Original Article

INVITRO ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST PATHOGENS CAUSING URINARY TRACT INFECTION (UTI).

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ABSTRACT:

The present study was done to compare the antibacterial activity of *Coriandrumsativum*, *Trigonella foenum-graecum, Menthapiperita*and *Spinaciaoleracea*against pathogens *(Escherichia coli, Klebsiella, Pseudomonas aurogenosa, Staphylococcus aureus)* that causes UTI. Leaves and stems of all the plants were used. The disk diffusion method was applied for antibacterial assay. Ciprofloxacin was used as a standard. The study revealed that the aqueous extracts of all the plants had higher antibacterial activity against bacterial pathogens as compared to ethanol extracts. *Mentha piperita* showed higher zone of inhibitions whereas *Spinacia oleracea* showed lower zone of inhibition values among all the plants against bacterial strains.

KEYWORDS: *Coriandrumsativum*,*Trigonella foenum-graecum, Menthapiperita, Spinaciaoleracea,* Antibacterial activity.

INTRODUCTION:

From the literature, it was noted that bacterial strains that are involved in UTI are *Escherichia coli, Klebsiella, Pseudomonas aurogenosa, Staphylococcus aureus.* Symptoms of UTI includes a frequent urge to urinate, pain and pressure at the upper side of pubic, pain on urination, difficulty in urination, fatigue and pyuria. Signs of UTI are cloudy or milky urine that may be pink or reddish colored if blood appears in urine. When infection affects the kidneys signs of urinary tract infection are fever, nausea, vomiting and flank pain that results in acute pyelonephritis. For UTI risk factors are catheter use, mode of birth control pills, diabetes, menopause, urinary tract obstruction due to enlarged prostate, strictures, stone and tumor[5].

Phytochemical studies have revealed that bioactive compounds like tannins, terpenoids, alkaloids, flavonoids, glycosides, saponins are present in plants having antimicrobial activity[15].

Coriandrum sativum commonly known as coriander belongs to family Apiaceae. Found in regions such as southern Europe, North Africa, and southwestern Asia. Coriander is a soft plant with length 20 inches. The leaves are lobed at base, slender and feathery higher at the stem. Flowers vary in color from white to pale pinkish. Fruit is globular 0.12 to 0.20 inches in diameter, which is often used as spice.[9]

Trigonella foenum-graecum commonly known as fenugreek belongs to the family Leguminosae It is widely grown in central Asia, in certain parts of Australia, central Europe, northern Africa and North America. It's an annual plant of leaf size 1 to 4 cm in length. Flowers are 6 to 7mm long and yellow in color[8].

Spinacia oleracea commonly known as spinach. It belongs to a family called Amaranthaceous and native to region like central and southwestern Asia. Leaves of spinach are ovate or triangular in shape ranges from 2 to 30 cm in length and 1 to 15 cm broad. Flowers are yellow green with diameter 3 to 4mm[16]

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*Mentha piperita*is also called as peppermint belongs to family Labiate. Cultivated in Asia, Europe, North America and North Africa, but today grown all over the world. It is a perennial, herbaceous plant of height usually ranges from 50 to 90 cm[11].

MATERIALS AND METHODS:

Collection and drying of plants:

1-2 kilograms of *Coriandrum sativum*, *Trigonella foenum-graecum, Mentha piperita*and *Spinacia oleracea* were collected from the local market of Sargodha. The leaves and stems of all the plants were washed with running tap water for 2 to 3 times and shade dried. After drying, they were grinded with grinding machine to make a powder. Grinded powder was then stored in a sterile container and used for the extraction process[3].

Preparation of extracts:

Extracts were prepared by maceration. Grinded powdered was allowed to macerate in the extracting solvents such as water and ethanol. Mixture of solvent and powdered plant was blended in an electric blender so that they are mixed properly[10]. To prepare the aqueous and ethanol extract of all plants 40 grams of dried powder of all these plants was weighed in a closed glass container and mixed separately with 100 ml of distilled water and 120 ml of ethanol in a glass container. The mixture of powdered plant and solvents was allowed to stand for 48 hours at room temperature. Within this time period the mixture was agitated frequently in order to dissolve the soluble material into solvents. After complete mixing the mixture was filter with muslin cloth then with wattman no 1 filter paper[3]. 10 micro liter of each extract was used for antibacterial assay.

Preparation of inoculums:

Muller Hinton medium was used. The test pathogens like *E.coli, Pseudomonas, Staphycococus*and*Klebsiella* were isolated clinically and sub cultured on a medium. These agar plates containing bacterial pathogens were incubated at 37 degree centigrade for 24 hours. From this fresh culture, standard size ($10^8$to 10^9CFUper ml) inoculum was prepared.

