

Antimicrobial Activity of Leaf extracts of *Antidesma menasu* Miq.

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Abstract: The present study aimed to evaluate the antibacterial and antifungal activity of the leaf extracts of *Antidesma menasu* against four bacteria namely, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexneri* and *Escherichia coli* as well as two fungi viz., *Candida albicans* and *Cryptococcus neoformans*. The results indicated the maximum zone of inhibition in ethanol extract of *Antidesma menasu* against *Staphylococcus aureus* and the results reveals that the leaves have a potential against bacterial activity.

Index terms- Antibacterial, *Antidesma menasu*, Antifungal, *Staphylococcus aureus*

I INTRODUCTION

India is rich with wild natural resources and motherland of many herbal plants in which some highly potent and considered as life savings drugs. Medicinal plants are the wealthy source of antibacterial agents and curatives [1]. They have been recognized as a valuable source of therapeutic components for centuries and about 60% of the world's population are known to use the traditional medicines derived from medicinal plants. In the last few years, plant extracts and phytochemicals with known antimicrobial properties have been investigated by a number of researchers in different countries to understand their properties, efficacy and safety [2]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action. In recent years, multiple drug resistant has developed due to indiscriminate usage of existing antimicrobial drugs in treatment of infectious diseases. In addition to this, antibiotics are sometimes associated with adverse effects on the hosts like hypersensitivity. Therefore there is a need to develop, alternative antimicrobial drug for the treatment of infectious diseases from other sources such as plants [3]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulteration and side effects. Therefore, there is a need to search new infection-fighting strategies to control microbial infections [4].

The history of infection and infectious diseases is as old as mankind. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compound as anti-microbial agent [5]. The reference of microorganisms is available in the oldest manuscripts of Ayurveda and Vedas as well. Plant derived substances have recently become of great interest owing to their versatile applications [6].

Infectious diseases are the world's leading cause of premature death, killing almost 50,000 people every day. Morbidity and mortality due to diarrhoea continues to be a major problem in many developing countries, especially amongst children. Infections due to a variety of bacterial etiologic agents such as pathogenic *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus* are most common [7].

Euphorbiaceae family in the plant kingdom is a complex heterogeneous family consisting of about 322 genera and 8900 species in the world [8]. Various plant members are used in different types of herbal preparations from the ancient to present day. The antibacterial study of the leaf extract of *Euphorbia hirta* showed highest activity against *Escherichia coli* and *Enterobacter aerogenes* [9]. *Euphorbia milli* also known as crown of thorn and its milky latex is used to treat abdominal oedema, constipation and sprains relieve. Chloroform extract of the leaves of this plant showed positive antimicrobial activity against *Aspergillus fumigatus*, *Staphylococcus aureus*, and *Bacillus subtilis* [10]. *Sapium sebiferum* plant extract has a broad pharmacological effects such as antibacterial, anti-inflammatory, blood pressure, cholesterol etc. *Flueggea leucopyrus* is one of the medicinally used bushy weed and it has been used in preparations of traditional medicines for the treatment of cough, hay asthma, bowel complaints, disinfections, laxatives, diarrhoea, gonorrhoea, constipation, mental illness, and kidney stones. An aqueous ethanolic extracts of leaf of *Flueggea leucopyrus* had shown antibacterial activity against both Gram positive and Gram negative bacteria [11].

Antidesma is a homogeneous genus in the family Euphorbiaceae. There are about 170 different species of *Antidesma* in the world and many species from this genus have been used traditionally for medicinal purposes. The methanol extract of *A. ghaesembilla* has been reported to possess antimicrobial, antioxidant and cytotoxic activity while *A. madagascariense* is reported to have significant antimicrobial activity and the ethanol extract of *A. bunius* is reported to have cytotoxic activity [12]. *Antidesma venosum* is used in traditional medicine to treat a diversity of conditions, including hookworm infestation, gonorrhoea, malaria and bilharzias, schistosomiasis, abdominal disorders, dysentery and cut wounds. Extracts of this plant are reported to possess anti-inflammatory activity and have also been used for the treatment of diarrhoea, anaemia and lack of appetite, tuberculosis and

