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Research Article

Evaluation of Preliminary Phytochemical Constituents and Antibacterial Activity of Edible Plants against Urinary Tract Infection Causing Bacteria in Children

Abstract

The present study is aimed to determine the preliminary phytochemical screening and antibacterial activity of acetone extract of the edible plants, *Solanum nigrum (L.), Murraya koenigii* (L.), *Sesbania grandiflora* (L.) against urinary tract infection causing bacteria in children. These edible plant extracts were checked for their antibacterial activity by the agar disc diffusion method. The preliminary phytochemical screening of this extract revealed the presence of alkaloids, proteins, amino acids, anthraquinone glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins and steroids. In the Kirby-Bauer disc diffusion method, Ciprofloxacin showed the high zone formation against all the isolated bacterial strains. Among all the extracts, *Murraya koenigii (L.)* from winter season showed maximum inhibitory activity for all the isolated bacterial strains. The minimal inhibitory concentration values of the acetone extract of these three edible plants from two different seasons against the isolated bacterial strains were observed. The acetone extracts of these edible plants have a broad spectrum of antibacterial activity and support the traditional use of these plants as medicines.

Introduction

Urinary tract infections affect about 3 percent of children every year. UTIs account for more than 1 million visits to pediatricians' offices every year. These are a very common infection that occurs when bacteria enter into the urinary bladder and multiply anywhere along the normally sterile urinary tract. Most of the infections are caused by bacteria normally present on the skin or in the intestinal tract that invades the urinary tract [1]. It is remaining a major clinical problem over 50 years after the introduction of antimicrobial chemotherapy. The common infection is referred as bacteriuria, which is the multiplication of bacteria in urine within the renal tract. A concentration of greater then10^5 organisms/ml is regarded as significant bacteriuria. Pyuria is the presence of W.B.C (polymorphous) in the urine. And Hematuria is the presence of R.B.C in urine [2]. The common urinary tract bacterial pathogens identified in patients are gram negative bacteria such *Escherichia coli* being the most common one followed by the *Proteus mirabilis*, *Klebsiella species* and *Enterococcus species* and other aerobic gram-negative bacteria of the *Enterobacteriaceae* family include *Citrobacter species* and *Salmonella species* also cause urinary tract infections [3].

Urinary tract infections are treated with bacteria - fighting drugs called antibiotics.

Oral antibiotics such as trimethoprim,losporins, nitrofurantoin, or a fluoroquinolonesubstantially shorten the time to recovery. All are equally effective for both short and long-term cure rates. Resistance has developed in the community to all of these medications due to their widespread use [4]. traditional medical methods, especially the use of medicinal plants still plays a major role in the developing countries. Thus the use of plants as medicine is an ancient practice common to all societies, especially in Indian and African society. However, 80% of the world's population uses plants their primary source of medication and in view of the fact that the antibiotics are sometimes associated with adverse side effects to the host including hypersensitivity, immunosuppressive and allergic reactions. It is of interest to develop alternative antimicrobial drugs such as plants sources used for the treatment of infectious diseases [5].

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Solanum nigrum L. commonly known as Black Nightshade is a dicot weed in the Solanaceae family. Flowers are small and white with a short pedicellate and five widely spread petals. Fruits are small, black when ripe. *S. nigrum* is found mainly around the waste land, old fields, ditches, and roadsides, fence rows, or edges of woods and cultivated land. It is a common plant found in most parts of Europe and the African continent [6].

The plant has a long history of medicinal usage. This plant's leaves are used to treat mouth ulcers. The boiled extracts of leaves and berries are also used to alleviate the patient's discomfort in liver-related ailments including jaundice. Chinese experiments confirm that the plant inhibits the growth of cervical carcinoma. It is antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic. It is also used to treat meningitis, chronic intestinal toxemia. In Northern India, the boiled extracts of its leaves and fruits are used to alleviate the discomfort caused by liver-related ailments, even in jaundice. The leaves of black nightshade plant strongly promote perspiration, when ingested in small amounts. The juice of the herb or an ointment prepared from it is externally applied to cure certain skin problems and tumors. The juice of the stalk, leaves, and roots of black nightshade was used as a wound healing agent [6].

