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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIY OF ACALYPHA INDICA AND EUPHORBIA HIRTA OF FAMILY EUPHORBIACEAE AGAINST SOME PATHOGENIC ORGANISMS

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A B S T R A C T

Plant and plant-based products are the base for many of the modern pharmaceuticals that we use today for various alignments. The objective of the study was to determine the bioactive chemical constituents and to evaluate the extracts of *Acalypha indica* and *Euphorbia hirta* for *in vitro* antimicrobial activities. Preliminary phytochemical analysis of the crude extracts of *Acalypha indica* revealed the presence of alkaloids, flavonoids, phenolic compounds and saponins. *Euphorbia hirta* revealed the presence of anthroquinones, flavonoids, phenolic compounds and saponins.The antimicrobial activity was determined by disc diffusion technique using eight reference bacterial strains *vi,: Bacillus subtilis*, *Escherichia coli*, *Enterococcus feacials*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Klebsiella pneumonia and Proteus vulgaris. E*thyl acetate extract of *Acalypha indica* exerted mean zone of inhibition in the range of 10-23mm. *Euphorbia hirta* exhibited zone of inhibition from 18-31mm for the solvent ethyl acetate. Methanol, ethanol, diethyl ether and distilled water extract of *Acalypha indica* were bactericidal against tested bacteria. The MIC and MBC of both the plant extracts ranged between 30-90mg/ml. The antimicrobial activities of these extracts is currently being undertaken to identify the secondary metabolites responsible for the bioactivity will be studied further.

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INTRODUCTION

The medicinal plants are of great interest to human health. plant based medicines have been a part of traditional healthcare in the most part of the world for thousands of years (Chariandy *et al.*, 1999: New man et al 2000).Plants contain numerous biologically compounds, many of them have been shown to exhibit antimicrobial properties and therefore were in use as antimicrobial drugs in traditional medicines. Plants used in traditional medicine contain a very vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of WHO, more than 80% of the world's populations depend on traditional medicine for their primary healthcare needs. Knowledge of the phytochemical is desirable not only for the discovery of health care products, but also in disclosing new sources of economic materials like alkaloids, tannins, oils, gums etc (Farnsworth, 1966). The systematic screening of plant extracts or plant derived substances still remains an interesting strategy to find new lead compounds in many plant species.

The success story of modern medicine lies in the continuous search for new drugs to counter the challenges posed by resistant strains of bacteria. There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants (Muhammad and Muhammad, 2005; Falodun *et al.*, 2006; El- Mahmood and Amey, 2007) and such reports had attracted the attention of scientists worldwide (Falodun *et al.*, 2006; Lai *et al.*, 2008). Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation (Adedapo *et al.*, 2005). This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs. The largest genus of family Euphorbiaceae is Euphorbia with 1600 species. It is characterized by the presence of white milky latex which is more or less toxic.

One such species is *Euphorbia hirta* which is widely used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and

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respiratory diseases (asthma, bronchitis, hay fever, etc.), and conjunctivitis. Hypotensive and tonic properties are also reported in *E. hirta*. The aqueous extract exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities. The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils

E. hirta belongs to the plant family *Euphorbiaceae* and genus Euphorbia. It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to blong- lanceolate, acute or subacute, dark green above, pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds. (Williamson EM *et al.,* China 2002; Prajaptati ND *et al.,* 2003.,Kirtikar KR, Basu BD *et al.,* 2003). The alcoholic extract of whole plant shows hypoglycemic activity in rats.(Sood SK *et al.,* 2005). It has a sedative effect on the genitor-urinary tract.

Similarly *Acalypha indica* Linn belongs to the same family is a common weed in many parts of Asia. It is an annual herb, about 80 cm high and commonly found in waste lands or fields. This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (Varier, 1996). In the present study, an attempt has been made to enrich the knowledge of anti bacterial activity of *Acalypha indica and Euphorbia hirta* plant extracts against pathogenic bacteria like, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus epidermis, Bacillus subtilis and Proteus vulgaris* which were reported to cause wide infections.

