ABSTRACT
Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents; hence resistance to older and newly produced drugs is on the rise. The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Thus the present study was undertaken to investigate the antibacterial activity of crude flower extracts of *Combretum indicum* (L.) DeFilipps against gram-positive as well as gram-negative human pathogenic bacterial strains. Agar well diffusion assay method was used to determine the antibacterial activity of the extracts. The different solvent extracts have shown marked inhibition against the tested human pathogenic bacterial strains. The results showed that *Staphylococcus aureus* was highly susceptible to the extracts as compared with the *Pseudomonas aeruginosa*, *Streptococcus* sp. and *Salmonella typhimurium*, bacterial strains. The results also showed that the methanol extract of *Combretum indicum* was the most effective as the widest inhibitory zone was observed compared to the ethanol as well as aqueous extracts. The commercially available, standard antibiotic streptomycin offers relatively higher inhibition as compared to the tested different solvent extracts. This revealed that the crude flower extracts of *Combretum indicum* have antibacterial activity against the treated bacterial strains, probably could be used to control infections associated with these human pathogenic bacteria.

KEYWORDS: Antibacterial, *Combretum indicum*, Solvents, Zone of Inhibition. Human Pathogens.
INTRODUCTION

The medicinal use of plants is probably as old as mankind. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified.[1] The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants is found in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge.[2] In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. The indigenous system of medicine, namely Ayurvedic, Siddha and Unani have been in existence for several centuries. From over 3, 00,000 species of higher plants to occur in nature, only about 2 percent have been screened so far.[3]

Plants are a rich source of antimicrobial agents.[4] An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria. Such compound is said to have antibacterial activity.[5] About 60 to 90% of populations in the developing countries use plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases.[4, 6, 7] Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties.[8, 9] Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses.[10, 11]

The global prevalence of infectious diseases caused by bacteria is a major public health problem.[6, 12] The bacterial agents, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Proteus vulgaris is caused several human infections.[13] The recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents and are prompting a revival in research on the antimicrobial role of plants against resistant strains due to comparable the safety and efficacy.[4, 14]

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide.[15] Various parts of the plants like root, bark, seed and leaves have been an important source of medicine for thousands of years. In recent years a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria. India has a rich flora of medicinal plant species that are widely distributed throughout the country.[16]
Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi-synthetic resources. It has been reported that plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs.\textsuperscript{[2]} There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.\textsuperscript{[17]}

*Combretum indicum* (L.) DeFilipps commonly known as Rangoon Creeper is an excellent vine for outdoor gardens belonging to family Combretaceae. It is a strong climber, ligneous vine that can reach from 2.5 m to up to 8 m (Figure 1). This plant is indigenous in Africa, Indo Malaysian region and cultivated all over India. Flowers numerous, pendent, 7.5 cm long, 3.8 cm wide. At first they are white in color, then they become deep red.\textsuperscript{[16]}

The selection of crude plant extracts of screening programs has the potential of being more successful in the initial steps than the screening of pure compounds isolated from natural products.\textsuperscript{[18]} The present study was planned to evaluate the antibacterial activity of *Combretum indicum* flower extracts against selected human pathogenic bacterial strains.

*Figure 1: Photo of *Combretum indicum* (L.) DeFilipps plant*
MATERIALS AND METHODS

Plant collection
Fresh flowers of *Combretum indicum* (L.) DeFilipps were collected from my outdoor garden, Rohtak, Haryana, and brought to the laboratory in polythene bags. Rinsed the flowers twice with distilled water and shade dried on a clean sheet for one week at room temperature. It was made into small pieces using sharp, sterile scissors and powdered using sterile mortar and pestle.

Extract preparation
Extraction was done at room temperature by the simple extraction method. 100 gm coarse powdered plant material was immersed in 200 ml of different solvent (methanol, ethanol and aqueous) contained in 500 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for 5 days. They were first filtered with double layered muslin cloth and then through Whatman No. 1 filter paper, and the march was discarded. The filtrate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. The dried extract was dissolved in solvent Dimethyl sulphoxide [DMSO] in a ratio of 100mg/ml to determine the antibacterial activity by agar well diffusion assay (AWDA) method.[19]

Source of microorganisms
Four bacterial strains, including both gram-positive as well as gram-negative bacteria were used in the present study: *Pseudomonas aeruginosa* (MTCC4673), *Staphylococcus aureus* (MTCC6908), *Salmonella typhimurium* (MTCC3224) and *Streptococcus* sp. (MTCC9724) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The bacterial strains were grown in nutrient broth medium, pH 7.0 and incubated for overnight at 37ºC. Stock cultures were maintained on a nutrient agar slant pH 7.0 at 4°C until needed. The media components were purchased from Hi-media, Mumbai, India.