The Curry Tree *(Murraya koenigii)* is tropical to the sub-tropical small tree in the family Rutaceae, which is native to India. It produces the leaves known as Curry leaves or Sweet Neem leaves. It is a small tree, growing 4–6 m tall, with a trunk up to 40 cm diameter. They are highly aromatic. The flowers are small, white, and fragrant. The small black shiny berries are edible, but their seeds are poisonous. These are also used as a herb in ayurvedic medicine. Their properties include much value as an antidiabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc. Curry leaves are also known to be good for hair, for keeping them healthy and long. The leaves bark and roots of *Murraya koenigii* (L.) plant can be used as a tonic and a stomachic. The bark and the roots are used as a stimulant by the physicians. They are also used externally to cure eruptions and the bites of poisonous animals. The green leaves are stated to be eaten raw for curing dysentery, and the infusion of the washed leaves stops vomiting [7].

All parts of *Sesbania grandiflora (L.)* are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruit. In Folk medicine, it is reported to be aperients, diuretic, emetic, febrifuge, laxative, and tonic. Agati is a folk remedy for bruises, catarrh, dysentery, eyes, fevers, headaches, smallpox and sore throat. Different parts of this plant are used in the siddha system of Indian traditional medicine for the treatment of a wide spectrum of ailments including anemia, bronchitis, fever, headache, ophthalmic, nasal catarrh, inflammation, leprosy, gout and rheumatism. In addition, *S. grandiflora* is mentioned as a potent antidote for tobacco and smoking-related diseases. Thus, the various parts of *Sesbania* are used as medicine for many diseases and disorders [8].

Materials and Methods

Clinical samples

A total of 52 Urine Samples were collected using sterile containers (from Hi-Media, Mumbai) from the children of age between 2-10 years from the Government hospital in Salem District, Tamil Nadu, and India. The collected samples were immediately transferred to the laboratory and processed. The collected sample details have been shown in table 1.

Identification of bacterial strains

The collected urine samples were allowed to serial dilution. The 10^{-5} to 10^{-7} dilutions were plated on different media, namely Nutrient agar medium and different selective media such as MacConkey agar medium, Bismuth sulfite (BSA) agar medium, Eosin Methylene Blue (EMB) agar medium, Xyline Lactose Deoxycholate (XLD) agar medium, Urinary Tract infection (UTI) agar medium and incubated at 37ºC for 12 - 24 hours. The incubated agar media plates were studied for Morphological characteristics, staining reaction, Biochemical characteristics, Antibacterial activity of standard antibiotics and edible plant extracts.

Antibacterial activity of antibiotics

Antibacterial activity using the selected antibiotics was determined by Kirby-Bauer agar disc diffusion method using Muller-Hinton agar medium. The sterile cotton swab was dipped into a well-mixed Nutrient broth; (containing bacterial cultures incubated in the shaker for eight hours at 37°C) excess inoculum was removed by pressing the swab against the inner wall of the culture tube. The entire agar plates were swabbed horizontally, vertically and an outer edge of the plate to ensured heavy growth over the entire surface. All the culture plates were allowed to dry for about five minutes. The selected antibiotics used in the study includes, Amikacin (30µg/disc), Amoxicillin (30µg/disc), Ampicillin (25µg/disc), Ciprofloxacin (30µg/disc), Cloxacillin (10 µg/disc), Erythromycin (15µg/disc), Gentamycin (30µg/disc), Kanamycin (30µg/disc), Nalidixic acid (30µg/disc) and Streptomycin (25µg/disc). After the inoculation, the different antibiotic discs were placed on the medium using sterile forceps. Then the plates were incubated at 37ºC for 18-24 hours. After the incubation, a clear zone of inhibition around the disc was measured and the results were noted.

Plant sources and its solvent extract

The Plant sources [*Solanum nigrum* (L), *Murraya koenigii* (L) and *Sesbania grandiflora* (L.)] from winter season (January) and summer season (May) were collected from Ayothyapattanam,

Table 1: Total Number of Urine Samples.

Sex	Age (in Years)	Total Number of Samples		
Male	0-5	8	19	52
	6-10	11		
Female	0-5	15	33	
	6-10	18		

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Salem District, Tamil Nadu and India. The collected plant species were identified and authenticated by Dr. R. Selvaraj, Professor of Botany, Annamalai University, Annamalai Nagar-608 002. All the plant sources were washed with running tap water and then finally washed with distilled water to remove the dirt. The plant parts were dried under shade for seven days then they were kept in hot air oven for four to six hours at 50°C to remove excess moisture. The dried plants were separately crushed softly to make powder form using mixer grinder. That crushed powder was loaded into the clean dry soxhlet apparatus tightly using the soft metal rod. Then, the apparatus was run to get plants extract with acetone. And the apparatus with acetone was run until to get clear solvents in the side tube. The time was measured to get clear solvent in the side tube. Now the acetone extract of the three plants contains active ingredients. Then, the extracts of three plants from two seasons were evaporated using rotary vacuum evaporator to remove the solvents [9].

