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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS AGAINST URINARY TRACT INFECTIOUS PATHOGENS

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ABSTRACT

The antibacterial activity of extract of medicinal plants, namely, *Rhizophora apiculata*, *Phyllanthus emblica*, *Avicennia marina*, *Acalypha indica* and *Withania somnifera* was evaluated against urinary tract infectious pathogens *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*. As compared to ethanol, acetone extract showed broad-spectrum activity. The multidrug-resistant (MDR) bacteria *Enterococcus faecalis* was inhibited by the acetone extract of *Phyllanthus emblica* fruit whereas the other two resistant bacteria *Staphylococcus aureus* and *Escherichia coli* were inhibited by both ethanol and acetone extract of all the species. Biochemical analysis revealed the presence and confirmation of the organism. Further studies using different solvents for extraction are necessary to confirm that medicinal plants are a better source for the development of novel antibiotics.

INTRODUCTION

Urinary Tract Infections (UTI) are the second most common bacterial infections that occurs anywhere in the urinary tract which includes like kidneys, ureters, bladder and urethra. UTI occur in patients of all ages, but is more frequent among women when compare to men due to their physiology. A 2010 report indicated that 3.1% of urgent care visits for UTIs. It has been estimated that 150 million people were infected with UTI per annum worldwide. It may involve only the lower urinary tract or may involve both upper and lower tract. The term cystitis (bladder infection) has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness. The term phylonephritis (kidney infections) has been uses to describe upper UTI, which includes high fever and flank pain in addition to the symptoms of lower UTI (Gibson, 2012). The bowel movement is act as the main source of floral organism to colonize the urinary system and later it results into the infection. Urinary instrumentation such as catheters serves as a major source of infection. Most of the pathogens follow the ascending route of transmission from the lower urinary tract (urethra) to the upper urinary tract (kidneys). UTI's are more common during pregnancy because of changes in the urinary tract. The uterus sits directly on top of the bladder. As the uterus grows, its increased weight can block the drainage of

urine from the bladder, causing an infection. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Nair et al., 2005) The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body. Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever, bronchitis, etc (Dagmar Janovska et al., 2003). Mangroves are shrubs or small trees that grow in coastal saline or brackish water. The term "mangrove" refers to an assemblage of tropical trees and shrubs that grows in the intertidal zone. Mangroves include approximately 16 families and 40 to 50 species (Chapman et al., 1976). Mangroves serve as nursery habitats for many species of fish and invertebrates that spend their adult lives on coral reefs, Sediment trapping to sustain offshore water quality for coral reefs, Protection for inland sites from storm surges and flooding, Building materials, Traditional medicines, Firewood and Food (Kathiresan et al., 2001)

Avicennia marina

Avicennia marina, commonly known as grey mangrove or white mangrove, is a species of mangrove tree classified in the

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plant family Acanthaceae (formerly in the Verbenaceae or Avicenniaceae). As with other mangroves, it occurs in the intertidal zones of estuarine areas (Rippey *et al.*, 2004)

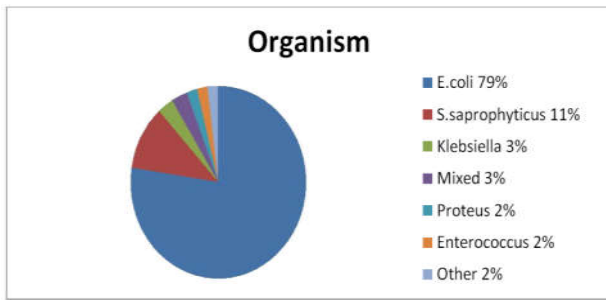


Fig. 1. Common uropathogens in UTI



Fig. 2. *Avicennia marina*

Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Lamiales
Family	: Acanthaceae
Genus	: Avicennia
Species	: marina

Rhizophora apiculata

Rhizophora apiculata is called “bakhawlalaki”, in the Philippines, "Randho" in the Maldives, 'Duroc' in Vietnam, Garjan in India, as well as other vernacular names (Premanathan, 1999).



Fig. 3. *Rhizophora apiculata*

Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Rosidae
Order	: Rhizophorales
Family	: Rhizophoraceae
Genus	: Rhizophora
Species	: apiculata

Withania somnifera

Withania somnifera, known commonly as ashwagandha, Indian ginseng, or wintercherry, is a plant in the Solanaceae or nightshade family. *Ashwagandha* is used for arthritis, anxiety, trouble sleeping (insomnia), tumors, tuberculosis, asthma, a skin condition marked by white patchiness (leukoderma), bronchitis, backache, fibromyalgia, menstrual problems, hiccups, and chronic liver disease (Mishra *et al.*, 2000).



Fig. 4. *Withania somnifera*

Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Solanales
Family	: Solanaceae
Genus	: Withania
Species	: somnifera

Phyllanthus emblica

Phyllanthusemblica, also known as emblic, emblicmyrobalan, myrobalan, Indiangooseberry, Malacca tree, or amla from Sanskrit amalika is a deciduous tree of the family Phyllanthaceae (Krishnaveni *et al.*, 2010). It is one of the most important plants in the traditional Ayurvedic medical system as well as in other traditional health systems for immunomodulatory, anti-inflammatory, antiulcer, hepatoprotective, and anticancer actions. However, there is very limited clinical evidence to support the use of emblica for any indication (Pole and Sebastian, 2006).

Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Rosidae
Order	: Euphorbiales
Family	: Euphorbiaceae
Genus	: Phyllanthus
Species	: emblica



Fig. 5. *Phyllanthus emblica*

Acalypha indica

It is also known as Indian acalypha, Indian nettle, Indian Copper leaf or three-seeded mercury. It is known in various names across regions – Kuppikhokli (Hindi), Kuppigida (kannada), Kuppameni (Malayalam), Kuppaimeni (tamil), araotong (Philippines) (Jagatheeswari, 2013). *Acalypha indica* has been used widely in Indian Ayurvedic medicine for treating various ailments. The leaves, roots and young shoots of *Acalypha indica* are found to have powerful medicinal value and used in various alternative medicinal form in Philippines (Khare, 2003).



