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THE ANTIMICROBIAL ACTIVITY OF *PARGULARIA DAEMIA* LEAVES EXTRACT AGAINST BACTERIAL PATHOGENS ISOLATED URINARY TRACT INFECTIONS

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ABSTRACT

The leaves of *Pargularia daemia* belonging to family Asclepiadaceae was investigated to evaluate in vitro antimicrobial activity of methanolic extract against urinary tract infection causing pathogens consisting of *Staphylococcus aereus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Candida albicans,* and *Aspergillus niger*. The major Urinary Tract Infection (UTI) causing bacterial and fungal pathogens under investigation were tested by the disc diffusion assay method and the minimum inhibitory concentration was evaluated. Methanolic extract exhibited a significant and broader spectrum of inhibition followed by petroleum ether, aqueous and chloroform extract against bacterial pathogens. The results of antifungal activity revealed that *Pargularia daemia* extract inhibited fungal pathogens significantly with a broader spectrum of inhibition. An attempt has been made to compare the activity of extract with most potent standard antibiotics (Baccteria - ciprofloxacin (25 µg/disc; Fungi - clotrimazole (10 µg/disc) effective against selected UTI causing pathogens under test.

KEYWORDS: Urinary Tract Infection, Antimicrobial activity, Antibiotics, *Pargularia daemia.*

INTRODUCTION

Urinary Tract Infection (UTI) represents one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today (Raju and Tiwari, 2004). More than 95% of UTI are caused by single bacterial species E. coli which is the most

frequently infecting organisms (Kebira et al., 2009). However, many other bacteria can also meet and lead to infections for example, Klebsiella, Pseudomonas, Enterobacter, Proteus, Staphylococcus, Mycoplasma, Chlamydia, Serratia and Neisseria spp. It is reported that about 35% of healthy women suffer symptoms of urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency (Hootan 2003).

A study realized in at the Avicenne Teaching Hospital (Marrakech, Morocco) from 2010 to 2012 showed that Escherichia coli and K. pneumoniae have been reported to be the most common organisms causing UTI (Bouamri et al., 2015). This is demonstrated by the prevalence of these two pathogens in the epidemiology of both nosocomial and community-acquired UTIs. Although *E. coli* is a more common cause of UTIs (63%) and the prevalence of *K. pneumoniae* species has been 22%, and it was isolated in 10% and 28% of the urine samples in the Meknes (Lahlou et al., 2009) and Rabat (Tlamcani et al., 2009) regions respectively. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance (Rosina et al., 2009). However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates, 2002). For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against microbe strains with ultimate goal to provide efficient drugs to the patient. Finding healing powers in plants is an ancient idea.

People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory (Rosina et al., 1999). The present study, realized in the first time in Morocco, made an attempt to find out the chemical composition and the antimicrobial activity of *Pargularia daemia* (Tamil: Velliparuthi) leaves as well as to compare its inhibitory effect versus commercial antibiotics (Baccteria - ciprofloxacin (25 µg/disc; Fungi - clotrimazole (10 µg/disc) against five bacteria's urinary tract infections bacterial pathogens isolates.

MATERIALS AND METHODS

Plant materials

The leaves of *Pargularia daemia* were collected in January 2017 from Manimandapam, Thanjavur, Thajavur district, Tamil Nadu, India.

Preparation of alcoholic extract

The leaves of *Pargularia daemia* were first washed well and dust was removed from the leaves. The leaf was dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Microorganisms

The antimicrobial activity of *Pargularia daemia* essential oil was tested against *Staphylococcus aereus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Candida albicans,* and *Aspergillus niger* isolated from patients having urinary infection in nephrology service at SPL Hospital at Thanjavur, these bacteria and fungi were identified and confirmed by classical biochemical gallery and the API (bioMérieux, France). The commercial antibiotics (Baccteria -ciprofloxacin (25 µg/disc; Fungi - clotrimazole (10 µg/disc) were used.