Preliminary phytochemical screening

The preliminary phytochemical screening method was performed using the standard procedure for the identification of its active chemical constituents includes alkaloids, flavonoids, Anthraquinone glycosides, tannin and phenolic compounds, carbohydrates, saponins, proteins and amino acids [8].

Characteristics features of plant extracts

The appearance and amount of the extracts of each plant were observed and measured using electronic balance. A loop full of each different plant extracts were streaked on sterile nutrient agar plates to check the presence of any microbes.

Preparation of discs using plant extracts

The Observing capacity of 5 mm sterile disc (*HIMEDIA*) was selected ranges from 10µl to 50 µl. For the preparation of the stock solution, 10 mg of each different crude extract was dissolved in 1 ml of DMSO. From these stock, 10µl, 20µl, 30µl, 40µl and 50 µl was added on the sterile discs to get 100µg, 200µg, 300µg, 400µg, and 500µg respectively of plant extracts. Then these prepared discs were used for Antibacterial activity against the isolated bacterial strains [10].

Antibacterial activity of plant extracts

Antibacterial activity using plant extracts were determined by agar disc diffusion method using Muller-Hinton agar medium. The sterile cotton swab was dipped into a well-mixed Nutrient broth, (containing bacterial cultures incubated in a shaker for eight hours at 37°C) excess inoculums was removed by pressing the swab against the inner wall of the culture tube. The entire agar plates were swabbed horizontally, vertically and the outer edge of the plate to ensured heavy growth over the entire surface. All the culture plates were allowed to dry for about five minutes. Then the prepared discs with compounds were placed on an upper layer of the inoculated plates using sterile forceps. The equal distance was maintained between each disc. The disc was gently pressed down aseptically with the help of alcohol flamed forceps. All the plates were incubated for 24 hours at 37°C. Then, the presence of a zone of inhibition could be measured on the plates [11].

Residual effects of solvents and DMSO

To find the residual effects of acetone and DMSO, 10ml of acetone and DMSO were evaporated separately until to get 1ml of residue. Then the residues were added as small drops using micropipette on the sterile disc by keeping the disks on the hot plate at 50°C to remove excess residues. Then the discs were kept in Muller-Hinton Agar medium plates swabbed with overnight broth culture of isolated strains. One empty sterile disc was kept to check whether it possess any inhibitory activity.

Determination of minimal inhibitory concentration

Minimal inhibitory concentration (MIC) was determined for each plant extract showing antibacterial activity against test pathogens. Broth dilution method was followed for determination of MIC values. Plant extracts were suspended in acetone to make 10 mg/ml final concentration and serially diluted and added to the respective tubes containing nutrient broth media. Thereafter, 100µl of inoculum (for bacteria 1×10^8 CFU/ml) was added to each tube. The tubes were incubated at 37°C for 24 hrs. The MIC values were taken as the lowest concentration of the extracts in the tube that showed no turbidity after incubation [12].

Results

Isolated bacterial strains

The identification of isolated bacterial strains was performed on the basis of Colony morphology on different media included as Nutrient agar medium, MacConkey agar medium, EMB agar medium, UTI agar medium, XLD agar medium, BSA agar medium. Gram staining reaction and biochemical analysis were also performed and the results have been shown in table 2,3.

Antibacterial activity of antibiotics

The antibiotic sensitivity test was performed by the Kirby-Bauer Disc Diffusion technique using standard antibiotic discs against the isolated bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Salmonella typhi*. Ciprofloxacin showed the high zone formation against all the isolated bacterial strains. The antibiotic Sensitivity pattern of the isolated bacterial strains was recorded. The results have been shown in table 4.

Characteristic features of plant extracts

The plants *Solanum nigrum* (L.), *Murraya koenigii* (L.), *Sesbania grandiflora* (L.) was extracted using acetone. The obtained extracts appeared as green colored semisolid paste form with viscosity. The nature of the plant extracts and the amount of yield have been shown in table 5.

