WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 8, 591-600. Research Article ISSN 2277– 7105

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF COMMERCIALLY AVAILABLE MEDICINAL PLANTS AGAINST UTI PATHOGENS

S. A. Bhutada*

Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon-423603, (India).

Article Received on
25 Feb. 2018,

Revised on 18 March 2018,
Accepted on 08 April 2018,

DOI: 10.20959/wjpr20188-11675

***Corresponding Author**
Dr. S. A. Bhutada
Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon-423603, (India).

ABSTRACT

The herbal products today symbolize shelter in contrast to the synthetics that are regarded as unsafe to human and environment. In the present study the Aqueous, ethanol, methanol and acetone extract of whole plant of *Cyperus rotundos* (Nagarmotha), *Pedalium murex* (Gokharu), *Curculigo orchioides* (Kalimusali) *Ipomoea turpethum* (Nishotar) were prepared, preliminary phytochemical test were carried out. The antibacterial activity of was determined by disk diffusion method against *Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Protease vulgaris and Staphylococcus aureus.* Phytochemical analysis of the solvent extracts revealed the presence of alkaloids, glycosides, flavonoids, steroids, tannins and reducing sugars. All the solvent extracts of the plants showed the significance antibacterial activity against *Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Protease vulgaris and Staphylococcus aureus.* The occurrence of these biologically active chemicals in the selected plants may justify their wide usage in the treatment of urinary tract infection.

KEYWORDS: Medicinal plants, phytochemical analysis, antibacterial activity, bacterial UTI pathogens.

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The plant- derived medicines are relatively safer than synthetic drugs and offering profound therapeutic benefits by providing alternative and

effective treatment for chronic disorders and various diseases (Tambekar and Dahikar 2011). More than 1500 herbal preparations are sold in India as dietary supplements or ethnic traditional medicine to treat the diseases but only a few of them have been scientifically explored for its antibacterial potentials (Tyler 2000 and WHO 2000). About 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 (Sharma and Singh 2011). Reports are available on the use of several plant byproducts which posses' antimicrobial properties, on several pathogenic bacteria and fungi (Kohli, 2010).

Urinary tract infection is an important cause of morbidity and mortality in Indian subjects affecting all age groups across the life span. Urinary tract infection can be defined by the presence of significant quality of bacteria in the urine along with signs and symptoms of infection. Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Although urine contains a variety of fluids, salts and waste products, it usually doesn't have bacteria in it. When bacteria get in to the bladder or kidney, it multiplies in the urine and cause UTI which is also often called cystitis. Another kind of UTI is kidney infection known as pyelonephritis and is much more serious. However, since asymptomatic colonization of the urinary tract can occur, other features such as the presence of inflammatory markers or follow up cultures may be needed to definitely diagnose a UTI (Jagadeesan *et al.*, 2013)**.**

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 because an infection 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 *et al.*, 2003).

Nowadays multiple drug resistance microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Recently, it has been demonstrated that many human pathogenic bacteria

have developed resistance against several synthetic drugs. Available reports on lesser efficacy and more side effects of synthetic drugs need to search an alternative medicine. According to World Health Organization (WHO, 1985) medicinal plants would be the best source to obtain a variety of drugs. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Hence the attempt of study was to evaluate the antibacterial activity and phytochemical analysis of aqueous extracts from four medicinal plant i.e. *Cyperus rotundus*, *Curculigo orchioides*, *Ipomoea turpethum*, *Pedalium murex* against UTI pathogens.

MATERIALS AND METHODS

Samples collection

In the present work, 4 herbal plant powders were collected from Ayurvedic shop and used for phytochemical analysis and antibacterial activity against bacterial pathogens. The name of the selected plants with botanical names, local names, family and indications listed below:

Table 1: List of Commercially Available Medicinal plant used in the study.

S.N	Scientific Name	Local Name	Family	Indication
1	*Cyperus rotundus*	Nagarmotha	*Cyperaceae*	appetizer, digestive stimulant, anthelmintic, diuretic, galactopurificator, anti-inflammatory, antidiabetic,
2	*Curculigo orchioides*	Kalimusli	*Amaryllidaceae*	asthma, impotency, jaundice, skin, urinary and venereal diseases, demulcent, diuretic
3	*Ipomoea turpethum*	Nishotar	*Convolvulaceae*	hepatitis, ntoxication, abdominal tumors, ulcers,wounds, worm infestation, pruritus,skin disorders, cough, asthma,
4	*Pedalium murex*	Gokharu	*Pedaliaceae*	demulcent, diuretic, gonorrhea, dysuria, incontinence of urine, cough, asthma, pain, cures skin disease, piles and leprosy

Preparation of extracts

The aqueous extract was prepared by adding 20 g of herbal preparations in 200mL distilled water, heated at 60^0C for 2h, filtered through cloth and the filtrate was evaporated on sand bath. The dry mass was then stored at 4^0C. The organic solvent extract was prepared by adding 20g herbal preparation (powder) in 200mL of acetone or methanol separately in

screw-capped bottles; shaken at 190-220 rpm using a rotary shaker. After 24h of shaking, the extract was filtered, evaporated in vacuum and dried using rotary evaporator at 60^0C.[16] Dried extracts were stored in labeled sterile screw capped bottles at 4^0C and later used for the in *vitro* study.

