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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND PATHOGENIC INHIBITION ACTIVITY OF *PEDALIUM MUREX* (L.) AGAINST URINARY TRACT INFECTION BACTERIA

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ABSTRACT

Main objectives of the present work to estimate the phytochemical profile and antibacterial activity of whole plant Pedalium murex (L.) with highly medicinal value against urease producing pathogens. Plants are the effective source of both traditional and recent medicines. Nowadays, medicinal plants were used to treat most unbearable disease among humans such as Urinary tract infections (UTI). The extraction was done by using Traditional Healer Method (THM) and different solvent such as ethanol, methanol, ethyl acetate and petroleum ether by using standard procedures. The antibacterial assay was carried by using agar well method with different Pathogens. The ethyl acetate and petroleum ether extracts of P. murex (L.) (500 µg/ml) showed higher antibacterial activity against gram positive bacteria and gram negative bacteria using micro-dilution method. Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of ethyl acetate extract showed the value of 625, 62.5, 125, 125 and 15.125 µg/ml against the E. coli, Staphylococcus aureus, Salmonella typhi, Proteus mirablis, Bacillus licheniformis, Bacillus cereus respectively and the petroleum ether extract has 0.39, 12.5, 6.25, 1.56 and 1.56 respectively. MBC value found only in ethyl acetate against S. aureus (>125) and B. cereus (>31.25). From the present work, we conclude that the ethyl acetate and Petroleum ether have potential of antibacterial activity and contain secondary compounds which responsible for biological activities. Due to the presence of phyto-components in the plant extract it may control the bacterial growth either in high concentration/long durations and it may have the ability to control the UTI bacteria.

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INTRODUCTION

Urinary tract infection (UTI) is the second most common type of infection in the body. There are about 150 million urinary tract infections for every year throughout the world. It is a bacterial infection that can affects any part of urinary tract (Stamm and Norrby, 2001). It is mainly caused by Gram negative bacteria which include *E. coli*, Klebsiella, Enterobacter and Proteus species and also Gram positive bacteria Staphylococcus sp. plays an important role in the Urinary tract infection (Kunin, 1997). About 35% of healthy women suffer with the symptoms of Urinary tract infection and each year about 5% of women get suffer with the trouble of painful urination (dysuria) and rate of recurrence (Hootan, 2003 and Dhanalakshmi and Selvi, 2013). The occurrence of UTI is superior in women as compared to Men due to the presence of shorter urethra which allowing bacteria faster entry to the bladder (Dhanalakshmi and Selvi, 2013). The bacteria which form infection may enter into urethra and bladder with a compromised body resistance mechanism and reduced urine flow and move to the bladder mucosa, colonize, develop and cause irritation; this causes painful, burning, occurrence and hurry of urination, nocturia, foul smelling, unclear urine and haematuria (Mishra *et al*., 2015). In recent years, the problem of the treatment to human pathogenic bacteria is growing frequently and to utilize the drugs in future aspect is indecisive (Musgrove *et al*., and Maurya and Singh, 2015). For that reason, the humanity goes into some alternative and valuable medicine predominantly from natural source is main goal for providing efficient medicine to the affected people. Medicinal plants are a great source of traditional and orthodox medicines that have profitable value throughout the world and it has potential to develop drugs for therapeutic uses. The use of medicinal plants to treatment specific disease has been in vogue from very old times. This medico-lore is passed over generations habitually all over the humanity. Nature has several medicinal plants which contain natural compounds and