Preliminary phytochemical analysis

The results of preliminary phytochemical analysis of acetone extract of *Solanum nigrum (L.)*, *Murraya koenigii* (L.) and *Sesbania grandiflora* (L.) showed that the presence and absence of tannins, carbohydrates, alkaloids, flavonoids, saponins, glycosides and amino acids with a slight difference between

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the two seasons. The alkaloids, carbohydrates, tannins and flavonoids are present in all the three plants from the two seasons. Saponins are present only in *Solanum nigrum* (L.) and *Murraya koenigii* (L.) in both the seasons and absent in both the seasons of *Sesbania grandiflora* (L.). Steroids are absent in all the three plants from two seasons. The presence of secondary metabolites by preliminary phytochemical screening was recorded and the results have been shown in table 6.

Residual effects of solvents and DMSO

DMSO and acetone did not show any inhibitory effect against the isolated bacterial strains. So, the inhibitory effect of the solvents such as acetone and DMSO was negligible. The residual effects of solvent and DMSO were recorded and the results have been shown in table 7.

Antibacterial activity of plant extracts

Antibacterial activity of acetone extracts of *Solanum nigrum (L.)*, *Murraya koenigii (L.)*, *Sesbania grandiflora (L.)* from winter and summer season against the isolated bacterial strains were performed. *Murraya koenigii (L.)* from winter season showed maximum inhibitory activity for all the isolated urinary tract bacterial strains. In the concentration of 500µg, it showed a maximum zone of inhibition which is 16mm for *Escherichia coli;* 18mm for *Klebsiella pneumoniae*; 17 mm for *Proteus mirabilis and* 15mm for *Salmonella typhi. Solanum nigrum (L.)* from winter season also possessed 16mm for *Salmonella typhi* which is more effective than other extracts. The zone of inhibition formed by these plant extracts against the isolated bacterial strains was recorded and the results have been shown in table 8.

Table 2: Morphological and Biochemical Characterization of Bacterial Isolates.

S. No	Name of the Tests	Result and Observation			
		Escherichia coli	Klebsiella pneumoniae	Salmonella typhi	Proteus mirabilis
1	Gram staining	Gram Negative Rod	Gram Negative Rod	Gram Negative Rod	Gram Negative Rod
2	Motility Determination	+	+	+	+
	Biochemical Tests				
3	Catalase Test	+	+	+	+
4	Oxidase Test	-	-	-	-
5	Indole Test	+	-	-	-
6	Methyl Red Test	+	+	+	+
7	Voges Proskauer Test	-	-	-	-
8	Citrate Utilization Test	-	+	+	+
9	Triple Sugar Iron Agar Test	Acid slant and acid butt	Acid slant and acid butt	Alkaline slant, acid butt with gas	Alkaline slant with acid butt
10	Urease test	-	+	-	+

Table 3: Cultural Characterizations of Isolated Bacterial Strains.

S.No.	Selective media	Results and observation
1	Eosin Methylene Blue Agar Medium	Green- black colonies with metallic sheen (*Escherichia coli)*
2	MacConkey Agar Medium	Pink color colonies (*Klebsiella pneumoniae)*
3	Urinary Tract Infection Agar Medium	Brown color colonies (*Proteus mirabilis*)
4	Bismuth Sulfite Agar (BSA) Medium	Black color colonies (*Salmonella typhi*)
5	Xylose Lysine Deoxycholate Agar Medium	Red color colonies with black spots (*Salmonella typhi)*

Table 4: Antibacterial activity of Antibiotics against Isolated Bacterial Strains.

S.No	Name of the Antibiotics	Conc. of Antibiotics (µg/disc)	Zone of inhibition (in mm)			
			Escherichia coli	Klebsiella pneumoniae	Proteus mirabilis	Salmonella typhi
1	Amikacin	30	18mm	17mm	15mm	17mm
2	Amoxicillin	30	22mm	19mm	28mm	16mm
3	Ampicillin	30	-	-	19mm	19mm
4	Ciprofloxacin	30	32mm	26mm	36mm	33mm
5	Cloxacillin	10	-	10mm	-	11mm
6	Erythromycin	25	-	-	-	-
7	Gentamycin	30	13mm	12mm	19mm	18mm
8	Kanamycin	30	18mm	14mm	13mm	17mm
9	Nalidixic acid	30	13mm	17mm	10mm	19mm
10	Streptomycin	30	11mm	10mm	14mm	15mm

Table 5: Yield of Acetone Extract of Three Plants from Two Different Seasons.

