



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research  
Vol. 9, Issue, 8(D), pp. 28534-28538, August, 2018

**International Journal of  
Recent Scientific  
Research**

DOI: 10.24327/IJRSR

## Research Article

# EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF EUPHORBIACEAE MEMBERS AGAINST HUMAN PATHOGENS

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DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0908.2476>

### ARTICLE INFO

#### Article History:

Received 6<sup>th</sup> May, 2018  
Received in revised form 10<sup>th</sup> June, 2018  
Accepted 24<sup>th</sup> July, 2018  
Published online 28<sup>th</sup> August, 2018

#### Key Words:

Euphorbiaceae, antibacterial activity, Phytoconstituents, phenols, flavanoids

### ABSTRACT

The members of Euphorbiaceae are cosmopolitan comprises about 8000 known species belonging to 340 genera with an array of growth from including herbs, shrubs, trees, geophytes and multiple succulent forms. The present study was carried out on the evaluation of preliminary phytochemical and antibacterial activity of ethanolic extracts of Euphorbiaceae members such as *Euphorbia milii*, *Euphorbia hirta*, *Euphorbia pulcherrima*, *Euphorbia tithymaloides*, *Euphorbia prostrata* and *Emblica officinalis* against human pathogens in Dehradun, Uttarakhand. The plant materials were collected from different places of Dehradun, Uttarakhand, dried at room temperature for the crude drug extraction by using ethanol. Phytochemical screening of these extracts showed the presences of number of secondary metabolites like alkaloids, carbohydrates, flavonoids, proteins and phenolic compounds while absence of amino acids, glycosides and saponins. Different extracts of the plants were assayed for antibacterial activity against the pathogenic bacterial strains. Out of six plant extracts only two plants such as *Emblica officinalis* and *Euphorbia milii* were showing maximum activity against all the bacterial strains. Therefore, Minimum Inhibitory Concentration (MIC) of these two plant extracts were determined against the pathogenic bacterial strains showing zone of inhibition  $\geq 10$ mm. The presence of secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins and phenolic compounds in all the selected plant extracts and MIC of *Emblica officinalis* and *Euphorbia milii* possess good antibacterial activity. Thus, in future the plant extracts could be exploited to control various bacterial infections such as urinary track infection, food poisoning, skin diseases etc.

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### INTRODUCTION

Plants have a great potential for producing new drugs of large benefit to mankind. The World Health Organization (WHO) estimates that 80% of the world's inhabitants rely mainly on traditional medicines for their health care (Anonymous, 1998). The tribal and rural people of various parts of India are highly depending on medicinal plant therapy for meeting their health care needs. This attracts the attention of several botanists and plant scientists who directing vigorous researches towards the discovery or rediscovery of several medicinal plants along with their medicinal remedies for various diseases (Khare *et al.*, 2004). Many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is

therefore, should be utilized to combat the diseases causing pathogens (Kamali *et al.*, 2010; Lalitha *et al.*, 2010; Hussain *et al.*, 2011). Many medicinal plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today the search for natural compounds rich in antimicrobial properties are escalating due to their medicinal importance in controlling many diseases. The rapid emergence of multiple drug resistant strains of pathogens to current antimicrobial agents has generated an urgent intensive search for new antibiotics from medicinal plants (Chopra *et al.*, 1997). Human infections constitute a serious problem especially in tropical and subtropical countries. The use of antibiotics and chemically synthesized medicines cures microbial infections very fast but they may also disturb the natural immunity of the body and cause variety of side effects. This has aroused interest in

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plant products which may partially support or substitute synthetic drugs. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistance pathogens (Ahmed *et al.*, 2001). Hence, researchers have been recently paid attention to safer phytochemical and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Pavithra *et al.*, 2010; Warriar *et al.*, 1995). The members of Euphorbiaceae are cosmopolitan comprises about 8000 known species (Radcliffe-Smith *et al.*, 2001) belonging to 340 genera (Webster *et al.*, 1994) widely distributed throughout both hemispheres that comprises an impressive array of growth forms including herbs, shrubs, trees, geophytes and multiple succulent forms. Despite the morphological diversity, the plants are characterized by specialized, highly reduced flowers, inflorescence (Cytium) (Horn *et al.*, 2012; Riina *et al.*, 2013). Most of members are lactiferous producing a white latex that contains number of secondary metabolites (Jassbi *et al.*, 2006; Pintus *et al.*, 2010; Gunawardana *et al.*, 2015). Which possess antiproliferative activity (Xu *et al.*, 1998), cytotoxicity (Fatope *et al.*, 1996), antimicrobial activity (Murugan *et al.*, 2007), anti-inflammatory activity (Asmawi *et al.*, 1993) etc. Tribes and local people used these plants for remedy against various diseases and disorders such as skin diseases, gonorrhoea, migraines, intestinal parasites, warts, fever and for mediating pain perception. Thus, the plant species belonging to Euphorbiaceae family which are commonly found in diverse habitats of Dehradun, Uttarakhand, India are selected for the present study to evaluate their preliminary phytochemical analysis followed by antibacterial characterization against human pathogens.