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support health and alleviate illness. The World Health Organization has estimated that 80% of people use plant extracts as folk medicine in conventional therapies (WHO, 1985, Ibrahim, 2011 and Hero, 2013). The concentration has been made for developing the new therapeutic drug that shrink the increasing resistance among the microorganism (Edith *et al*., 2005 and Mako *et al*., 2012). It has several advantages related to safety, availability, and minimizing the risk of side effects and addiction due to the usage of medicinal plants. This condition enforced scientists to search for new antimicrobial substances from medicinal plants (Valsaraj *et al*., 1997). The medicinal importance of a plant is due to the presence of bioactive compound such as primary and secondary metabolites and these active compounds usually present in the storage organs of the plants like fruits, roots, seeds, bark, leaves etc. *Pedalium murex* (L.) is a luscious herb, commonly called as Yanai Nerunji in Tamil Nadu, India, which is a member of Pedaliaceae family. *P. murex* (L.) is mainly used in the treatment of disorders of urinary systems such as gonorrhea, incontinence of urine, dysuria, etc. (Subramanian and Nair, 1972; Satyavathi *et al*., 1987; Haravey, 1996; Chopra, 1999; Shukla, 2004 and Sermakkani, 2011). It acts as antibilious agent, present in the root decoction and it is used to promote lochial discharge from juice of fruits. It is also used to control white discharge due to excessive body heat in the decoction of leaf. The secondary metabolite, gums and mucilage is present in large amount in *P. murex* (L.). and it purifies the blood and removes stone in the bladder. According to Unani system of medicine, *P. murex* (L.) is used for the treatment of diuretic and it enriches blood, increases mensural flow, gargles for mouth and aching gums, cures severe pain at the base of bladder (strangury), cures pain in muscles and joints of lower back (lumbago), tonic, appetizer etc (Hemalatha *et al*., 2011). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin *et al*., 1985). Moreover, they are cheaper and safer, while they are non-toxic and biocompatible. Aim of our present study is to detect the *in–vitro* antibacterial activities and also investigates the most effective antibiotic against urinary pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant

The whole plant of *Pedalium murex* (L.) was collected from Madurai, Tamil Nadu, India and then transferred to A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur, Tamil Nadu, India. It was taxonomically authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph College (Autonomous), Tiruchirapalli, Tamil Nadu, India. The voucher specimens are deposited at the Rapinat herbarium and the voucher number is RHPM SR 001.

Extraction of plant

Traditional method

Traditional Healers Method (THM) of extract was prepared by vigorously stirring the fresh whole plant (200 g) into the water continuously up to 10 min. The extract turned into sticky in nature. Then the resulted extracts were filtered and concentrated by lyophilizer. The final yield of extract was 9.2 % (w/w) in terms of plant material.

Solvent extracts

Whole plants of *Pedalium murex* (L.) was collected, cleaned, shade dried. It was powdered by an electrical blender and passed through 20 m mesh filter for the extraction. Five fifty gram (550 g) of powdered plant was extracted separately with 1:2 w/v of different solvents using Soxhlet apparatus. The extract was concentrated at 45 °C using rotary vacuum evaporator under reduced pressure. The yield of extract was 15.1% w/w in terms of dried starting material (Chopra *et al*., 1992).

Reagents used

The solvents and chemicals used as GR grade and were obtained from MERCK, India. From Hi – media (Mumbai, India), the nutrient agar were collected. Penicillin and Ampicillin were used as standard drug (antibiotics).

Preliminary Phytochemical Screening

The presence of chemical constituents such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins in the plant *Pedalium murex* (L.) were qualitatively analysed by the standard methods (Harborne, 1984).

Antibacterial Activity

Pathogens

For this study, both Gram Positive (*S. aureus* (MTCC 1430), *B. licheniformis* (MTCC 113), *and Bacillus cereus* (MTCC 143)) and Gram Negative (*Escherichia coli* (MTCC 433), *Salmonella typhi* (MTCC 733), *and Proteus mirablis* (MTCC 425)) bacteria were used to determine the antibacterial activity.

Preparing Inoculums

Bacterial broth was prepared by dissolving 1.3 g of nutrient broth (NB) in 100 ml of distilled water. Then, took a loopful of bacterial culture from the slant and inoculate bacteria in the broth medium. Then, incubate the culture broth for 18 - 24 hrs at 37 °C.