Candida infections. Petroleum ether and dichloromethane fractions of the roots of this particular plant shows a promising activity against Gram positive bacteria and thus support the use of this plant for the treatment of conditions associated with bacterial infections such as cut wounds, chest infections and some types of diarrhoea [13]. Leaves of *Antidesma acidum* Retz. are used for treatment of stomach ache of children and in case of digestion. In the Western Ghats region leaves are used against dysentery and in case of appetizer [14]. *Antidesma madagascariense* is an indigenous and an endemic plant that has always been used in folkloric medicine among the local population of the Mascarene Islands for the treatment and management of various ailments. A decoction of the leaves of *A. madagascariense* has been traditionally used to treat dysentery. A bath in the leaf decoction has been reported to alleviate skin infections, rheumatic and body aches. The different crude extracts of *A. madagascariense* exhibited potent antimicrobial activity which was found to increase with increasing polarity [15].

Antidesma menasu is a folklore medicinal plant belongs to family Euphorbiaceae. This is a shrub or small tree; leaves of these plants are used by folklore practitioners in joint pains and inflammatory swelling [16][17]. It is found commonly in Belgaum, Chikmagalur, Kodagu, Hassan, Mysore, Uttara Kannada, Shimoga, Dakshina Kannada of Karnataka and Coimbatore, Dharmapuri, Dindigul, Kanniyakumari, Nilgiri, Salem, Theni, Tirunelveli, Virudhunagar of Tamil Nadu during rainy seasons.

The objectives of the present study was to predict the antibacterial activity of the leaf extract of *A. menasu* against few selected bacteria namely *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexneri* and *Escherichia coli* as well as antifungal activity against *Candida albicans* and *Cryptococcus neoformans*.

II MATERIALS AND METHODS

Collection of plant: The leaves of *Antidesma menasu* was collected from Alike, Dakshina Kannada district, Karnataka during September 2014 and authenticated from Department of Pharmacognosy, S.D.M Research Center, Udupi.

Preparation of extracts: The leaf samples were cleaned, dried in shade and coarsely powdered using an electric blender and stored in an air tight container. Leaf extracts were prepared by using soxhlet [18].

Fifteen grams of coarsely powdered leaf material was extracted for 24 hours by using soxhlet apparatus with water as well as ethanol separately. The process was repeated until to get required amount of extract. Aqueous extract was obtained by evaporating water from the extract by using boiling water bath. Ethanol was recovered from ethanol extract by distillation and dried. These extracts were stored in air tight containers and preserved at -4°C until further use.

Culture media and strains: The pathological strains used for antimicrobial study were obtained from Alva's College of Medical Laboratory Technology, Moodbidri, Karnataka, India. They include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Candida albicans* and *Cryptococcus neoformans*. For antibacterial study peptone water and Mueller Hinton Agar and for antifungal study Sabouraud Dextrose broth and Sabouraud Dextrose agar (HiMedia Labs Pvt. Ltd., Mumbai, India) were employed.

Determination of antibacterial activity: The antibacterial activity of the leaf extracts of *Antidesma menasu* was determined by following agar well diffusion method [19]. The ethanol extract as well as aqueous extract were tested against bacteria and fungi. Bacterial strains were sub cultured overnight at 37° C in peptone water and fungal strains were subcultured to Sabouraud dextrose broth. The cultures were swabbed on to the plates containing Mueller Hinton Agar for bacteria and Sabouraud dextrose agar for fungi. Wells were made by using cork borer and 50µl of each sample with different concentrations were inoculated. Ciprofloxacin (5µg) and Methicillin (5µg) disks were used as standard antibiotic disks (C1) for bacteria and Fluconazole (25µg) disks were used as standard disk for fungi. Distilled water and ethanol served as negative control (C2) with respect of extract types. The plates were incubated at 37°C for 24 hours for bacteria and at 37°C for two days for fungal strains. The diameters of zone of inhibition were measured.

