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ANTIMICROBIAL ACTIVITY OF STEM BARK OF *COMBRETUM ALBIDUM* G. DON; A TRADITIONAL MEDICINAL LIANA

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ABSTRACT: Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. In the present scenario, emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. *Combretum albidum*, a traditional medicinal liana, used by rural peoples, villagers and tribals residing in different parts of Maharashtra, Tamil Nadu and Kerala, to treat various ailments. The Muthuvans, Chinnar tribe of Idukki district, Kerala is using stem bark of this plant as a remedy for Jaundice. The present study carried out to check the antimicrobial activity of water and methanol extracts of stem bark of *C. albidum*. The result showed significant antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhimurium.*

INTRODUCTION: lndian subcontinent excels in plant biodiversity having several medicinal properties. It enables traditional healers to develop effective therapies against various ailments. It has been estimated that nearly 80% of the world population depends on plant based medicine for their health care and similar trends were ported from lndian where a large population of rural peoples and tribals rely heavily on the use of traditional plants for physical and psychological health needs. Due to safety and cost effectiveness the usage of plants in traditional as well as modern medicine is on the increase [1].

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Infectious diseases are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and on-going epidemics of human immunodeficiency virus (HIV) infections [2, 3].

This situation provided impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [4]. Plant medicines are used on a worldwide scale to prevent and treat infectious diseases [5].

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found *in vitro* since they have antimicrobial properties and may serve as an alternative, effective, cheap and safe antimicrobial for the treatment of microbial infections [6]. Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance [7].

The Muthuvans, Chinnar tribe of Idukki district, Kerala is using *C. albidum* as a remedy for Jaundice. *Phyllanthus amarus* and *P. airy-shawii* are very common in this area and they are aware of the use of these plants, even though the tribe is using water extract of stem bark of *C. albidum* as an effective remedy for both normal and severe jaundice [8]. *C. albidum*, commonly known as Manjakody, is a large woody climbing, deciduous shrub, up to 30 m high; its distribution is restricted to semi-evergreen and deciduous forests, along river banks of Peninsular India and Sri Lanka.

The macro- and, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation and HPTLC profiles of sequential extraction of stem bark were studied[9]. The preliminary phytochemical screening of leaf, stem, flower and fruit in various extract *i.e. petroleum ether*, *benzene*, *chloroform*, *acetone*, *ethanol* and *water* were also reported [10]. Its bark used for treating skin diseases [11], liver protection [8, 9]. The leaf of this plant is anti-ulcer in activity [12].

A decoction of fruit is taken for dysentery and diarrhea [13]. One to two tea spoon of fruit powder is burnt with a pinch of alum powder and the fumes inhaled once a day at night before sleep up to 15-18 days to cure cough and cold [14]. Antibacterial screening of medicinal plant extracts from Western Ghats by microtitreplate resazurin reduction assay showed no antibacterial activity of flowers of *C. albidum* against *Staphylococcus aureus*, *Pseudomonos aeruginosa*, *Bascillus subtilis* and *Escherischia coli* [15].

The present study carried out to check the antimicrobial activity of water and methanol extracts of stem bark of *C. albidum*, a traditionally important medicinal liana.

MATERIALS AND METHODS:

Collection and Authentication: The plant collected from Chinnar, Idukki district of Kerala, during the month of March-April and authenticated at Taxonomy Division, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala. The voucher specimen (Col. No. 5545) and raw drug (Col. No. CMPR/RD 211) were deposited at CMPR herbarium and raw drug depository respectively. One part of the stem bark were shade dried, powdered and passed through 20 mesh sieve and stored in an airtight container for further use.

Antibacterial Activity:

Preparation of Plant Extract: The stem bark were thoroughly washed, dried in shade and powered. 100g of the dried stem bark were successfully extracted using Soxhlet apparatus with methanol as solvent. After two days of extraction the solvent was evaporated using vacuum and the residue obtained was used for the studies. The extract was dissolved in Dimethyl sulfoxide (DMSO) and used antibacterial screening at the concentration of 1g/5ml. The water extract was prepared by weighing 100mg powder of stem bark by boil with 300ml of distilled water in a water bath for 24 hours, filtered and evaporated. The extract obtained was dissolved in sterile distilled water at a concentration of 1g/5ml and used for antibacterial screening.