Bacterial pathogens

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and use in the present study. The bacteria rejuvenated in Nutrient broth (Hi-media Laboratory, Mumbai, India) at 37^0C for 18h and then soaked at 4^0C in Nutrient Agar. Subcultures were prepared from the stock for bioassay. The inoculums size of the bacteria culture was standardized according to the National committee for clinical Laboratory Standards (NCCLS, 2002) guidelines. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attended a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephloturbidometer.

Table 2: List of Bacterial pathogens.

SN	Bacterial pathogen	MTCC No.
1	Escherichia coli	739
2	Protease vulgaris	426
3	Staphylococcus aureus	96
4	Klebsiella pneumoniae	109
5	Salmonella typhi	733

Preparation of disc for antibacterial activities: The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 5 mg of each extract. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial Activity using disc diffusion method: The modified paper disc diffusion[17] was employed to determine the antibacterial activity of both aqueous and organic solvents extract of herbal preparations. Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in sterile

distilled water or various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37°C. The antibacterial activity was evaluated for 5mg/disc and diameter of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (15-20mm) and mild (12-15mm) and less than 12mm was taken as inactive. Antimicrobial Sensitivity Index (ASI) was calculated by following formula

Antimicrobial Sensitivity Index for Herbal preparation	=	Total zone of growth inhibition / No. of antimicrobial agents tested × no. of bacterial Pathogens

Phytochemical Analysis

Phytochemical tests were carried out in the aqueous condition with the help of distilled water, when plants powdered were mixed with water using standard procedures to identify the constituents of selected plants powdered. In identifying the some components some chemicals were used other than distilled water.

Detection of Carbohydrate

Fehling`s test: 1 ml of extract was boiled on water with 1ml each of Fehlings solutuion A and B. The colour change was observed. A red precipitate indicates presence of sugar.

Detection of Saponin

Distilled water 2 ml was added of each plant extracts and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1cm foam indicates the presence of saponin.

Detection of Phenols

Lead acetate test: The extract (5 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white Precipitates indicated the presence of phenols.

Detection of Proteins

The extract was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

Biuret test: An aliquot of 2 ml of filtrate was treated with drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. The pink color in ethanol layer indicated presences of proteins.

Detection of Amino acid

Ninhydrin test: Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. The color change was observed. A characteristic purple color indicated the presence of amino acids.

Detection of Terpenoid

Chloroform (2 ml) and concentrated Sulphuric acid was added carefully to 0.5 ml of extract. Formation of red brown color at the interface indicated the presence of terpenoids.

Detection of Steroids

A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated Sulphuric acid was added in it, indicates the presence of steroids.

Detection of Tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Alkaloids

About 50 mg of Solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

RESULTS AND DISCUSSION

The uses of herbal medicines are increasing as dietary supplements to fight or prevent common diseases. It has great demand for primary health care because plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc. are thought to be non-toxic, have less side effects and easily available at affordable cost. Plants phytochemical components like Tannins, Saponins, Phlobatannins, Terpenoids, Flavonoids, Glycosides, steroids etc are used in many pharmaceutical and drugs fields. These are the either drugs from any plants material that destroy or inhibit the growth of bacteria act as chemotherapeutic

agents. It has also the ability to prevent or treat bacterial infections. Phytochemical screening of various extracts of the *Cyperus rotundus, Curculigo orchioides, Ipomoea turpethum and Pedalium murex*, fractions showed the presence of most important phytoconstituents. The medicinal value of the selected plants can be correlated due to the presence of various bioactive chemical constituents.

