



Evaluation of Antibacterial Potential of *Spinacia oleracea* L. against Urinary Tract Pathogens

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ABSTRACT

Plants contain a wide range of natural drugs. Thus the present study investigated the effect of different solvent extract of *Spinacia oleracea* on some urinary tract pathogens *in vitro*. The well diffusion method was used in determining the antibacterial efficacy of plant extract against test microorganisms. Here four polar solvents namely, water, ethanol, methanol, acetone, and four non-polar solvents such as, petroleum ether, chloroform, hexane, benzene were used. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated for all solvent extracts of *Spinacia oleracea* against each pathogen that recorded antibacterial activity and found the significant bactericidal MIC ranged between 1 to 40 mg/ml and MBC ranged between 10 to 50 mg/ml against UTI causing bacteria. The results predict that the polar solvent extract of this plant can be used as natural antibacterial agent to cure urinary tract infection (UTI).

Keywords: Antibacterial activity, MBC, MIC, *Spinacia oleracea*, Urinary Tract Infection.

INTRODUCTION

Plants are wealthy sources of antimicrobial agents as they contain a different variety of phytoconstituents. The past few decades have seen increasing scientific interest in the both growth of plant tissue culture and the commercial development of this technology as means of producing that valuable phytochemicals.¹ These types of natural drugs are always a better substitute of synthetic drugs. Thus numerous drugs have entered the international pharmacopoeia through ethnobotany and traditional medicine.^{2,3} For centuries, medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate. Elsewhere, many potent drugs have been purified from medicinal plants including anti-malarial, anti-cancer, anti-diabetic and antibacterial compounds.⁴ The medicinal value of a plant lies on bioactive phytochemical constituents that produce a definite physiological action on the human body.⁵ These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.⁶ The most important of these bioactive constituents of plants are alkaloids, tannins and flavonoids.⁷

Spinacia oleracea Linn. (Family-Chenopodiaceae), is commonly known as "Spinach". It is an erect herb with about 30-60 cm height. It is native to South-West Asia and cultivated throughout world as vegetables. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic effects.^{8,9} It is a rich source of vitamin A (especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B₂, calcium, potassium, vitamin B₆, folic acid,

copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids.¹⁰ Spinach also packed with a number of antioxidants components like polyphenols, flavonoids and carotinoids which are shown to possess anti-inflammatory effects, anti-mutagenic potential, anti-neoplastic effects, as well as chemo-preventive activities.^{11,12}

The potential of development of new antimicrobials from plants are rewarding towards mankind. Hence in present study the sensitivity of bacteria causing urinary tract infection (UTI) to the plant *Spinacia oleracea* was evaluated.

MATERIALS AND METHODS

Plant Materials

Fresh plant leaves of *Spinacia oleracea* were collected from Chennai, Tamil Nadu. The leaves are thoroughly washed through running water and dried under shade for 4-6 d. The dried leaves are ground to fine powder and stored in sterile container for further use.

Preparation of plant extract

To study the antibacterial potential of *Spinacia oleracea* four polar solvents namely, water, ethanol, methanol, acetone and four non-polar solvents such as, petroleum ether, chloroform, hexane, benzene were used. 20 g of dried powder extracted by 200 ml of each solvent by using Soxhlet extractor for 5 h at a temperature not exceeding the boiling point of the solvent.¹³ At the end of extraction, the extracts were filtered through Whatman No. 1 filter paper, concentrated and kept in airtight container at 4°C.



Preliminary Phytochemical Screening

The presence of phytoconstituents of *Spinacia oleracea* was evaluated using each solvent extract by Phytochemical screening as per standard procedure.^{14,15}

Test microorganisms

The bacteria used in this study included *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29213, *Klebsiella pneumoniae* ATCC 13883, frequent urinary tract infection (UTI) causing pathogens. The pure cultures of bacteria were grown on nutrient agar and slants were maintained at 4°C. Nutrient agar (pH 7.2-7.4) was also used for routine susceptibility testing of nonfastidious bacteria.

Preparation of bacterial inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) (HiMedia, Mumbai, India) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell density of about 1×10^7 CFU/ml.

Screening of antibacterial activity

Antibacterial assay was carried out on Muller Hinton Agar (MHA). Screening of antibacterial activity was performed by well diffusion technique.¹⁶ The MHA plates were seeded with 1% of the standardized inoculum of each test organism. Once agar was solidified, 6 mm diameter uniform well was cut using sterile standard cork borer on the surface of the MHA. The cut agar discs were removed by vacuum device. 100 µl of different concentrations (25, 50, 75 and 100 mg/ml) of plant extracts were introduced using sterilized dropping pipettes into the each well and allowed to stand for 1 h at room temperature to diffuse and incubated at 37°C for 24 h. The Inhibition Zone Diameter (IZD) was measured by antibiotic zone reader to nearest mm.¹⁷ Ampicillin was used as positive standard control antibiotic. Three replicates were maintained for each extract against each of the test organism.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the plant extracts that did not permit any visible growth of the inoculated test organism in broth culture in each case.¹⁸ The plant extracts with various concentrations were introduced into different test tubes, each tube was inoculated with an overnight culture *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae* diluted to give a final concentration of 10^6 cells/ml. The tubes were incubated at 37 °C for 24 h. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom or turbidity in the broth.

