Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(2): 183-186

Received: 24-01-2017
Accepted: 25-02-2017

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Phytochemical Screening and antimicrobial study of *Euphorbia hirta* extracts

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Abstract
Medicinal plants have biologically compounds which are used for treating various human diseases and also play an important role in curing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents involve chlorophyll, proteins sugar and amino acids whereas secondary constituents contain terpenoids and alkaloids. Due to the presence of these secondary constituents medicinal plants show antifungal, antibacterial and anti-inflammation activities. The present study was designed to investigate the phytochemical screening and antimicrobial activities of *Euphorbia hirta* extracts. Phytochemical screening revealed the presence of alkaloid, flavonoid, saponin, terpenoid, steroid and sterols in the extracts of aerial part of *Euphorbia hirta*. Methanol, chloroform and hexane extract of leaf and fruit were tested against *Proteus mirabilis, Listeria monocytogenes, Clostridium absonum, Aspergillus niger, Aspergillus fumigates, Arthrographis cuboidea* by the agar disc diffusion method.

Keywords: Phytochemical, antimicrobial activity, Euphorbia hirta

1. Introduction
India has tremendous wealth of aromatic and medicinal plants. In current days medicinal plants play a key role as pillar of traditional healthcare systems of medicine in many developing countries. Since from the ancient times, several drugs have been formulated using the bioactive compounds present in these medicinal plants [1]. According to World health organization (WHO) more than 80% world's population depends on medicines derived from these medicinal plants for primary health care needs. The use of medicinal plants as a source for relief from illness can be traced back over since before recorded history. These phytomedicines are safe and environment friendly [2]. Phytomedicines have become increasingly popular and their use is widespread. Plants produce a varied range of bioactive molecules these are called phytochemicals, Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that make them may have little need for them [3, 4]. These secondary metabolites are synthesized naturally in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components making them rich sources of different types of phytochemicals. Mostly, these phytochemicals are secondary metabolites like flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenol compounds, etc [5]. These compounds extracted from different parts of plant. The amount of phytochemical compounds differ significantly from species to species and even from plant to plant, depending on the age and different ecological and climatic conditions. In current years, phytochemicals which have unknown pharmacological activities have been widely investigated as a source of phytomedicine [6].
Today there is growing awareness in chemical composition of plant based medicines. A large number of bioactive constituents have been isolated and studied for medicinal activity [7-9]. During the last two decades, the pharma industry has made massive investment in pharmacological and chemical researches all over the world to discover much more potent drugs, quite, a few new drugs. Plants have effectively passed the tests of commercial screenings [10, 11]. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents a systematic investigation was under taken to screen the Phytochemical and antimicrobial activity of leaf and flower of *Euphorbia hirta*. Euphorbia hirta is an important plant for medicinal herb. Euphorbia hirta belong to genus Euphorbia and family Euphorbiaceae [12]. It is a small annual herb and it is

common to tropical countries. It can grow to a height of 40cm [13]. Euphorbia hirta is a popular herb in practitioners of traditional herb medicine. Euphorbia hirta is also called asthma herb and pill bearing spurge [14, 15]. The steam of Euphorbia hirta is slender and other reddish in color and covered with yellowish bristly hair especially in young part of Euphorbia hirta. The leaves of Euphorbia hirta are arranged oppositely and are usually reddish or greenish underneath measuring about 5cm long [16-18].

2. Materials and methods

2.1 Plant Collection and Authentication

The leaves and flower of *Euphorbia hirta* was collected from the garden of Uttaranchal university, Dehradun and authenticated by Botany department of FRI, Dehradun. The leaves and flower of collected plant material were washed thoroughly 2-3 times with running water and once with sterile distilled water.

2.2 Preparation of extract

Shade-dried plant leaves and flower chopped into small pieces by using mortar and pestle, grinded into powdered form. The powdered plant material was subjected to sequential solvent extraction by soxhlet extraction method. The extraction was done with different solvents in their increasing order polarity such as hexane chloroform and methanol. All the extracts were evaporated using rotary evaporator and the percentage yield was thus recorded. Dried extracts were stored in airtight containers for further studies. Concentrated extracts were subjected to various chemical tests in order to detect the various phytoconstituents.

2.3 Phytochemical screening

The concentrated extracts of selected plant was subjected to different chemical tests for the detection of different phytoconstituents using standard methods [19, 20].

(i)Test for saponins

Crude extract when mixed with 5ml distilled water in a test tube then it was shaken briskly. The formation of stable foam which indicate the presence of saponins.

