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Research article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *EUPHORBIA HIRTA* AND *CALOTROPIS PROCERA* AGAINST MDR PATHOGENS

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ABSTRACT: The present study is carried out by evaluation of Euphorbia hirta and Calotropisprocera against MDR cultures. In preparation of plant extract methanol, ethyl acetate, acetone and Hot water were used as a solvent. Further phytochemical analysis and MIC was performed. *In Euphorbia hirta* the best results were observed in methanolic extract against *P.aeruginosa, S.aureus* and *E.coli* as compare to *Calotropisprocera.* The leaves and stems were used as a sample part for antibiogram analysis. The least concentration was obtained 4.6 mg/ml against E.coli and 1.66 mg/ml against S.aureus in Euphorbia hirta leaves while MIC of ethylacetate extract is observed 0.75 mg/ml against *E.coli,* 1.66 mg/ml against S.aureus and 4.6 mg/ml against P.aeruginosa of *Calotropisprocera* leaves.
Keywords: Phytochemical analysis, Antibiogram and MIC.

INTRODUCTION

Many centuries medicinal plants have been used for curing disease, bubonic plague, tuberculosis, malaria, and recently, the human immune deficiency virus acquired immunodeficiency syndrome pandemic, have affected substantial portions of the human population, causing significant morbidity and mortality [1,2].

Due to which these herbs are staging a comeback as these herbal product signify the safety in contrast to the synthetics that are regarded as unsafe to human as well for the environment.

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species at one time or others were used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. The economic importance of medicinal plants is much more to countries such as India than to rest of the world. *Euphorbia hirta* (Asthma weed) is a very common annual herb [3,4].This hairy plant grows up to 2' in height.it has numerous small flower clustered together with opposite oblongs leaves, which have a toothed margin. The young yellow fruit is a small hairy capsule with 3 reddish- brown wrinkled seeds. There is milky latex in all parts of the plants. *Calotropisprocera* (Madar) is a neglected medicinal weed. It is taxonomically known as *Calotropis* belonging to the family *Asclepiadaceae*. In English it is commonly known as milk weed or swallowwort. It is a common wasteland plant which gains not much recognition from animals and human beings.

The present work is carried out by Evaluation of *Euphorbia hirta* and *Calotropisprocera* against MDR pathogens.

MATERIALS AND METHODS

A Plant samples were collected from Visheshkh and, Gomtinagar (Lucknow) and the used parts are stems and leaves.

Solvent used

Organic solvents were used for the preparation of plant extracts. Secondary metabolites were needed from plants which were organic in nature and were used to dissolve secondary metabolites. During extraction solvents diffuse into the solid plant material and solubilize compounds with similar polarity.The followingfoursolvents were used for plant extract preparation are these are: Methanol, Ethyl Acetate, Acetone andHot water.

Extract preparation:

5 gm of each dried plant leaves was grinded in mortar and pestle.Then grinded sample is mixed with 50ml 0f ethanol, methanol, Acetone and Water in the ratio (1:10).They were kept in beaker for 3-4 days in dark. These were then filtered through Whattman's filter paper. Add DMSO to filtrate, evaporation solvent at room temperature up to thick solid residue.

Tested microorganisms:

Bacterial cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD Life Sciences, Lucknow. One gram positive culture- *Staphylococcus aureus* (MTCC 2940) and two gram negative cultures- *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC739) were used.