## MATERIAL AND METHODS

### Location of the experiment and climatic conditions

The present experiment was carried out at Botany laboratory in the Department of Life Sciences, Shri Guru Ram Rai Institute of Technology & Science, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. Dehradun, capital of Uttarakhand, located between the latitude 29°55' and 38°31'N and longitude 77°35' and 78°20'E, covering an area of 2002.4sq.Km with an elevation of 2000m above the sea level. The average annual rainfall is 2073.3mm.

### Material

The material for the present study comprised of important members such as *Euphorbia milii*, *Euphorbia pulcherrima*, *Euphorbia prostrata*, *Euphorbia hirta*, *Euphorbia tithymaloides*, *Emblica officinalis* of Euphorbiaceae family, that grow in different places of Dehradun, Uttarakhand.

### Experimental methodology

#### Collection and processing of plant material

Plant material of all the important members of the Euphorbiaceae family were collected from the different places of Dehradun, Uttarakhand and were dried in shade at 25°C to

35°C for 20-25 days and crushed to coarse powder by using grinder. The dried plant materials were stored in paper bags.

#### Extraction of crude drug

The dried plant materials were subjected to infusion in ethyl alcohol. About 50gm accurately weighted plant samples were taken into beakers (500ml) and about 250ml of ethanol were also taken and filled into the beaker, soaking the extracts for 48 hours, at room temperature. After that, the contents were filtered through muslin cloth into a 250ml beaker and their solvents were evaporated on the water bath till they were finally reduced to dryness to get dry extracts. The extracts were then transferred to previously weighted vials and were stored in the dark place until they were screened for their phytochemical and antibacterial activity.

#### Percentage yield

Formula for percentage yield of the extracts:

Percentage yield (%) = weight of extract /weight of powdered drug taken ×100

#### Phytochemical analysis

The various extracts of Euphorbiaceae family were subjected to preliminary qualitative phytochemical investigation. The various tests were performed for the estimation of protein, amino acids, carbohydrates, alkaloids, phenol, flavonoids, saponins and glycosides.

#### Antibacterial assay

An antibacterial is a substance that kills or inhibits the growth of bacteria. Antibacterial drugs either kill bacteria or prevent the growth of bacteria.

#### Source of bacterial strains

The antibacterial assay of different extracts of Euphorbiaceae family were performed against five pathogenic bacterial strains i.e. two Gram positive – *Staphylococcus aureus*, *Bacillus cereus* and three Gram negative – *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. All the pathogenic bacterial strains were procured from Shri Guru Ram Rai Medical College, Patel Nagar, Dehradun, Uttarakhand.

#### Antibacterial assay method

The antibacterial assay was performed by the disc diffusion method using pre sterilized disc made from whatman filter paper of diameter 6mm. The disc diffusion method tests the effectiveness of antibiotics on some specific microorganisms. Here we use this method to screen out the antibacterial activity of the plant extracts against the particular bacterial strains. 50µl of inoculum was spread on the solidify agar surface with the help of spreader. Then by dipping the pre-sterilized filter paper discs in a known concentration of the plant extracts and discs were then placed on an agar growth medium containing a confluent lawn of bacteria. Then the petri plates were shifted into the incubator for 24 hours at 37°C temperature for proper incubation. Next day check the growth if there is absence of bacterial growth around the discs containing the plant extracts, it indicates that the respective

plant extract shows antibacterial activity against that particular bacterial strain.

