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INVITRO EVALUATION ON ANTI - MICROBIAL ACTIVITY OF *CORDIA MONOICA* (ROXB) LEAF EXTRACT

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ABSTRACT

OBJECTIVE: The aim of the present study was to evaluate the antimicrobial activity of various solvent extracts of *Cordia monoica* leaves.

METHOD: Ethanol, Ethyl acetate and Petroleum ether extracts of this plant were evaluated against bacterial strains *Escherichia coli*, *Pseudomonas aeruginsoa*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Streptococcus pyogens*. Fungal strains *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces cerevisea* using disc diffusion method.

RESULT: The results of this study showed that the ethanol leaf extract exhibited better antimicrobial activity against bacterial strains. The fungal strains were susceptible to petroleum ether extract as compared to other extracts. The extracts were compared with standards like Gentamycin and Chloramphenicol for anti-bacterial activity and Nystain and Greseofulvin for antifungal activity. The extracts showed remarkable inhibition of zone of bacterial and fungal growth and results were comparable with that of standard drugs against the organism tested.

CONCLUSION: In conclusion, leaf extract of *Cordia monoica* showed significant antimicrobial activity.

INTRODUCTION

Microorganisms have evolved a number of defense mechanisms against antimicrobial agents. There is an increase in the resistance of newly produced drugs. This has led to the screening of several medicinal plants for their antibacterial and antifungal activity. The secondary metabolites produced from plants have been directly used as precursors or lead compounds in the pharmaceutical industry. [1]In developing countries the use of traditional medicine remains wide spread. It is believed that the herbal remedies are naturally superior to synthetic drugs. The use of traditional medicine doesn't impact any side effects to human beings. [2]

Medicines are derived from plants either in simple form or in more complex form of crude extracts, mixtures, etc. According to World Health Organization, about 60-85% of the population depends on plants for their primary health care. [3] There is a rising interest in the use of plant derived drugs than synthetic drugs for their cost effectiveness.[4] There are many historical evidences for the use of herbs by primitive people. [5]

Antibiotics have adverse effects like hypersensitivity, immune suppression and allergic reaction on host cell. [6] There is a constant need for development of alternative antimicrobial drugs from medicinal plants due to the antibiotic resistance in bacteria. There are numerous research works that report on the antimicrobial activity of different herbal extract in different regions of the world. [7-10]The emergence of Multidrug resistant bacteria has established a situation in which there are options for infections with certain micro organism. [11] Along with bacterial infections, fungal infections also cause significant cases of mortality and morbidity despite advances in medicine and emergence of new antifungal agents.[12, 13] Many plants have been reported to have antifungal activity. [14, 15, 16, 17] Plants exhibit beneficial medicinal effects due to the combination of secondary metabolites present in them. These secondary metabolites include phenolic compounds, steroids, tannins and alkaloid. [18, 19] These compounds are complex, specific and found to have heterogenicity in wild species. They exert action by resembling signal transduction molecules, ligands, hormones or neurotransmitters and exhibit effect on humans due to their similarity in their potential target site. Therefore, it is important to screen plants for their biological activity. *Cordia monoica* is a small tree which belongs to Borangiacea family. It is also known as Snot berry or Sand paper Saucer berry. [20] The tree is widely distributed in India, Srilanka and Africa. [21, 22, 23] The leaves of *Cordia monoica* are alternate and broadly obovate,

oblong to almost circular. The leaves are rough on the upper surface, hence the name sand paper tree. [21] The leaves have many uses in traditional medicine. The leaves are used to treat eye diseases, leprosy, MICH, [24] stomach disorders and for the removal of retained placenta. [24, 25]

MATERIALS AND METHODS

COLLECTION OF PLANT

The leaves of *Cordia monoica* have been collected from Marudamalai hills, Western Ghats Tamil Nadu India. It was identified and authenticated at Botanical Survey of India.

PREPARATION OF EXTRACT

The leaves of *Cordia monoica* were shade dried, powdered and extracted with solvents. The solvents used include ethanol, petroleum ether and ethyl acetate. 50 g of the powdered leaf sample was taken and the extracts were prepared with soxhlet apparatus using 100 ml of each solvent. The obtained extract was filtered with Whatman No 1 filter paper and evaporated to dryness. The extract was then weighed and dissolved in minimal volume of dimethyl sulfoxide. [26] Further the extract is used for anti-microbial activity.

ANTIMICROBIAL TESTING

TEST CULTURE

The microorganisms used in the study were obtained as pure culture isolates. The test Culture includes three different gram positive (*Bacillus subtilis, Streptococcus aureus* and *Streptococcus pyogens*) and gram negative bacteria (*Pseudomonas aeuroginosa, Salmonella typhi* and *Escherichia coli*). In fungal strains, Yeast includes *Candida albicans* and *Saccharomyces cerevisea* and molds include *Aspergillus niger* and *Aspergillus flavus*.

The bacterial strains were grown in Muller- Hinton agar (MHA) plates at 37°C, where as yeasts and molds were grown in Sabouraud dextrose agar and Potato dextrose agar (PDA) media at 28°C for 7 days. The stock cultures were maintained at 4° C.