MATERIAL AND METHODS

Plant material

The plants were collected from in and around of Tiruchengode, Namakkal Dist, Tamilnadu, India during the month of August to November 2009. The plant was identified and authenticated from standard resources. The plant was then brought to laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in an air tight –container for further use.

Preparation of extract

Acalypha indica and *Euphorbia hirta* plant powder (100gm) were taken separately and exhaustively extracted by soxhlet extraction method using each of the following solvents: ethyl acetate, methanol, ethanol and Diethyl ether. The mixture was heated slowly at 400ºC for 16h in an oven and filtered through several layers of muslin cloth. The filtrate was again filtered by using Whatman no.1 filter paper and concentrated to 1/5 of the original volume by evaporation in shaded conditions and stored at 4^0C.

Preliminary Phytochemical screening of crude extracts

The phytochemical components of the medicinal plants were screened using the methods. The extracts were analyzed for, the presence of saponins, steroid, cardiac glycosides, anthraquinones, tannins, flavonoids, alkaloid, terpenoids and balsam (gum).

Test for terpenoids (Salkowski test)

To 0.5 g of each the extract was added 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for anthraquinones

About 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered using Whatman filter paper No.1. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of 10% of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for flavonoids

Two methods were used to test for flavonoids. First, 10% of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled with 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black colouration.

Test for alkaloids

About 0.5 g of extract was diluted in 10 ml of 1% aqueous hydrochloric acid, boiled and filtered. Two ml of dilute ammonia was added to 5 ml of the filtrate. Further five ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was observed indicating the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted in 5 ml of water was added and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was mixed. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear

below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer.

Determination of Antimicrobial activity

Disc diffusion Assay

Antibacterial activities of *Acalypha indica* and *Euphorbia hirta* plant extracts were investigated by disc diffusion method (Bauer and Kirby, 1996). The Muller – Hinton Agar (MHA) plates, containing an inoculums size of 10^6 colony-forming units (CFU) / ml bacteria were spread with a sterile cottons swab. Then disc (6 mm diameter) impregnated with 25 µL of each extract at a concentration of 100mg/ml were placed on inoculated plates. Similarly, a disc with 25µL distilled water served as negative control, 10 µg / disc of streptomycin was also used as positive control for the bacteria. The plates were allowed to stand for 30min for pre-diffusion of the extracts to occur and then incubated at 37ºC for 18 to 24 h for bacteria and the zone of inhibition including the diameter of disc were measured to the nearest mm. The mean of triplicate results was calculated.

Maximum inhibitory concentration (MIC)

The MIC of the plant extracts was determined on solid medium (nutrient agar) using method of Siddiqui and Ali (1997). Standardized suspensions of the test organism was inoculated into a series of sterile tubes with nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Minimum bactericidal concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was subcultured onto extract free agar plates, incubated for another 24 h at 37°C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

The preliminary phytochemical screening revealed the chemical nature of the constituents present in the plant extracts. It is be used to identify the presence of bioactive compounds that could be used in the synthesis of useful drugs. The results of phytochemical screening and microbial activities of the plant *Acalypha indica* and *Euphorbia hirta* showed the presence of secondary metabolites (Table-1)

Phytochemical analysis of *Acalypha indica* showed the presence of tannins, steroids, saponins, cardiac glycosides, terepens, alkaloids and absence of anthroquinones, catachol and triterpenoids. Phytochemical analysis of *Euphorbia hirta* revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, steroids and flavonoids (Table-1)

Table 1 Phytochemical analysis of *Acalypha indica* and *Euphorbia hirta*.