Minimum Inhibitory Concentration is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of microorganisms after overnight incubation. In the experiments, the MIC value of extracts were determined only against those bacterial strains which shows high activity during the preliminary antibacterial testing, MIC analysis was performed by serial dilutions of the active concentrated plant extracts in pure DMSO to achieve the decreasing concentration range of the 1000mg/ml to 31.25mg/ml. By using the different concentration of the plant extracts i.e. the zone of inhibition around the discs with lowest concentration to which the bacterial strains (organism) was susceptible would be determined as MIC of the plant extracts against the particular bacterial strains.

## RESULTS AND DISCUSSION

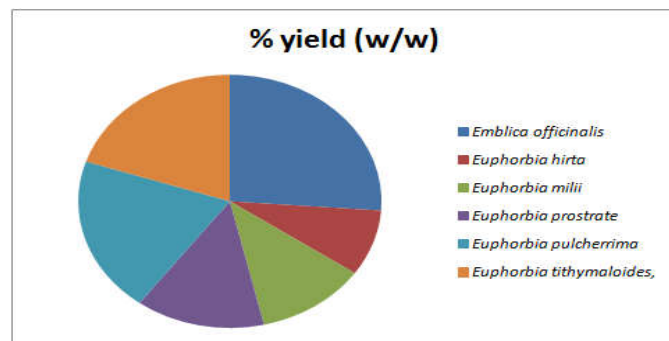
Euphorbiaceae is one of the largest family of flowering plants, comprising about 8000 known species (Radcliffe-Smith *et al.*, 2001) belong to 340 genera (Webster *et al.*, 1994). Members of Euphorbiaceae family are widely distributed throughout both hemispheres and range in morphology from large desert succulents to trees and even some small herbaceous plant types. On the basis of available literature and review focusing mainly on various biological activities such as antiproliferative activity (Xu *et al.*, 1998), cytotoxicity (Fatope *et al.*, 1996), antimicrobial activity (Murugan *et al.*, 2007), antipyretic-analgesic activity (Hezarah *et al.*, 2005) etc. The experimental methodology that has been adopted for the present study includes ethanol extraction, concentration of the extracts followed by preliminary phytochemical characterization and antibacterial screening against pathogenic bacterial strains. Besides this, these plants are of great medicinal values and are used by tribes and local people for remedy against various diseases and disorders such as skin diseases, gonorrhoea, migraines, intestinal parasites, warts, fever and for mediating pain perception. The plant species belonging to family Euphorbiaceae included in the present study commonly found in gardens, roadsides, moist and shady places in Dehradun, Uttarakhand. Findings of the present study were discussed under the following heads:

### Yield and Appearance of crude extracts

For the extraction of total crude extract, organic solvent ethanol was used. The appearance of crude yield varies from light green to dark green in studied Euphorbiaceae members. Percentage crude extract ranges from 2.82%-7.4%. The lowest yield was in *Euphorbia tithymaloides* and highest percentage yield was in *Emblia officinalis*.

**Table 1** Appearance and yield of crude extracts of important members of Euphorbiaceae family

S. N.	Plant Sample	Appearance of crude extract	Weight of extracts(gm)	% yield (w/w)
1.	<i>Emblia officinalis</i>	Light Green	3.7	7.4
2.	<i>Euphorbia hirta</i>	Dark green	1.19	2.38
3.	<i>Euphorbia milii</i>	Light Green	1.67	3.34
4.	<i>Euphorbia prostrata</i>	Dark Green	1.96	3.92
5.	<i>Euphorbia pulcherrima</i>	Dark Green	2.81	5.62
6.	<i>Euphorbia tithymaloides</i>	Light Green	2.82	5.64



**Figure no.1** Graphical representation of percentage yield of Crude extracts of important members of Euphorbiaceae family

### Phytochemical characterization

The term phytochemical is generally used to refer to those chemicals which are derived from the plant source. Alkaloids, tannins, flavonoids, saponins, glycosides, terpenoids, steroids etc. are some classes of phytochemicals. They not only play different role in plants but also are biologically active to cure various ailments. These compounds show various biological activities like anticancer, antioxidants, anti-inflammatory activities etc (Kamali *et al.*, 2010; Lalitha *et al.*, 2010; Hussain *et al.*, 2011). Preliminary qualitative phytochemical screening of important members of the Euphorbiaceae family confirms the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, and proteins in all the six wild and ornamental studied plants. Carbohydrates were present in five plant extracts but not present in *E. tithymaloides*. Proteins were present in five extracts except *E. pulcherrima*. Glycosides and Amino acids were totally absent in all the plant extracts. Saponin was present in two extracts namely – *E. tithymaloides* and *E. pulcherrima* but absent in other four studied Euphorbiaceae members. Similarly, on the basis of previous literature on Euphorbiaceae members such as *E. helioscopia*, *E. milii*, *E. prostrata* revealed that alkaloids, flavonoids, glycosides and saponins were present in them (Singh *et al.*, 2017; Kamurthy *et al.*, 2015; Qaisar *et al.*, 2012).