III RESULTS AND DISCUSSION

In the solvent extraction method, 15.29% extract yield was obtained with ethanol and 18% yield with water/aqueous. The ethanol extract has shown zone of inhibition against *Staphylococcus aureus*. The minimum inhibition concentration (MIC) was observed at the concentration of 300µg (Table 1). Other bacteria have not shown any zone of inhibition against ethanol extract. No antibacterial activity was observed in aqueous extract. The methanolic leaf extracts of *Antidesma madagascariense* exhibited inhibitory effects against *Enterococcus faecalis*, *S. aureus*, Methicillin-resistant *S. aureus* (MRSA) and *Candida albicans* [15]. Similarly, in another study by Hassan et al [8], the methanolic and ethanolic extracts of *Sapium sebiferum* showed significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. An aqueous ethanolic extracts of leaf of *Flueggea leucopyrus* had shown antibacterial activity against both Gram positive and Gram negative bacteria [11]. Both aqueous as well as ethanol extracts have not shown any antifungal activity against *Candida albicans* as well as *Cryptococcus neoformans*.

Table 1: *Staphylococcus aureus* showing different zone of inhibitions at different concentrations of ethanol extract of *Antidesma menasu*

Sl.No	Test organism	Concentration	Diameter of zone of inhibition	
			Methicillin(5 μ g)	Ethanol
1.	<i>Staphylococcus aureus</i>	300 μ g	2cm	0.5cm
		350 μ g		0.6cm
		400 μ g		0.8cm
		500 μ g		1.2cm
		1000 μ g		1.7cm

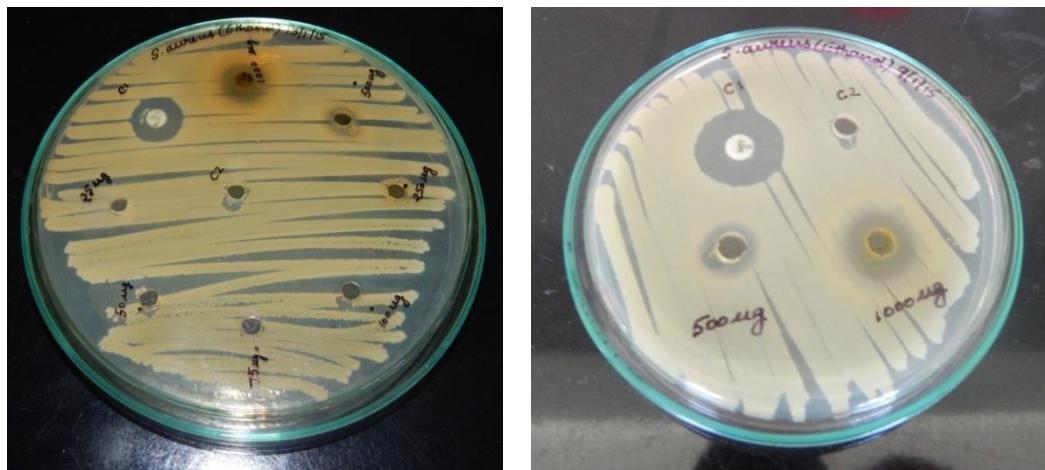


Fig. 1: Petri plate showing zone of inhibition at different concentrations of ethanol extract of *Antidesma menasu*



Fig. 2: Petri plate showing Minimum Inhibition Zone at the concentration of 300 μ g/ml.

IV CONCLUSION

In the present study, crude aqueous and ethanolic leaf extracts of *A. menasu* were tested against four bacterial strains and two fungal strains. Among this, the ethanol extract has got significant antibacterial activity on *Staphylococcus aureus*. The above results proved antibacterial activity of *Antidesma menasu*.

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