Antibiotic Susceptibility Testing

Antibacterial activity of plant *Pedalium murex* (L.) extracts were carried out by a modified well in agar method (Sinclair, J.B. and Dhingra, 1995 and Ahmad *et al*., 1998). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with Twenty four hour old broth culture of respective bacteria. consequently, using sterile borer, well of 0.5 cm diameter was made into the each agar plate and then 20, 30 and 40 µl containing 500 µg/ml concentration of each extract (THM, Ethanol, Methanol, Ethyl acetate and Petroleum ether) in

aseptic condition filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs at 37 °C. The results were recorded by measuring the diameter of inhibitory zone using a transparent meter rule at the end of the 24 – 48 hrs. Penicillin and Ampicillin were the standard drugs for antibacterial activities, respectively.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts

Broth dilution test is used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Pedalium murex* (L.) extract against the bacteria. Fresh 24 hrs cultures of the test bacteria were grown in NB (50 µl bacterial cultures in 1 ml NB). They were diluted using two fold serial broth dilution with different concentrations of plant extracts. Final concentration of the plant extracts ranging from 500 – 15.8 µg/ml were added to the test tubes, they contained the bacterial cultures for knowing the inhibitory concentration in a particular tube inhibiting the bacterial growth (final inoculums of 10^6 bacteria). The antibiotic drug of penicillin and ampicillin were used as positive control ranging from 50 – 0.1587 µg/ml. All tubes were incubated at 37 °C for 24 hrs. The tubes were examined for visible turbidity and optical density of cultures was determined at 620 nm using nutrient broth as a control. Control tubes without the tested extracts were assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC. The least concentration showing no visible growth on agar subculture was recorded as MBC value method (Adiguzer *et al*., 2005).

RESULTS

Phytochemical screening

The phytochemical estimation of various extracts of plant *P. murex* (L.) was analyzed for the presence of secondary metabolites such alkaloids, Saponin, tannins, flavonoid and protein, Gum and Mucilage, phenols, amino acids, glycosides, Fats and Oils and anthraquinone and the results are represented in Table. 1. From the result, more number of phytochemical present in all extracts. In the THM extract, most of the secondary metabolites were presented except Saponin, Flavonoids, Anthraquinone and Quinone. In the alcoholic extract of ethanol and methanol, moreover all phytochemical were present except the phenolic compound. But in the extracts of Petroleum ether and Ethyl acetate all the phytochemical compounds were present which has more activity than the other extracts.

Antibacterial activity

The antibacterial activity was carried out by using THM, methanol, ethanol, ethyl acetate and Petroleum ether extracts by agar well method showed in Table 2, which revealed that ethyl acetate and petroleum ether extracts showed significant activity against pathogenic bacteria were used in the test. Other extracts such as THM, methanol, and ethanol showed less significant result in the zone of inhibition when compared with positive control. Ethyl acetate and petroleum ether extracts showed moderately significant antibacterial activity when compared with ampicillin and penicillin. Among human pathogens *Escherichia coli* (MTCC 433), *Staphylococcus aureus* (MTCC 1430), *Salmonella typhi* (MTCC 733), *Proteus mirablis* (MTCC 425), *Bacillus licheniformis* (MTCC 113) and *Bacillus cereus* (MTCC 143) were sensitive to ethyl acetate and petroleum ether extracts. Some of the microbes such as *Staphylococcus aureus*, *Bacillus licheniformis* are not susceptible against THM. At the same time the *S. aureus*, *Bacillus cereus* were not controlled by the ethanol extract. Unfortunately, *Salmonella typhi* was inhibited by the most of the extract except the methanol extracts and it proof the efficient activity of the plant *P. murex* (L.).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts

Due to the inhibition effect of ethyl acetate and petroleum ether extracts against the pathogenic bacteria, they were again tested their MBC and MIC concentration which showed the MIC values were 625, 62.5, 125, 125 and15.125 against the *E. coli, S. aureus, S. typhi, P. mirablis B. licheniformis, B. cereus* respectively in the ethyl acetate extracts and for petroleum ether had 0.39, 12.5, 6.25, 1.56 and 1.56 respectively (Table 3). MBC value found only in ethyl acetate against *S. aureus* (>125) and *B. cereus* (>31.25).