**Table no 2** Phytochemical analysis of members of Euphorbiaceae

SN	Test	EO	EH	ET	EP	EPU	EM
1	Amino acid	-	-	-	-	-	-
2	Alkaloids	+	+	+	+	+	+
3	Carbohydrates	+	+	-	+	+	+
4	Flavonoids	+	+	+	+	+	+
5	Glycosides	-	-	-	-	-	-
6	Phenolic compounds	+	+	+	+	+	+
7	Proteins	+	+	+	+	-	+
8	Saponins	-	-	+	-	+	-

Note: + and – sign indicate the presence or absence of a compound respectively

**EO**– *Emblia officinalis*, **EH** – *Euphorbia hirta*, **ET** – *Euphorbia tithymaloides*, **EP** – *Euphorbia prostrata*, **EPU** – *Euphorbia pulcherrima*, **EM** – *Euphorbia milii*.

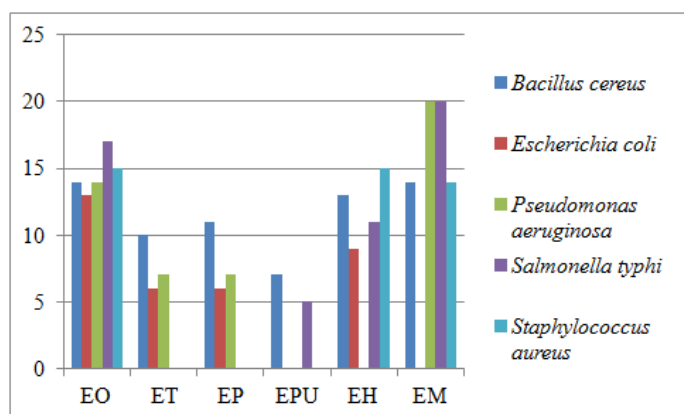
### Antibacterial assay

Different extracts of Euphorbiaceae family showed antibacterial activity against all the bacterial strains that are highly pathogenic to human beings causing several diseases such as food poisoning, urinary tract infection, pneumonia,

fever etc. Among the extracts assayed, two extracts were found most active that showed antibacterial activity against most of the studied bacterial strains. *E. tithymaloides* also showed inhibition activities against all the studied bacterial strains which ranged from 6mm (*E. coli*) to 17mm (*S. typhi*). *Emblica officinalis* showed inhibition activities against all the five studied pathogenic bacteria. *Euphorbia milii* showed maximum zone of inhibition i.e. 20mm against *S. typhi* and *P. aeruginosa* followed by *B. cereus*. Whereas no zone of inhibition was reported against *E. coli* bacteria. *E. tithymaloides* and *Emblica officinalis* also revealed the same 17mm zone of inhibition against *S. aureus*, *S. typhi* respectively. *E. tithymaloides* also showed the inhibition whereas *E. prostrata* and *E. pulcherrima* members showed minimum inhibition activities against most of the studied bacterial strains.

**Table no. 3** Zone of Inhibition (mm) against pathogenic bacterial strains (Preliminary antibacterial test for all plant extracts)

SN	Bacterials strains	EO	ET	EP	EPU	EH	EM	Positive control (Streptomycin)	Negative control (DMSO)
1	<i>Bacillus cereus</i>	14mm	10mm	11mm	07mm	13mm	14mm	32mm	-
2	<i>Escherichia coli</i>	13mm	06mm	06mm	-	09mm	-	30mm	-
3	<i>Pseudomonas aeruginosa</i>	14mm	07mm	07mm	-	-	20mm	17mm	-
4	<i>Salmonella typhi</i>	17mm	-	-	5mm	11mm	20mm	32mm	-
5	<i>Staphylococcus aureus</i>	15mm	-	-	-	15mm	14mm	12mm	-



**Figure no.2** Graphical representation of preliminary antibacterial screening of important members of Euphorbiaceae family

Minimum Inhibitory Concentration (MIC) is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent (Andrews *et al.*, 2001). An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge *et al.*, 2003). As the zone of inhibition greater than 13mm showed by *Emblica officinalis* and *E. milii*, therefore MIC was carried out with the crude extract of these two Euphorbiaceae members. MIC analysis was performed by serial dilution of the concentrated ethanol extract in pure DMSO to achieve a decreasing concentration range of 1000mg/ml to 31.25mg/ml.