Fig 6. *Acalypha indica*

Classification:

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Euphorbiales
Family	:	Euphorbiaceae
Genus	:	Acalypha

Aim

The study involves in the evaluation of antibacterial activity of selected medicinal plants against multidrug resistant isolates of urinary tract infection. The main objective 1. To determine the solvent for extraction by using various solvents. 2. To perform biochemical test to identify the organism. 3. To identify the multidrug resistant isolates of urinary tract infectious

pathogens. 4. Study of antibacterial effect of plant crude extract on isolates of UTI.

MATERIALS AND METHODS

Chemicals and Media

Chemicals and solvents used in this study were of laboratory and analytical grade. Growth media for antimicrobial screening were obtained from Hi-Media.

Sterilization

Growth media and glasswares used in the study were autoclaved at 121°C at 15 lbs/sq.inch pressure for 70 minutes (Hugo *et al.*, 1999).

Ethyl acetate: Cleaning of glass wares

All the glasswares (Borosil and Corning) were immersed in cleaning solution for 3 hr. Then, the glassware were washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water. The cleaned glassware were dried in hot air oven and stored. Cleaning solution (Mahadevan and Sridhar, 1996)

Selection of medicinal plants for antibacterial study

Five plants with known medicinal properties such as *Avicennia marina* (stem), *Rhizophora apiculata* (leaf, root), *Withania somnifera* (root), *Acalypha indica* (leaf) and *Phyllanthus emblica* (fruits).

Collection of plant materials

The leaves of mangroves plants, viz., *Avicennia marina* and *Rhizophora apiculata* were collected from Ramnad District (Latitude: 9.4071343 Longitude: 78.7022678), Tamilnadu India. *Phyllanthus emblica*, *Withania somnifera* and *Acalypha indica* were collected from the Country medicine shop, Chennai. The plant materials were washed thoroughly with running water and finally rinsed with sterile distilled water. Then they were shade dried at room temperature for one week. The dried plant was crushed into fine powder with the help of a mechanical grinder and refrigerated in sealed vials until further use.

Preparation of the plant extracts

The collected medicinal plants leaves were dried under shade and then powdered with mechanical grinder. The obtained plant powder (10gram) was soaking with four organic solvents (100ml) viz., ethyl acetate, hexane, acetone and ethanol successively to get ethyl acetate, hexane, acetone and ethanol extracts for 72hrs (Rios *et al.*, 2007). The suspension was then filtered through Whatmann (No.1) filter paper. The filtrate was transferred into vials and allowed to evaporate using rotary evaporator until completely dried (Parekh and Chanda, 2006). Finally the filtered extract was air dried and then it was stored at -20°C until further use. The crude extract weres weighed and dissolved in 10% dimethyl sulfoxide (DMSO). It was stored at 4 °C in airtight for further studies (Sharma, *et al.*, 2009).

Study design: UTI patients showing symptoms of lower and

upper urinary tract infection above 20 years of age.

Isolation of Bacteria

Collected isolates were inoculated into MacConkey agar plates followed by blood agar plate and identified in UTI agar plate after that incubated at 37°C for 24 hours. The isolates were identified by using standard protocol (Kersters, 2005). Identified and pure isolates were maintained in nutrient agar slants and incubated at 37°C for 24 hours. The isolates were subculture periodically in UTI agar. They were stored in LB broth for further studies.

Biochemical test for identification of bacteria

Grams Staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process (Lockhart, 1995).

Motility

A method for microscopic examination of organisms suspended in a drop on a special concave microscope slide. Cells exhibit a wide range of movement. These movements include migration of cells along a surface or through a tissue, or movement of components within cells (Tittsler and Reese, 1936).

Citrate Utilization Test

Citrate utilization test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic (NH₄H₂PO₄) is the sole fixed nitrogen source. Streak the Simmon's Citrate Agar (SIM medium) slant back and forth with a light inoculum picked from the center of a well-isolated colony (Vaughn *et al.*, 1950).

Mannitol Sorbitol Test

This type of medium is both selective and differential. The MSA will select for organisms such as Staphylococcus species which can live in areas of high salt concentration. An inoculum from a pure culture is transferred aseptically to a sterile tube of phenol red mannitol broth. The inoculated tube is incubated at 35-37°C for 24 hours and the results are determined (Holding and Collee, 1971).

Triple Sugar Iron Test

Triple sugar iron agar test is used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentatively and produce hydrogen sulfide (H₂S). It contains 10 parts of lactose: 10 parts of sucrose: 1 part of glucose and peptone. Inoculate culture by first stabbing through the centre of the TSI medium to the bottom of the tube and then streak

the surface of the slant (McKee *et al.*, 2012).

Urease Test

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive bacteria from other Enterobacteriaceae (Baird-Parker, 1963). The broth medium is inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant is streaked with the test organism.

Methyl Red Test

Methyl Red (MR) test determines whether the microbe performs mixed acids fermentation when supplied glucose. Types and proportion of fermentation products produced by anaerobic fermentation of glucose is one of the key taxonomic characteristics which help to differentiate various genera of enteric bacteria. An inoculum from a pure culture is transferred aseptically to a sterile tube of MRVP broth. The inoculated tube is incubated at 35-37°C for 24 hours (Ljutov, 1961).

Voges-Proskauer Test

Voges-Proskauer is a double eponym, named after two microbiologists working at the beginning of the 20th century. They first observed the red color reaction produced by appropriate culture media after treatment with potassium hydroxide. It was later discovered that the active product in the medium formed by bacterial metabolism is acetyl methyl carbinol (A product of the butylenes Glycol Pathway (Ljutov, 1963). An inoculum from a pure culture is transferred aseptically to a sterile tube of MRVP broth. The inoculated tube is incubated at 35-37°C for 24 hours.

