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ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *CISSUS PALLIDA*

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ABSTRACT: Herbs have been widely used for centuries as remedies for diseased human conditions as they have certain phytochemicals of diseases curing nature. The present study explains the antimicrobial (antibacterial & antifungal) potential of dried stem wood of *Cissuspallida* methanolic extract against bacterial strains of *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa*, and *Aspergillus niger*, *Aspergillus fumigates*, *Monosculus purpure* fungal strains by Disc diffusion method. The test extract was compared with Ciprofloxacin 5 µg for bacterial organisms and Fluconazole 20 µg for fungal organisms. The extract showed significant antibacterial and antifungal activity which was confirmed by the zone of inhibition studied.

INTRODUCTION: Humankind has been subject to infection by microorganisms since before the dawn of recorded history. One presumes that humankind has been searching for suitable therapy for nearly as long. This was a desperately difficult enterprise given the acute nature of most infections and the nearly total lack of understanding about their origins that was prevalent until the last century. One can find indications in old medical writings of folkloric use of plant and animal preparations, soybean curd, moldy bread and cheese, counter infection with other microbes.

The slow development of public health measures, and an understanding of the desirability of personal cleanliness, but these factors were erratically and inefficiently applied and, when they were applied, often failed¹. Until the discovery of bacteria 300 years ago and the subsequent understanding of their role in infection about 150 years ago, there was no hope for rational therapy.

Until, recently, chemotherapy of fungal infections has lagged far behind chemotherapy of bacterial infections. This lack of progress has resulted, in part, because the most common fungal infections in humans have been relatively superficial infections of the skin and mucosal membranes and potentially lethal deep-seated infections have been quite rare². The number of effective antimicrobials available is quite small compared to those available to treat bacterial infections, but research in this area is quite active³.

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Several new agents have been introduced in the last few years. In the present study, *Cissus pallida*⁴ methanolic extract of its stem wood was screened against certain gram-positive, gram negative and fungi organisms to evaluate its antimicrobial potential.

MATERIALS AND METHODS:

Collection of Plant Material: The stem and wood part of *Cissus pallida* was collected from Nallamalla forest region, Kurnool, Andhra Pradesh in June. Plant material was thoroughly washed with water to remove adhered particles and debris and shade dried. The air-dried plant was powdered using Pulveriser and passed through sieve no.20. The plant specimens were identified and authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University Tirupathi, Andhra Pradesh, India.

Extraction: About 1 kg of powdered plant material was extracted by Soxhlet extractor using methanol as a solvent. Percentage of the extract was determined. The extract was dried under reduced pressure using rotator vacuum evaporator afforded semisolid extract⁵.

Phytochemical Analysis: The methanolic extract was subjected to systematic qualitative phytochemical screening to identify the phytoconstituents⁶.

Antimicrobial Screening:

Microorganism Strains: Bacterial strains of *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa*, and *Aspergillus niger*, *Aspergillus fumigates*, *Monascus purpures* fungal strains were used for the study. Ciprofloxacin and Fluconazole were used as the standard drugs.

Preparation of the medium: Peptone: 0.6g%, Tryptone: 0.4g%, Yeast extract: 0.3g%, Beef extract: 0.15g%, Dextrose: 0.1g%, Agar: 1.75g%, Distilled water q.s to 100 ml. The pH of the medium was adjusted to 6.5 - 6.6 before autoclaving the media. The media was autoclaved at 15lb of pressure per square inch at 121 °C for 15 min. A stock solution of the respective plant extract at the concentration of 100 g/ml was prepared using 10% DMSO for the samples. Stock solutions were diluted further with DMSO.

10% DMSO was used as the control. Then the antimicrobial activities of the extracts of different concentration and standard drug ciprofloxacin, Fluconazole were determined by the conventional disc diffusion method against different strains. For the sensitivity test, the wells were filled with different concentrations of the extracts. Then the plates were incubated at 37 °C. After 24 h were determined by measuring the zone of inhibition. The results (zone of inhibition) obtained were mean of three readings in each case⁷.

RESULTS AND DISCUSSION: The extract showed significant antimicrobial action against the organism used by disc diffusion methods. The organisms *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa* were shown 10, 11, 10, 9, 10/100µgm of disc compared with ciprofloxacin 5µgm/disc and *Aspergillus niger*, *Aspergillus fumigates*, *Monascus purpures* fungal strains shown 8, 11, 11/100µgm of disc of the extract compared with Fluconazole 20 µgm/disc. The zone of inhibition was shown in the **Fig. 1-8**.

Herbs and herbal materials were the potential useful lead for the development of novel chemotherapeutic agents. *The in-vitro* screening was the primary step in the screening of the novel chemotherapeutic agents.

TABLE 1: ANTIBACTERIAL ACTIVITY OF *CISSUS PALLIDA* EXTRACT

S. no.	Organisms	Ciprofloxacin (5µg/disc)	<i>Cissus pallida</i> (100µg/disc)
1	<i>Bacillus subtilis</i>	14 mm	10 mm
2	<i>Klebsiella pneumoniae</i>	15mm	11mm
3	<i>Staphylococcus aureus</i>	20 mm	10 mm
4	<i>Escherichia coli</i>	17 mm	9 mm
5	<i>Pseudomonas aureginosa</i>	20 mm	10 mm

The spectrum of activities of the herb majorly due to the presence of phytoconstituents in the particular plant used as a health remedy in the folklore medicine. Phyto molecules from the herb can be used as antimicrobials for the treatment of various microbial infections. Natural products with different medicinal herbs were used in the folk practice to prevent microbial infections⁸.

