631	61.944 ± 0.2499
120	29.782 ± 0.2226	66.722 ± 0.160	82.982 ± 0.2186
160	42.494 ± 0.2049	87.954 ± 0.2657	91.224 ± 0.1988
200	56.226 ± 0.1718	92.144 ± 0.2759	94.842 ± 0.1930
IC ₅₀	181.86 µg/ml	63.7 µg/ml	47.8 µg/ml

Fig. 1: Antioxidant activity of different extracts of *Euphorbia hirta*.

Fig. 2: GC-MS Chromatogram of ethyl acetate leaf extract of *Euphorbia hirta*.

Table 4: GC-MS analysis of ethyl acetate leaf extract of *Euphorbia hirta*

S. No	RT	Name of the compound	Molecular formula	Molecular weight
1	12.17	Desulphosinigrin	279.31008 g/mol	C ₁₀ H ₁₇ NO ₆ S
2	14.27	thyl iso-allocholate	436.62456 g/mol	C ₂₆ H ₄₄ O ₅
3	16.00	9,12,15-Octadecatrienoic acid, 2- [[trimethylsilyl]oxy]-1- [[[(trimethylsilyl)oxy] methyl] ethyl ester, (ZZZ)	278.4296 g/mol	C ₁₈ H ₃₀ O ₂
4	17.23	Pentadecanoic acid, 14-methyl, methyl ester	270.4507 g/mol	C ₁₇ H ₃₄ O ₂
5	17.62	1-Monolinoleoylglycerol trimethylsilyl ether	498.8863 g/mol	C ₂₇ H ₅₄ O ₄ Si ₂
6	19.07	16-Octadecenoic acid, methyl ester	296.48794 g/mol	C ₁₉ H ₃₆ O ₂
7	25.35	Ethyl iso-allocholate	436.62456 g/mol	C ₂₆ H ₄₄ O ₅
8	28.27	21-Acetoxy-6a, 11a-dihydroxy-16a, 17a-propylmethylenedioxypregna- 1,4-diene-3,20-dione	488.56994 g/mol	C ₂₇ H ₃₆ O ₈

the plant extracts were referred as phytoanticipins and phytoprotectants (Salehi *et al.*, 2019). In the present study the analysis of aqueous, methanol and ethyl acetate extracts of *Euphorbia hirta* revealed the presence of various bioactive compounds such as steroids, terpenoids, tannins, coumarins, alkaloids, cardiac glycosides, carbohydrates, flavonoids and phenols and these bioactive compounds could be responsible for the recorded antibacterial activity against the tested organisms. The number of clinical infections were increased due to the development of resistance among pathogenic organisms against the standard antibiotics (Priscila *et al.*, 2007). Nowadays, it is common to use antibiotics frequently to treat minor health problems, which results in the development of resistance among pathogenic organisms and the occurrence of drug resistance was reported to be very high in Asia Pacific Region (Al Farraj *et al.*, 2020). Thus, it is essential to find plant-based drugs with hydrophobic nature that can react with the cell membrane of bacteria and

increase the permeability and thereby damage the cells along with the change in the organisation of mitochondria (Tiwari *et al.*, 2009).

Euphorbia hirta plant extracts were tested against gram-positive and gram-negative bacteria using the disc diffusion method. The *S. aureus* infection is one of the common sources of food borne infection, while *E. coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* were reported to induce gastroenteritis diseases (Mustafa *et al.*, 2018). The ethyl acetate mediated *Euphorbia hirta* extracts were effective against all the tested organisms. Gill and Holley (2006) have reported that the plant metabolites such as terpenoids, alkaloids and flavonoids will interact with the cell membrane proteins and enzymes causing damage to the membrane which results in the dispersion of flux of protons out of the cells leading to the death of the cell. The results of present study coincides with the findings as the maximum amount of alkaloids, terpenoids and phenolic compounds

such as flavonoids and tannins were reported with ethyl acetate mediated *Euphorbia hirta* extract.