Indole Test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. An inoculum from a pure culture is transferred aseptically to a sterile tube of SIM or tryptone broth. SIM should be stabbed all the way to the butt carefully to disturb the medium as little as possible. The inoculated tube is incubated at 35-37°C for 24 hours (Powers *et al.*, 1977).

Oxidase Test

This test is used to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative Enterobacteriaceae and oxidase positive Pseudomonadaceae. A nutrient medium is streaked with bacteria. After colonies have arisen, individual colonies are removed using a sterile, non-metallic instrument (pre-sterilized plastic loop or sterile wooden splint). The cells are rubbed into a moistened strip impregnated with oxidase reagent. This chemical takes the place of oxygen as a recipient for the electrons from the oxidase cytochrome. The additional electrons turn the oxidase reagent from colorless to purple. If oxidase is not present, no color change is observed (Tarrand *et al.*, 1982).

Catalase Test

This test is used to identify organisms that produce the enzyme, catalase. This enzyme detoxifies hydrogen peroxide

by breaking it down into water and oxygen gas. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result. A small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production (Taylor *et al.*, 1972).

Coagulase test

The coagulase test differentiates strains of *Staphylococcus aureus* from other coagulase-negative species. *S. aureus* strains are capable of coagulating plasma in the tube test and will produce clumps of cells in the slide test. Emulsify one or two colonies of *Staphylococcus* on blood agar plate on each drop to make a smooth suspension. The test suspension is treated with a drop of citrated plasma and mixed well with a needle. Clumping of cocci within 5-10 seconds is taken as positive (Sperber *et al.*, 1975).

Antibiotic susceptibility test

Identified isolates were tested for antimicrobial susceptibility test by the standard Kirby Bauer's disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar (Hi-media) and the antibiotic disc were placed and the plates were incubated at 37°C for 24 hours. After 24 hours, the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standard Institute guidelines (CLSI-2016). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococci* ATCC 29212, *Staphylococcus* ATCC 25923 and *Klebsiella pneumoniae* ATCC 700603 were used for quality control. The following standard antibiotic discs were used for the isolates.

Assessment of antibacterial activity of the medicinal plant extracts

Disc diffusion method

Disc diffusion method was followed to detect antibacterial activity of leaves extracts prepared from *Avicennia marina* and *Rhizophora apiculata*, *Phyllanthus emblica*, *Acalypha indica*, *Withania somnifera* (Bauer, 1966). The disc size of 6mm and they were loaded with 0.01mg of crude ethanol dissolved in 5% Dimethyl Sulphoxide (DMSO) at the concentration of 0.01mg/ml to obtain 1µg/disc. 0.05mg of crude ethanol dissolved in 5% Dimethyl Sulphoxide (DMSO) at the concentration of 0.05mg/ml to obtain 5µg/disc. 0.1mg of crude ethanol dissolved in 5% Dimethyl Sulphoxide (DMSO) at the concentration of 0.1mg/ml to obtain 10µg/disc. 0.5mg of crude ethanol dissolved in 5% Dimethyl Sulphoxide (DMSO) at the concentration of 0.5mg/ml to obtain 50µg/disc (Sharma *et al.*, 2009).

RESULTS

21 bacterial isolates were recovered and the biochemical tests revealed that, these isolates belong to 5 species. Of these *E. coli* is the predominant one (28%), *S. aureus* (23%), *K. pneumoniae* (19%), *E. faecalis* (19%) and *P. aeruginosa* (9%).

Antimicrobial susceptibility

Results of antimicrobial susceptibility test showed marked differences among bacterial isolates in their susceptibility and resistance patterns to a particular antibiotic. *Pseudomonas*

aeruginosa strain 1 is resistant to all the antibiotics except amikacin, *Staphylococcus aureus* strain3 is sensitive to vancomycin, gentamycin and chloramphenicol, *Klebsiella pneumoniae* strain 3 are sensitive to ciprofloxacin, *Enterococcus faecalis* strain 2 is resistant to all the antibiotics except vancomycin and norfloxacin. *Escherichia coli* strain 5 is sensitive to only ampicillin. The result of the present study reveals that, the *Staphylococcus aureus*, shows total susceptibility of 32% of all the acetone extract of plants and 4% susceptibility of all the ethanol extract of plants. *Escherichia coli* showed 63% total susceptibility of all the acetone extract of plants and 33% susceptibility of all the

Table 1. Botanical information of medicinal plants

Plant name	Family	Common name	Parts used
<i>Avicennia marina</i>	Acanthaceae	Grey mangrove	Stem
<i>Rhizophora apiculata</i>	Rhizophoraceae	Garjan	Root
<i>Withania somnifera</i>	Solanaceae	Ashwagandha	Root
<i>Acalypha indica</i>	Euphorbiaceae	Kuppameni	Leaf
<i>Phyllanthus emblica</i>	Phyllanthaceae	Amla	Fruit

Table 2. Selection of solvents for extraction

Polar	Non-Polar
Acetone	Hexane
Ethanol	Ethyl acetate

Table 3. List of antibiotics used

Antibiotics	Symbol	Disc Content
Cephotaxime	Ce	30 mcg
Erythromycin	E	15 mcg
Gentamycin	G	10 mcg
Piperacilin	Pi	10 mcg
Chloramphenicol	C	30 mcg
Ciprofloxacin	Cf	5 mcg
Co-trimoxazole	Co	23 mcg
Tetracycline	T	30 mcg
Amikacin	Ak	30 mcg
Ceftazidime	Ca	30 mcg
Imipenem	I	10 mcg
Ampicillin	A	10 mcg
Nitrofurantoin	Nf	300 mcg
Vancomycin	Va	30 mcg
Penicillin G	P	10 units
Norfloxacin	Nx	10 mcg

