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RESEARCH ARTICLE

Appraisal of *Cymbopogon citratus* (Lemon grass) for Antibacterial Activity Against Uropathogens

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A B S T R A C T

Urinary tract infections (UTI) are one of the major public health concerns in both genders, but variations in the anatomy, physiology and behaviour of urogenital and reproduction tract make women more susceptible. UTI is more prevalent and severe in women of all ages and in older men because of multi-drug resistant strains and high recurrence, it has become an important socioeconomic burden. Due to the microbial resistance, several life-threatening side effects, repeated high doses, high cost and low effectiveness of these antibiotics motivated the researchers to explore natural remedies for UTI therapy. The purpose of the research was to evaluate the antibacterial effect of *Cymbopogon citratus* (*C. citratus*), against uropathogens isolated from UTI patients, mainly include *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*). Isolates were confirmed through conventional biochemicals techniques. Ethanolic extract of *C. citratus* was evaluated against isolates through disc diffusion method and minimum inhibitory concentration was also determined. Ethanolic extract of *C. citratus* was phytochemically characterized through high profile liquid chromatography (HPLC). Antibacterial susceptibility was determined by measuring zone of inhibition (ZOI) and *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae* showed average14.0, 13.0, 13.0 and 8mm ZOI against ethanolic extract, respectively. HPLC showed flavonoids and phenolics components present in ethanolic extract of *C. citratus.* In mouse model *C. citratus* also decreased the significant number of uropathogens. This study reports the role of lemon grass for treating UTI and provides new remedy for the treatment of UTI.

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INTRODUCTION

Microbial infections, including *Escherichia coli* (*E. coli*), *Enterococcus faecalis* and *Klebsiella* species have been found to be the main causes of urinary tract infection (UTI). Various signs including painful urination or dysuria, haematuria, urinary urgency, burning, frequent urination, nausea and vomiting observed in UTI (Anderson *et al.*, 2004). UTIs are among the most common conditions requiring medical treatment with 6-10% of all young women with bacteriuria. The incidence of UTIs increases (25-50%) with age in females aged 80 years and older have bacteriuria (Hung *et al*., 2009). UTIs occur as a result of interactions between the uropathogens and host. The uropathogens initially bind to the epithelial surface, colonize and spread throughout the mucosa causing tissue damage. Pathogens can ascend into the urinary bladder after the initial colonization period, leading to symptomatic or asymptomatic bacteriuria. Further progression may lead to renal impairment and

pyelonephritis. Specific virulence factors residing on the uropathogen's membrane are responsible for bacterial resistance to the normally effective defense mechanisms of the host. Bacterial adhesions and their associated epithelial binding sites have recently been identified and natural mechanisms of anti-adherence are currently being investigated (Jafari *et al*., 2012). According to statistical calculations, the association between UTIs caused by *E. coli* in the female ($P<0.05$) was significant (Jahandeh *et al.*, 2015). Reported research showed 80% of UTIs are caused by *E. coli* and 10-15% caused by *S. saprophyticus.* In United States and other region of the world *Enterococci*, *Klebsiella*, *Enterobacter* and *Proteus mirabilis* rarely cause uncomplicated cystitis and pyelonephritis (Ronald, 2002).

Eternal antibiotic resistance in bacteria leads to the alterations in antibiotics for the control of bacterial infections (Younas *et al*., 2019). Moreover, antibiotics impose many side-effects on the host gut flora, hypersensitivity and immunosuppression (Patel, 2007). The pharmaceutical industries developed latest generations of antibiotics for the treatment of resistant bacterial strains (Barrata *et al*., 1998). Antibiotics resistant strains were reported first time in Brazil from 1980s and now it persists worldwide. Until now various strategies has been developed including modified drugs but natural bioactive antimicrobial agents proved to be a good alternate for the treatment of bacterial infections with very few limitations (Buckova *et al*., 2018).

The herbal medicines have greater antimicrobial activity due to presence of different bioactive chemicals like Allicin, flavonoids, terpenoids, tannins, alkaloids etc. (Fayyaz *et al*., 2019; Mahmood *et al*., 2019; Abbas *et al*., 2019). Different plant materials have been reported to possess immunostimulant (Mahmood *et al*., 2014), antiviral (Aslam *et al*., 2014), antibacterial (Arshad *et al*., 2017; Yasmin *et al*., 2020) and antiprotozoan activity (Abbas *et al*., 2017; Zhang *et al*., 2020), while some plant material extracts have been reported to consist up of biofuel (Fatima *et al*., 2016). *C. citratus* plant extract also reported as an excellent source of various bioactive compounds which can be used to treat UTIs. It can be utilized as a remedy for ophthalmia, intestinal sickness, elephantiasis pneumonia and vascular scatters. Analysts found that *C. citratus* has characteristics of nervous disease preventing agent, bactericidal, antiseptic, astringent, antioxidant, fungicidal and sedative characteristics (Ronald, 2002).

