

Phytochemical analysis, antioxidant and antibacterial potential of some selected medicinal plants traditionally utilized for the management of urinary tract infection

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Abstract: Recent studies on prevalence of urinary tract infection indicate that approximately one third population of the world has been suffering from this disease. The current study was designed to evaluate the antibacterial activity of aqueous-ethanolic extracts (30/70) of *Tribulus terrestris* (TT), *Vaccinium macrocarpon* (VM), *Cuminum cyminum* (CC), *Rheum emodi* (RE), *Piper cubeba* (PC) and their compound formulation “Crano-cure” against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus saprophyticus* and *Proteus mirabilis* through disc diffusion method and agar well methods compared with standard Ciprofloxacin. DPPH radical scavenging methods were applied for antioxidant activities and phytochemical analysis was also performed to detect the phytoconstituents. All the plants exhibited potent antibacterial strength while Crano-cure showed most potent results comparable with that of standard drug. The zone of inhibition produced by disk diffusion test was 26±0.34, 26±0.75, 26±0.00, 18±0.64, 22.5±0.52, 29±0.39, 32±0.00 mm and for agar well diffusion test 23±0.67, 22±0.46, 23±0.77, 20±0.00, 22±0.46, 24±0.52, 33±0.00 mm against *Tribulus terrestris*, *Cuminum cyminum*, *Rheum emodi*, *Piper cubeba*, *Vaccinium macrocarpon*, crano-cure and ciprofloxacin. Similarly, percentage inhibition for antioxidant potential was 78.74, 24.57, 58.75, 20.23, 88.88, 90.12 and 92.35 respectively. The tested plants exhibited remarkable antibacterial and antioxidant activities.

Keywords: Phytochemical analysis, antioxidant, antibacterial, UTI, medicinal plants.

INTRODUCTION

Urinary tract is the most prone part of the body for infectious diseases as it is directly intact with external environment via pathway for liquid waste disposal. The urinary tract infection (UTI) is one of the most common infections which can involve upper and or lower urinary tract causing pyelonephritis (renal pelvis), urethritis (urethra), cystitis (urinary bladder) and prostatitis (prostate gland) respectively. Urinary tract infection presents clinically with varying features depending upon the site of infection such as dysuria, frequent micturation, and urinary incontinence in case of lower UTI whereas pyrexia, lumbar pain and occasionally hematuria in upper UTI (Bao *et al.*, 2017). The incidence of UTI in one of the literature is 0.7% and the essential hazard aspects for UTI are age, previous history of UTI, diabetes mellitus, sexual activity, obesity and structural anomalies. Around 150 million individuals build up a urinary plot contamination annually (Ullah *et al.*, 2018). *Escherichia coli* (*E. coli*) and *Staphylococcus saprophyticus* (*S. saprophyticus*) are most important pathogen which causes UTI (Kang *et al.*, 2018; Hashemzadeh *et al.*, 2021). Urinary tract infection caused by *E. coli*, *K. pneumoniae*, *E. faecalis*, *S. saprophyticus*

and *P. mirabilis* mostly followed by blood-borne infections (Pannu *et al.*, 2020). The incidence of UTI is more common in female rather than in male due to the very close presence of urethra to the anus (Paude *et al.*, 2018).

There is increased resistance to antibiotics which is leading to recurrent infection and also the associated side effects (Tandogdu Z and Wagenlehner FM., 2016). Therefore, the interests are diverted towards alternative natural remedies, which at various levels have proved efficacy and safety. Medicinal plants have also played role in the management of UTI having less adverse actions, cheap, easy available with minimal bacterial resistance (Yatoo *et al.*, 2018). Medicinal plants gained popularity now days worldwide as WHO reported that 80% of world population depends upon herbal medications (Aziz *et al.*, 2017). The medicinal plants contains phytoconstituents more importantly secondary metabolites which have therapeutic effects in different diseases. Traditionally many medicinal herbs were used in UTI such as *Tribulus terrestris*, Cranberry, cucumber seeds etc. Cranberry juice has pivotal role in controlling the UTI (Shaheen *et al.*, 2019). It contains sialic acid which has ability to reduce inflammation as well as pain relieving potential (Luczak *et al.*, 2018). *Rheum emodi* have phytoconstituents like carbohydrate, phenols,

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anthrones, flavonoids, anthraquinones, lignans, oxanthrone ethers and esters, stilbenes and oxalic acid (Singh *et al.*, 2018). Piper species contains compounds such as amides, terpenes, benzoic acids, chromenes, phenylpropanoids, lignans, phenolics and a series of alkaloids (Andriana *et al.*, 2019).