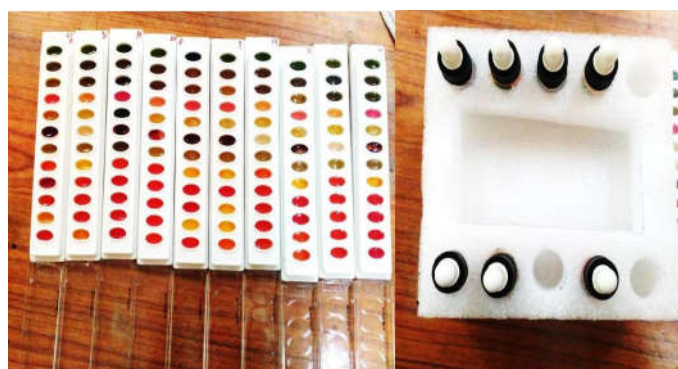


Fig 7. Biochemical kit (HIMEDIA)

ethanol extract of plants. *Klebsiella pneumoniae* showed 40% of all the acetone extract of plants and 3% susceptibility of all the ethanol extract of plants. *Pseudomonas aeruginosa* showed 65% of all the acetone extract of plants and 5% susceptibility of all the ethanol extract of plants. *Enterococcus faecalis* showed 3% of susceptibility of all the acetone extract of plants