Bacterial cultures: Bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. The microorganims used for the present study include *Escherichia coli* (MTCC40), *Klebsiella pneumonia* (MTCC3384), *Proteus mirabilis* (MTCC425), *Pseudomonas aeruginosa* (MTCC741), *Bacillus subtilis* (MTCC 441), and *Salmonella typhimurium* (MTCC). All the bacterial cultures were maintained in nutrient agar and stored at 4^{o}C.

Preparation of inocula: Several colonies were transferred to sterile peptone water (5 ml) from the sub cultured organism. The suspensions were mixed for 15 seconds to ensure homogeneity and subsequently diluted to match the turbidity of a 0.5 McFarland standard (i.e. OD = 0.12–0.15 at k = 530 nm, corresponding to 1–5 x 106 CFU/ml).

Antimicrobial screening: The antimicrobial assay was performed by two methods viz. agar disc diffusion method [16] and agar well diffusion method [17]. Mueller Hinton Agar (MHA) was prepared in plates as the media for test bacteria. The bacterial inoculum was spread evenly on the surface of the MHA plates using a sterilized cotton swab. For agar disc diffusion method, sterile filter paper discs (6mm) were saturated with different concentrations of the test compound, allowed to dry and introduced on the upper layer of the seeded agar plate.

For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.6cm). 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37°C. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. Sterile distilled water served as negative control. The result was obtained by measuring the zone diameter. The experiment was done thrice and the mean values are presented. The results were compared with the standard antibiotics nitrofurantoin (300mg/disc), chloram- phenicol (30mg/disc), cephalexin (30mg/disc) and gentamicin (10 mg/disc) [7].

RESULT AND DISCUSSION: Emergence of multidrug resistance in pathogenic bacteria as well as undesirable side effect of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The results of the present study reveal the significant dose dependent antibacterial activity of aqueous and methanol extract of *C. albidum*. Both the extracts significantly inhibited the growth of all the bacterial strains tested. The alcohol extract was found to be more efficient in inhibiting the bacterial growth than the water extract. **Table 1, Figure 1 and 2** showed the results of antibacterial potential of *C. albidum* water and methanol extracts. *P. mirabilis* was found to be the most sensitive and *E. coli* the least sensitive organism to both the extracts.

The higher concentration of the extract used (200µl) inhibited the growth of *P. mirabilis* by 37 and 31 for water and methanol extract respectively. The same concentration of the extracts showed a growth inhibitory zone measuring 16 and 22 for water and methanol extract respectively towards *E. coli*. The extract at all the concentrations significantly inhibited the growth of *S. typhi*. 200 µl of the water and methanol extract inhibited the growth of *S. typhi* by 21 and 29 mm respectively. The growth inhibitory activity of the extract towards *S. typhi* is of great importance as typhoid remains still a chronic illness. The extract also showed an efficient inhibition of the growth of *K. pnuemoniae, B. subtilis and P. aeruginosa* dose dependently.

TABLE 1: ANTIBACTERIAL ACTIVITY OF STEM BARK OF *C. ALBIDUM* WATER EXTRACT

Organism	Different concentration of the extract			
	50µl	100 µl	150 µl	200 µl
Escherichia coli	13	14	15	16
Klebsiella pneumonia	19	20	21	25
Proteus mirabilis	25	28	30	31
Pseudomonas aeruginosa	11	12	16	17
Bacillus subtilis	15	17	18	20
Salmonella typhimurium	18	19	20	21