STANDARD USED: Chloramphenicol, Gentamycin, Nystain and Greseofulvin

DISC DIFFUSION METHOD

ANTI BACTERIAL ASSAY

The anti bacterial activity was studied against *E.coli, P.aeuroginosa, S.typhi, S. aureus, S. pyogens* and *B.subtilis* by disc diffusion method [27, 28, 29 30]. The Muller- Hinton agar was prepared, sterilized and poured into petri dishes. The media was inoculated with test bacteria

($1x10^8$ bacteria/ml) using cotton swabs. Then a disc (6mm) was saturated with 100pt of the plant extract, dried and introduced on the upper layer of the seeded agar plate. For each bacterial strain Chloramphenicol and Gentamycin were used as standard. All petri dishes were incubated at 37°C for 24 hours. Microbial growth was determined by measuring the diameter from the end of growth to the disc at one end of the beginning of growth at the other end including the diameter of the disc. The results were again obtained by measuring zone of inhibition. The experiment was repeated three times and the mean values were recorded.

ANTI FUNGAL ASSAY

Antifungal activity was performed with mold and yeast using disc diffusion method. [31] The Potato dextrose agar and Sabouroad Dextrose agar were prepared and poured in petridishes. The media was spreaded with fungal spores (1x1.0 spores/ml). In each petridish a disc of 6mm diameter was saturated with 100 pt of the plant extract, dried and placed on the seeded culture plate. Nystain and Greseofulvin were used as standard for fungal strains. All the petridishes were allowed for incubation at 28C for 7 days. After incubation time, media was observed for zone of inhibition which is measured in milli meter. The experiment was repeated for three times. The mean value was recorded.

RESULTS

In the present investigation, antimicrobial activity of leaf extract against 6 bacterial strains and 4 fungal strains were recorded. The diameter of Zone of inhibition was measured in mm. [32]. The greater diameter indicates more activity in plant extract tested against the colony of organism. [33, 34]. The results of the antibacterial activity (Table I) revealed that all the bacterial strains were more sensitive to ethanol extract rather than ethyl acetate and petroleum ether extract. The maximum zone of inhibition was achieved with ethanol extract for *Pseudomonas aeuroginosa* species (28 mm). The ethyl acetate exhibited better activity with *E.coli, P.aeuroginosa* and *Bacillus subtilis*, while petroleum ether showed lower activity for the bacterial strains. *S. aureus* was more susceptible with ethyl acetate, while *S.typhi* and *S.pyogens* were moderately effective against ethanol extract. The result of antifungal activity (Table II) indicates that petroleum ether exhibited maximum inhibitory activity against *Saccharomyces cerevisea* and *Candida albicans*. The ethanol and ethyl acetate showed lower inhibitory activity for fungal strains than bacterial strains. The ethanol leaf extract showed low inhibitory activity against *Aspergillus niger* where as ethyl acetate showed minimum zone of inhibition for *Aspergillus flavus.*

TABLE I: ANTI BACTERIAL ACTIVITY OF DIFFERNET EXTRACTS OF *CORDIA MONOICA* (ROXB) LEAVES

EXTRACT	ZONE OF INHIBITION (mm)					
	B1	B2	B3	B4	B5	B6
ETHANOL	21.0±0.02	17.2±0.06	18.0±0.58	19.3±0.22	13.22±0.09	11.2±0.03
ETHYL ACETATE	17.5±0.65	14.2±0.52	10.5±0.23	13.6±0.06	12.6±0.06	9.2±0.36
PETROLEUM ETHER	10.3±0.35	11.2±0.65	7.3±0.59	8.2±0.36	9.7±0.22	5.6±0.95
CHLORAMPENICOL	22±0.03	18.3±0.34	21±0.34	20±0.58	17±0.81	15±0.11
GENTAMYCIN	25±0.76	22±0.54	15±0.28	24±0.61	15±0.17	18±0.06

All are mean values of triplicates

B1- *Psuedomonas aureoginosa* B2- *Bacillus subtilis*

B3- *Escherichia coli* B4- *Staphylococcus aureus*

B5- *Salmonella typhi* B6- *Streptococcus pyogens*

TABLE II: ANTI FUNGAL ACTIVITY OF DIFFERNET EXTRACTS OF *CORDIA MONOICA* (ROXB) LEAVES

EXTRACT	ZONE OF INHIBITION (mm)			
	F1	F2	F3	F4
ETHANOL	6.2±0.06	5.33.±0.58	1.36±0.11	7.05±0.06
ETHYL ACETATE	5.03±0.03	3.23±0.31	2.09±0.84	4.36±0.67
PETROLEUM ETHER	9.66±0.86	7.48±0.19	4.24±0.03	8.84±0.37
NYSTAIN	20.23±0.33	17.39±0.48	16.05±0.05	18.39±0.28
GRESEOFULVIN	17.08±0.19	16.34±0.23	15.09±0.23	20.08±0.48