DISCUSSION

Universally, the bacterial infections are an important cause of morbidity and mortality. Encountering a bacterial infectivity is a usual part of the creature's life. New treatment can be enhanced constantly developing with the advances in medicinal science. The exploration for antimicrobials from environmental plant source has expected much attention and efforts to discover compounds that can perform as right antimicrobials agent to change the synthetic ones (Sen and Batra, 2012). In most of the common cases, the microbes do not involve directly to cause disease. It allows the bacteria to overcome the natural protective mechanisms of the body through the damage of the skin, leading to infections (Ejaz *et al*., 2014). In the present situation, the use of medicinal herbs and conventional medicine in economical way for increasing concern in cost of health (Dharma *et al*., 2014). The use of plant and its parts, with this the extracted phytochemical compound for the preclusion and healing for various ailments has been applied from time immemorial. It is mandatory to inspect and authenticate various indigenous drugs along with better understanding of their biological and pharmacological properties (Dharma *et al*., 2014). Phytocompounds extracted from the plant source can serve as a prototype to develop less lethal and efficient drug in controlling the development of microorganism (Kelmanson *et al*., 2000; Ahmad and Beg, 2001 and Sen and Batra, 2012). Principally, it involved in the separation and discovery of the secondary metabolites extracted from the plants and used as the active ingredients in medical preparations (Taylor *et al*., 2001). Nowadays, most of the people interested to consume drugs with safe, effective

from natural products as extracts or plant oils that is alternative to the commercial synthetic medicine. These antimicrobial drugs have enormous potentially effective therapeutic value which involve in the treatment of infectious disease caused by microbes (Aiyegoro and Okoh, 2009). These Secondary metabolites are act as a protective mechanism against microorganisms, insects and other herbivores (Zablotowicz *et al*., 1996). In the present study, Traditionally and crude extracts of the whole plant *P. murex* L. material obtained in polar and non-polar solvents were tested against Gram Negative and Gram Positive organisms. The presence of medicinally active compound in the plant such as Coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins which can be responsible for the observed antimicrobial property. The active component alkaloids contain ∝ - tocopherol and Isatin which act as antioxidant and antifungal properties. Saponin also present in this plant which contains antifungal, anticancer, anti inflammatory and antifungal properties are due to certain enzymes from the cell and leakage of proteins (Zablotowicz *et al*., 1996 and Shihabudeen *et al*., 2010). Tannin also one of the secondary metabolites which contain antimicrobial and antioxidant properties (Peteros and Mylene, 2010) is for binding to proline rich proteins and obstruct with the protein synthesis (Shimada, 2006 and Shihabudeen *et al*., 2010). Flavonoids contains Dinatin – 7 glucuronide, Diosmetin – 7 – glucuronide, Pedalitin (Subramanian and Nair, 1972; Peteros and Mylene, 2010; Oliver, 1980; Shukla and Thakur, 1983 and Akpan *et al*., 2012) and also hydroxylated phenolic substance which can be derived from plant act as effective antimicrobial substances against a wide array of microorganisms act as antimicrobial, anti allergic and anti-cancer properties and their activity is based on their ability to bind with extracellular and soluble proteins also with bacterial cell walls (Marjorie, 1999; Shihabudeen *et al*., 2010 and Imran *et al*., 2015). The primary antioxidants or free radical scavenger's properties are present mainly in the metabolites such as tannins and flavonoids which are major groups of compounds (Polterai, 1997). Diosgenin is a steroid compound it plays a vital role in antimicrobial activities which indicates the mechanism of steroids specifically combine with membrane lipid and exerts its action by causing leakages from liposomes (Epand, 2007 and Shihabudeen *et al*., 2010). Coumarins are responsible against gram positive bacteria and also fungal infection which could be recognized as antimicrobial activity (Hoult and Paya, 1996). Anthraquinones also has antimicrobial properties (Hoult and Paya, 1996), antidepressant, antiparasitic (Pieters and Vlietinck 2005) and bacteriostatic (Pieters and Vlietinck, 2005). The plant contains gum and Mucilage which act as suspending agent used in pharmaceutical adjuvant (Yeole *et al*., 2010). These secondary metabolites play a vital role in the antimicrobial activity against human pathogenic bacteria (Hemalatha *et al*., 2011 and Imran *et al*., 2015).