S.No	Name of the Plant Extracts	Colour	Form	Extract weight (in grams)
1	*Solanum nigrum.Linn (s)*	Green	Oily paste	4g
2	*Solanum nigrum.Linn (w)*	Green	Oily paste	4.7g
3	*Murraya koenigii.Spreng (s)*	Green	Oily paste	6g
4	*Murraya koenigii.Spreng (w)*	Green	Oily paste	5.8g
5	*Sesbania grandiflora.Linn (s)*	Green	Oily paste	5.6g
6	*Sesbania grandiflora.Linn (w)*	Green	Oily paste	6.2g

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration values of the acetone extract of these three plants [*Solanum nigrum* (L.), *Murraya koenigii* (L.), *Sesbania grandiflora* (L.)] from two different seasons were observed. The MIC value of *Solanum nigrum* (L.) from two different seasons for *Escherichia coli* was 500µg/ml. For *Klebsiella pneumoniae*, the MIC value was 250µg/ml in the winter season and 500µg/ml in a summer season. For *Proteus mirabilis* and *Salmonella typhi*, *the* MIC value was 250µg/ml in both of the seasons. *Murraya koenigii* (L.) had a MIC value of 250µg/ml in both the seasons for all the four isolated bacterial strains. *Sesbania grandiflora* (L.) had a MIC value of 500µg/ml in both seasons for *Klebsiella pneumoniae* and *Proteus mirabilis.* For *Escherichia coli*, the MIC value was 250µg/ml in winter and 500µg/ml in summer. For *Salmonella typhi*, the MIC value was 500µg/ml in summer and 250µg/ml in winter. The values were observed and the results have been shown in table 9.

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Discussion

Urinary tract infections affect about 3 percent of children every year. Throughout childhood, the risk of a UTI is 2 percent for boys and 8 percent for girls. Most of the infections are caused by bacteria normally present on the skin or in the intestinal tract that invades the urinary tract [1]. Urinary tract infection occurs more frequently in female than men. This is remaining a major clinical problem over 50 years after the introduction of antimicrobial chemotherapy [13]. From this present study, a total of 52 urine samples were collected. Among them, 48 samples showed the presence of *E.coli*, *K.pneumoniae*, *P.mirabilis and S.typhi*. The similar work with 33 UTI samples from those samples they found UTI caused gram-negative bacteria such as *E.coli*, *K.pneumoniae*, *P.vulgaris*, *P.aeruginosa*, *Enterobacter sp* [14].

Table 6: Preliminary Phytochemical Analysis.

S.No	Constituents / Tests	Solanum nigrum (winter)	Solanum nigrum summer)	Murraya koenigii (winter)	Murraya koenigii (summer)	Sesbania grandiflora (winter)	Sesbania grandiflora (summer)
1	**Alkaloids**						
	Mayer's test	+	+	++	+	+	+
	Dragendorff's test	-	-	-	-	-	-
	Hangers test	+	+	+	+	+	+
	Wagers test	-	-	-	-	-	-
2	**Proteins & Aminoacids**						
	Millon's test	-	-	-	-	-	-
	Ninhydrin test	+	+	+	+	++	++
	Biuret test	-	-	-	-	-	-
3	**Anthraquinone glycosides**						
	Borntragers test	+	+	-	-		-
4	**Flavonoids**						
	Shinoda's test	+	+	+	+	+	+
	Ferric chloride test	+	+	+	+	+	+
5	**Tannins & Phenols**						
	Ferric chloride test	++	++	++	+	+	+
	Lead acetate test	++	++	+	+	+	+
	Gelatin contains Nacl test	++	++	+	+	+	+
6	**Carbohydrates**						
	Molisch's test	+	+	+	+		
	Barfoed's test	+	+	+	+	+	+
	Fehling test	+	+	+	+	+	+
7	**Saponins**						
	Frothing test	+	+	+	+		-
8	**Steroids**						
	Liebermann-Burchard test	-	-	-	-		-

Table 7: Residual effects of Solvent and DMSO.

S.No	Solvents	Zone of Inhibition (In mm) For 10ml Residues
1	Acetone	0mm
2	DMSO	0mm
3	Sterile discs	0mm

Table 8: Antibacterial Activity of Acetone extracts of Edible plants against Urinary Tract Isolated Bacterial Pathogens.