The use of medicinal plants as key drugs to sustain human health is emphasized by the World Health Organization (WHO). Brazil, Latin America and Argentina have gradually increased the use of medicinal plants. About 80% of people in developing countries use medicinal plants as conventional remedies. Therefore, these plants should be studied in order to better understand their properties, safety and efficiency (Cimanga *et al*., 2002). Many medicinal plants has been investigated phytochemically for the bioactive compounds and their therapeutic use bioactive compounds including tannins and phenols are growth inhibiters for pathogenic bacteria (Cui *et al*., 2016). Identifying new and effective strategies as an alternative treatment will be the foremost priority. Medicinal plants and essential oil of plants showed significant bactericidal and antioxidant activity (Naik *et al*., 2018). Multi drug resistant bacteria cause long term acute infections which fails antibiotic therapy for the control of pathogens (Patel, 2007).

Recent studies on medicinal plants and their therapeutic use is quite helpful to solve the antibiotic resistance issue of bacteria. The infectious diseases are leading cause of diseases and one third of the total deaths are attributed to infectious diseases. The multi-drug resistant bacteria are reported to be associated mainly with human diseases epidemics. The emerging antibiotic resistance against antibiotic trigger the use of herbal medicine as an alternative.

This present study was conducted to evaluate the bioactive compounds of *C. citratus* and their antimicrobial activity against UTIs causing bacteria. *C. citratus* readily grow in Asia and no such previous work related to antimicrobial activity against UTIs causing bacteria is available in literature. This study helped to evaluate the role of *C. citratus* for treating UTIs and provide new dimension in medical field.

MATERIALS AND METHODS

Plant extract and Phytochemical Anaylysis: *Cymbopogon citratus* plants were collected from local nurseries and confirmed from a Botanist. Leaves were air dried and crushed to powdered form. Ethanolic extract (80%) of *C. citratus* leaves was made through conventional method by using soxhlet apparatus (Redfern *et al.*, 2014). High performance liquid chromatography (HPLC) was performed for the phytochemical analysis of ethanolic extract of *C. citratus.* By adding the plant extract sample in HPLC grade at 0.1mg/µl concentration and strained through 0.2 millipore membrane filter. It was then subjected on RP-18 column. The fractions correlating to maximum peaks with fixed retention time were collected by using a fraction collector. It was performed by using two LC-10AT pumps (Shimadzu).

Collection of Bacterial culture and *In-vitro* antibacterial analysis: UTIs causing bacteria including gram positive (*Staph. aureus*, *K. pneumoniae*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*) cultures and confirmation was done through biochemical testing. Antibacterial activity of ethanolic extract of *C. citratus* was performed through disc diffusion method. Bacterial culture compared with 0.5% MacFarland standard having 1.5×10^8 CFU/ml grown as a lawn culture on Mueller-Hinton agar plates. Filter paper discs dipped in 50ul extract for overnight and used to evaluate their antibacterial activity against selected uropathogens. Discs placed on Mueller-Hinton agar plates with bacterial lawn and incubated at 37ºC for 24 hrs. Zones of inhibition (ZOI) were measured for results. Recommended antibiotics discs used as positive control and PBS dipped filter paper disc used as negative control for each bacterium (Cui *et al*., 2016). ZOI were compared with negative and positive control.

Minimum Inhibitory Concentration (MIC): MIC of *C. citratus* ethanolic leaves extract was performed in micro dilution plate. 50µl of extract with two- fold serial dilution

and 50µl of nutrient broth were added from well no 3 to 12 in micro dilution plate. Then 20 µl of bacterial inoculums was added in each well. Well no 1 and 2 were maintained as control negative and positive containing nutrient broth (50µl) + bacterial inoculum (20µl) and antibiotic + nutrient broth (50µl) + inoculum (20µl) respectively. After incubation of 24 hours at 37ºC, turbidity change was observed.