All are mean values of triplicates

F1-*Sacchoromyces cerevisae* F2- *Aspergillus niger*

F3- *Aspergillus flavus* F4- *Candida albicans*

DISCUSSION

The antibacterial activity of different extracts of *Cordia monoica* leaves were screened by disc diffusion method. Three gram negative bacteria strains, *E.coli*, *P.aueroginosa* and *S.typhi* and three gram positive bacteria, *B.subtilis*, *S. aureus* and *S.pyogens* were selected for this study. *E.coli* bacteria cause meningitis, joint infections, urinary tract infections, gastroenteritis and skin infections. [35, 36] *P.aueroginosa* is most infectious for lung, urinary tract and kidneys. It causes pneumonia and if the colonies are found, it may result in fatal condition [37] *Staphylococcus* are threat to both animals and humans.[38, 39, 40] It spreads pneumonia at slow rates. [41]*Proteus* and *Bacillus* also play role in infectious diseases. The ethanol leaf extract showed highest activity for bacterial strains. The maximum zone of inhibition is attained for *Pseudomonas aeuroginaosa* (30

mm). *P. aueroginosa* (Gram negative bacteria) is one of the difficult micro organisms to be managed by many antibiotics due its nature of cell wall. [42] *Cordia monoica* leaves showed better results and it may be due to the higher diffusion rate or the degree of sensitivity of the tested micro organisms to the extract is higher. *Cordia monoica* leaves may contain compounds that can be used to control diseases caused by *P.aeuroginosa*. The different rate of inhibition could be due to the molecular size of the phytochemical compounds present in the extracts. [30]

Among gram positive bacteria maximum activity is seen with Staphylococcus aureus. Similar results have been reported by Kugo *et al* [42] the antibacterial activity of chloroform and methanol leaf and stem extract of *Cordia monoica* showed maximum zone of inhibition (12-21mm) against *Staphylococcus aureus*. The GC -MS analysis of ethanol extract of *Cordia monoica* leaves also justifies presence of bioactive compounds responsible for antibacterial activity. [24]

In fact Gram negative bacteria develop multi drug resistance to many of the antibiotics in the market where *E.coli* is prominent.[43-45] Still the extracts of the *Cordia monoica* leaves showed more pronounced activity for gram negative than gram positive. The reason is attributed to the sensitivity difference between gram positive and gram negative bacteria. This is mainly due to the morphological constitution between these microorganisms, Gram negative bacteria has thicker cell wall made up of phospholipid membrane which makes it impermeable for antimicrobial chemical components. The gram positive bacteria have only outer peptide glycan layer that is not an effective impermeable barrier. Therefore gram negative organisms are easily susceptible to antimicrobial agents than gram positive.[46-48] Inspite of this permeability difference *Cordia monoica* leaf extract still exerted some degree of inhibition against gram negative organism as well.

The fungal strains were also tested against *Cordia monoica* leaf extract which exhibited maximum zone of inhibiton with petroleum ether and ethyl acetate for yeast and mold. The results were in agreement with Kugo *et al*,[42] The anti-fungal activity of stem and leaf extract with chloroform gave maximum inhibition for *Saccharomyes cerevisea*. The ethyl acetate extract of *Cordia monoica* leaves also reported to have bioactive compounds responsible for antimicrobial activity. In the recent years there were many reports on anti microbial activity.

Stem bark of *Cordias sinensis* showed antibacterial activity against *S.aureus* (19mm) and *Proteus vulgaris* (15mm).[44] Flowers of *Cordia sebestena* showed 6-13mm zone of inhibition

against bacterial strains (*E.coli, K.pneumonia, P.aueroginaosa, S.aureus, B.cereus* and *S.pyogens*).[49] *Cordia dichotama* leaf and fruit extract showed moderate activity against *S.aureus* and minimum activity for *Candida albicans* [50]. Similar reports for *Cordia dichotoma* have been exhibited with methanol and butanol extract against *E.coli, P. aeuroginosa, S. aureus* and against *A.niger, candida albicans*. [11]

Cordia macleodii showed 3mm zone of inhibition against *Streptococcus* and *Staphylococcus aureus* but it is resistant to *K. pneumonia*. For molds the zone of inhibition is 1mm. [51]

Cordia myxa showed highest inhibition with *S.aureus* and *E.coli* but no activity is reported for fungal strains. [52] *Cordia latifolia* also exhibited maximum activity against *S.aureus*. [36]. *Cordia gilletii* roots extracts showed antibacterial activity against 8 bacterial strains. The maximum zone of inhibition was with *S.aureus* and *E.coli*.[53]. Also, *Cordia curassavica* was tested against 13 bacterial strains and 5 fungal strains. *Sarcina lutea* and *V. cholera* was more sensitive for essential oils. [54]

CONCLUSION

The use of plants in folk medicine plays major criteria for cost effectiveness and safe alternate to treat infectious diseases. In the current investigation it is clear that *Cordia monoica* leaf extracts showed anti microbial activity against both bacterial and fungal strain in compare to standard drugs. This may form the basis for selection of this plant for further development and discovery of new drugs. The antimicrobial activity may be increased when the bioactive compounds are purified and proper dosage is determined for proper administration.

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