(ii) Test for flavonoids

Crude extract when mixed with 10ml distilled water, 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate solution then added 1ml concentrated sulphuric acid. Indication of yellow color shows the presence of flavanoids.

(iii) Test for steroids

The crude extract of selected plant was dissolved in 0.5mL dichloromethane to prepare a dilute solution and then 0.5 mL of acetic anhydride was added followed by four drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

(iv) Test for tannins

Curde extract of plant was mixed with small amount of water and heated on water bath. The mixture was filtered and ferric chloride was added drop by drop to the filtrate. A dark green appear which indicates the presence of tannins.

(v) Test for Alkaloids

Curde extract was dissolved with 2ml of 1% HCl and heated gently. Wagners and Mayers reagents were added to the mixture. Turbidity of the resulting precipitate was taken as confirmation for the presence of alkaloids.

(vi) Test for carbohydrate

Both Felhing A and Felhing B solution were mixed in equal volume. These reagent are added in crude extract and smoothly boiled. A brick red precipitate is appeared at the bottom of the test tube and indicate the presence of reducing sugar.

2.4 Becterial culture

The human becteria such as *Streptococcus mutans, Streptococcus aureus, Clostridium absonum, Listeria monocytogenes, Escherichia coli* and *Proteus mirabilis* were obtained from culture collection center department of biotechnology of Uttaranchal University Dehradun and were maintained in Nutrient agar at 4 ºC for experiment studies. The different fungus strains such as *Arthogrophis cuboida, Aspergillius fumigates* and *Aspergillius nigar* were isolated from potato dextrose agar.

2.5 Preparation of standard culture inoculums of test organism

The colonies of different bacteria and strains of different fungus were inoculated in the 20ml nutrient broth and incubated for 24- 72hours.

2.6 Assay of anti-bacterial activity-

Assay of anti-bacterial activity of leaf and flower extract of *Euphorbia hirta* was done by Disc Diffusion method. In this method 20ml of sterilized Mueller Hinton Agar was poured into sterile petri plates, after solidification, 120µl of bacterial culture poured on the plates and the culture was spread on plates using spreader. Then, the Whatmans filter paper discs (6mm in diameter) were kept over the agar plates using sterile forceps at various concentrations. Concentrated solvent was used as negative control. The anti-bacterial assay plates were kept incubator, where all the plates were incubated at 37ºc for 24hours. The diameter of inhibition zone was noted down.

2.7 Assay of anti-fungal activity

Assay of anti-fungal activity of leaf and flower extract of *Euphorbia hirta* was done by Disc Diffusion method. In this method 20ml of sterilized Mueller Hinton Agar was poured into sterile petri plates, after solidification, 120µl of fungus culture poured on the plates and the culture was spread on plates using spreader. Then, the Whatmans filter paper discs (6mm in diameter) were kept over the agar plates using sterile forceps at various concentrations. Concentrated solvent was used as negative control. The anti-fungus assay plates were kept incubator, where all the plates were incubated at 37ºc for 24hours. The diameter of inhibition zone was noted down

3. Results and discussion

Plants are playing crucial role in traditional Indian system of medicine. Studies on medicinal plants are gaining consensus in recent years in India and Abroad. In the present study, three different extracts (ethanol, chloroform, hexane) of leaves and flower of *Euphorbia hirta* were subjected to qualitative phytochemical analysis to explore its anti-microbial activity for its therapeutic applications.

The percentage yields of extracts and the phytochemical constituents of the plants are shown in table 1 and 2 respectively. The highest yield of leaves extract was found when extraction was done with ethanol and the lowest in case

of hexane. The highest yield of flower and leaves extract was found when extraction was done with ethanol and the lowest in case of hexane this is most probably due to change in the polarity of solvents. Ethanol is the highest and hexane is the lowest polar solvent. The result of our study clearly indicate that for extraction ethanol is the best solvent

Table 1: Plant samples with yield of extraction (%)

Plant Sample	Ethanol Extract (%)	Chloroform Extract (%)	Hexane Extract (%)
Leaves (1)	5.29	4.72	1.84
Flower (2)	3.26	2.54	1.63