Shanmugapriya *et al.* (2012) have reported that ethyl acetate extract has moderate antibacterial activity against clinical isolates. In contrast to the above findings, the results of the present study showed that the ethyl acetate extract possess potential antibacterial activity against all the tested organisms. The antibacterial activity of the extracts was due to the presence of major bioactive compounds (Navarro *et al.*, 1996).

Reactive oxygen species are found to be responsible for many diseases. It is essential to neutralize these free radicals and ROS formed in human body by several endogenous and exogenous factors to prevent the onset of tissue damages (Bulbul *et al.*, 2011). The plant metabolites were considered as the best antioxidant agents to scavenge the free radicals. In the present study the DPPH free radical scavenging activity was performed with aqueous, methanol and ethyl acetate extracts of *Euphorbia hirta*.

The results obtained in the present study showed that the ethyl acetate extract possess a strong radical scavenging activity with an IC₅₀ value of 47.8 µg/ml followed by methanol extract with IC₅₀ value of 63.7 µg/ml. The observed results agree with the work of Bakr *et al.* (2012), who stated that the ethyl acetate fraction of *Euphorbia hirta* showed potential free radical scavenging activity with lowest IC₅₀ value. The phenolic compounds present in the plants were responsible for 98.89% of antioxidant activity (Basma *et al.*, 2011). The results suggested that the presence of flavonoids and other phenolic compounds supports the above findings with highest antioxidant activity recorded with the tested extracts. The phenolic compounds play a major role in the direct antioxidative activity due to the presence of hydroxyl groups (Yen *et al.*, 1993). The antioxidant potential of aqueous extract was very meagre, which may be due to the presence of lesser amount of phenolic compounds, which

confirms the findings that the phenolic compounds are more responsible for the antioxidant potential of the plant extracts.

The GC-MS spectrum of ethyl acetate extract confirms the presence of eight major compounds with different retention times (RT). The presence of components in GC-MS correlated with their biological functions. The traditional knowledge on the medicinal plants can give way for the identification of new drugs with modern screening techniques (Balamurugan *et al.*, 2012). The compounds identified with reference to GC-MS analysis may be responsible for the antibacterial efficacy of the ethyl acetate extract against gram-positive and gram-negative bacteria tested in this study. The presence of Desulphosinigrin (Sosa *et al.*, 2016), Ethyl iso-allocholate (Huang *et al.*, 2011; Muthulakshmi *et al.*, 2012), Pentadecanoic acid, 14-methyl, methyl ester (Beschi *et al.*, 2021) and 1-Monolinoleoylglycerol trimethyl-silyl ether (Meenakshi *et al.*, 2012) were reported to possess broad spectrum antimicrobial activity. Desulphosinigrin, 9,12,15-Octadecatrienoic acid, 2- [(trimethylsilyl)oxy] - 1- [((trimethylsilyl)oxy) methyl]ethyl ester, (ZZZ), Pentadecanoic acid, 14-methyl, methyl ester were reported to possess antioxidant activity (Krishna *et al.*, 2012; Elezabeth and Arumugan, 2014; Hussein, 2016; Tian *et al.*, 2018) The present results are correlated with the presence of bioactive compounds in the ethyl acetate extract and their potential antibacterial activity observed in the present study. It is clear from the present results that the tested plant *Euphorbia hirta* consists of enormous bioactive compounds with the potential of pharmacological constituents and phyto-constituents responsible for antibacterial and antioxidant activities. Thus, the identification of different phytochemical components from ethyl acetate extract of *Euphorbia hirta* exhibits the significant medicinal properties of the plant.

Conclusion

Ethyl acetate mediated crude extracts of *Euphorbia hirta* was tested for its antibacterial and

antioxidant activities and the results showed that this plant has potential for these activities. The GC-MS analysis of the ethyl acetate extract reveals the presence of specific compounds which can be isolated, characterised and can be used as a reliable and potential therapeutic medicine.

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