Antibacterial assay:

To examine the antibacterial activity of plants, the disc diffusion susceptibility method was used. Sterilized petri dishes were taken and molten culture media was poured on petri dishes. After the solidification of culture media, (Muller Hinton) bacterial inoculums were applied to the surface of media plates (By streaking method). 5 microgram of composed disc of ciprofloxacin was used as standard. Then the dried test samples discs and standard antibiotic ciprofloxacin were placed on inoculated agar surface and incubated in incubator at 37 degree centigrade temp for 16 to 24hrs prior to determination of results. After incubation period, petri dishes were removed from incubator and zone of inhibitions of all the plants extracts were measured from each petri dish by scale, in millimeters[7]. Antibacterial assay was performed in six replicates. After antibacterial assay data was tabulated and analyzed statistically.

Statistical analysis:

Data obtained after antibacterial assay was analyzed statistically by using ANOVA (Analysis of variance) technique.

Results:

Disc diffusion method was used for antibacterial assay. Zone of inhibitions were measured in mm. For example against *Escherichia coli* aqueous extract of *Mentha piperita* had higher zone 17±0.37 mm that was close to standard drug ciprofloxacin zone i.e. 20±0.31 mm where as *Coriandrum sativum*, *Trigonella foenum-graecum* and *Spinacia oleracea* had lower zones as compare to *Mentha piperita* and standard. Same is the case with *Pseudomonas aurogenosa* and *Staphylococcus aureus,* against which aqueous extract of *Mentha piperita*had higher zones as compare to other plants such as15.17±0.31mm against *Pseudomonas aurogenosa* and 19.5±0.43mm against *Staphylococcus aureus.* Whereas in case of *Klebsiella* ethanol extract of *Mentha piperita* has higher zones i.e. 15.17±0.31mmas compare to ethanol extract of other plants and aqueous extracts of all plants. According to table aqueous and ethanol extract of mint has

shown maximum zone of inhibition against *Pseudomonas aurogenosa, Staphylococcus aureus, Escherichia. coli* and *klebsiella* as compare to other plants extracts. Among all the observed zones of *Mentha piperita*, aqueous extract of *Mentha piperita* has maximum zone against *Staphylococcus aureus* i.e.19.5±0.37 that is close to standard antibiotic ciprofloxacin. Aqueous and ethanol extracts of mint are statistically similar against *Escherichia. coli.* Likewise Aqueous and ethanol extracts of mint are also statistically similar against *Pseudomonas aurogenosa* and *klebsiella.* Aqueousextract of coriander has also shown higher zones against *Escherichia.coli.* Coriander aqueous extracts are statistically similar against *Pseudomonas aurogenosa, Staphylococcus aureus.* Aqueous and ethanol extracts of coriander are also significantly similar against *klebsiella.* When we analyze fenugreek it has shown maximum zone against *Escherichia. coli.* Aqueous and ethanol extracts of fenugreek are also statistically similar against *klebsiella* and *Pseudomonas aurogenosa, Staphylococcus aureus.* Spinach has also presented maximum zone against *Escherichia.coli.* Against *Pseudomonas aurogenosa* both ethanol and aqueous of spinach are statistically similar while aqueous extract of spinach against *Staphylococcus aureus* and ethanol extract of spinach against *klebsiella* are also statistically similar.

