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Int. J. Drug Dev. & Res.
| July - September 2014 | Vol. 6 | Issue 3 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

In vitro Antibacterial and Antioxidant Activities of *Apium graveolens* l. Seed extracts

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Abstract:

The present study was aimed to study the antibacterial and antioxidant activity of different solvent extracts viz., Methanol, Diethyl ether and aqueous of *Apium graveolens* seeds. Uropathogens isolated from UTI samples, were tested against 10 different antibiotics that are commercially used for the treatment. The antibiotic resistant bacteria *Escherichia coli* and *Pseudomonas aeruginosa* isolated from Urinary Tract infected patients were used to determine the inhibitory potential of *A.graveolens* seed extract by using agar diffusion well method. From the analysis, methanol extract was showed highest inhibition, against bacterial pathogens when compared with other solvent extracts and standard antibiotics. The antioxidant activity of *A.graveolens* seed extracts was carried out 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay method. Among the different extract tested Methanol extract was showed higher antioxidant activity than that of standard Gallic acid. Therefore, *A.graveolens* seed extract exhibiting enormous significance in therapeutic aspects and can applied for treating the UTI.

Keywords: *A .graveolens*, Antibacterial, Antioxidant

Introduction

Urinary tract infection (UTI) is the second most common infection found in Worldwide and estimated that 150 million people affected per year. UTIs account for more than 8 million visits to physician's offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually. UTIs are most microbial infection that affects any part of urinary tract. Nearly 95% of cases of UTIs are caused by bacteria, which are typically multiply at the opening of the urethra and travel up to bladder. UTI is commonly caused by Gram-negative bacteria that belong to the family Enterobacteriaceae members which include *Escherichia, Klebsiella, Pseudomonas, Enterobacter* and P*roteus* and Gram-positive *Staphylococcus* sp. *E.coli* is one of the most common bacteria capable of causing UTI infection in humans. Now-a-days antibiotic resistant bacteria are growing problem of interest so that screening of new antibiotic with resistant to bacteria is necessary.

Free radicals initiating auto oxidation, react with cell membrane, and induce lipid-peroxidation cause inflammation, which may result as important pathological mediators in many clinical disorders. Reduction of their radicals by antioxidant molecule is leucial to the protection of cells against various disorders. Free radicals may also be a contributory factor in a progressive decline in the function of the immune system. Natural antioxidants have attracted great attention from consumers over the world due to their lower toxicity than synthetic antioxidants [1]. Medicinal plants exhibiting antioxidant activities have been employed as the source of therapeutic agents.

There are thousands of species of medicinal plants used globally for the cure of different infections

caused by microbes. *Apium graveolens* L. is commonly known as celery belongs to the family Apiaceae; Biennial herbaceous plant grows to 1m tall, seeds are broad and egg shaped. *A.graveolens* has wide commercial significance all over the world especially in Europe, North America, India, Iran and Pakistan[2]. The major components of *A. graveolens* are alkaloids, glycosides, terpenoids, flavonoids, tannins and polyphenols. It is rich in β –carotene, folic acid, vitamin C, magnesium, potassium, silica, sodium and fiber. Seeds of this plant have been used in Ayurvedic medicine and have been found useful in herbal medicine for the treatment of urinary calculi, gut diseases, relief of flatulence, various painful states, reduction of visceral spasms, and stimulation of the smooth muscle of the womb [3]. Hence, the study was focused on to screen the efficiency of *A.graveolens* seed extracts against antibiotic resistant uropathogens and to determine the antioxidant activity.

MATERIAL AND METHODS

Collection of plant sample

Apium graveolens seeds were collected from Ooty hills station. Tamilnadu. India and authenticated by the expert of botanical department.

Extraction procedure

The shade dried seeds were pulverized in to a moderately fine powder. Hundred grams of plant seed powder was extracted separately with methanol and diethyl ether using soxhlet apparatus. Aqueous extract also prepared by using standard methods. The extracts containing solvents evaporated under reduced pressure at 45ºC using rotary evaporator (Buchi R-210, Germany). The dried extract obtained was kept in desiccators at 20ºC until further use.