Antibacterial susceptibility assay:

Extracts obtained were evaluated for their potential antibacterial activities by the standard agar well diffusion assay also called cup plate method [6].First of all prepare nutrient broth for pathogens *(E.coli, S.aureus and P.aeruginosa).* Prepare 50 ml nutrient agar, pour in petriplates (100mm) and allowed to solidify.Spread 50 µl of inoculum from suspension culture of test organisms and prepare 3 wells of 5mm diameter in a triangular fashion with the help of micro-tips and marked as A (positive control), S (Sample) and DW (negative control).Incubate the plates at 37 ^{0}C for 24 hours under strict aseptic conditions to ensure consistency of all findings. Measure the zone of inhibition which was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Minimum inhibitory concentration (MIC)

MIC is an important diagnostic laboratory test which is generally performed in microbiology. It is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As much as the value of MIC will be low it will show that antimicrobial agent have better potential against microorganisms. It is the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms (or organisms) [5]. For performing MIC of single extract, twelve washed test tubes were taken.Each test tube was filled with 3ml of culture media (*i.e.*, nutrient broth (NB) for bacteria and potato dextrose broth (PDB) for fungus culture).The test tubes were then autoclaved properly. After autoclaving, the test tubes were taken to the LAF where they were allowed to cool for few minutes. Out of the twelve test tubes, six were treated as control (*i.e.*, Blank- without any culture) and remaining six was treated with culture (pathogens) to obtain the MIC value. For each culture test tube there was a control test tube. In the 1st test tube of both the control and cultured test tube, 500µl of extract was added and mixed properly. The test tubes were then serially diluted till the 6th test tube and finally 500µl was discarded from the last one (*i.e.*, 6th test tube).After the serial dilution, except the six control test tubes the other six were inoculated with 20µl of pathogenic culture .Finally the test tubes with culture were incubated in shaker incubator for overnight and the controls were stored in refrigerator. After the incubation period, the MIC values were determined by taking the Optical Density (OD) of the samples serially (*i.e.*, from 1st to 6th test tube) at 600nm in spectrophotometer.

Phytochemical analysis

Phytochemicals are the main constituents of any plants samples, which are responsible for secondary metabolites also. The other work of these phytochemical are flavouring, coloursetc [7, 8].

Reducing Sugar : Take1 ml or 1 gm of plant sample in a test tube and add 10 ml deionized water then add few drops of fehling solution (1 ml fehling solution A and B) heat at 100 °C In a water bath.Brick red colour precipitate showed positive result.

- **Taninns:** Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. Ablue-green or black coloration indicated the presence of phenols and tannins.
- **Phlobatannins :** Take 2 ml plant sample in attest tube and add 10 ml deionized water and boil at 100 °C with few drops of 1 % HCl.Deposition of red precipitation gives positive result.
- **Saponin :** Take 5 ml of aq. extract and then add 2ml chloroform followed by addition of 3 ml conc. Sulphuric acid, observed the reddish brown interface for presence of terpenoids.
- **Flavonoids:** Take 1 ml of sample and add 1 % NH_3 solution if yellow colour observed, showed presence of flavonoids then after this take ethanolic or aq. extract and add 10 ml DMSO then heat it followed by adding Mg and addconc. HCl,red colour red colourshowed flavonoids.
- **Polyphenol :** Take 2 ml ethanolic extract of plants sample and add folin-ciocalteu reagent and 9 ml of distilled water again add sodium carbonate solution,vortex to mix then kept test tube in dark and mix O.Dat 760 nm.

RESULTS

Graph 1: Showed that the *Euphorbia hirta* was showing best results as compare to *Calotropisprocera.*