Minimum Bactericidal Concentration (MBC): To check the MBC, wells showing no visible growth in MIC, 20ul of mixture were transferred onto fresh nutrient agar plate with micropipette by spread plate method and incubated at 37ºC for 24 hours.

***In-vivo* testing of extract in mouse model:** Experimental mouse model as reported previously (Hung *et al*., 2009; Cui *et al*., 2016) was used to evaluate the *in-vivo* effect of 80% ethanolic leaves extract of *C. citratus.* Albino mice (7-9 weeks) of age were injected with 1×10^7 CFU in 50µl PBS bacterial culture and after three days treatment for 6 days with 50 µl *C. citratus* extract. *C. citratus* extract was injected intraperitoneally. Three days post treatment mice were sacrificed and bacterial titer were measured from homogenized tissues of bladders. 0.2 ml of homogenized tissues were poured on LB medium and bacterial titter after 24 hours was measured by using the formula:

CFU per ml= number of colonies $\times 10^{\text{dilution}}$ × total volume

RESULTS

Confirmation of bacteria: The bacteria isolated from UTI patient were characterized through conventional biochemical methods presented in Table 1.

Phytochemical Anaylysis of extract: HPLC showed the phenolics and flavonoids compounds present in ethanolic leaves extract of *C. citratus.* The quantity and positive control are presented in Table 2. Fig. 1 presents the retention time of different flavonoids and phenolic compounds. Structures of compounds drawn through chembio draw 16 software are shown in Fig. 2.

Table 1: Biochemical identification of bacterial strains

Bacterial Strains	Selective media	Gram staining	Catalase test	Oxidase test	Triple sugar iron test	Citrate test	Methyl red
Staph. aureus	Manitol salt agar	+ve.	+ve	-ve	A/A	+ve.	+ve.
E. coli	MacConkey agar	–ve.	+ve.	-ve	A/A, gas+H_2S	-ve.	+ve.
P. aeruginosa	Cetrimide agar	–ve.	+ve.	+ve	K/K,gas-,no H_2S	+ve	-ve
K. pneumoniae	MacConkey agar	–ve.	+ve.	-ve	A/A,gas+,No H_2S	+ve.	-ve.

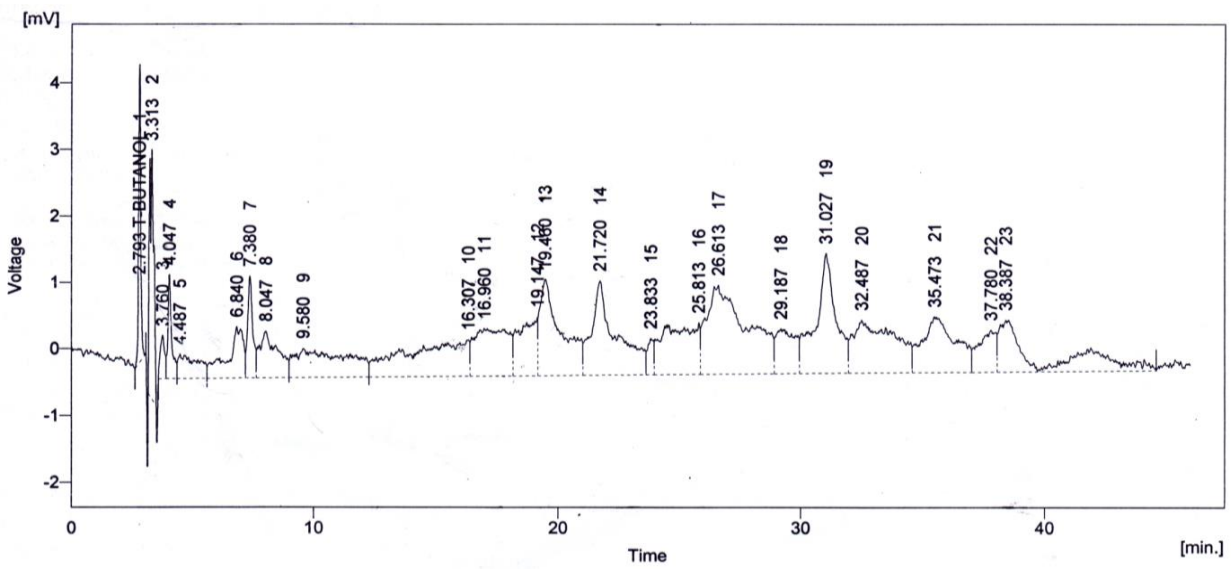
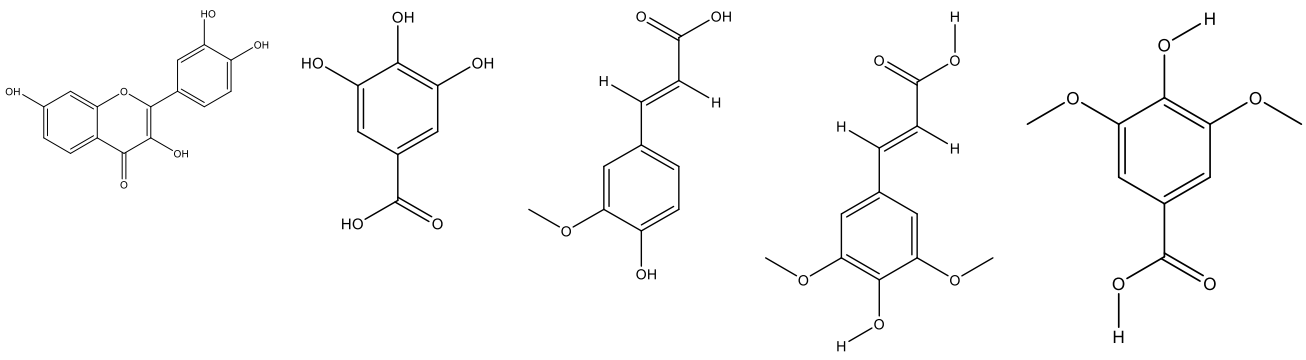