Urinary tract infection (UTI) is the widespread infections standing next to upper respiratory infection with a rising fighting to antimicrobial agents. These have an effect on patients in all age of groups and sexes. Most common resistant bacteria involved in UTI include *E.coli, Enterobacter*, *Klebsiella, Proteus, Salmonella, Serrata and Pseudomonas species.* The most important microbes linked with urinary tract infection are *Escherichia coli* have been recognized in many countries. The incidence of UTI is greater in women than men and it is normally present in the intestine, but they sometime enter into the urinary tract. So it is important to evacuate this kind of pathogens by using plant based drugs. The microbial studies of the plant extracts showed the most promising antimicrobial activity showed the prospective for the innovation of novel drugs from plants. In our present study, the ethyl acetate and petroleum ether extracts shows significant effects against urinary tract infection bacteria. Among human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi, Bacillus licheniformis,* and *Bacillus cereus* were sensitive to ethyl acetate and petroleum ether extracts compare to other extracts. The bioactive compounds which are present in the plant *Pedalium murex* (L.) show inhibitory effects against pathogenic bacteria. The microorganisms used in these study such as *E.coli* which act as agent for urinary tract infection (Bichler *et al*., 2002) and it also cause diarrhea, sepsis and meningitis (Adegoke *et al*., 2010). *Staphylococcus aureus* is the major human pathogen which causes food poisoning and infections such as septicaemia (Adegoke *et al*., 2008 and Adegoke *et al*., 2010), skin infection (Adegoke *et al*., 2008) and urinary tract infection (Bichler *et al*., 2002). From the present study, revealed that the ethyl acetate extracts shows significant activity against these microorganisms which contain several bioactive components may serve as antibacterial activity. While work done by Anjana Sharma (2009) using aqueous extract of plant *Terminalia chebula* and *Zinziber officinale observed maximum zone was 6 mm and 9 mm,* Aliyu *et al* (2008) by using aqueous extract of plants *Vernonia blumeoides* and *Phyllanthus amarus* against *UTI pathogens* observed the maximum zone of inhibition is 11mm and 13mm and also Munish Jaryal (2012) observed the aqueous leaf extract of *Euphorbia hirta, Erythrophleum suaveolens* and methanolic leaf extract of *Thevetia peruviana* showed maximum zone was 14 mm and 15 mm respectively which is also less efficient than our studied plants. Nevertheless, not many information are existing on the plants for rising commercial drug for applications to overcome antibiotic resistant in the resistant strains. Hence, in this analysis ethyl acetate extract shows higher antibacterial activity against human pathogenic related to urinary tract infection bacteria that are challenging against many antibiotic drugs. The antimicrobial effectiveness of the medicinal plants is believed due to the presence of secondary metabolites such as tannins, saponins, phenolic compounds, essential oils and flavonoids (Aboaba and Efuwape, 2001 and Khan *et al*., 2009). The technical information of plant extracts *Pedalium murex* (L.) was provided in the present study and also useful for the traditional healers to cure some bacterial infection in the humans and also search for cheap and effective natural excipient.

Conclusion

From the present work, we conclude that all of the plant extracts tested in this research had potential antibacterial action against the reference strains. Our results support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can support the folkflore claim along with the

development of new antimicrobial drugs from the plants. Further investigation is essential to find the bioactive compounds present in these plants with their full range of efficiency. Further, the separation of major compound and crystal studies in the plant *Pedalium murex* (L.) is in under progress which was responsible for potent activity against occurrence of kidney stone studies.

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Compliance with ethical standards

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors

Informed Consent

Informed consent does not require for this study.

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