Plant Sources with two seasons	Acetone Extract Concentration (µg/disc)	Zone of Inhibition (in mm)			
		Isolated Bacterial Pathogens			
		Escherichia coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Salmonella typhi*
Solanum nigrum (winter)	100	0 mm	0 mm	0 mm	0 mm
	200	0 mm	5 mm	4 mm	7 mm
	300	8 mm	7 mm	7 mm	10 mm
	400	11 mm	11 mm	13 mm	13 mm
	500	14 mm	15 mm	15 mm	16 mm
Solanum nigrum (summer)	100	0 mm	0 mm	0 mm	0 mm
	200	0 mm	0 mm	5 mm	5 mm
	300	7 mm	6 mm	7 mm	7 mm
	400	11 mm	9 mm	9 mm	10 mm
	500	12 mm	13 mm	12 mm	12 mm
Murraya koenigii (winter)	100	0 mm	0 mm	0 mm	0 mm
	200	8 mm	7 mm	7 mm	6 mm
	300	11 mm	11 mm	12 mm	9 mm
	400	14 mm	16 mm	14 mm	12 mm
	500	16 mm	18 mm	17 mm	15 mm
Murraya koenigii (summer)	100	0 mm	0 mm	0 mm	0 mm
	200	7 mm	5 mm	5 mm	0 mm
	300	11 mm	9 mm	9 mm	6 mm
	400	12 mm	12 mm	12 mm	9 mm
	500	14 mm	15 mm	17 mm	12 mm
Sesbania grandiflora (winter)	100	0 mm	0 mm	0 mm	0 mm
	200	6 mm	0 mm	6 mm	0 mm
	300	9 mm	7 mm	10 mm	6 mm
	400	11 mm	11 mm	13 mm	10 mm
	500	14 mm	14 mm	16 mm	13 mm
Sesbania grandiflora (summer)	100	0 mm	0 mm	0 mm	0 mm
	200	0 mm	0 mm	0 mm	4 mm
	300	7 mm	6 mm	7 mm	7 mm
	400	10 mm	10 mm	11 mm	10 mm
	500	14 mm	14 mm	13 mm	12 mm

Identification and screening was carried out to detect the presence of bacteria present in the urine sample *using* the biochemical characterization, motility determination and colony formation on differential culture media like Nutrient agar, MacConkey agar, Eosin Methylene Blue (EMB) agar, Urinary Tract Infection (UTI) agar, Bismuth Sulphite (BSA) agar and Xylose Lysine Deoxycholate Deficient (XLD) agar. Likewise, the similar related work has been identified by Ravikumar S, et al. [15] and isolated the gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* using the biochemical characterization and growth on different selective medium. The same results have been identified Linda

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Table 9: Minimal Inhibitory Concentration (MIC) of Acetone extract of *Edible plants* against from different seasons against isolated bacterial strains.

Plant Extracts	MIC for Escherichia coli (µg/ml)									
	1.95	3.90	7.81	15.6	31.2	62.5	125	250	500	1000
Solanum nigrum (w)	+	+	+	+	+	+	+	+	β	-
Solanum nigrum (s)	+	+	+	+	+	+	-	+	β	-
Murraya koenigii (w)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (s)	+	+	+	+	+	+	+	β	-	-
Sesbania grandiflora (w)	+	+	+	+	+	+	+	β	-	-
Sesbania grandiflora (s)	+	+	+	+	+	+	+	+	β	-
	MIC for *Klebsiella pneumoniae* (µg/ml)									
	1.95	3.90	7.81	15.6	31.2	62.5	125	250	500	1000
Solanum nigrum (w)	+	+	+	+	+	+	+	β	-	-
Solanum nigrum (s)	+	+	+	+	+	+	+	+	β	-
Murraya koenigii (w)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (s)	+	+	+	+	+	+	+	β	-	-
Sesbania grandiflora (w)	+	+	+	+	+	+	+	+	β	-
Sesbania grandiflora (s)	+	+	+	+	+	+	+	+	β	-
	MIC for *Proteus mirabilis* (µg/ml)									
	1.95	3.90	7.81	15.6	31.2	62.5	125	250	500	1000
Solanum nigrum (w)	+	+	+	+	+	+	+	β	-	-
Solanum nigrum (s)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (w)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (s)	+	+	+	+	+	+	+	β	-	-
Sesbania grandiflora (w)	+	+	+	+	+	+	+	+	β	-
Sesbania grandiflora (s)	+	+	+	+	+	+	+	+	β	-
	MIC for Salmonella typhi (µg/ml)									
	1.95	3.90	7.81	15.6	31.2	62.5	125	250	500	1000
Solanum nigrum (w)	+	+	+	+	+	+	+	β	-	-
Solanum nigrum (s)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (w)	+	+	+	+	+	+	+	β	-	-
Murraya koenigii (s)	+	+	+	+	+	+	+	+	β	-
Sesbania grandiflora (w)	+	+	+	+	+	+	+	+	β	-
Sesbania grandiflora (s)	+	+	+	+	+	+	+	β	+	+

Note: + = Growth. - = No Growth. β =MIC value

MD, et al. [1] as most of the gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Proteus sp*, *Enterobacter sp* and *Pseudomonas sp* by using biochemical characterization and different selective medium.