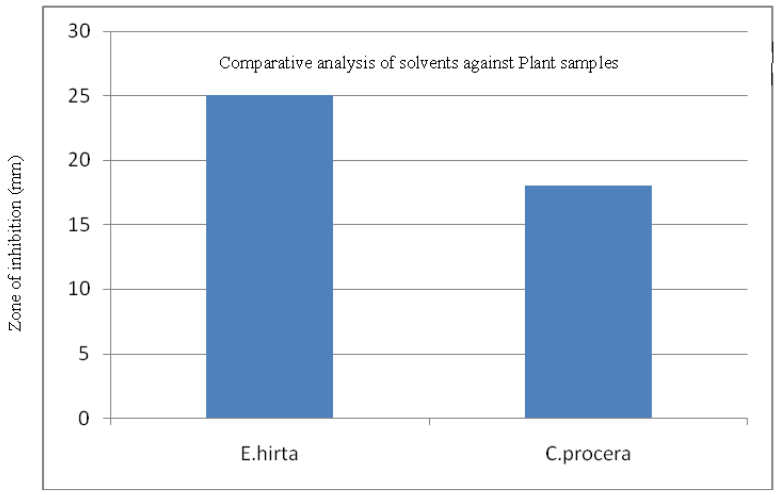

Table 1: Antibacterial Activity of *Euphorbia hirta* Leaves

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl Acetate	Methanol	Hot water extract
Escherichia coli	20	23	25	19
Staphylococcus aureus	24	25	23	16
Pseudomonas aeruginosa	21	22	22	15

Fig.No.1: Antibacterial activity of *Euphorbia hirta* leaves

Table 1 and Fig 1 showed that maximum antibacterial activity obtained against methanolic extract of *Euphorbiahirta* leaves.

Table 2: Antibacterial activity of *Euphorbia hirta* stems

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl Acetate	Methanol	Hot water extract
Escherichia coli	0	0	15	0
Staphylococcus aureus	0	0	13	0
Pseudomonas aeruginosa	0	10	0	0

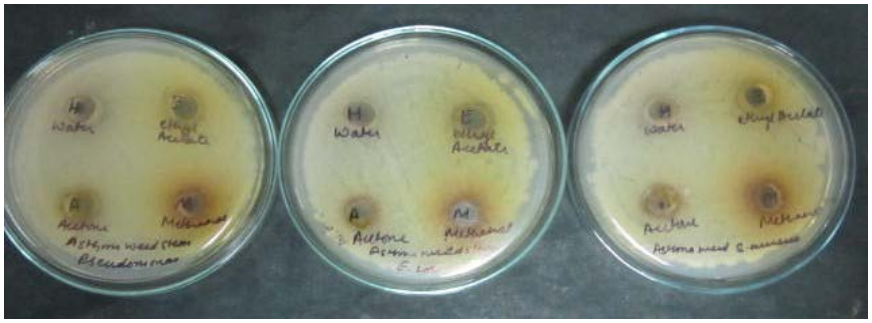

Fig 2: Antibacterial activity of *Euphorbia hirta* stem

Table 2 and Fig 2 showed that maximum antibacterial activity obtained against methanolic extract of *Euphorbia hirta*stems.

Table 3: Antibacterial Activity of *Calotropisprocera* leaves

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl Acetate	Methanol	Hot water extract
Escherichia coli	12	17	18	10
Staphylococcus aureus	13	15	16	0
Pseudomonas aeruginosa	0	16	15	0

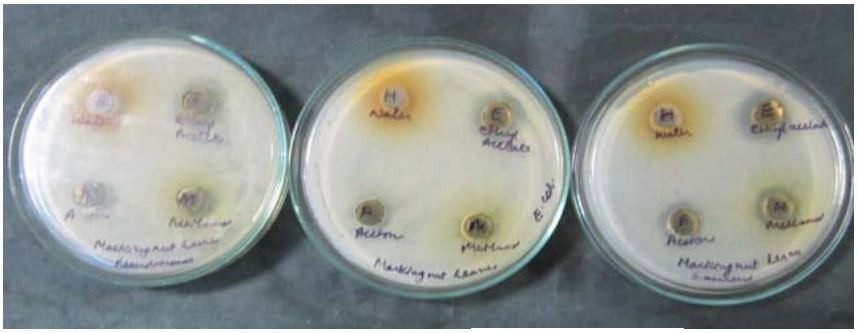

Fig 3: Antibacterial Activity of *Calotropisprocera* leaves

Table 3 and Fig 3 showed that maximum antibacterial activity obtained against methanolic extract of *Calotropisprocera* leaves