Sample collection

In Thanjavur District totally 250 urine samples were randomly collected from different age groups of females viz., 0-15, 15-20, 20-30, 30-40 and above 40 and each group shares 50 samples each. The collected urine samples were stored in an airtight container and transported to the laboratory.

Isolation & Identification

The collected urine samples serially diluted and were subjected to spread plate technique in nutrient agar medium. The inoculated plates were incubated at room temperature at 24-48 hrs. After incubation the development of colonies on the medium observed and was transferred in a selective medium, sub cultured and the pure culture were maintained. The organisms isolated from those samples were further identified by morphological and biochemical characteristics. The purified colonies were identified by growing in a selective medium of MacConkey agar and EMB agar medium. Among the different types of bacterial cultures, the antibiotic resistant bacteria were selected for further study.

Antibiotic assay activity

E.coli and *P.aeruoginosa* isolated from urinary tract infected samples were tested against 10 different antibiotics, which are commonly used to treat the UTI. The antibiotics used in the current study were Penicillin, Gentamycin, Ampicillin, Ciprofloxacin, Fluroquinolone, Cephalosporin, Nitrofurantoin, Amoxillin, Tetracycline, Trimethoprim. Inoculums of bacterial pathogen were swapped in Muller Hinton agar medium; the discs were placed, after incubation, the halos around the disc were measured.

Agar diffusion method

Methanol, diethyl ether and aqueous extracts of plant seeds were tested against antibiotic resistant uropathogens by agar diffusion well method. Inoculums of the pathogen for the assay were prepared in nutrient broth. Muller Hinton agar medium was poured in to the petriplates and allowed for solidification. After solidification, 0.1 ml of inoculums was swapped and 6mm diameter wells in duplicate plates were made with the help of a sterile cork borer in the medium. In each well 100µl of the different solvent extracts were added separately. All the plates were incubated at room temperature and the zone of inhibition were recorded [4].

Antioxidant assay

The method of Shirwaikar *et al.* [5] used for the determination of scavenging activity of DPPH free radical. Powdered seed materials extracted with methanol, diethyl ether and aqueous using maceration and clear extracts obtained after filtration using Whatman No.1 filter paper. The solvents were removed using evaporator at 40^{0}C. Two ml of extracted sample and Gallic acid as a standard were dissolved in distilled water at different concentrations such as 20, 40, 60 and 80µg/ml separately. One ml extract solutions mixed with 2ml of freshly prepared DPPH (0.1mM) in methanol, diethyl ether and crude. The mixture was incubated at 25^{0}C for 30mins in dark and then absorbance was read at 517nm using UV-visible Spectrophotometer. Methanol was used to calibrate the Spectrophotometer. Total antioxidant activity (TAA) was expressed as the percentage inhibition of the DPPH radical and was determining by using the following equation.

Inhibition (%) = A_o(absorbance of the control) – A_s (absorbance of the sample) / A_o (absorbance of the control) × 100

Result and Discussion

Microorganisms isolated from UTI patients

In this study, 250 numbering of urine samples were collected from females in different age groups. Among them 200 samples were found Urinary Tract Infection and the percentage of infection was 92, 52, 100, 88 and 68, respectively from different age groups (Fig: 1). The isolated UTI samples contain 5 different organisms such as *E.coli* (42.25%), *Pseudomonas* (27%), *Klebsiella* (18.25%) *Proteus* (8.25%) and *Staphylococcus* (4.25%) (Fig: 2). *E.coli* and *P.aeruginosa* had highest percentage of pathogens found in this study. Similarly Al-Hadad[6] reported that *E.coli* is one of the most common bacteria capable of causing infections in human's particularly urinary tract infection.