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis* and *Escherichia coli* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* and *Aspergillus flavus,* were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States. Death from infectious disease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, an increase of 58% (Pinner et al. 1996).

It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US (Pinner et al. 1996). This is alarming given that it was once believed that we would eliminate infectious disease by the end of the millenium. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most dramatic increases are occurring in the 25–44 year old age group (Pinner et al. 1996). These negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention (Fauci 1998).

Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These ‘secondary compounds’ instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Liu, 2003). The aim of this study is to will be determined the phytochemical compounds present in the medicinal plant with the aid of analytical techniques and evaluation of their antimicrobial activity.

The phytochemical characters of the *Pargularia daemia* plant investigated and summarized in Table-1. Methanol extract of *Pargularia daemia* leaves showed that the presence of steroids, saponins, triterpenoids, phenolics, carbohydrate, glycosides, flavonoids, tannin and protein. Significant amount of Flavonoids (168mg/gm) and phenol (300mg/gm), saponin (33mg/gm) and terpenoids (50mg/gm).

Table. 1: Phytochemical screening of *Pargularia daemia.*

S.No	Phytochemical analysis	100% Methanol	Quantitative analysis (mg/gm)
1	Tannin	+	-
2	Phlobatannins	-	-
3	Saponin	+	33
4	Flavonoids	++	168
5	Steroids	++	-
6	Terpenoids	-	50
7	Tritrpenoids	+	-
8	Alkaloids	-	-
9	Carbohydrate	-	-
10	Protein	++	-
11	Anthroquinone	-	-
12	Polyphenol	+	300
13	Glycoside	+	-

(+) Presence; (-) Absence; (++) Higher concentrations

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003). This situation forced scientists to search for new antimicrobial substances with plant origin. In the present study to evaluate the antimicrobial activity against UTI causing microbes.

The extract of *Pargularia daemia* leaves showed optimum activity against all tested UTI causing pathogens. However, results of the disc diffusion method as indicated in **Table 2**, Methanolic extract inhibited significant and broader spectrum of inhibition against the UTI pathogens. Methanolic extract showed the maximum inhibition was 12 mm at 150µl while minimum inhibition was 10.20 mm at 50 µl against *Staphylococcus aereus*. Similarly, the maximum inhibition was 7.20 mm, 11 mm, 10.50 mm at the concentration of 150µl against *Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis* respectively while the minimum inhibition was 3 mm, 10 mm, 8.20 mm at the concentration of 50µl against *Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis* respectively. The maximum inhibition was 10.10 mm and 8.50 mm, at the concentration of 150µl against fungi as *Candida albicans,* and *Aspergillus niger* respectively while the minimum inhibition was 5.70

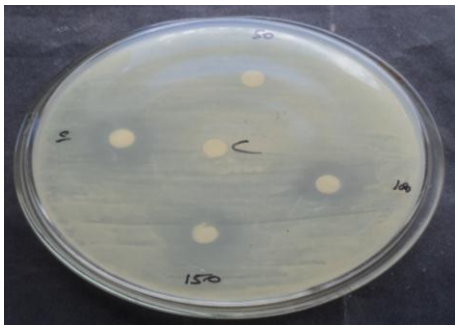
mm and 7.50 mm at the concentration of 50µl against fungi as *Candida albicans*, and *Aspergillus niger* respectively. The standard inhibition was 14.50 mm *Staphylococcus aereus*, 9 mm *Escherichia coli*, 12.20 mm *Klebsiella pneumonia* and 13.50 *Proteus mirabilis* for bacteria while 14 mm *Candida albicans*, and 8.70 mm *Aspergillus niger* for fungi.

Table. 3: Antimicrobial activities of *Pargularia daemia* against selected UTI casuing microbes.