Table 4-Antibacterial Activity of *Calotropisprocera* stems

Pathogens	Zone of inhibition (mm)			
	Acetone	Ethyl Acetate	Methanol	Hot water extract
Escherichia coli	0	13	0	0
Staphylococcus aureus	0	0	13	0
Pseudomonas aeruginosa	0	1	1	0

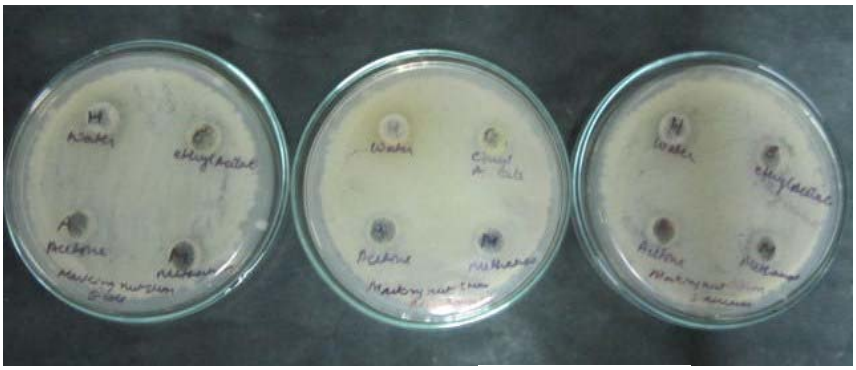

Fig.4: Antibacterial Activity of *Calotropisprocera* stem

Table 4 and Fig 4 showed that maximum antibacterial activity obtained against methanolic extract of *Calotropisprocera* stems.

MIC : Extracts found to have inhibitory effects were further tested for determination of minimum inhibitory concentration (MIC) by serial dilution method against susceptible bacterial species.

Table 5: *Euphorbia hirta* leaves against test organisms

TEST-TUBES	Conc. Of extracts mg/ml	OD of ethyl acetate extract against *E.coli*	OD of methanolic extract against *E.coli*	OD of methanolic extract against *S.aureus*
1 (BLANK)	1.66	0.04	0.07	0.00
2	27.6	0.00	0.17	0.00
3	4.6	0.38	0.00	0.31
4	0.75	0.25	0.02	0.29
5	0.12	0.18	0.00	0.33
6	0.02	0.25	0.33	0.32

MIC of ethyl acetate is observed 27.6 mg/ml against *E.coli* and In methanolic extract- 4.6 mg/ml against *E.coli* and 1.66 mg/ml against *S.aureus* in *Euphorbia hirta* leaves.

Table 6: *Euphorbia hirta* stems against test organisms

TEST-TUBES	Conc. Of extracts mg/ml	OD of ethyl acetate extract against *P.aeruginosa*	OD of methanolic extract against *S.aureus*
1 (BLANK)	1.66	0.12	0.04
2	27.6	0.06	0.29
3	4.6	0.12	0.31
4	0.75	0.19	0.28
5	0.12	0.00	0.26
6	0.02	0.25	0.28

MIC of methanolic extract observed 0.04 mg/ml against *S.aureus*, For ethyl acetate MIC observed 0.12 mg/ml against *P.aeruginosa*.

Table 7: *Calotropisprocera* leaves against test organisms

TEST-TUBES	Conc. Of extracts mg/ml	OD of ethyl acetate against *E.coli*	OD of ethyl acetate against *S.aureus*	OD of ethyl acetate against *P.aeruginosa*
1	1.66	0.34	0.00	0.65
2	27.6	0.39	0.36	0.32
3	4.6	0.37	0.34	0.00
4	0.75	0.00	0.00	0.00
5	0.12	0.08	0.08	0.24
6	0.02	0.08	0.05	0.31

MIC of ethylacetate extract is observed 0.75 mg/ml against *E.coli*, 1.66 mg/ml against *S.aureus* and 4.6 mg/ml against *P.aeruginosa* of *Calotropisprocera* eaves.