Fig 1: UTI in females with different age groups

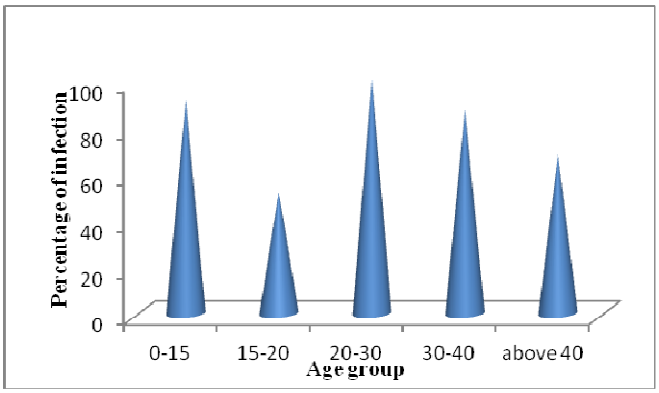
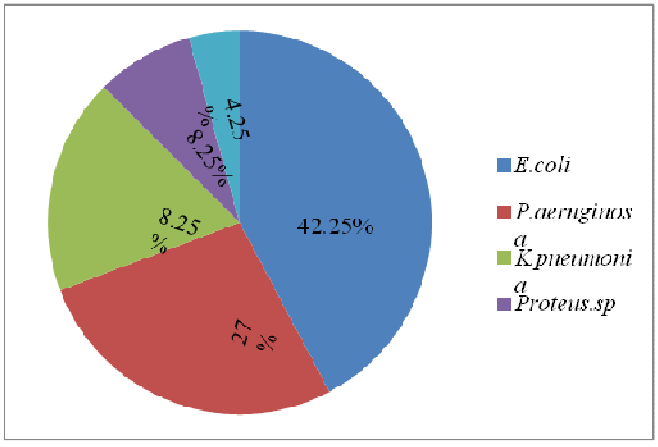


Fig 2: Percentage distributions of Uropathogens



Antibacterial activity of commercial antibiotics

Among the ten different types of antibiotics used in the study, *E.coli* resistant to ampicillin, cephalosporin, ciprofloxacin, Gentamycin, Penicillin and sensitive to Amoxillin, Fluroquinolone, Nitrofurantoin, Trimethoprim *P.aeruginosa* resistant to Amoxillin, Cephalosporin, Ciprofloxacin, Gentamycin and sensitive to Ampicillin, Fluroquinolone, Nitrofurantoin, Tetracycline and Trimethoprim. Mohamad *et al.* [7] reported the antibiotic sensitivity tests that showed *E. faecalis* and *P.aeruginosa* were sensitive to Cefotaxin, Amoxicilln, Norfloxacin, Trimethoprim and resistant to Amikacin. Pathogens resistant or sensitive to different antibiotics are depends upon the nature of species or strain variations.

Table 1: Antibiotic assay activity against Uropathogens

S. No	Antibiotics	Zone of inhibition(mm)	
		E.coli	*P.aeruginosa*
1	Amoxillin	5	-
2	Ampicillin	-	4
3	Cephalosporin	-	-
4	Ciprofloxacin	-	-
5	Fluroquinolone	5	3
6	Gentamycin	-	-
7	Nitrofurantoin	7	6
8	Penicillin	-	-
9	Tetracycline	-	4
10	Trimethoprim	6	5