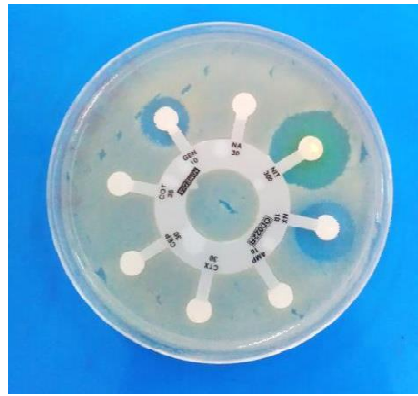


Fig 8. Antibiotic sensitivity test



Fig 9. Antibacterial activity of plant extract against UTI isolates

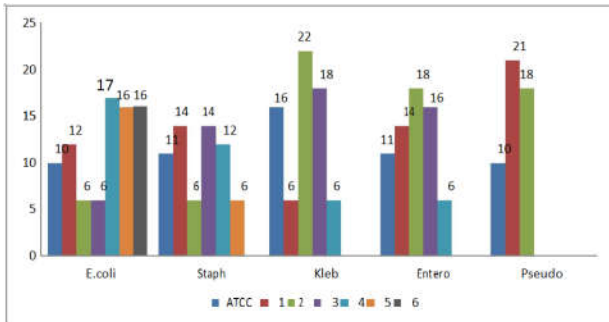


Fig 10. Activity of *Phyllanthus emblica* (Acetone) against UTI pathogen

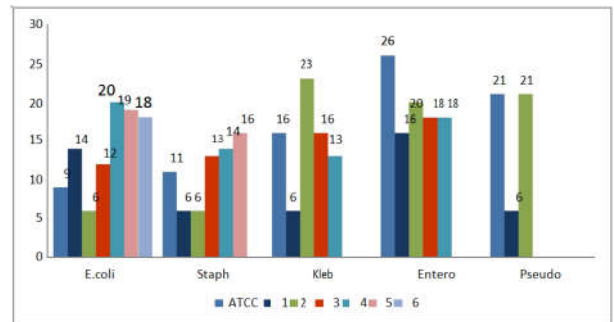


Fig 11. Activity of *Phyllanthus emblica* (Ethanol) against UTI pathogens

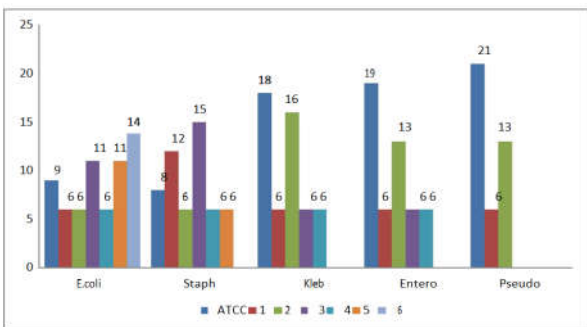


Fig 12. Activity of *Withania somnifera* (Acetone) against UTI pathogens

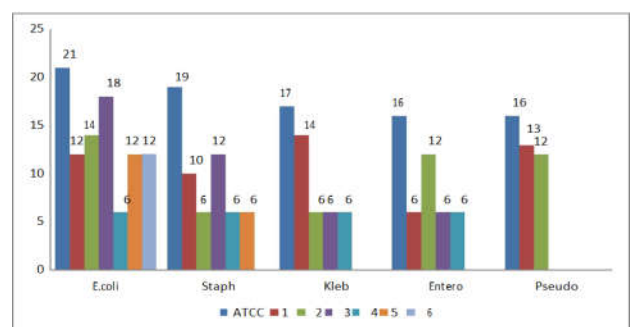


Fig 13. Activity of *Withania somnifera* (Ethanol) against UTI pathogens

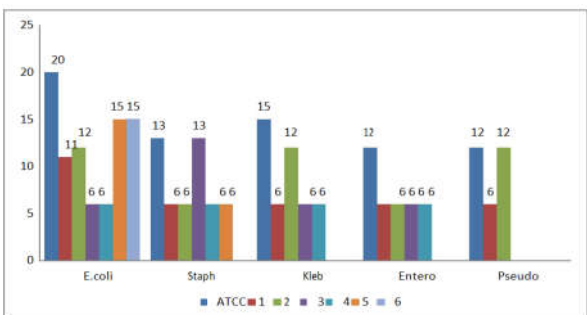


Fig 14. Activity of *Acalypha indica* (Acetone) against UTI pathogens

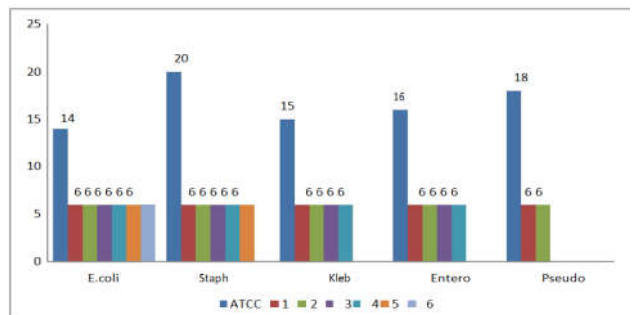
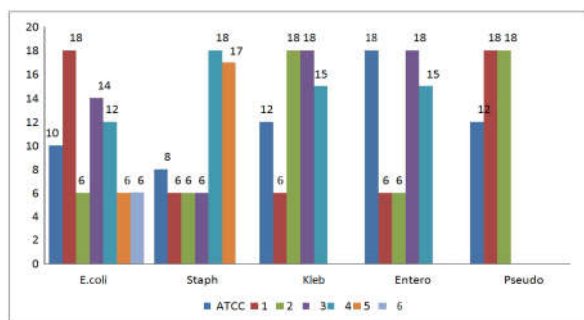
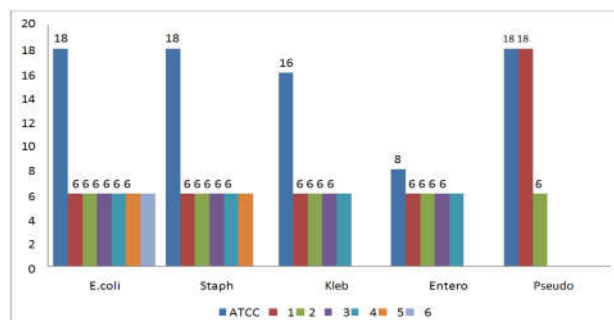
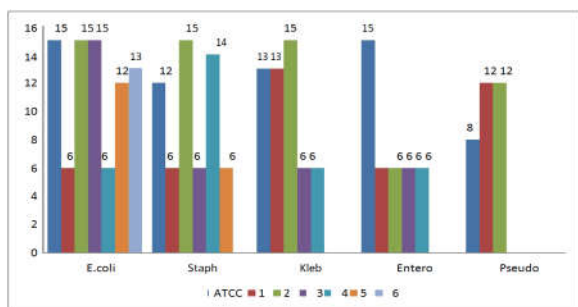
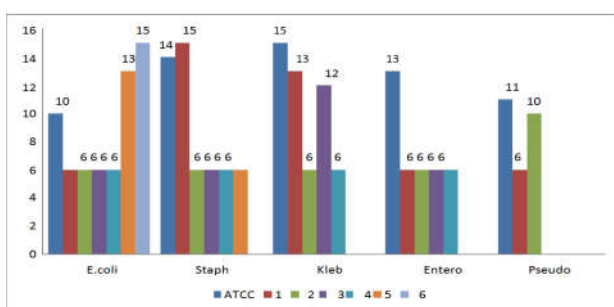


Fig 15. Activity of *Acalypha indica* (Ethanol) against UTI pathogens

Fig. 16. Activity of *Avicennia marina* (Acetone) against UTI pathogensFig. 17. Activity of *Avicennia marina* (Ethanol) against UTI pathogensFig. 18. Activity of *Rhizophora apiculata* (Acetone) against UTI pathogensFig. 19. Activity of *Rhizophora apiculata* (Ethanol) against UTI pathogens

and 2% susceptibility of all the ethanol extract of plants. Thus, it is clearly understood from the result *Pseudomonas aeruginosa* showed higher susceptibility among other UTI pathogens. The leaf extract was tested for the antimicrobial activity against the antibiotic resistant pathogens. The fruit extract of *P. emblica* (26 ± 0.84 mm) followed by root extract of *W. Somnifera* (22 ± 0.11 mm) f, leaf extract of *A. indica* (20.02 ± 0.02 mm), stem extract of *A. marina* (18 ± 0.04) and root extract of *R. apiculata* (15 ± 0.05). Acetone and ethanol extracts of *Phyllanthus emblica* showed antimicrobial activity against all the isolates *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Staphylococcus aureus* while hexane and ethyl acetate extract was not found to be active against any of the isolates.

Antibacterial susceptibility pattern (plant extract)

Sensitivity pattern of *Phyllanthus emblica*

The zone of inhibition obtained from the extract of *P.emblica* acetone and ethanol was plotted in the Fig.10 and Fig.11 respectively.

Sensitivity pattern of *Withania somnifera*: The zone of inhibition obtained from the extract of *W. somnifera* acetone and ethanol was plotted in the Fig.12 and Fig.13 respectively.

Sensitivity pattern of *Acalypha indica*: The zone of inhibition obtained from the extract of *A. indica* acetone and ethanol was plotted in the Fig.14 and Fig.15 respectively.

Sensitivity pattern of *Avicennia marina*: The zone of inhibition obtained from the extract of *A. marina* acetone and ethanol was plotted in the Fig.16 and Fig.17 respectively.

Sensitivity pattern of *Rhizophora apiculata*: The zone of inhibition obtained from the extract of *R. apiculata* acetone and ethanol was plotted in the Fig.18 and Fig.19 respectively.