Determination of minimum bactericidal concentration (MBC)

The minimum bacterial concentration was determined by adopting standard methods.^{19,20} To determine MBC, 10 µl

of broth medium from each well of MIC tested plate was taken and incubated on Nutrient agar at 37°C for 24 h. The MBC endpoint is defined as the lowest concentration of antimicrobial agent that kills >99.9% of the initial bacterial population where no visible growth of the bacteria was observed on the NA plates.

Statistical analysis

The antibacterial activity was determined by measuring the diameter of zone of inhibition that is the mean of three replicates. The results were expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

The *in vitro* antibacterial activity of different extract of *Spinacia oleracea* was evaluated against bacteria responsible for urinary tract infection (UTI). Four polar and four non-polar solvents were used to extract the photochemical components.

Phytochemical analysis

The qualitative phytochemical screening of *Spinacia oleracea* using different solvent extract revealed that it possesses various secondary metabolites and biochemical compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenol, steroids, carbohydrates and amino acids (Table 1).

Phytochemical screening of the crude extracts showed the presence of steroids in aqueous, ethanol, methanol and chloroform extracts where as other extract showed negative result. In case of terpenoids, it is present in methanol, petroleum ether and hexane extract. Saponins are present in all polar solvents and in only one non-polar solvent i.e. chloroform. But phenolic compounds are present in all extracts. The aqueous, methanol and chloroform extract showed positive results for flavonoids, and methanol, ethanol and chloroform extract showed positive results for alkaloids. In the case of tannins, tannins are present aqueous, methanol, ethanol and benzene extract where as all four remaining solvents showed negative result. Carbohydrates and amino acids are present in almost all polar solvents than the non-polar solvents. For carboxyl acids, except acetone extract all other exhibited negative result whereas for glycosides, except acetone extract all other showed positive result. The ethanol extract only showed positive results for anthraquinones. The above screening result revealed that all polar solvent extracts contain a wide range of phytoconstituents than the non-polar solvent extracts. Among the polar solvents, methanolic extract showed better results.

Antibacterial assay

The antibacterial efficacy of *Spinacia oleracea* using different solvent extract was determined against three most UTI causing bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*. All the solvent including polar and non-polar solvents extracts of the plant indicated that the plants exhibited antimicrobial



activity against the tested microorganisms at four different concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. The potent sensitivity of the extract was obtained against the three bacteria tested

and the zone of inhibition was recorded for *Escherichia coli* (Figure 1), *Enterococcus faecalis* (Figure 2), and *Klebsiella pneumoniae* (Figure 3).

Table 1: Phytochemical constituents of leaf extract of *Spinacia oleracea* in different extract

Phytochemical components	Water	Ethanol	Methanol	Acetone	Petroleum ether	Chloroform	Hexane	Benzene
Steroids	+	+	+	-	-	+	-	-
Terpenoids	-	-	+	-	+	-	+	-
Saponins	+	+	+	+	-	+	-	-
Phenols	-	+	+	+	+	+	+	+
Flavonoids	+	-	+	-	-	+	-	-
Alkaloids	-	+	+	-	-	+	-	-
Tannins	+	+	+	-	-	-	-	+
Carbohydrates	+	+	+	-	-	+	-	-
Amino acids	-	+	+	+	-	-	-	-
Carboxyl acids	-	-	-	+	-	-	-	-
Glycosides	+	+	+	-	+	+	+	+
Carbonyl	+	+	+	+	-	-	-	-
Anthraquinone	-	+	-	-	-	-	-	-

Table 2: Minimum inhibitory concentration (MIC) of different extracts of *Spinacia oleracea* against test pathogens (mg/ml)

Test pathogens	Water	Ethanol	Methanol	Acetone	Petroleum ether	Chloroform	Hexane	Benzene
<i>E.coli</i>	5	10	1	15	40	15	25	40
<i>E.faecalis</i>	5	1	1	10	40	15	20	25
<i>K.pneumoniae</i>	5	10	1	10	20	25	30	40

Table 3: Minimum bactericidal concentration (MBC) of different extracts of *Spinacia oleracea* against test pathogens (mg/ml)

Test pathogens	Water	Ethanol	Methanol	Acetone	Petroleum ether	Chloroform	Hexane	Benzene
<i>E.coli</i>	15	20	10	35	50	35	50	50
<i>E.faecalis</i>	10	10	15	20	45	40	40	35
<i>K.pneumoniae</i>	15	20	10	25	40	45	45	50