S.No	Preliminary Phytochemicals	Study Plants	
		Acalypha indica	Euphorbia hirta
1.	Alkaloids	+	-
2.	Anthroquinone	-	+
3.	Catachols	-	-
4.	Flavonoids	+	+
5.	Phenolic Compounds	+	+
6.	Saponins	+	+
7.	Steroids	-	+
8.	Tannins	-	+
9.	Triterpenoids	-	-

Note: (+) - present, (-) Absent

ANTI BACTERIAL ASSAY

Antibacterial activities of the plants were determined by extracting the plants with various solvents like ethyl acetate, diethyl ether, methanol and ethanol. The extract were subjected to preliminary screening for antimicrobial activity against eight pathogenic bacteria *Bacillus subtilis, Escherichia coli, Enterococcus feacials, Pseudomonas aeroginosa, Staphylococcus aureus, Staphylococcus epidermis, Klebsiella pneumonia, Proteus vulgaris.*

The antimicrobial activity of *Acalypha indica* was illustrated in (Table-2) Ethyl acetate extract (100mg/ml) of the plant displayed very fair antibacterial activity against the selected gram positive organism and gram negative bacteria. The zone of inhibition ranged from 10mm-27mm.The standard antibiotics streptomycin was found to have inhibition zone in the range of 10-44mm. Diethyl ether, methanol, ethanol extract of the plant showed very mild elevation. Distilled water was taken as negative control which did not show any zone of inhibition.

The observed antimicrobial activities of various extracts of E.hirta were expressed in (Table-3). Ethyl acetate extracts (100mg/ml) displayed good antibacteraial against Gram positive andGram negative bacteria. Inhibition zone ranged from 31-13mm). the zone of inhibition range from 12-31mm. the methanol extract exhibited zone of inhibition at the range of 12-31mm the standard streptomycin was found to have inhibition zone in the range 30-56mm) at the concentration of 10µg/disc.Distilled water was used as the control which did not show any zone of inhibition. The antibacterial activity of *Acalypha indica* was very low when compared to *Euphorbia hirta*

The inhibition zone exhibited by the extract of the plant *Euphorbia hirta* against *Bacillus subtilis, Pseudomonas aeroginosa, Staphylococcus aureus Enterococcus faecalis* justified that the plant can be used in the treatment of spores,bores on open wounds. Gram-negative bacteria showed weakest inhibition zones than gram positive ones, which may be related to the presence of a thick phospholipids and lipopolysaccharide outer membrane layer that protects them from environmental factors and makes them highly resistant, even to synthetic antibiotics. Difference in activities of the plant may be due to the extraction capability of various bioactive compounds available. Resistant microorganisms do not indicate the

Table 2 Antimicrobial activity of various extracts of Acalypha indica

Organism	Diameter of zone of inhibition (mm)				Standard Antibiotic	Control
	Ethyl acetate	Diethyl ether	Methanol	Ethanol	Streptomycin	Distilled water
Staphylococcus Epidermis	15	-	-	-	10	-
Escherichia coli	10	-	-	-	52	-
Klebsiella Pneumonia	12	-	-	-	41	-
Staphylococcus aureus	-	27	-	-	46	-
Enterococcus Faecalis	23	-	-	-	-	-
Pseudomonas aeruginosa	15	-	-	-	32	-
Bacillus subtilis	20	14	-	-	45	-
Proteus vulgaris	12	-	-	-	22	-

Table 3 Antimicrobial activity of various extracts of Euphorbia hirta

Organism	Diameter of zone of inhibition(mm)				Standard Antibiotic	Control
	Ethyl acetate	Diethyl ether	Methanol	Ethanol	Streptomycin	Distilled Water
Staphylococcus Epidermis	18	-	15	-	12	-
Escherichia coli	12	-	23	-	56	-
Klebsiella Pneumonia	26	-	24	-	39	-
Staphylococcus aureus	27	-	26	-	45	-
Enterococcus Faecalis	29	-	12	-	-	-
Pseudomonas aeruginosa	23	-	31	-	32	-
Bacillus subtilis	31	9	28	-	47	-
Proteus vulgaris	21	27	14	-	30	-