DISCUSSION

The results of the present study clearly showed that, extracts from *Phyllanthus emblica* showed antimicrobial activity against tested pathogenic strains including antibiotic resistant strains. The effectiveness of the active compounds

present in the plant extracts showed growth inhibition. Most of the urinary tract infection isolates are resistant to the CLSI recommended drugs. *Pseudomonas aeruginosa* UTI isolates was resistant to all the recommended drugs except Amikacin. The side effect of amikacin includes kidney dysfunction, hearing loss when taken high dose (Neuman *et al.*, 1982). *Staphylococcus aureus* UTI isolates was resistant to all the recommended drugs according to CSLI guidelines except Vancomycin, Gentamycin and Chloramphenicol. The side effects of Gentamycin include kidney dysfunction secondary to acute tubular necrosis, neuromuscular blockade and ototoxicity (Yunis, 1989). Serious and fatal blood dyscrasias are known to occur after the administration of Chloramphenicol. *Klebsiella pneumoniae* UTI isolates were resistant to all the recommended drugs except Ciprofloxacin, which causes stomach upset, diarrhea, vomiting, headache and restlessness (Johansson *et al.*, 2014). *Enterococcus faecalis* UTI isolates were resistant to all recommended drugs except Vancomycin and Norfloxacin. Side effect of Norfloxacin are dizziness, fainting, fast and pounding heartbeat, sudden pain or swelling near joints, dark coloured urine, sore throat and skin rash (Mellor *et al.*, 1985). *Escherichia coli* UTI isolates were resistant to all the recommended drugs except Ampicillin. Side effects of Ampicillin includes Diarrhea, nausea and vomiting, swelling of the tongue, thrush or yeast infection (Bachev *et al.*, 1974). All plants studied showed antibacterial activity. This could justify their use in treatment of microbial infections in man and livestock. Acetone extracts showed higher activity compared to ethanol extracts on bacteria. *Phyllanthus emblica* extracts showed broad spectrum antibacterial activity against all the UTI isolates under study. Similarly Parekh and Chanda, 2006 observed that aqueous extract of *A. indicum* was not effective against *K. pneumoniae*, *E.coli* and *P. pseudoalkaligenes*.

It has been reported that Amla possesses spasmolytic (relieves cramps and spasms), purgative (laxatives), expectorant (brings up mucus and relieves cough or congested chest), anti-bacterial, hypoglycemic (lowers high blood glucose levels), hypolipidemic (lowers high cholesterol levels), anti-pyretic

(treats fever) and protects liver. (Singh and Sharma, 2012). Hence it is clearly found that *Phyllanthus emblica* has no toxic effect and it can be extrapolated to humans. Because of its cooling nature, amla is a common ingredient in treatments for a burning sensation anywhere in the body and for many types of inflammation and fever; these are manifestations of pitta (fire) agitation (Singh et al., 2012). Amla has been considered the best of the Ayurvedic rejuvenative herbs, because it is tridosaghna.

Conclusion

Among the five plants *Avicennia marina*, *Rhizophora apiculata*, *Withania somnifera*, *Acalypha indica* and *Phyllanthus emblica*, best antibacterial activity against Urinary tract infection isolates and control strains was observed in Acetone extracts of *Phyllanthus emblica*. Extracts of *Withania somnifera* also showed appreciable activity against UTI isolates and control strains. Rest of the three plant extracts was showed very mild activity. *Phyllanthus emblica* is highly regarded as a universal panacea in the Ayurvedic medicine. It is one of the most versatile plants having a wide spectrum of medicinal activities. This versatile medicinal plant is the unique source of various types of compounds has diverse chemical structure. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *Phyllanthus emblica* could be considered for the control of various diseases including urinary tract infections, since it showed better activity in comparison to synthetic drugs.

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REFERENCES

- AmFam, 2000. "Urinary Tract Infections During pregnancy", *Physician*, Vol.61, No.3, pp.713-720.
- Ananthan, S. and Subha, A. 2005. "Cefoxitin resistance mediated by loss of a porin in clinical strains of *Klebsiella pneumoniae* and *Escherichia coli*", *Indian Journal of Medical Microbiology*, Vol.23, pp.120-128.
- Bachev, S, Petrova. L. and Voicheva. V. 1974. "Experimental studies on the teratogenic effect, acute and chronic toxicity of ampicillin", *Savremenna Med.*, Vol.25, No.4, pp.29-32.
- Baird-Parker, A. C. 1963. "A classification of micrococci and Staphylococci based on physiological and biochemical tests", *Microbiology*, Vol.30, No.3, pp.409-427.
- Bauer. A. W, Kirby. W. M, Scherris. J. C. and Turck. M. 1996. "Antibiotic susceptibility testing by a standardized single disk method", *Am J ClinPathol.*, Vol.45, pp.493-496.
- Beyene and Wonde Wosen, 2011. "Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital", *Southwest Ethiopia -Ethiopian Journal of Health Sciences*, Vol.21, No.2, pp.141-146.
- Busch, R. and Huland, H. 1984. "Correlation of symptoms and results of direct bacterial localization in patients with urinary tract infections," *The Journal of Urology*, Vol.132, No.2, pp.282-285.
- Chapman and Valentine Jackson, 1976. "Mangrove vegetation," *Vaduz.: J. Cramer*, Vol.21, p.581.
- Craig and Jonathan, C. 1996. "Effect of circumcision on incidence of urinary tract infection in preschool boys," *The Journal of Pediatrics*, Vol.128, No.1, pp.23-27.
- Dagmar Janovska, Katerina Kubikova and Ladislav Kokoska, 2003. "Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine". *Czech Journal of Food Science*, Vol.21, pp.107-110.
- Ely Rodrigues, Tilvi Supriya, and Naik, C. G. 2004. "Antimicrobial activity of marine organisms collected off the coast of South East India," *Journal of Experimental Marine Biology and Ecology*, Vol.9, pp.121-127.
- Fenwick, E. A, Briggs, A. H. and Hawke, C. I. 2000. "Management of urinary tract infection in general practice: a cost-effectiveness analysis," *Br J Gen Pract.*, Vol.50, pp.635-639.
- Foxman, B. 2002. 'Epidemiology of urinary tract infections: incidence, morbidity, and economic costs'. *Am J Med.*, Vol.113, pp.5S-13S.
- Foxman, B. and Betsy, 2002. "Epidemiology of urinary tract infections: incidence, morbidity, and economic costs," *The American Journal of Medicine*, Vol.11, No.2, pp.5-13.
- Gibson, 2012. "Urinary tract infection update." *Am. J. Clin. Med.*, Vol.9, pp.82-86.
- Govindarajan, M. 2008. "Studies on effect of *Acalypha indica* L. Euphorbiaceae leaf extracts on the malarial vector, *Anopheles stephensi* Liston Diptera: Culicidae," *Parasitology research*, Vol.10, pp.691.
- Gupta, Kalpana, Thomas Hooton, M. and Walter Stamm, E. 2001. "Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections," *Annals of Internal Medicine*, Vol.13, pp.41-50.
- Handley and Margaret Anne, 2002. "Incidence of acute urinary tract infection in young women and use of male condoms with and without nonoxynol-9 spermicides," *Epidemiology*, Vol.13, No.4, pp.431-436.
- Harvard, B. J. 1987. "Part II: Staphylococcus Bacteria, Chapter 12.In: Clinical and pathogenic Microbiology". Washington, D.C: The C.V. Mosby Company Vol.15, pp.231-244.
- Holding, A.J. and Collee J.G. 1971. "Chapter I Routine Biochemical Tests," *Methods in Microbiology*, Vol.6, pp.1-32.
- Hooton and Stamm, 1997. "Diagnosis and treatment of