Antibacterial activity

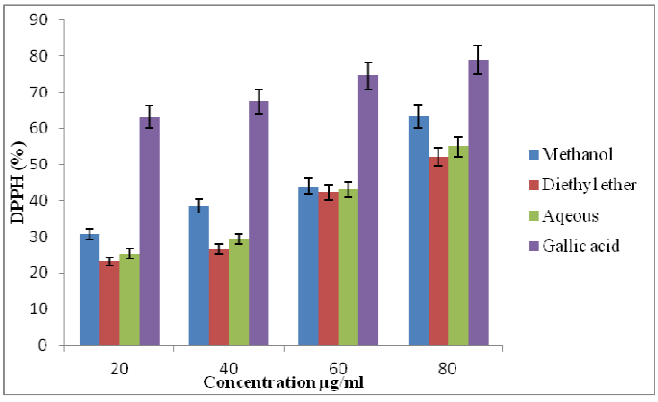
Methanol extract of seeds exhibited better antibacterial activities than those of Diethyl ether and aqueous extract. The methanol extract exhibited 16mm zone for *E.coli,* which is the maximum zone of inhibition followed by diethyl ether (15mm), and aqueous (5mm) was found as effective for inhibition and the Methanol extract exhibited 17mm zone for *P. aeruginosa* that is the maximum zone of inhibition, subsequently diethyl ether (10mm) and aqueous (8mm) extracts. The antibacterial activity of herbal compounds extracted from plants depends upon the type of solvent used for extraction. Seed extracts of *A.graveolens* was showed the broad spectrum of antibacterial activity on the tested microorganisms. It was clear from that Methanol is the most effective against bacterial strains than the other macerated forms. This study was corroborates with the findings of Ramesh *et al* [8] found that the inhibitory activity was maximum in the methanolic extracts of Euphorbiaceae plants against urinary tract pathogens of *E.coli, K. pneumonia* and *P.aeruginosa*. Similarly antibacterial activity against *E.coli*, *S.aureus* and *P. aeruginosa* was exhibited in *Chlorophytum borivilianum* [9]. The fatty oil extracted from *A.graveolens* seeds is used in many medicinal preparations as an antispasmodic which is used for smooth muscle contraction, thus bladder it prevent the spasms of Urinary bladder. Inhibitory effect of this medicinal plant extract exhibited activity against UTI pathogens is similar to the previous results obtained by the researchers.

Antioxidant activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical scavenging activity [10]. In DPPH radical scavenging assay, antioxidant reacts with DPPH, and convert it to yellow colored α, α-biphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant activities. In this test, the methanol extract of *A.graveolens* seeds exhibited profound antioxidant activities. At 80µg/ml concentration, the antioxidant activity was highest in methanol extract of seeds (63.28 ±

0.86%) followed by Diethyl ether (54.04± 0.21%) and aqueous extract (52.97± 0.64%). Likewise in the entire concentrations methanol extract of the plant seed had highest antioxidant activity (Fig:3). Therefore, the presence of such compounds may be responsible for the antioxidant activity found in the methanol extract. Luteolin and additional flavonoids have been shown to reduce the release of reactive oxygen species and to increase expression of enzymes (superoxide dismutase) that protect against oxidative damage thereby displaying antioxidant properties [11]. In addition, the flavonoids apigenin and luteolin exhibit high biological activity and pronounced anti –inflammatory effects [12]. Recently, Zhou *et al,* [13] analyzed that the alcoholic extract of celery seed and phthalide dimer isolated from this extract inhibited *Helicobacter pylori* in vitro [14]. These pharmacological effects suggest that *A.graveolens* may be potentially used in therapeutic applications for the treatment of a wide range of inflammatory and microbial infections.

Fig 3: Antioxidant activity



Conclusion

In the present study, the antibacterial screening of three different extracts of *Apium graveolens* seeds showed varying degree of activity against UTI pathogenic bacteria such as *E.coli* and *P.aeruginosa* cultured in vitro. Pathogens were showed various levels of inhibition by the commercially available antibiotics. The antioxidant activity reflected by the DPPH radical scavenging assay were clearly observed in the methanol extracts of seeds. Among the three extracts, the methanol extract of seeds was very effective for both antibacterial as well as antioxidant activity. This study proved that *A.graveolens* seeds were potential for therapeutic agents.

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Article History: ----------

Date of Submission: 16-09-2014

Date of Acceptance: 29-09-2014

Conflict of Interest: NIL

Source of Support: NONE