In the present study, Ciprofloxacin showed high inhibitory activity against all the isolated bacteria. Similar work was done by Olerumi et al. [16] observed that the Ciprofloxacin and Oflaxin could inhibit the growth of gram-negative bacteria. The extraction of crude extracts from *Solanum nigrum* (L.), *Murraya koenigii* (L.) and *Sesbania grandiflora* (L.) from two different seasons by acetone was performed to detect the secondary metabolites. The similar work was carried out by Prajakta. J. Patil et al. [17] and they used methanol for crude extraction to detect the presence of secondary metabolites. Another similar work was carried out by Venkatesan et al. [18]. They used ethanol and petroleum benzene for extraction of *Solanum nigrum (L.)* to detect the secondary metabolites.

In the present study, the preliminary phytochemical analysis was performed and the result showed the presence of alkaloids, tannins, phenolic compounds, carbohydrates, amino acids and flavonoids and saponins. Similar work was carried out by Avalaskar et al. [8] as the phytochemical analysis was done by preliminary analysis from the methanolic extract of *Sesbania grandiflora*. It showed that the presence of alkaloids, glycosides, sugars, amino acids and steroids.

Antibacterial activity of acetone extracts of *Solanum nigrum (L.)*, *Murraya koenigii (L.)* and *Sesbania grandiflora (L.)* from two different seasons against isolated strains was done by using disc diffusion method. From that study, acetone extract of *Murraya koenigii (L.)* from winter season showed high activity (*E.coli* 16mm, *K.pneumoniae* 17mm, *P.mirabilis* 15mm, *S. typhi* 16mm) than other extracts against all organisms. Similar work was carried out by Abishek mathur et al. [19]. From their study, they reported that *Murraya koenigii (L.)* showed high inhibitory activity against *E.coli* and *K.pneumoniae* which is 26 and 22 mm respectively. Another work was performed in *Ocimum bacillicum*

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L. by M. B. Outarra, et al. [20]. In their study, they reported that the *Ocimum bacillicum* (L.) showed a high zone of inhibition against *E.coli*, *P.mirabilis* and *S.typhi* which is 24, 27and 21 respectively.

In the present study, the minimal inhibitory concentration was observed against the bacterial isolates. The extracts showed a good activity against all the isolated bacterial strains. *Murraya koenigii* (L.) showed 250µg/ml in both the seasons for all strains which is more effective than all other strains. Similar work was carried out by Usman H, et al. [5]. They observed MIC of *Tribulus terrestris* (L.) extract against *Escherichia coli* which was 1.250mg/ml.

The plants, *Solanum nigrum (L.)*, *Murraya koenigii (L.)*, *Sesbania grandiflora (L.)* have been involved in our investigation which acts as a nonantibiotic alternative for preventing urinary tract infection. Meanwhile, all the three edible plants are effective in winter season than the summer season. The difference in the antibacterial activity between the two seasons is due to the variation in a number of secondary metabolites present in the plants. Using these edible plants leads to reducing the amount of antibiotics prescribed for the treatment of UTI and preventing drug resistance. This study demonstrated that the extracts from the leaves of *Solanum nigrum (L.)*, *Murraya koenigii (L.)*, *Sesbania grandiflora (L.)* act as a modern medicine for UTI bacteria. The advanced pharmacological screening of these edible plants using the modern tool may lead to some new drug.

Conclusion

The present research study suggested that the acetone extracts of the selected edible plants possess the broad spectrum antibacterial activity against the isolated urinary tract infection causing bacterial strains. This study revealed that the edible plant sources can also be effective as the modern medicine to inhibit the growth of pathogenic urinary tract bacteria and devastating the antibiotic resistance. Further studies should be needed with these edible plants for the structural elucidation of bioactive compounds to formulate a new drug to treat the urinary tract infections.

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References

1. Linda MD, Shortliffe D (2002) Host pathogenesis in urinary tract infection. *Int J Antimicrob Agents* 17: 245-251.

2. Meyhoff H, Nordling J, Gammelgaord P, Vejlsgaard R (1981) Does antibacterial ointment applied to urethral meatur in women prevent cystitis. *Scand J Urol Nephrol* 15: 81-83. Link: https://goo.gl/WivwUn

3. Hasan Ejaz, Aizza Zafar, Anwar N, Cheema TA, Humaira Shehzad (2006) Prevalence of bacteria in Urinary tract infection among children *Biomed* 1: 22.