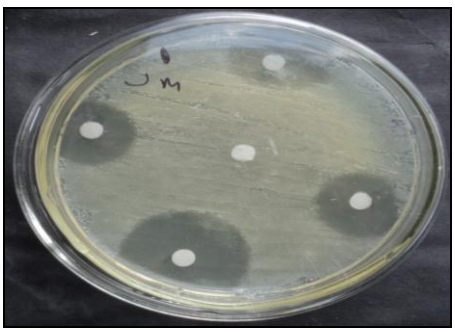
Microbial Organism	50µl	100 µl	150 µl	Standard	Control
Escherichia coli (mm)	3±0.21	7±0.49	7.20±0.50	9±0.63	0
Staphylococcus aereus (mm)	10.20±0.71	11±0.77	12±0.84	14.50±1.01	0
Klebsiella pneumonia (mm)	10±0.70	10.50±0.23	11±0.77	12.20±0.87	0
Proteus mirabilis (mm)	8.20±0.57	9.50±0.66	10.50±0.73	13.50±0.94	0
Candida albicans (mm)	5.70±0.40	10±0.70	10.10±0.71	14±0.98	0
Aspergillus flavus(mm)	7.50±0.52	8±0.56	8.50±0.59	8.70±0.61	0

Values were expressed as Mean ± SD.

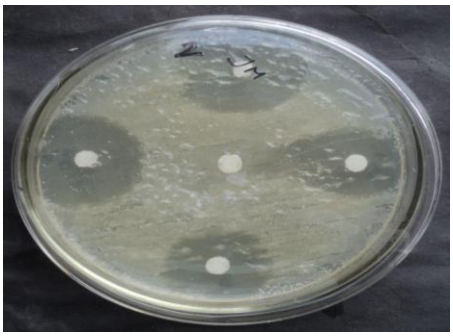
Bacterial standard – Chloromphenical; Fungal standard - Fluconazole; Control: Water



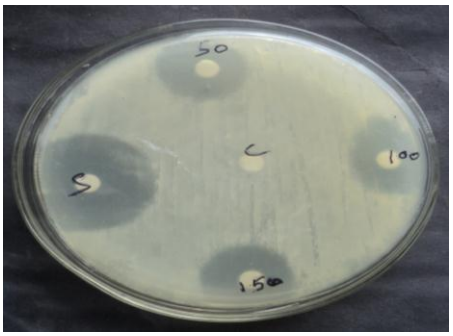
Escherichia coli



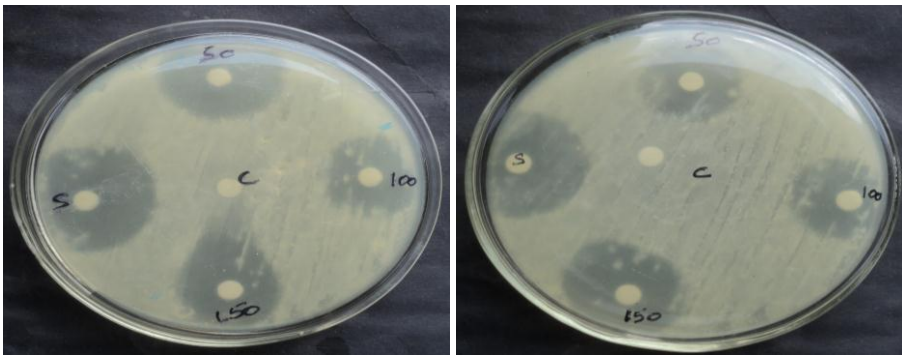
Staphylococcus aureus



Klebsiella pneumonia



Proteus mirabilis



Candida albicans *Aspergillus flavus*

Fig. 1: Antimicrobial activities of *Pargularia daemia.*

The findings of the present investigation revealed that the methanolic extract of *Pargularia daemia* leaf was found to be against various UTI causing pathogens and fungal strains. Further investigation needs for isolation and characterization of active phytoconstituents from extract which may yield few more compounds with greater antimicrobial potential.

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