Antibacterial activity by agar well diffusion assay (AWDA) method
The antibacterial activity of the crude flower extracts (methanol, ethanol and aqueous) of *Combretum indicum* against two gram-positive as well as two gram-negative bacterial strains were evaluated by agar well diffusion assay method.[20] For this, a well (6 mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with 5 ml soft agar, seeded with a target strain (~10^6 cfu/ml). Aliquots (100 μl) of the test compound were introduced into the well and the plates were incubated overnight at 37 ºC. The diameters of
the inhibition zones were measured in millimeters (mm). For each bacterial strain, the dissolving solvent DMSO and streptomycin (50 µg/ml) were used as negative and positive controls respectively.

**Statistical analysis**

The experiment was carried out in three independent sets, each consisting of three replicates. Values shown here represent mean ± standard error of the mean (SEM).

**RESULTS AND DISCUSSION**

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in human beings. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs has increased and has now become a global concern.[21] Due to the increase of resistance to antibiotics, there is a pressing need to develop new and innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as a standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.[22, 23] Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infection.[17] The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[23, 24]

Many reports are available on the antibacterial, antifungal, antiviral, anthelmintic, antimolluscal and anti-inflammatory properties of plants.[25, 26] Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

There are many literatures reporting the ethnomedicinal values of *Combretum indicum*.[27-29] but there is little scientific proof for further using this plant commercially or in a more effective form. Therefore, in order to evaluate the antibacterial activity of *Combretum*
*Combretum indicum* flower extracts against both gram-positive as well as gram-negative human pathogens, assessed by AWDA method. The results of the present study exhibited the excellent antibacterial activity against treating human pathogenic bacterial strains as shown in Figure 2.

![Antibacterial activity of flower extracts of *Combretum indicum* (L.) DeFilipps](image)

**Figure 2: Antibacterial activity of flower extracts of *Combretum indicum* (L.) DeFilipps**

Among the treated four different bacterial strains, *Staphylococcus aureus* was the most sensitive pathogen with the inhibition zone (mm) 20 (methanol), 18 (ethanol), 15 (aqueous). However the *Salmonella typhimurium* exhibited less sensitivity as compared to the other treated pathogens with the inhibition zone (mm) 15 (methanol), 12 (ethanol), 10 (aqueous). *Pseudomonas aeruginosa* as well as *Streptococcus* sp. shows intermediate sensitivity with the zone of inhibition (mm) 17 (methanol), 15 (ethanol), 12 (aqueous) and 18 (methanol), 16 (ethanol), 14 (aqueous) respectively. The results of the present study revealed that commercially available, used standard antibiotic streptomycin offers relatively higher antibacterial activity as compared to different tested solvent extracts with the inhibition zone (mm) 22 (*Staphylococcus aureus*), 20 (*Streptococcus* sp.), 19 (*Pseudomonas aeruginosa*) and 17 (*Salmonella typhimurium*). However, no zones of inhibition were observed against DMSO solvent. The results indicate that the tested different solvent extracts showed the significant
antibacterial activity against both gram-positive and gram-negative human pathogenic bacterial strains with various degrees.

It is known that the successful prediction of extracting compounds from plant material is largely dependent on the type of solvent used in the procedure for the extraction. The traditional practitioners make use of water as a primer solvent, but in the present study, the methanol extract of *Combretum indicum*, was the most effective as the widest inhibitory zone was observed as compared to the ethanol as well as aqueous extract used. This may be due to better solubility of the active compounds in organic solvents.[30] Many studies have revealed that the methanol extract inhibited the growth of treated bacteria more than the aqueous extract.[31, 32] The present investigation also supports the above observations.

In the present investigation, antibacterial activity of the *Combretum indicum* crude flower extracts against treating two gram-positive and two gram-negative human pathogens are an indication that there is a possibility of discovering an alternative antibiotic substance in these plants for the emerging of newer antibacterial agents and carry out further pharmacological evaluation.

**CONCLUSION**

The results of the present study indicate that the crude flower extract of *Combretum indicum* (L.) DeFilipps showed remarkable antibacterial activity against two gram-positive (*Staphylococcus aureus* and *Streptococcus* sp.) as well as two gram-negative (*Pseudomonas aeruginosa* and *Salmonella typhimurium*) human pathogenic bacterial strains. The data revealed that the methanol extract showed better antibacterial activity as compared to other tested solvent extracts. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The demonstration of a broad spectrum of antimicrobial activities of the plants used in this study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibilities for the treatment of diseases caused by these tested pathogens. Further research has to be conducted on the activity of the extracts against a wider range of bacteria as well as fungi.

**COMPETING INTERESTS**

The author declares that he has no competing interests.
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