4. Sree Bhushan R, Tiwari B (2001) Identification and characterization of UTI pathogens *J Bacteriol* 107: 718-735.

5. Usman H, Abdul FI, Landon AA (2007) Phytochemical and antimicrobial evaluation of *Tribulus terrestris* L.(Zygophyllaceae) growing in Nigeria *Bio* 2: 244-247. Link: https://goo.gl/FStiLx

6. Atanu FO, Ebiloma1 UG, Ajayi EI (2010) A review of the pharmacological aspects of *Solanum nigrum* Linn. *Biotech Molecular bio Rev 6*: 1-7. Link: https://goo.gl/3q0sup

7. www.wikipedia.org/wiki/Murraya koenigii /Solanum nigrum/Sesbania grandiflora.

8. Avalaskar AN, Itankar PR, Joshi VS, Agrawal M, Vyas J (2011) Phytochemical and TLC Studies of ethanolic extract of *Sesbania grandiflora. Int J Pharm Res* 3: 1346-1349. Link: https://goo.gl/BQueCy

9. Arun Thangavel, Senthilkumar Balakrishnan, Aarthy Arumugam, Senbagam Duraisamy, Sureshkumar Muthusamy (2014) Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents and anti-bacterial activity of *Aerva lanata* (L.) leaves *Afr J Pharm Pharmacol* 8: 126-135. Link: https://goo.gl/QYSH3L

10. Arun T, Senthilkumar B, Purushothaman K, Aarthy A (2012) GC-MS determination of bioactive components of *Phyllanthus amarus* (L.) and its antibacterial activity *J Pharm Res* 5: 4767-4771. Link: https://goo.gl/kaaL7p

11. Saranya MS, Arun T, Iyappan P (2012) *Invitro* antibacterial activity and preliminary phytochemical analysis of leaf extracts of *Argemone Mexicana* Linn–A medicinal plant *Int J Curr Pharm Res* 4: 85-87. Link: https://goo.gl/hyVw4v

12. Sharma B, Kumar P (2009) Extraction and Pharmacological Evaluation of Some Extracts of *Tridax procumbens* and *Capparis deciduas. Int J App Res in Nat prod* 1: 5-12. Link: https://goo.gl/2H3pEs

13. Mohammad R, Kambiz Diba (2010) Invitro activity of cranberry extract against etiological agents of Urinary tract infection. *Afr J Pharm Pharmacol* 4: 286-288. Link: https://goo.gl/aipaG7

14. Anjana S, Rani V, Padmini R (2009) Antibacterial Activity of Some Medicinal Plants Used by Tribals Against Uti Causing Pathogens *World Appl sci J* 7: 332-339. Link: https://goo.gl/vSlnB6

15. Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi A (2006) Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens *Int J Med Sci* 2: 94-99. Link: https://goo.gl/t4qiyr

16. Oluremi BB, Idowu AO, Olaniyi JF (2011) Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a Teaching Hospital in Southwestern Nigeria *Afr J Microbiol Res* 5: 3658-3663. Link: https://goo.gl/Sa7aaH

17. Prajakta J, Patil, Jai S Gosh (2010) Antimicrobial activity of *Catharanthus roseus* – A detailed study *Bri J Pharmacol and Toxicol* 1: 40-44. Link: https://goo.gl/wWbJhr

18. Venkatesan D, Karunakaran CM, Kumar SS (2009) Studies on Phytochemical constituents, functional group identification and antimicrobial activity of *Solanum nigrum* (Solanaceae) *Ethnobot Leaflets* 13: 1485-1503. Link: https://goo.gl/umD04E

19. Abhishek M, Dua.VK, Prasad KS (2010) Antimicrobial Activity of Leaf Extracts of *Murraya Koenigii* against Aerobic Bacteria Associated with Bovine Mastitis *Int J Chem Environ Pharm Res* 1: 12-16. Link: https://goo.gl/QFozn2

20. Ouattara MB, Konate KM, Kiendrebeogo, Ouattara N, Compaore M, et al. (2011) Antibacterial Potential and Antioxidant Activity of Polyphenols of *Sesbania grandiflora Curr Res J Bio.Sci* 3: 351-356. Link: https://goo.gl/Idzb39

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