Table 3: Qualitative phytochemical analysis Pedalium murex, Curculigo rchioides Ipomoea turpethum and Cyperus rotundus

S.N	Parameters	*Pedalium murex*	*Curculigo orchioides*	*Ipomoea turpethum*	*Cyperus rotundus*
1	Alkaloids	+	-	+	-
2	Carbohydrate	+	+	+	+
3	Saponin	-	+	+	+
4	Phenols	+	+	+	+
5	Proteins	+	-	-	-
6	Amino acid	+	+	+	-
7	Terpenoid	-	+	+	+
8	Steroids	-	+	+	+
9	Tannins	+	+	+	+
10	Flavonoids	+	-	-	-

Antibacterial Activity

In the present study, Medicinal plants powder **such** as *Ipomoea turpethum, Pedalium murex, Cyperus rotundus* and *Curculigo orchioides,* were screened for antibacterial potential against UTI bacterial pathogens. They exhibited significant antibacterial activity against *S. aureus, P. vulgaris, E. coli, K. pneumoniae* and *S. typhi.*

Antibacterial activity of herbal aqueous extract was observed that *Ipomoea turpethum* was moderate antibacterial activity against *S. aureus* (15 mm), *E. coli* (15 mm), *S. typhi* (16 mm), *P. vulgaris* (17 mm) and *K. pneumoniae* (18 mm). *Cyperus rotundus* was moderate antibacterial activity against *P. vulgaris* (15 mm), *S. typhi* (16 mm), *K. pneumoniae* (16 mm), *S. aureus* (20 mm) and *E. coli* (18 mm). *Curculigo orchioides* was moderate antibacterial activity against *E. coli* (13 mm), *S. aureus* (15 mm), *S. typhi* (15 mm), *P. vulgaris* (17 mm) and *K. pneumoniae* (19 mm). *Pedalium murex* was moderate antibacterial activity against *P. vulgaris* (16 mm), *K. pneumoniae* (17 mm), *S. aureus* (18 mm), *S. typhi* (18 mm) and *E. coli* (19 mm). Whereas, the Ampicillin shown the moderate antibacterial activity against *E. coli* (16 mm), *P. vulgaris* (18 mm), *S. aureus* (19 mm) and *K. pneumoniae* (19 mm) and strong against *S. typhi* (20 mm).

The zone of inhibition against *E. coli* and *S. typhi* was (19 mm and 18 mm) respectively, which is moderate and highest zone of inhibition showed by *Pedalium murex* comparing with *Ipomoea turpethum, Cyperus rotundus* and *Curculigo orchioides*. The zone of inhibition against *P. vulgaris* was (17 mm) which is moderate and highest zone of inhibition showed by *Ipomoea turpethum* and *Curculigo orchioides* comparing with *Cyperus rotundus* and *Pedalium murex*. The zone of inhibition against *S. aureus* was (20 mm) which is strong and highest zone of inhibition showed by *Cyperus rotundus* comparing with *Pedalium murex, Ipomoea turpethum*, and *Curculigo orchioides*. The zone of inhibition against *K. pneumoniae* was (19 mm) which is strong and highest zone of inhibition showed by *Curculigo orchioides* comparing with *Cyperus rotundus, Pedalium murex* and *Ipomoea turpethum*.

Table 4: Zone of inhibition aqueous extracts against different bacteria (Avarage Zone of Inhibition in mm) remove mm from table.

Medicinal plants extract	*E. coli*	*P. vulgaris*	*S. aureus*	*S. typhi*	*K. pneumoniae*
Cyperus rotundus	18	15	20	16	16
Curculigo orchioides	13	17	15	15	19
Ipomoea turpethum	15	17	15	16	18
Pedalium murex	19	16	18	18	17
Positive	16	18	19	28	18
The antibacterial activity was classified as strong (>20 mm), moderate (15-20 mm) and mild (12-15 mm) and less than 12 mm was taken as inactive.					

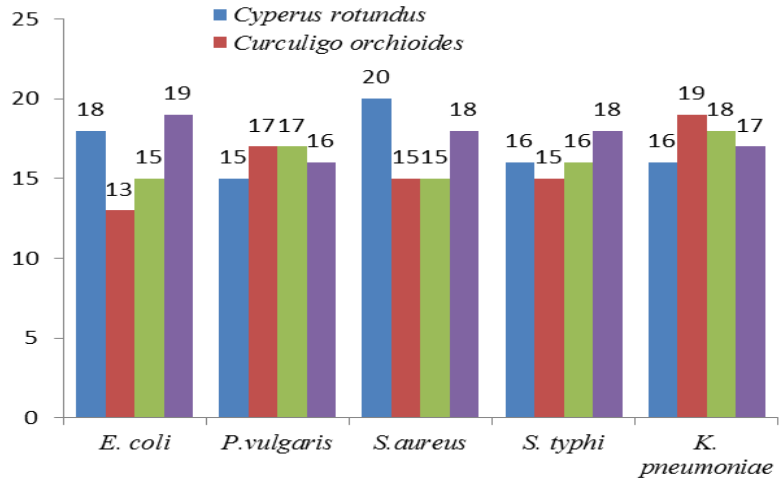

Fig. 1: Antibacterial response of medicinal plants.