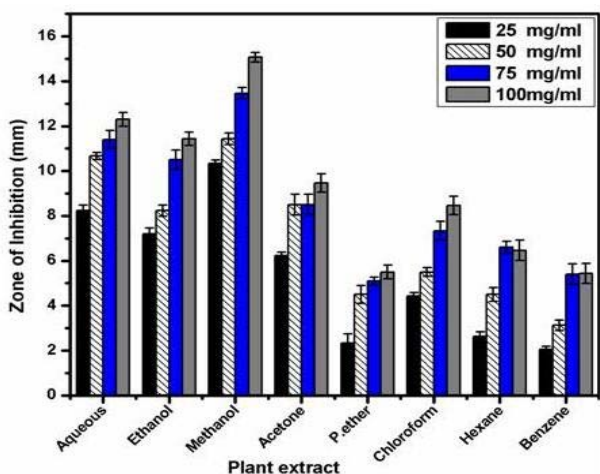


Figure 1: Antibacterial potential of *Spinacia oleracea* against *Escherichia coli*

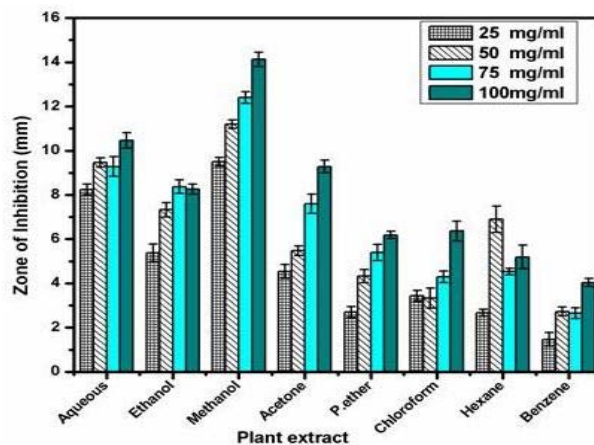


Figure 2: Antibacterial potential of *Spinacia oleracea* against *Enterococcus faecalis*

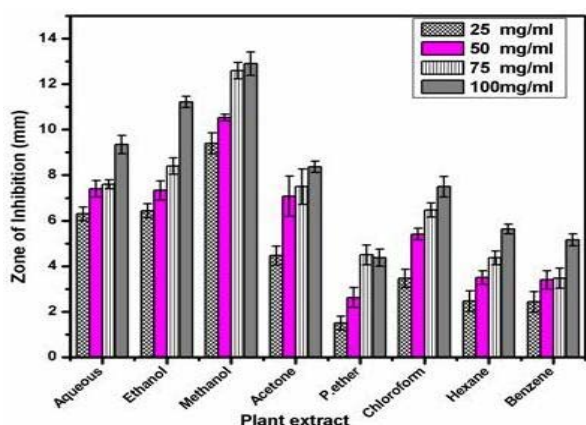


Figure 3: Antibacterial potential of *Spinacia oleracea* against *Klebsiella pneumoniae*

Natural products of plant origin have played significant role in the search of therapeutic drugs, such as quinine from cinchona.²¹ Search of new antimicrobials is very important in recent time considering the escalating levels of antibiotic resistance among pathogenic bacteria.^{22,23} In the present investigation, the active phyto-components of *Spinacia oleracea* in methanol extract produced higher sensitivity for all pathogens, whereas ethanol, aqueous and chloroform extract showed moderate sensitivity and other extracts exhibited minimum zone of inhibition. This least sensitivity against these bacteria may be due to the absence of potent bioactive compounds. For methanol extract, maximum zone of inhibition was found 15 mm, 14 mm, and 13 mm at the concentration 100 mg/ml of plant extract against *E. coli*, *E. faecalis*, and *K. pneumoniae* respectively, compared to all other solvent extracts. The aqueous extract (100 mg/ml) also showed satisfactory against *E. coli* (12 mm), *E. faecalis* (10 mm), and *K. pneumoniae* (9 mm). The MIC of all eight extracts against the selected pathogens was determined (Table 2). The most effective minimum inhibitory concentration was found 1 mg/ml of methanolic extract against all three pathogens, *E. coli*, *E. faecalis*, and *K. pneumoniae*. Also the MBC values were evaluated against those bacteria (Table 3) and the very effective result for minimum bactericidal concentration was found 10 mg/ml of methanolic extract against *Escherichia coli* and *Klebsiella pneumoniae*, and 10 mg/ml of aqueous and ethanolic extract against *Enterococcus faecalis*.

The non-polar solvents, especially petroleum ether and benzene extract of plant exhibited very poor effect on these pathogens. The methanolic extract of *Spinacia oleracea* was found to be highly active against UTI pathogens because might be the presence of large number of bioactive compounds such as steroids, tannins, alkaloids, flavonoids, terpenoids, saponins, glycosides, carbohydrates, amino acids, carbonyl compounds, phenolic compounds compared to other solvent extracts. The inhibitory effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation.²⁴ These results confirmed the antibacterial of *Spinacia*

oleracea and it can be used as phytomedicine to treat the infection in urinary tract.

CONCLUSION

To determine the potential and promote the use of herbal medicine, antibacterial efficacy of *Spinacia oleracea* was evaluated. It was revealed that presence of a wide range of bioactive compounds in polar solvent extract of this plant was responsible for the inhibition of the urinary tract infection. It was seen that methanolic extract of *Spinacia oleracea* has lowest MIC and lowest MBC values for each test organisms showing that it is most effective as compared to other extracts. Thus *Spinacia oleracea* can be used as non-toxic, cheaper and natural antimicrobial agents.

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