Table 8:*Calotropisprocera* stems against test organisms

TEST-TUBES	Conc. Of extracts mg/ml	OD of methanolic extract against *E.coli*	OD of methanolic extract against *S.aureus*	OD of methanolic extract against *E.coli*
1	1.66	0.16	0.13	0.08
2	27.6	0.16	0.16	0.06
3	4.6	0.13	0.16	0.04
4	0.75	0.18	0.21	0.10
5	0.12	0.16	0.24	0.04
6	0.02	0.19	0.25	0.00

MIC of methanolic is observed 1.66 mg/ml against *S.aureus*4.6 mg/ml against *E.coli*, and *P.aeruginosa* and 0.02 mg /ml against *E.coli* of *Calotropisprocera* stem.

Table 9: Phytochemical Tests for *Euphorbia hirta*

Euphorbia hirta		
Compounds	**Leaves**	**Stem**
Reducing sugar	+	+
Taninns	+	+
Phlobataninns	-	-
Saponin	+	+
Flavonoids	-	-
Polyphenol	1.70	1.53

Table 9 showed that reducing sugar, tannins and Saponins are present in leaves and stems of *Euphorbia hirta*.

Table 10: Phytochemical Tests for *Calotropisprocera*

Calotropisprocera		
Compounds	**Leaves**	**Stem**
Reducing sugar	+	+
Taninns	-	-
Phlobataninns	-	-
Saponin	+	+
Flavonoids	-	-
Polyphenol	0.99	0.60

Table 10 showed that reducing sugar and Saponin are present in leaves and stem of *Calotropisprocera*.

DISCUSSION

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary [9, 10]. Plant Extracts were prepared from dried samples of *Euphorbia hirta,* and *Calotropisprocera* using ethyl acetate, acetone, methanol and water as solvents. The antibacterial activity of *Euphorbia Hirta*, and *Calotropisprocera* extracted using different solvents showed varying degree of response towards selected pathogens [11,12]. Methanolic extract of *Euphorbia hirta* leaves showed maximum zone of inhibition against *E.coli, S.aureus and P.aeruginosa* that is of 25 mm. *Calotropisprocera* leave .The phytochemical analysis of plant *Euphorbia hirta* studied showed the presence of reducing sugar, tannins, saponin, and polyphenol. The phytochemical analysis of plant *Calotropisprocera* studied showed the presence of reducing sugar, saponin, and polyphenol. Earlier literature indicate that medicinal plants are the backbone of the traditional medicine and plant based antimicrobials represent vast unused source for medicine and further exploration of plants antimicrobials needs to occur. With extensive use of antibiotics and other antimicrobial agents, more and more of the clinical multidrug- resistant (MDR) pathogens appeared, and the degree of resistance has become progressively serious. At the same time, because of difficulty in developing chemical synthetic drugs and because of their side-effect, scientist are making more efforts to search for new drugs from plants resource to combat MDR microbial infection [13].

CONCLUSION

The result of Antibacterial susceptibility assay shows promising evidence for the antibacterial effect *of Euphorbiahhirta*, and *Calotropisprocera* have the wide spectrum of antimicrobial activity on the bacteria.These have various medicinal values and has been used since earliest time as a medicine for curing various diseases. Traditional medicinal are now the mainstay of drug recovery, for the treatment of emerging and old diseases.Each part of the plant has some medicinal value and is thus commercially exploitable. It is now and considered as a valuable source of several unique products for the medicines against various disease and also for the development of some industrial products. The present review includes comprehensive information on the chemical constituents, biological activities of important compounds, pharmacological actions, medicinal applications.

For improvement of the health of people there is a need to establish the necessary expertise for development of traditional medicines and deliberate efforts should be made to encourage local industrial production of herbal medicines and these herbal products signify the safety in contrast to the synthetics that are regarded as unsafe to human as well for the environment.

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