Fig. 1: Chromatogram of HPLC analysis of Lemon grass extract.



A. Quercetin structure B. Gallic acid C. Ferulic acid D. Sinapic acid Syringic acid

Fig. 2: Chemical structures of different alkaloids isolated from Lemon grass extract.

Table 2: Antibiotic susceptibility testing, zone of inhibition at 50ul of *C. citratus* extract in three replicates and their mean with comparison to positive control

Bacterial spp.	Zone of inhibition (mm)			Mean	Antibiotic (positive control)
E. coli	15	13	15	14	16
K. pneumoniae	11	8	7	8	20
Staph. Aureus	15	11	14	13	22
P. aeruginosa	11	16	14	13	25

***In-vitro* antibacterial activity of extract:** Plant extract of *C. citratus* was evaluated through disc diffusion method. ZOI compared with positive controls (antibiotics), ampicillin is used for *S. aureus, E. coli, K. pneumonia* and gentamycin was used for *P. aeruginosa*. *C. citratus* has proved to be more effective against *P. aeruginosa, Staph. aureus* and *E. coli* and less effective against *K. pneumoniae* in disc diffusion method. The largest ZOI were observed against *E. coli* (14mm) and *P. aeruginosa* (13mm). Table 2 showed all the mean ZOI against uropathogens.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): MIC of plant extract performed in micro dilution plate. Minimum concentrations inhibiting the growths of *E. coli, Staph. aureus, P. aeruginosa* and *K. pneumoniae* were 6.25, 0.8, 3.125 and 6.25µl, respectively. All the details of MIC are given in Table 3. 20µl from each MIC determined well evaluated through spread plate method on agar plates and all showed same MBC values as MIC.

***In-vivo* testing of extract in mouse model:** After 6 days of treatment, all the animals were sacrificed and bladders were homogenized in PBS. Bacterial titer was significantly decreased in both gram negative and positive uropathgens are presented in Table 5.