absence of bioactive constituents, nor that the plant is inactive,rather,active compound(s)could be found in insufficient quantity in the extracts to show activity at the tested concentration. Lack of activity can thus only be proven by using large doses on the other hand there could also be other constitutes extracting antagonistic effects on the bio active compounds for the methods used for extraction could not extract all the bio active compounds.Differences in zone of inhibition exhibited by the same plant with different extracts may be due to the various bio active compounds extraction of specific solvents which might be influenced by the various methods of extraction (Hot / Cold, acidic / basic, fresh / dry plants.etc.,)

Minimum inhibitory concentrations

The minimum inhibitory concentrations of the plants extracts on the test isolates are shown in (Table-4) the MIC values ranged from 50 – 80 mg / ml for Acalypha indica The lowest MIC was recorded against E.coli with a concentration of 50 mg / ml, followed by Proteus vulgaris, Staphylococcus epidermis, Staphylococcus aureus and K pneumonia with concentrations of 60 mg / ml. Furthermore, the extract had a MIC of 70 mg / ml against Bacillus subtillis and Pseudomonas aeruginosa (80mg /ml). Earlier studies reported that the MBC values can either be the same or higher than the corresponding MIC values(10), but in the study. In the present study MBC values which are obtained after plating under various dilution of the plant extracts were more reliable than the MIC values, obtained using turbidity as a measure of growth.

The minimum inhibitory concentration of Euphorbia hitra on the test isolate is shown in (Table-4). The MIC values ranged from 10-80mg/ml. The lowest MIC were recorded against Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtillis and Proteus vulgaris with concentration of 10mg/ml, 20mg/ml,30mg/ml and 40mg/ml respectively. Similarly the MBC values ranged from 20-80mg/ml.

Minimum inhibitory and Bactericidal concentrations of Euphorbia hirta leave extract

The lower MIC and MBC values indicate higher efficacy. Thus the MBC values exhibited by the plant extract against E.coli and Pseudomonas aeroginosa is of potential importance in the health care delivery systems, since it could be used as an alternative to orthodox antibiotics in the treatment of infectious diseases caused by this microbes which frequently develop resistance to known antibiotics.MIC and MBC of Ethyl acetate extract of Acalypha indica and Euphorbia hirta MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration;

The potent extract of Acalypha indica and Euphorbia hirta warrants further investigation and rapid development to add to the list of currently available antimicrobial agents. Certainly, this could decrease cost, improve efficacy and decrease mobidity and mortality.

Microorganism	E.hirta MIC (mg/ml)	E.hirta MBC(mg/ml)	A.indica MIC (mg/ml)	A.indica MBC(mg/ml)
Bacillus subtilis	70	80	40	50
Escherichia coli	50	70	50	60
Enterococcus faecalis	80	90	10	20
Pseudomonas aeroginosa	70	60	70	80
Staphylococcus aureus	60	70	20	30
Stapylococccus epidermis	60	70	60	70
Klebisella pneumonia	60	70	30	40
Proteus vulgaris	60	70	40	50

The preliminary phytochemical screening of E. neriifolia leaf extracts has revealed the presence of secondary metabolites of therapeutical importance. The major phytochemicals found were phlobatannins, saponins, flavonoids, phenols, terpinoids and cardenolids. However, all extracts tested showed the absence of sterols, anthoquinones and cardiac glycosides (kumara swamy *et al.,* 2011).

The screening and scientific evaluation of plant extracts against microbes may provide new antimicrobial substances. Also plant based antimicrobials it's have enormous therapeutic potential (Iwu *et al.,* 199).

Antibiotics provide the main basis of for the therapy of bacterial infection. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been the continuing search for newer and more potent antibiotics. Report of infectious diseases, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade as witnessed and increase in the investigation of plant material as a source of human disease management.