Vaccinium macrocarpon or Cranberries contains various group of chemically active compounds which may include proanthocyanins, anthocyanins, catechins, flavonols, , phenolic acids (hydroxybenzoic acid like vanillic acid), hydroxycinnamic acid (coumaric acid, ferocious acid, cinnamic acid and caffeic acid), precursor of vitamin A that is Beta carotene and vitamin C (Sintara *et al.*, 2018). *Cuminum cyminum* seeds contain volatile oils, proteins and other medicinally important chemical compounds like pinene, oleoresin, terpinene that have therapeutic effects against many diseases (Singh *et al.*, 2017). *Tribulus terrestris* possess active constituents which incorporate chlorogenins well as gitogenin with diosgenin, kaempferol 3-glucoside, kaempferol 3-rutinoside along with tribuloside (Semerdjieva IB and Zheljaskov VD, 2019).

Tribulus terrestris, *Vaccinium macrocarpon*, *Cuminum cyminum*, *Rheum emodi* and *Piper cubeba* and a compound formulation of all these medicinal plants was designed for antibacterial and antioxidant effects in the current research work.

MATERIALS AND METHODS

Collection and identification of plant material

The plants were purchased from local market of Bahawalpur. The plant material was taxonomically identified and authenticated by Dr. Ghazala H. Rizwani, Director Research of Hamdard University Karachi and voucher numbers were assigned as *Tribulus terrestris* Linn. 28617, *Vaccinium macrocarpon* Ait. 00046006, *Cuminum cyminum* L. 17558, *Rheum emodi* Wall. ex Meissn. 21473 and *Piper cubeba* L. 24786. To evaluate the synergetic effects of these plants, a compound formulation crano-cure was designed which contains following plants with given concentration.

Crano-cure capsule

Each 500mg capsule contains; *Tribulus terrestris* (TT) 125mg, *Vaccinium macrocarpon* (VM) 125mg, *Cuminum cyminum* (CC) 100mg, *Rheum emodi* (RE) 100mg and *Piper cubeba* (PC) 50mg. The combination of these plants and their ratios have been designed by the research team after interviewing the local practitioners and evaluating the literature (Mustafa *et al.*, 2017).

Extract preparation

The purchased plant ingredients were cleaned, washed and dehydrated underneath shadow. The crude plant

material was grinded to fine powder followed by soaking of 100 gm/ml in 70% aqueous ethanol for 72 hrs with occasional stirring and mixing. Serial filtration across muslin cloth and Whatman filter paper No.1 was performed prior to evaporation using rotary evaporator to get final semi-solid mass with percentage yield of 29.4% (w/w) (Balogun *et al.*, 2016).

Phytochemical analysis

The selected plants were analyzed qualitatively for presence of carbohydrates, proteins, flavonoids, glycosides, alkaloids, saponins, sterols, phenols and tannins using the standard procedures (Madhavan and Tharakan, 2017).

In vitro analysis

Antibacterial activity of individual plant and compound formulation at a concentration of 25(µg/ml), 50 (µg/ml), 100 (µg/ml), 250 (µg/ml) and 500 (µg/ml) was assessed. This activity was performed against *E. coli*, *K. pneumonia*, *S. saprophyticus* and *P. mirabilis* through disc diffusion method and agar well methods. The zone of inhibition was measured by both methods to analyze the antibacterial effects.