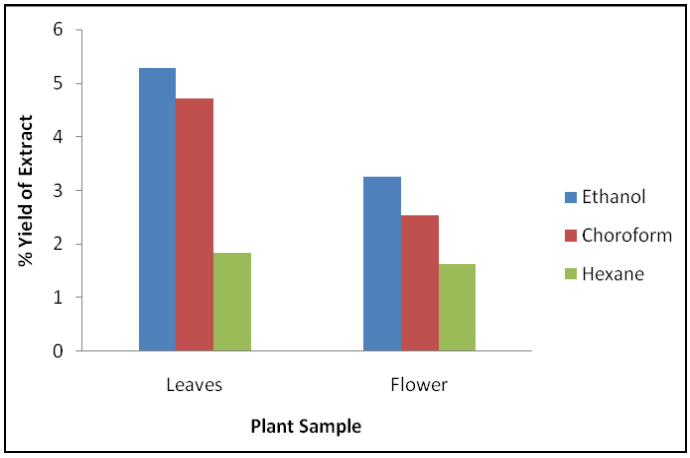

Table 2: Preliminary phytochemical analysis of flowers and leaves of *Euphorbia hirta*

Phytochemical constituents	Leaves Extract			Flower Extract		
	Ethanol	Chloroform	Hexane	Ethanol	Chloroform	Hexane
Alkaloids	+	+	_	+ +	+	-
Flavanoids	+	+	+	+	+	+
Terpenoids	+	+	_	+	+	-
Tannins	_	+	_	+	+	-
Saponins	+	_	_	_	_	-
Carbohydrate	+	+	-	+	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals

The result of the preliminary phytochemical screeing from leaves and flowers of *Euophorbia hirta* shows in table 2. The present study reveals the phytochemical screening and qualitative estimation of leaves of *Euphorbia hirta* showed the presence of alkaloid, flavanoid, tannin, terpenoid and carbohyrate in choroform. In ethanol the extract of leave of *Euphorbia hirta* show the presence of alkaloid, flavanoid, sponins, carbohydrate and terpenoids. The hexane extract was exhibited flavonoid in leave extract of *Euphorbia hirta*. The ethanol extract of flower showed the presence of alkaloid, flavanoid terpenoid, tannin and carbohydrate. The chloroform extract of the flower of *Euphorbia hirta* showed the presence of alkaloid, flavonoid, terpenoid, tannin and carbohyrate. The hexane extract of flower exhibited the presence of flavanoid.

3.1 Antibacterial activity

Antibacterial activity is that anything that destroys bacteria or suppresses their growth or their ability to reproduce themselves. Heat, chemicals such as chlorine and antibiotic drugs all have antibacterial properties. Antibacterial are used to treat bacterial infections. The toxicity to humans and other animals from antibacterial is generally considered low. Antibacterial have a negative impact on health.

Result of the antibacterial activity of the isolated extract by using different solvent (ethanol, chloroform, hexane) was showed in table 3. The dried leaf and flower extract of *Euphorbia hirta* shown to posses antibacterial activity. The antibacterial activity of ethanol, chloroform and hexane of extract of leaf and flower of *Euphorbia hirta* were inspected against the selected experiment pathogens such as *Streptococcus mutans*, *Streptococcus aureus*, *Proteus mirabilis*, *Clostridium absonum*, *Listeria monocytogenes* and *Escherichia coli* by disc diffusion method. The tested microbial organism shows varying degree of antibacterial activities in examined plant extract. Chloroform extracts of leaf showed maximum zone of inhibition against *S-mutans* (20mm) which is a gram positive bacteria that is the primary causative agent in the formation of dental cavities in human and animals. The hexane extract of leaf extract of *Euphorbia hirta* showed the maximum zone of inhibition against in *Clostridium* (10mm) which is also gram positive bacteria and cause food poisoning, pneumonia and brain abscess. The hexane extract of flower extract of *Euphorbia hirta* showed minimum zone of inhibition was observed *Clostridium* (4mm). The ethanol extract was exhibited moderate activity against *S-mutans* of flower of extract of *Euphorbia hirta*.

Table 3: Anti-bacterial activity of leaves and flowers of *Euphorbia hirta*

Microorganisms	Leaves Extract			Flower Extract		
	Ethanol	Chloroform	Hexane	Ethanol	Chloroform	Hexane
S-mutans	-	+	-	+	-	-
P-mirabilis	-	-	-	-	-	-
Clostridium	-	-	+	-	-	+
Listeria	-	-	-	-	-	-
E-coli	-	-	-	-	-	-
S-aureus	-	-	-	-	-	-

+ = indicates presence of antibacterial activity, - = indicates absence of antibacterial activity

3.2 Antifungal activity

Antifungal activity is that anything that kills fungi or inhibits their growth or control of fungi infection. Antifungal is used to treat infection caused by a fungus. Antifungal drugs include amphoterin, griseofulvin, the lesimidazo, nystatin, teebinafine and tolnaftate.