- uncomplicated urinary tract infection," *Infectious disease clinics of North America*, Vol.11, No.3, pp.551-581.
- Hooton, T.M. 1996. Prospective study of risk factors for symptomatic urinary tract infection in young women. *N Eng J Med.*, Vol.21, pp.468-474.
- Hugo, William Barry, Ayliffe, G. A. J and Allan Denver Russell, 1999. "Principles and Practice of Disinfection, Preservation, and Sterilisation". *Blackwell Science*, Vol.42, pp.421-425.
- Jagatheeswari, D. 2013. "Acalypha indica L-An important medicinal plant: A review of its traditional uses and pharmacological properties," *International Journal of Research in Botany*, Vol.3, No.1, pp.19-22.
- Johansson, Henrik, C. Lisa Janmar and Thomas Backhaus, 2014. "Toxicity of ciprofloxacin and sulfamethoxazole to marine periphytic algae and bacteria," *Aquatic Toxicology*, Vol.56, pp.248-258.
- Kathiresan, K. and Brian Bingham, L. 2001. "Biology of mangroves and mangrove ecosystems," *Advances in Marine Biology*, Vol.40, pp.81-251.
- Kerstens, 2005. "Bergey's manual of systematic bacteriology" Vol.23, pp.321-334.
- Khare, C. P. 2003. "Indian Herbal Therapy," Vol.11, pp.87-89.
- Knothe, H. 1983. "Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens," *Infection* Vol.11, No.6, pp.315-317.
- Krcmery, S. 1999. "Ciprofloxacin once versus twice daily in the treatment of complicated urinary tract infections," *International Journal of Antimicrobial Agents*, Vol.11, No.2, pp.133-138.
- Krcmery, S. Hromec, J. and Demesova, D. 2001. "Treatment of lower urinary tract infection in pregnancy," *International Journal of Antimicrobial Agents*, Vol.17, No.4, pp.279-282.
- Krishnaveni, Mani and Sankaran Mirunalini, 2010. "Therapeutic potential of Phyllanthus emblica amla: the ayurvedic wonder," *J Basic ClinPhysiolPharmacol.*, Vol.21, No.1, pp.93-105.
- Kunin and Calvin, M. 1994. "Urinary tract infections in males," *Clinical Infectious Diseases*, Vol.18, No.1, pp.1-10.
- Lindsay, E. N. 2001. "Urinary tract pathogens in complicated infection and in elderly individuals" *J.Infect.Dis.*, Vol.1, pp.S5-8
- Litza, J.A. and Brill, J.R. 2010. "Urinary tract infections",- *Prim Care*, Vol.373, pp.491-507.
- Ljutov, V. 1961. "Technique of methyl red test," *APMIS*, Vol.51.4, pp.369-380.
- Ljutov, V. 1963. "Technique Of Voges-Proskauer Test," *Apmis* Vol.58, No.3, pp.325-335.
- Lockhart, 1995. Use of urinary gram stain for detection of urinary tract infection in infants," *Annals of Emergency Medicine*, Vol.25, No.1, pp.31-35.
- Lowy and Franklin, D. 1998. Staphylococcus aureus infections," *New England Journal of Medicine*, Vol.39, No.8, pp.520-532.
- Lukasova, J. 2003. "A.Enterococci and antibiotic resistance", *Acta.Vet.Brono.*, Vol.72, pp.315-323.
- Mac Faddin and Jean, F. 1976. "Biochemical tests for identification of medical bacteria". Williams & Wilkins Co Vol.12, pp.231-243.
- Mahadevan, A. and Sridhar, R. 1996. "Methods in physiological plant pathology. 4th Edn". Sivakami Publications Chennai India Vol.9, pp.154-161.
- McKee, Amy and Fred Peyerl, 2012. "TSI assay utilization: impact on costs of Graves' hyperthyroidism diagnosis," *The American Journal of Managed Care*, Vol.18, No.1, pp.e1-14.
- Mellor, J. A, Mary Cafferkey, and Keane. C. T. 1985. "Vancomycin toxicity: a prospective study," *Journal of Antimicrobial Chemotherapy* Vol.15, No.6, pp.773-780.
- Mishra, Lakshmi-Chandra, Betsy Singh, B. and Simon Dagenais, 2000. "Scientific basis for the therapeutic use of Withania somnifera ashwagandha: a review," *Alternative Medicine Review*, Vol.5, No.4, pp. 334-346
- Monroe, Sara, and Ronald Polk, 2000. "Antimicrobial use and bacterial resistance," *Current Opinion in Microbiology*. Vol.3, No.5, pp.496-501.
- Murray and Barbara, E. 1990. "The life and times of the Enterococcus," *Clinical Microbiology Reviews*, Vol.3, No.1, pp.46-65.
- Nair R, Kalariya, T. Chhanda, S. 2005. Antibacterial activity of some selected Indian medicinal flora. *Turk J Biol.*, Vol.29, pp.41-7.
- Neuman, M. 1982. "Comparative study of the renal toxicity of amikacin alone and combined with fosfomycin," *International Journal of Clinical Pharmacology Research*, Vol.2, No.1, pp.9-20.
- Parekh, J. and Chanda, S. 2006. "Screening of aqueous L. Shinto, M. Dorr, K. Wells, C.A. Wenner and alcoholic extracts of some Indian medicinal L.J. Standish, 2000". A phase I trial of andrographolide plants for antibacterial activity Vol.32, pp.835-838.
- Pole and Sebastian 2006. 'Ayurvedic Medicine: The Principles of Traditional Practice. Singing Dragon.' Vol.11, pp. 126-127.
- Powers, Edmund, M. and Thomas, G. 1977. "Simplified 48-hour IMVic test: An agar plate method," *Applied and environmental microbiology*, Vol.34, No.3, pp.274-279.
- Premanathan, 1999. "Antiviral properties of a mangrove plant, Rhizophora apiculata Blume, against human immunodeficiency virus," *Antiviral Research*, Vol.44, No.2, pp.113-122.
- Ramesh hotchandani and Aggarwal, K.K. 2012. "Urinary Tract Infection In Women", Vol.23, No.4, p.324.
- Rathish Nair, R. 2005. "Puciniagranatum-A potential source as antibacterial drug", *Asian Journal of Microbiology, Biotechnology and Environmental Science*, Vol.17, pp.625 - 628.
- Rios JL, Recio M. C. and Vilar, A. 2007. "Screening methods for natural products with antimicrobial activity", a review of the literature. *J Ethnopharmacol.*, Vol.23, pp.127-149.
- Rippey, Elizabeth and Barbara Rowland, 2004. Coastal plants: Perth and the south-west region. ISBS,
- Roberts and Kenneth, B. 2011. "Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months," Vol. 32, No.3, pp.595-610.
- Sharma, Anjana, V. K. Patel and Padmini Ramteke, 2009. "Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections," *Indian Journal of Pharmaceutical Sciences*, Vol.7, No.2, pp136-139.
- Sharma, Anjana, V. K. Patel, and Padmini Ramteke. 2009. "Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens," *World Applied Sciences Journal*, Vol.7, No.3, pp.332-339.
- Singh et al. 2012. Phytochemistry, traditional uses and cancer chemopreventive activity of Amla Phyllanthus emblica:

- The Sustainer”, Vol.21, No.3, pp.321-341.
- Sobieszczyk, Magdalena, E. Jairam R. Lingappa and Juliana McElrath, M. 2011. “Host genetic polymorphisms associated with innate immune factors and HIV-1,” *Current Opinion in HIV and AIDS*, Vol.6.5, pp.427-434.
- Sperber, W. and Tatini, S. R. 1975. “Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*,” *Applied microbiology*, Vol.29.4, pp.502-505.
- Svanborg, C. and Godaly, G. 1997. Bacterial virulence in urinary tract infection”, *Infect Dis Clin N Am.*, Vol.11, pp.513-29.
- Tarrand, Jeffrey, J. and Gröschel, D. H. 1982. “Rapid, modified oxidase test for oxidase-variable bacterial isolates,” *Journal of Clinical Microbiology*, Vol.16.4, pp.772-774.
- Taylor, Welton, I. and David Achanzar 1972. “Catalase test as an aid to the identification of Enterobacteriaceae,” *Applied Microbiology*, Vol.24.1, pp.58-61.
- Thomas, J.G. 1995. “Urinary tract infections. In: Diagnostic Microbiology”, Eds. Mahon, C. R. and G. Manuselis Vol.34, No.3, pp.950-969.
- Tittler and Reese, H. 1936. “The use of semi-solid agar for the detection of bacterial motility,” *Journal of Bacteriology*, Vol.31.6, pp.575-578.
- Tsarong and Tsewang, J. 1994. “Tibetan Medicinal Plants Tibetan,” Vol.12, pp.213-214. (1950) “The utilization of citrate by *Escherichia coli*,” *Journal of Bacteriology*, Vol.60.2, pp.119-121.
- Warren, J. W. 1996. “Clinical presentations and epidemiology of urinary tract infections,” *Urinary tract infections: molecular pathogenesis and clinical management* ASM Press Washington Vol.2, pp.3-27.
- Welch and Rodney, A. 2006. “The genus *Escherichia*,” *The prokaryotes*. Springer New York, Vol.5, pp.60-71.
- Williams and Gabrielle, J. 2012. “Diagnosis and management of urinary tract infection in children,” *Journal of Paediatrics and Child Health*, Vol.48, No.4, pp.296-301.
- Winn and Washington, C. 2006. “Koneman's color atlas and textbook of diagnostic microbiology”, Ed. Elmer W. Koneman. Lippincott Williams & Wilkins, Vol.3, pp.34-42.
- Yüksel and Selçuk, 2006. “Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections,” *International journal of antimicrobial agents*, Vol.28.5, pp.413-416.
- Yunis, A. 1989. A. “Chloramphenicol toxicity: 25 years of research,” *The American Journal of Medicine*, Vol.87, pp.44-48.