Figures Showing the Zone of Inhibition of Extract and the Standard:



FIG. 1: EXTRACT TREATED AGAINST *BACILLUS SUBTILIS*

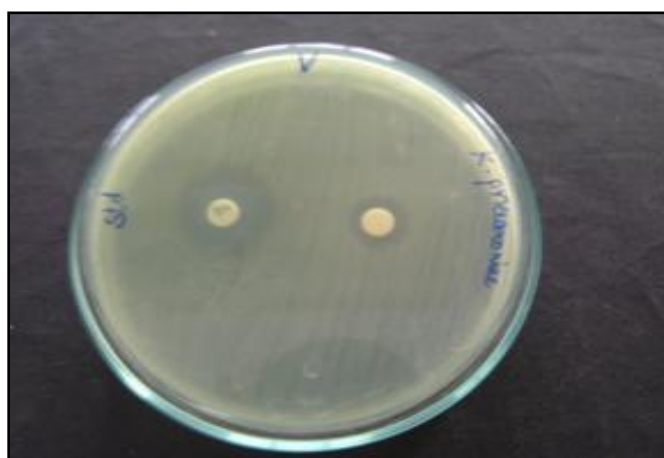


FIG. 2: EXTRACT TREATED AGAINST *KLEBSIELLA PNEUMONIA*

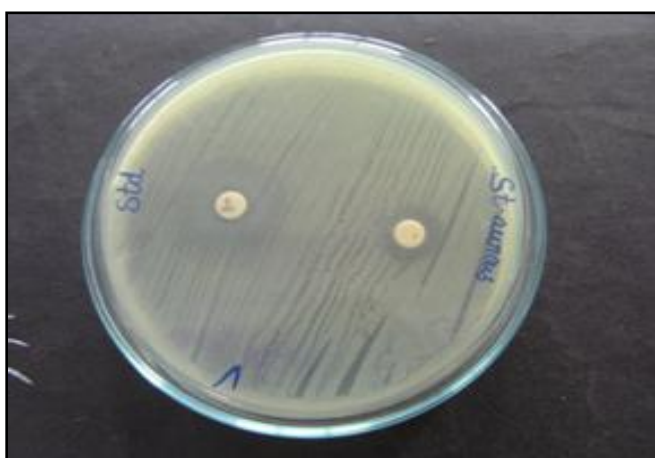


FIG. 3: EXTRACT TREATED AGAINST *STAPHYLOCOCCUS AUREUS*

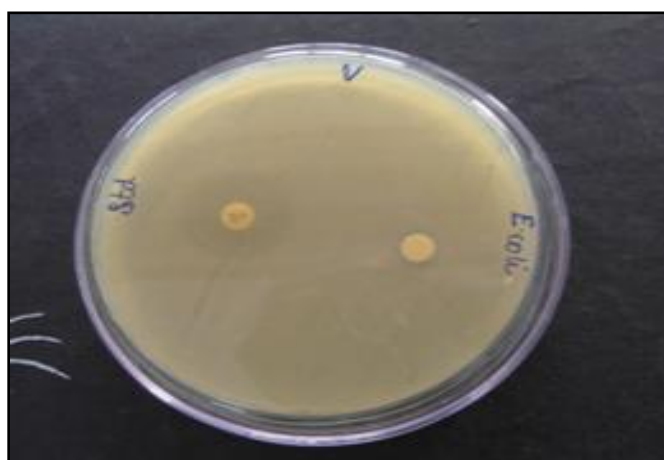


FIG. 4: EXTRACT TREATED AGAINST *ESCHERICHIA COLI*



FIG. 5: EXTRACT TREATED AGAINST *PSEUDOMONAS AUREGINOSA*

TABLE 2: ANTIFUNGAL ACTIVITY OF *CISSUS PALLIDA*

S. no.	Organisms	Standard Fluconazole (20µg/disc)	<i>Cissus pallida</i> (100µg/ disc)
1	<i>Aspergillus niger</i>	9 mm	8 mm
2	<i>Aspergillus fumigatus</i>	19 mm	11 mm
3	<i>Monascus purpureus</i>	20 mm	11 mm

Figures Showing the Zone of Inhibition of Extract and the Standard:**FIG. 6: EXTRACT TREATED AGAINST ASPERGILUS NIGER****FIG. 7: EXTRACT TREATED AGAINST ASPERGILUS FUMIGATUS****FIG. 8: EXTRACT TREATED AGAINST MONOSCUS PURPURES**

CONCLUSIONS: Herbs are an important source of potentially useful structures for the development of new chemotherapeutic agents. The primary step towards this research is the *in-vitro* antibacterial activity assay.

The broad spectrum antibacterial activities of the plant extract, possibly due to the identified phytoconstituents, further confirm its use as a health remedy in traditional medicine. Bioactive substances from the natural sources can be used in the antimicrobial agents for the treatment of various microbial infections.

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CONFLICT OF INTEREST: Nil

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