Result of the antifungal activity of the isolated extract by using different solvent (ethanol, chloroform, hexane) was

showed in table 4. The antifungal activity of ethanol, chloroform and hexane of extract of dried leaf and flower of *Euphorbia hirta* were inspected against the selected experiment pathogens such as *Arthogrophis cuboida*, *Aspergillius fumigates* and *Aspergillius nigar* by disc diffusion method. The extract of the dried leaf and flower of *Euphorbia hirta* does not show any antifungal activity against selected pathogens in any solvents.

Table 4: Anti-fungal activity of leaves and flowers of medicinal plant

Microorganisms	Leaves Extract			Flower Extract		
	Ethanol	Chloroform	Hexane	Ethanol	Chloroform	Hexane
Arthogrophis Cuboida	-	-	-	-	-	-
Aspergillius fumigates	-	-	-	-	-	-
Aspergillius nigar	-	-	-	-	-	-

4. Conclusion

Phyotochemical analysis and antimicrobial study of any selected plant species is a very significant way to establish that the selected plant species may be use as potent drugs. In our present study we select commonly found plant *Euphorbia hirta* which is easily available in our campus. It is a well-known medicine for inflammation of respiratory tract and for asthma as it has a special reputation for causing bronchial relaxation. It can also be used as diuretic and purgative action. The above points clearly illustrate that the plants studied here can be seen as a potential source of useful bioactive compounds. In this study, we found that the leaves and flowers extract of the plant contain large amount of alkaloids and flavanoids along with terpenoids, saponins, tannins and carbohydrate in small ration so these parts of the plant can be used as an important source of phytochemical and antimicrobial activity. On the basis of our antimicrobial study we find that our selected plant shows significant antibacterial activity against selected gram positive strains. In addition to antibacterial study we also perform antifungal activity against selected fungal strains but unfortunately isolated extracts of selected plant parts does not show any significant activity against selected fungal strains. So the study our results clearly indicate that we may use our plants as potent antibacterial drugs of natural origin. Further work will give emphasis to the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

5. References

1. Rahmati H, Salehi S, Malekpour A, Farhangi F. International Journal of Molecular Medicine and Advance Science. 2015; 11:9
2. Bansod S, Rai M. World Journal of Medicial Science. 2008; 3:81.
3. Suresh SN, Rathshkumar S, Rajeshwari V, Sagadevan P, Gayathri S, Vithya D. Esward International Journal of Pharmacy and Life Science 2012; 3:2209.
4. Afroz RST, Mondal B, Khan R, Ahmed S. International Journal of Research in Pharmacy and Chemistry. 2012; 2:1001
5. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar Ashad R. *Molecules.* 2013; 2:1001.
6. Yadav RNS, Agarwala M. Journal of Phytology. 2011; 3:10
7. Thilagavathi T, Arivindganth R, Vidhya D, Dhivya R. International Journal of Pharmacy. 2015; 6:246.
8. Edoga HO, Okwu DE, Mbaebie BO. Afr. Journal Biotechnol. 2005; 4:685.
9. Kimura Y, Akihisa T, Yuasa N, Ukiya M, Suzula T. Journal Nat. Prod. 2005; 68:807
10. Abubakar EM. Journal Medicinal Plants Research. 2009; 3:498
11. Hung L, Chen S, Yang M. Journal of Medicinal Plants Research. 2012; 6:5176
12. Akinnmade JF, Oyeleye OA, Journal Biotechnology. 2010; 9:5028
13. Dhass A, kumar S, DNSRBS, L.M, Journal Neural Transm, 2008; 115:35
14. Abu-Sayeed M, Ali MA, Bhattacharjee PK, Islam A, Astaq GRM, Khan M *et al*. Pakistan Journal of Science and Industrial Research. 2005; 48:122
15. Anuradha H, Srikumar BN, Shankaranarayana Rao BS. Journal of Neural Transmission. 2008; 115:35
16. Saravanan R, Dhachinamoorthi D, Senthilkumar K, Srilakshmi M, Divya Sri T. *IJRAP*, 2012; 3:439
17. Rajeh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Molecules. 2010; 15:6008
18. M. Hussain, U. Farooq, M. Rashid, H. Bakhsh, A. Majeed, I. A. Khan, S. L. Rana, M. Shafeeq-ur-Rahman, A. Aziz, International Journal of Pharma Science. 2014; 4:546
19. Harborne JB. Chapman and Hall London. 1998; 1:135
20. Sofowora A. Medicinal plants and Traditional medicine in Africa. 2nd Edition, John Wily and Sons, 1993; 6:56