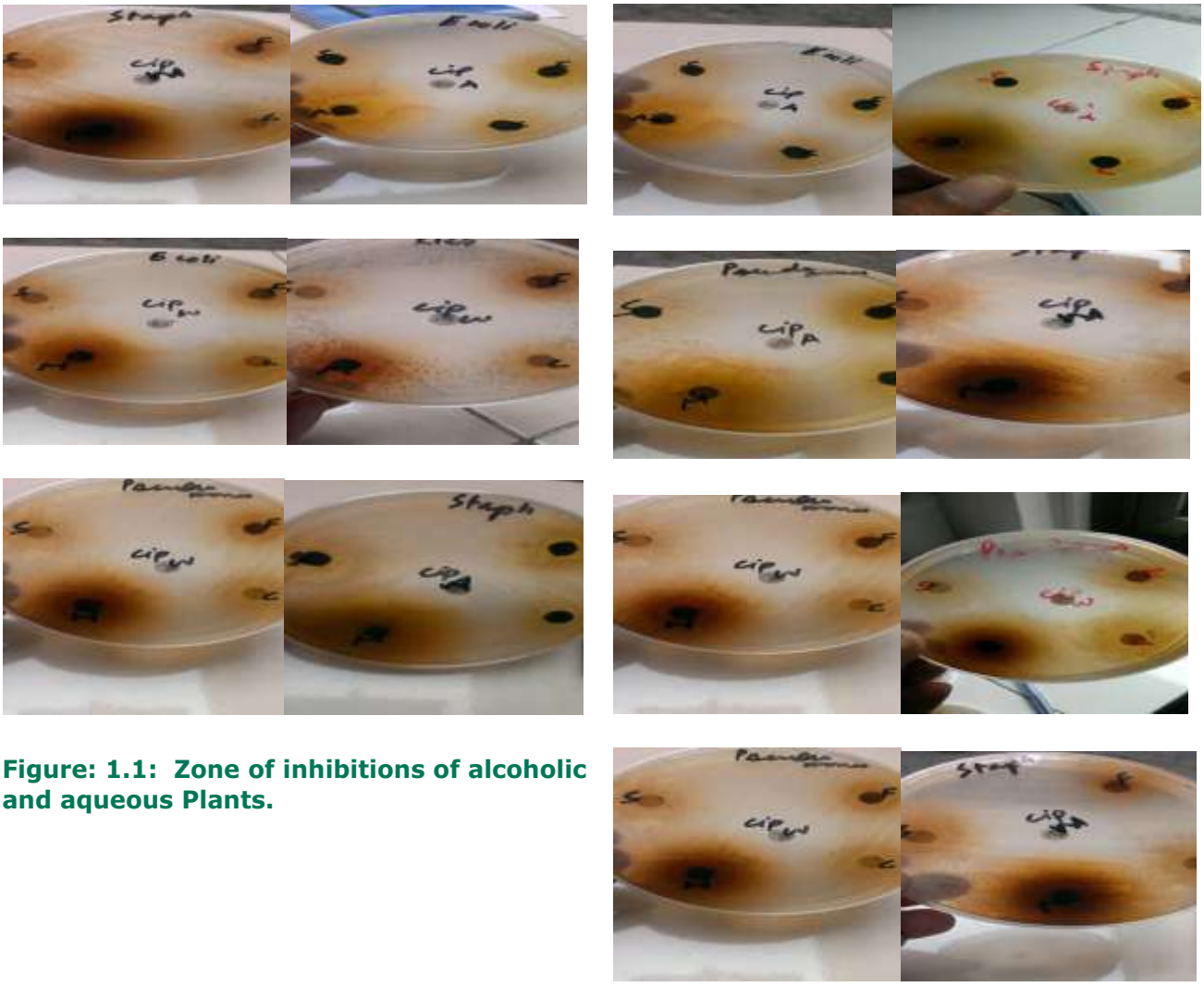

Figure: 1.1: Zone of inhibitions of alcoholic and aqueous Plants.

Table: 1.1 Analysis of variance.

Source of variation	Sum of squares	DF	Mean square	F	P
Intercept	42800.104	1	42800.104	77001.687	0.000
Solvents	218.504	1	218.504	393.111	0.000
Treatment	6488.292	4	1622.073	2918.272	0.000
Micro organisms	62.446	3	20.815	37.449	0.000
Solvent* treatment	67.475	4	16.869	30.349	0.000
Solvent* microorganisms	142.246	3	47.415	85.305	0.000
Treatment* microorganisms	449.408	12	37.451	67.378	0.000
Solvent* Treatment* Microorganisms	197.358	12	16.447	29.589	0.000
Error	111.167	200	0.556		
Total	50537.000	240			

Highly significant (P<0.01)

Table: 1.2 Comparison of antibacterial activity of aqueous and ethanol extracts of plants with standard drug ciprofloxacin.

	Ciprofloxacin	Extracts	Mint	Coriander	Fenugreek	Spinach
Ec	19.83±0.31a	Aqueous	17±0.31e	12.33±0.31d	14.17±0.31e	12.17±0.31d
		Ethanol	16.33±0.31e	8.17±0.31b	10.67±0.31bc	6.83±0.31a
Ps	25±0.31d	Aqueous	15.17±0.31d	10±0.31c	11.5±0.31c	8.17±0.31b
		Ethanol	9.5±0.31a	6.33±0.31a	10±0.31b	7.83±0.31b
St	22.83±0.31b	Aqueous	19.5±0.31f	9.17±0.31bc	12.17±0.31d	9±0.31c
		Ethanol	11.83±0.31b	6.67±0.31a	8±0.31a	6.5±0.31a
Kb	24±0.31c	Aqueous	13.5±0.31c	11.17±0.31d	8.33±0.31a	11.17±0.31d
		Ethanol	15.17±0.31d	12±0.31d	11±0.31c	9.5±0.31c