CONCLUSION

This study revealed the preliminary evidence of antibacterial interaction between the various solvent extracts of study plants, and the observed antibacterial effects on the isolates were associated with the presence of alkaloids, tannins, flavonoids, saponins,steroid, terpenoid, cardiac glycoside which have been showed to possess antibacterial activity. Therefore Acalypha indica and Euphorbia hirta extract can be used to discover active natural products that may serve to develop of new pharmaceutical agents to address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour. Bacterial infections can be treated with Acalypha indica and Euphorbia hirta, since it exhibited favorable antibacterial activities. Euphorbia hirta exhibited very good antibiotic activity than Acalypha indica. Hence it can be used as potent antibiotic agent against the treatment of pathogenic bacteria. It should be noted that plant should be consumed with small doses. There is need for further studies on the plant in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compound. Hence the present investigation clearly reveals the antibacterials nature of the plant E. hirta and A. indica suggest that this plants could be exploited in the management of diseases caused by this bacteria in human system.

References

Adedapo AA, Shabi OO, Adedokun OA (2005). Antihelminthic efficacyof the aqueous extract of *Euphorbia hirta* (Linn.) in Nigerian dogs. Vet. Arch. 75(1):39-47

Bauer.A.W,; Kirby, W.M.N,; Sherries, J.L.Antibiotics susceptibility testing a standing disc method Am.J.Clin Pathol.1996.45, 493-496.

Chariandy CM, Seaforth CE, Phelps RH (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J. Ethnopharmacol., 64: 265-27

El-Mahmood AM, Ameh JM (2007). *In-vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with Urinary tract infections. Afr. J.Biotechnol. 6(11): 195-200.

El-Mahmood 2009, A.M. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J. Med. Plants Res. 3, 498-505.

Fransworth NR (1966). Biological and Phytochemical screening of plants. J. Pharm. Sci., 35: 225-276

Falodun A, Okunrobe LO, Uzoamaka N (2006). Phytochemical screening and anti- inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn9Euphorbiaceae). Afr. J. Biotechnol. 5(6):529-531

Kirtikar KR 2003, Basu BD. Dehradun, India: Oriental Enterprises;. Indian medicinal plants with illustrations.

Muhammad HS, Muhammad S (2005). The use of *Lawsonia inermis*Linn. (henna) in the management of burn wounds infections. Afr.J.Biotechnol. 4(9):934-937

Newman DJ, Cragg GM, Snader KM (2000). The influence of natural products upon drug discovery. Nat. Prod. Reports, 17: 215-234.

Prajapati ND, Purohit SS, Sharma AK, Kumar T. Jodhpur, India: Agarbios; 2003. Handbook of Medicinal Plants. Vol. 3. New Delhi: Council of Industrial and Scientific Research; 2005. The Wealth of India (Raw material)

Siddiqui AA, Ali M (1997). Practical Pharmaceutical chemistry. Ist ed.,CBS Publishers and Distributors, New Delhi, pp. 126-131

Sood SK, Bhardwaj R, Lakhanpal TN. India: Scientific Publishers; 2005. Ethnic Indian Plants in cure of diabetes.

Trease GE, Evans WC (1989). Pharmacognosy (13th edn). English Language Book Society, Bailliere Tindall, Britain, pp. 378, 386-480.

Varier V.P.S. Indian medicinal plants: a compendium of 500 species Orient Longman. Publication, Madras, India, 1996; 134.

Williamson EM. China: Churchill Livingstone; 2002. Major Herbs of Ayurveda.

Kumara swamy. M Neeraj Pokharen, Santosh Dahal And Anuradha M (2011) Phytochemical and antimicrobial studies of leaf extract of Euphorbia neriifolia. Journal of Medicinal Plants Research, Vol. 5 (24) pp 5785-5788.

Iwu M, Angela RD, Chin OO (1999) New antimicrobials of plant origin a reprint from journal of janicked perspective on news crops and new uses, ASHS Press. Alexander.