On performing MIC analysis for ethanolic extract of *Emblica officinalis* the results revealed that all the selected bacterial strains i.e. *Pseudomonas aeruginosa*, *Bacillus*

*cereus*, *Escherichia coli* and *Salmonella typhi* were sensitive against 31.25mg/ml concentration of the extract whose MIC values were 31.25mg/ml while *Staphylococcus aureus* was sensitive against 62.5mg/ml concentration of the extract whose MIC was 62.5mg/ml.

**Table no. 4** Minimum Inhibitory Concentration (MIC) Analysis of *Emblica officinalis*

Bacterial strains	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
<i>Bacillus cereus</i>	18mm	12mm	11mm	10mm	8mm	6mm
<i>Escherichia coli</i>	13mm	11mm	10mm	9mm	7mm	5mm
<i>Pseudomonas aeruginosa</i>	17mm	11mm	8mm	7mm	7mm	6mm
<i>Salmonella typhi</i>	16mm	12mm	11mm	10mm	8mm	6mm
<i>Staphylococcus aureus</i>	16mm	13mm	11mm	7mm	6mm	-

On performing MIC analysis for the ethanolic extract of *E. milii*, the results revealed that all the selected bacterial strains i.e. *Pseudomonas aeruginosa* and *Bacillus cereus* were sensitive against the 250mg/ml concentration of the extract exhibiting 250mg/ml as their MIC value while *Salmonella typhi* was sensitive against 62.5mg/ml concentration of the extract whose MIC was 62.5mg/ml. No zone of inhibition was obtained around the disc impregnated with 31.25mg/ml concentration interpreting that all bacterial strains could resist this concentration of the extract. *Staphylococcus aureus* was sensitive against 125mg/ml concentration of the extract whose MIC was 125mg/ml. Similar studies were conducted by Bhaskara Rao *et al.*, (2010) and Ndam *et al.*, (2016) on *Euphorbia* species such as *E. hirta* and *E. golondrina* L.C. whereas Ndam *et al.*, (2016), antibacterial activities against *B. cereus*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* bacterial strains revealed significant results.

**Table no. 5** Minimum Inhibitory Concentration (MIC) Analysis of *Euphorbia milii*

Bacterial strains	1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
<i>Bacillus cereus</i>	17mm	10mm	6mm	-	-	-
<i>Pseudomonas aeruginosa</i>	20mm	12mm	10mm	-	-	-
<i>Salmonella typhi</i>	20mm	15mm	12mm	8mm	7mm	-
<i>Staphylococcus aureus</i>	14mm	10mm	9mm	7mm	-	-

## CONCLUSION

In the present study, ethanolic extracts of Euphorbiaceae members such as *Euphorbia milii*, *Euphorbia hirta*, *Euphorbia pulcherrima*, *Euphorbia tithymaloides*, *Euphorbia prostrata* and *Emblica officinalis* showed the presence secondary metabolites like alkaloids, carbohydrates, flavonoids, proteins and phenolic compounds while absence of amino acids, glycosides and saponins. Different extracts of the plants were assayed for antibacterial activity against the pathogenic bacterial strains. Out of six plant extracts only two plants such as *Emblica officinalis* and *Euphorbia milii* were showing maximum activity against all the bacterial strains. Thus, in future the plant extracts of euphorbiaceae members could be exploited to control various bacterial infections.

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#### How to cite this article:

Maneesha Singh and Sudha.,2018, Evaluation of Phytochemical and Antibacterial Activity of Euphorbiaceae Members Against Human Pathogens. *Int J Recent Sci Res*. 9(8), pp.28534-28538.DOI: <http://dx.doi.org/10.24327/ijrsr.2018.0908.2476>

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