Bacterial strain

The bacterial samples were taken from the FCBP, IAGS, Punjab University Lahore, Pakistan. The bacterial strains were allotted accession numbers from the culture bank University of Punjab as: *Escherichia coli* (011), *Klebsiella pneumoniae* (50), *Staphylococcus saprophyticus* (267) and *Proteus mirabilis* (043).

Culture preparation

8g of the nutrient broth of Merck, Germany was blended in 1 liter of distill water. Broth was autoclaved at 121°C for fifteen minutes at 15 Psi and was added in Erlenmeyer flasks along with fifty µl of bacterial culture were added in it. Flasks were placed on flat shaker for 24 hrs at room temperature at 200 rpm. Optical density of the culture was evaluated afterward 24hrs b/w 0.12-0.19 according to 0.5McFarland standard (Harathi *et al.*, 2017).

Broth dilution technique

Sterile 96 wells micro plates were used for antibacterial activity. 200µl was added in wells having 180µl suspension of bacterial culture and 20µl of ethanolic extract for pre read absorbance was determined at 540 nm. Petri dishes were incubated at 37°C for 24 hrs. After read was taken likewise just as the difference between pre read as well as after read was utilized as a record of bacterial movement. Outcomes were mean of triplicate (n=3, ±SEM). Ciprofloxacin was used as positive control as well as ethanol was used for negative control. All evaluations were triplicated (Srinivasan *et al.*, 2001). Percentage inhibition was calculated as:

$$PI = 100 \times (X - Y) / X$$

Where X= Negative control absorbance and Y= Test sample absorbance by bacterial culture.

MIC (Minimum inhibitory concentration) evaluation

For MIC serial dilutions were made of the test sample procedure such as describe previously. Software used for results was EZ-Fit5 Perrella Scientific Inc. Amherst USA.

Agar well diffusion assay

28 gm of Muller Hinton agar was dissolved in distilled water. Autoclaving was done at 121°C for 15 minutes at 15 Psi for sterilization purpose. Solidification is completed by putting Sterile Muller Hinton (20ml) agar into Petri dishes. Streaking of bacterial culture was complete on agar and dried. 4 holes of 6mm were made in agar present in every Petri dish. 20µl of ciprofloxacin was put in one hole and 20µl of extracted solution was added in unused three holes with the help of micro pipette. Total Petri dishes were protected at 37 °C for 24 hrs. At the end of incubation period the zone of inhibition were calculated to check antibacterial activity.

Antioxidant activity

DPPH radical scavenging assay

Antioxidant ability of the extracts was verified by DPPH radical scavenging assay (DRSA) (Jiao et al., 2015). A solution of 0.1mM DPPH was prepared in ethanol and 2.4 ml of this solution was mixed with 1.6ml of aqueous ethanolic extracts at different concentrations (25, 50, 100, 250 and 500µg/ml). The reaction mixture was vigorously shaken and kept in dark at RT for 30min. Spectrophotometer was used to measure absorbance of the mixture at 517 nm. Butylated hydroxytoluene (BHT) was used as reference. % DRSA was calculated as follows:

$$\%DRSA = \{(A_0 - A_1)/A_0\} \times 100$$

Where A_0 , and A_1 is the absorbance of control and extractives/standard respectively. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

STATISTICAL ANALYSIS

All data was expressed as mean±S.D of three replicates and analyzed by SPSS version 20. Tukey Post-Hoc One way analysis of variance (ANOVA) was performed for the determination of differences among mean. Values of $P < 0.05$ were regarded as significant.

RESULTS

Antibacterial activity

Antibacterial activity by disk diffusion method:

Anti-bacterial activity of individual plant and compound formulation was assessed at the concentration of 25, 50, 100, 250 and 500 (µg/ml) against *E. coli*, *K. pneumonia*, *S. saprophyticus* and *P. mirabilis* by disc diffusion

method. Ciprofloxacin was utilized as positive control. Zone of inhibition was measured by replicating the results three times and data was obtained in mean with ± SEM. The results are given in table 1.