Mean±S.E. sharing similar letter in a column are statistically non significant (P>0.05).
Ec: *Escherichia coli,* Ps: *Pseudomonasaurogenos,* St: *Staphylococusaureus,* Kb:*Klebsiella*

DISCUSSIONS:

The present study was done on plants exhibiting antibacterial activity on bacterial strains that are involved in UTI. Purpose of study was to investigate the antibacterial action of plants such as *Coriandrum sativum, Trigonella foenum-graecum, Mentha piperita, Spinacia oleracea* and to make a comparison of their antibacterial activity among themselves and with ciprofloxacin.

Phytochemical studies have revealed that bioactive compounds like tannins, terpenoids, alkaloids, flavonoids, glycosides, saponins are present in plants having antimicrobial activity[15]. Plants have economical, environmental and medicinal value, while some are taken in diet as source of vitamins and minerals.

UTI is the most common infection that affect humans. Uncomplicated infections in healthy women occur most commonly due to uropathogenic bacteria usually Escherichia coli that enter into the urinary bladder and overcome innate immunity, whereas complicated infections occurs due to functional

and anatomical abnormalities in urinary tract.

In the present study we have came to know that all the plants extracts possess antibacterial activity, but the magnitude of their activity varies for all pathogens. So these plants can be used remarkably in antimicrobial formulations against diseases for which only limited number of antibiotics are available. *Coriandrum sativum* also exbitsanti microbial, anti diabetic, myorelaxant, antiseptic, antiallergic, antiasthmatic, anti atherosclerotic, anticancer, emmenagogue, antihypertensive, lipolytic and possesses nerve-soothing property. Moreover it has well known healing properties[6].

Trigonella foenum-graecum has many medicinal values such as a demulcent, diuretic, tonic, emollient, carminative,antimicrobial, astringent, expectorant, restorative, aphrodisiac, and were also used to cure stomach irritation, mouth ulcers[8]. *Trigonella foenum-graecum* is also anti inflammatory, antipyretic and analgesic[1].

*Spinaciaoleracea*has carminative, laxative, diuretic, emollient, hypoglycemic, anthelmentic and antipyretic uses. It is also useful in certain conditions like inflammation of lungs, bowel, joints pain, fever, vomiting, flatulence, ring worm, scabies, jaundice, sore throat, sneezing and sore eyes. *Spinacia oleracea* also possesses antioxidant activity. Also has hepatoprotective effect[14]. It also enhances muscles performance. Same as *Spinacia oleracea* also has a protective effect on intestinal mucosa[13].

Mentha piperita shows different medicinal properties like antifungal, antiviral, analgesic, antibacterial and also reduces gas and indigestion. Has relaxing and cooling effect on skin when applied topically on rashes[2]. *Mentha piperita*can also be used in common cold because it contains menthol which acts as nasal decongestant[4]. It is a safe insecticide and a natural food preservative[12].

CONCLUSIONS:

Plants have economical, environmental and medicinal value, while some are taken in diet as source of vitamins and minerals. Antibacterial potential of plants such as *Coriandrum sativum*, *Trigonella foenum-graecum, Mentha piperita, Spinacia oleracea* was investigated against pathogens causing UTI. Bioactive constituents of these plants were active against pathogens causing UTI.

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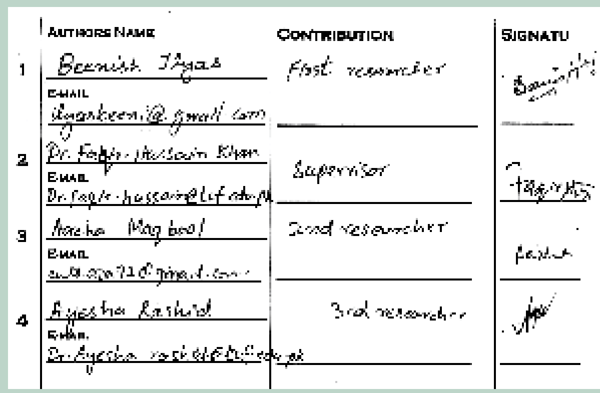

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Value of a man depends upon his courage; his veracity depends upon his self-respect and his chastity depends upon his sense of honor.

Hazrat Ali (Karmulha Wajhay)