As per antibacterial sensitivity index of herbal preparations (ASI), it was observed that the *Ipomoea turpethum, Pedalium murex, Cyperus rotundus* and *Curculigo orchioides* were showed significant antibacterial activity against UTI bacterial pathogens. *Pedalium murex*

shown highest antibacterial sensitivity index comparing *Ipomoea turpethum*, *Cyperus rotundus* and *Curculigo orchioides* against UTI pathogens.

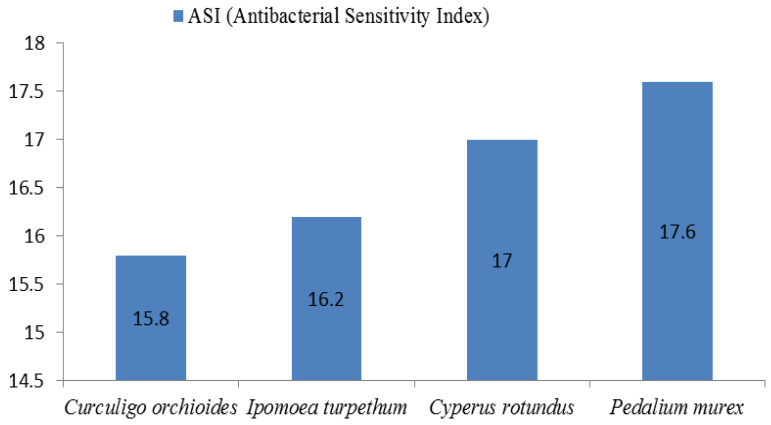

Fig. 2: Antibacterial sensitivity index of medicinal plants.

CONLUSION

In this research we observed the result for phytochemicals, it is inference that *Ipomoea turpethum*, *Pedalium murex*, *Cyperus rotundus* and *Curculigo orchioides* herbal plant powder contains the chemical constituents like Alkaloids, Saponins, Tannins, Flavonoids, Terpenoids, Carbohydrates, Protein, Amino acids, Steroids and Phenol. However, it is recommended that further work be carried out to isolate the bioactive constituents in *Ipomoea turpethum*, *Pedalium murex*, *Cyperus rotundus* and *Curculigo orchioides* using various extraction solvents with a view to characterize the presence of chemicals in such plants powders. These plants play very important role in the fields of medicine and pharmaceutical and also treat many infectious disease.

REFERENCES

1. Hootan T M. Urinary tract infection in adults, In: Johnson R.J., Feehally J, (Eds). Comprehensive clinical nephrology, 2nd ed, London: Mosby, 2003; 731-744.
2. Jagadeesan Sathya, Vidhya Natarajan and Rajitha. E. Vijayan. Antibacterial activity of selective plant extracts against urinary tract infection causing organisms. J. Microbiol. Biotech. Res., 2013; 3(3): 1-5.
3. Kebira A N, Ochola P, Khamadi S A. Isolation and antimicrobial susceptibility testing of Escherichia coli causing urinary tract infections. J. Appl. Biosci, 2009: 1320-1325.

4. Kohli K R, Nipanikar S U, and Kadbhane K P. a comprehensive review on *trivrit* [*operculina turpethum* syn. *ipomoea turpethum*]. International Journal of Pharma and Bio Sciences, Oct-Dec, 2010; 1(4): 443-452.
5. Sharma Surendra Kumar and Ajay Pal Singh Antimicrobial investigations on rhizomes of *Cyperus rotundus* Linn and Scholars Research Library, 2011; 3(3): 427-431.
6. Tambekar D. H. and S. B. Dahikar Screening of some herbal preparations used in Indian Ayurvedic Medicine for its antibacterial potentials. IJPSR, 2011; 2(2): 311-318.
7. Tambekar, D. H. and S. B. Dahikar. Antibacterial potential of some herbal preparation: an alternative medicine in treatment of enteric bacterial infection. *Int J Pharm Pharm Sci.,* 2010; *2(4): 176179.*
8. Tyler V: Herbal medicine: From the past to future, Pub Health Nutr, 2000; 3: 447-452.
9. WHO: 5th Programme Report, Programme for control of diarrhoeal diseases, Geneva. WHO Bulletin, 1985: 63: 557-772.
10. WHO:General guidelines for methodologies on research and evaluation of traditional medicine, World Health Organization, Geneva, 2000.
11. NCCLS (National Committee for Clinical Laboratory Standards). Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100S12. National Committee for Clinical Laboratory Standards. Villanova, Pa, 2002.