Antibacterial activities by well method

Antibacterial activity was evaluated by five dilutions (25, 50, 100, 250 and 500µg/ml) of individual plants extracts, compound formulation Crano-Cure and standard drug against mentioned bacteria. The zone of inhibition was measured by replicating results three times and data was obtained in mean with ± SEM. The results are given in table 2.

Antioxidant activity by DPPH radical scavenging activity

DPPH radical scavenging activity indicates the dose-response curve of DPPH radical scavenging activity of the aqueous ethanolic extracts of TT, VM, CC, RE, PC and Crano-cure matched with BHT. It was observed that the extract of VM had higher activity compared to the other extracts but yet less than Crano-cure that is comparable to standard drug. The results are given in table 3.

Phytochemical analysis

Phytochemical analysis exposed the occurrence of carbohydrates, glycosides, proteins, flavonoids, alkaloids, saponins, sterols, phenols and tannins in all. The presence and estimated concentrations of phytochemicals were variable but suspected for anti-bacterial and antioxidant activity of experimental plants (table 4).

DISCUSSION

Medicinal plants have been demonstrated to be very safe and effective and now essential for the development of new antimicrobial agents to cure infectious diseases and many other ailments. Therefore, herbal medicines possessing anti-infective constituents have been reported from India, Pakistan, Turkey, Japan, Taiwan and other different areas of the world (Dar et al., 2017). There is a great need for proper screening and scientific studies on these herbal medicines for their mechanism of action and therapeutic values.

Antibacterial potential by disk diffusion methods revealed that maximum inhibition was shown at 500µg/ml by all extracts, compound formulation “Crano-Cure” and by control drug where most sensitive organism was *E. coli* which was inhibited by TT, CC, VM with 26±0.34, 26±0.75, 22.5±0.52 mm respectively followed by *K. pneumonia* by PC and ciprofloxacin with 21±0.51, 33±0.37 mm respectively. Crano-cure exhibited marvelous antibacterial activity which is comparable to standard drug Ciprofloxacin at 29±0.39 mm against *E. coli* where as standard drug produced a zone of 32±0.00 mm for same organism. Antibacterial activity by well

Table 1: Antibacterial activity of *aqueous ethanolic* extracts of medicinal plants and compound formulation crano-cure against different bacteria by disk diffusion method

Plants (500 µg/ml)	Zone of inhibition in mm disk diffusion method (mm)			
	<i>E. coli</i>	<i>S. saprophyticus</i>	<i>K. pneumonia</i>	<i>P. mirabilis</i>
TT	26±0.34	25±0.96	21±0.43	18±0.23
CC	26±0.75	24±0.39	22±0.06	21.5±0.97
RE	26±0.00	27±0.41	21±0.0	19±0.73
PC	18±0.64	19±0.26	21±0.51	20±0.41
VM	22.5±0.52	21±0.0	19±0.36	22±0.64
Crano-Cure	29±0.39	28±0.48	25±0.51	23±0.0
Ciprofloxacin	32±0.00	31±0.43	33±0.37	30±0.48

Table 2: Antibacterial activities of *aqueous ethanolic* extracts of some medicinal plants and compound formulation crano-cure by well method against bacterial test organism

Plants (500 µg/ml)	Zone of inhibition in mm Agar Well method			
	<i>E. coli</i>	<i>S. saprophyticus</i>	<i>K. pneumonia</i>	<i>P. mirabilis</i>
TT	23±0.67	17±0.33	19±0.41	17±0.64
CC	22±0.46	19±0.42	20±0.43	19±0.33
RE	23±0.77	22±0.31	20±0.54	20±0.49
PC	20±0.00	18±0.47	18±0.18	19±0.37
VM	22±0.46	20±0.38	18.5±0.07	21±0.43
Crano-cure	24±0.52	23±0.46	21±0.34	22.5±0.08
Ciprofloxacin	33±0.00	31±0.47	32±0.42	30±0.71

Table 3: Antioxidant activity by DPPH radical scavenging activity

Plants (500 µg/ml)	Anti-oxidant activity	
	DPPH (%)	IC50
VM	88.88	40.50
TT	78.74	45.00
RE	58.75	80.00
CC	24.57	112.69
PC	20.23	120.00
Crano-cure	90.12	6.4
BHT	92.354	4.5