1gm/5ml zone diameter in mm

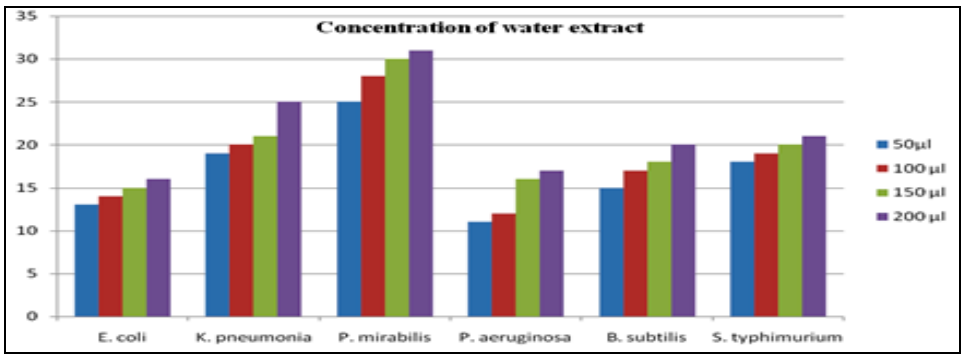

FIGURE 1: ANTIBACTERIAL POTENTIAL OF *C. ALBIDUM* WATER EXTRACT

TABLE 2: ANTIBACTERIAL ACTIVITY OF *C. ALBIDUM* METHANOL EXTRACT

Organism	Different concentration of the extract			
	50µl	100 µl	150 µl	200 µl
Escherichia coli	15	18	20	22
Klebsiella pneumonia	24	26	25	29
Proteus mirabilis	29	30	33	37
Pseudomonas aeruginosa	19	21	23	26
Bacillus subtilis	18	20	21	22
Salmonella typhimurium	23	24	25	29

1gm/5ml zone diameter in mm

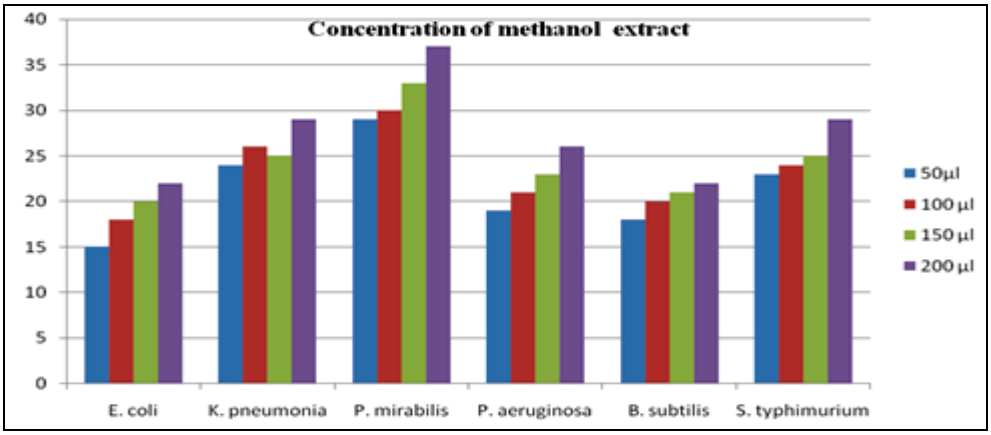

FIGURE 2: ANTIBACTERIAL POTENTIAL OF *C. ALBIDUM* METHANOL EXTRACT

The antibacterial activities of the extracts studied were compared with standard antibiotics. The zone of growth inhibition formed by the standard antibiotics was given in **Table 3**. The results signify that both the extracts possess more growth inhibitory activity than the standard antibiotics against all the tested organisms.

TABLE 3: ANTIBACTERIAL ACTIVITY OF STANDARD ANTIBIOTICS

Bacterial strains	Zone of Inhibition (mm)			
	FU	CP	CK	GM
B. subitilis	12	R	20	13
E. coli	14	R	R	8
K. pneumoniae	9	R	14	9
P. mirabilis	22	25	21	20
P. aeruginosa	R	R	R	R

FU: Nitrofurantoin, **CP**: Ciprofloxacin, **CK**: Chloramphenicol, **GM**: Gentamicin

The study thus reveals the effectiveness of the tested plant extracts against some pathogenic bacteria commonly associated with various human infections. *C. album* stem bark can be used as a potential source for the development of a phytomedicine to act against infectious bacteria. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from plants should be emphasized for the control of infectious diseases

CONCLUSION: Medicinal plants offer alternative remedies with tremendous opportunities. lndian subcontinent excels in plant biodiversity having several medicinal properties. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants.

The present study reveals the antibacterial activity of stem bark of *C. albidum* and can be treated against the infectious pathogens like *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhi.* The successful screening of this plant as a potential source of bioactive substances has urged to continue further tests in order to isolate the active ingredients involved.

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