Table 4: Phytochemical analysis of medicinal plants

Test applied for phytochemicals		TT	CC	RE	PC	VM
Carbohydrates	Molisch Test	A	P	P	A	P
Flavonoids	Alkaline reagent test	P	P	P	P	P
Protein	Biuret Test	P	P	A	A	A
Glycosides	Born Trager's test	P	A	P	A	P
Alkaloids	Mayer's Test	P	P	P	P	A
Saponins	Froth's Test	P	A	P	A	A
Sterols	Salkowski reaction	P	A	P	P	P
Phenols	Ferric chloride test	P	P	P	P	P
Tannins	Bromine water	A	A	P	P	A

P= present, A= absent

method was also performed where maximum inhibition was shown at 500 µg/ml by all extracts and control while most sensitive organism was *E. coli* inhibited by TT, CC, RE, VM, PC and Crano-cure with 23±0.67, 22±0.46, 23±0.77, 22±0.46, 20±0.00 and 24±0.52 mm respectively

followed by *S. saprophyticus* by RE with 22±0.31 mm and Ciprofloxacin with 33±0.00 mm, whereas *K. pneumonia* by RE with 20±0.54 and *P. mirabilis* by VM with 21±0.43mm.

The results of antibacterial potential from our selected medicinal plants are comparable with some other reports also. The antibacterial activity was assessed against pathogenic gram-positive and gram-negative microbes in a previous report (Mostafa *et al.*, 2018). Alcoholic concentrate along with cumin seed oil restrained the development of *K. pneumoniae* and its clinical segregates, lead to betterment in capsule expression, cell morphology as well as diminished urease action. This action of cumin was credited to carvone, cuminaldehyde, linalool along with limonene, though cumin oil having limonene, alpha-pinene, eugenol, as well as few additional minor active compounds have been recommended to add to the antimicrobial effect (Shivakumar *et al.*, 2010).

Antimicrobial action of ethanolic extract of *Tribulus terrestris* fruit was evaluated against 6 microbes *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus sanguis*, *Escheria coli*, *Actinomyces viscosus* and *Enterococcus faecalis* showing promising results (Soleimanpour *et al.*, 2015). In a current research work, antibacterial activity of 13 common flavonoids such as flavones, flavonols, flavanones) and 6 organic acids such as aliphatic and aromatic acids was evaluated. The MICs of selected plant substances were determined by the micro-dilution method using clinical strains of four species; *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* (Adamczak *et al.*, 2020).

In another study, ethanol extract of *Aloe vera* leaves and roots is applied on bacterial and fungal strains in different concentrations (15, 20, 25, 30µl). *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and some other bacterial strains were used in the study concluding that the application of plants in traditional medicine in the treatment of various diseases caused by these pathogenic strains (Danish *et al.*, 2020).

Furthermore, the scavenging activity of the extracts at a concentration of 500µg/mL determined in descending order as VM=88.88, TT=78.74%, RE=58.75, CC=24.57, PC=20.23 and for Crano cure it was 90.12% while at the same concentration, that of the BHT was 92.354%. IC₅₀ of aqueous ethanolic extracts were presented as: VM 40.50, TT 45.00, RE 80.00, CC112.69 and PC 120.00 µg/ml and for Crano cure and BHT (standard) IC 50 was 6.4 and 4.5µg/mL respectively.

Many studies have shown that plant polyphenols can be used as antioxidants against different oxidative stress-induced diseases. Several thousand polyphenolic molecules have been identified in higher plants, including edible ones (Boo YC 2019; Pawlowska *et al.*, 2019).

CONCLUSION

Results revealed that plants possess significant antibacterial and antioxidant activities. Therefore, based on these findings *Tribulus terrestris*, *Cuminum cyminum*, *Rheum emodi*, *Piper cubeba* and *Vaccinium macrocarpon* can be used for bacterial infections particularly UTI. Furthermore, compound formulation Crano-cure can be applied for the prevention of UTI in future clinical studies.

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