DISCUSSION

Antimicrobial effects of medicinal plants against UTIs causing bacteria and resistant strains proved to be an effective alternative to drugs. Increasing multidrug resistance in uropathogens demanded a safe substitute to drug therapies. Current study proved the antimicrobial effect of *C. citratus* (lemon grass) against selected gram negative and gram positive uropathogens. Results acquired from disc diffusion method and MIC showed the 80% ethanolic leaves extract of *C. citratus* can inhibit the growth of gram negative (*E. coli, P. aeruginosa, K. pneumoniae*) and gram-positive *Staph. aureus*. Similar observations reported by (Baratta *et al*., 1998; Cimanga *et al*., 2002; Pereira *et al*., 2004; Naik *et al*., 2010) against *C. citratus* oil. All the uropathogens were sensitive to ethanolic extract of *C. citratus* through disc diffusion method. *P. aeruginosa* previously reported resistant to *C. citratus* oil but 80% ethanolic leaves extract of *C. citratus* showed promising effect against *P. aeruginosa* in the current study, ZOI measured was 13mm as compared to positive control gentamycin 25mm. Previous studies of (Onawunmi *et al*., 1984; Duraipandiyan *et al*., 2006; Naik *et al*., 2010, Jafari *et al*., 2012) reported *P. aeruginosa* as least sensitive or resistant against *C. citratus* oil. Current study showed that the ethanolic leaves extract of *C. citratus* showed MIC value against gram negative bacteria from 3.125-6.25ul as compared with previously reported much weaker response 400mg/ml of methanolic extract against *P. aeruginosa* and *E. coli* (Duraipandiyan *et al*., 2006; Jafari *et al*., 2012; Naveed *et al*., 2013). Gram negative uropathogens were more resistant against *C. citratus* extract than gram negative bacteria. *Staph aureus* showed least MIC value of 0.8ul that inhibit the growth of bacteria in 96 well plate. As compared to other studies may the difference in techniques lead to the minor variability of the results. *P. aeruginosa* showed less resistant against 80% ethanolic leaves extract than methanolic extract, which is in agreement of Khan *et al*. (2012).

In current study HPLC showed the flavonoids and phenolic compounds were present in ethanolic extract that were most effective against uropathogens. Active compounds that include flavonoids, geranyl acetate, phenolic compounds, steroids and saponin were already reported in *C. citratus* oil by Duraipandiyan *et al*. (2006), Mothana *et al*. (2010), Hindumathy *et al*. (2011).

Various essential plant oils in synergism showed greater effect against uropathogens as compared to antibiotics as reported in previous studies (Sienkiewicz *et al*., 2012; Buckova *et al*., 2018). Ethanolic extract of *C. citratus* leaves can be recommended as alternative remedy for antibiotics to avoid the more damage from resistance strains. But further in vivo studies on animal models should be conducted for further improvement/refinement.

Table 3: Minimum inhibitory concentration (MIC) of different volumes of Lemon grass extracts against uropathogens

Well number	1	2	3	4	5	6	7	8	9	10	11	12
Plant extract dilution	-ve control	+ve control	50 µl	25 µl	12.5 µl	6.25 µl	3.125 µl	0.8 µl	0.4 µl	0.2 µl	0.1 µl	0.05 µl
E. coli	✓	×	×	×	×	×	✓	✓	✓	✓	✓	✓
S. aureus	✓	×	×	×	×	×	×	×	×	✓	✓	✓
P. aeruginosa	✓	×	×	×	×	×	×	✓	✓	✓	✓	✓
K. pneumoniae	✓	×	×	×	×	×	✓	✓	✓	✓	✓	✓

Table 4: Quantitative phytochemical analysis of extract and concentrations of different compounds

No. of series	Compound name	Retention time	Area (mVs)	Area %	Amount (ppm)
Standard	T- butanol (standard)	2.773	31.461	1.2	_____
Flavonoids	Quercetin	3.313	26.600	2.1	2.43
	Gallic acid	4.487	22.447	1.6	0.73
Phenolic compounds	Syringic acid	16.307	93.263	7.1	2.23
	Ferulic acid	21.720	99.395	7.6	1.14
	Sinapic Acid	26.613	156.00	11.9	2.212
	Gallic acid	4.487	22.447	1.6	0.73

Table 5: *In-vivo* testing of *C. citratus* 80% ethanolic leaves extract

Animals*	Bacterial inoculum	Bacterial titer after treatment**	Mean
1	*E. coli* 1×10^7 CFU/ml	1×10^4 CFU/ml	1×10^3 CFU/ml
2		1×10^3 CFU/ml	
3		1×10^2 CFU/ml	
1	*P. aeruginosa* 1×10^7 CFU/ml	1×10^5 CFU/ml	~1×10^4 CFU/ml
2		1×10^4 CFU/ml	
3		1×10^4 CFU/ml	
1	*Staph. aureus* 1×10^7 CFU/ml	1×10^3 CFU/ml	~1×10^2 CFU/ml
2		1×10^2 CFU/ml	
3		1×10^2 CFU/ml	
1	*K. pneumoniae* 1×10^7 CFU/ml	1×10^4 CFU/ml	~1×10^3 CFU/ml
2		1×10^3 CFU/ml	
3		1×10^3 CFU/ml	

*Three replicates of animal for each uropathgens were tested; **50ul of 80% ethanolic leaves extract of *C. citratus* for each animal.

Authors contribution: JL, XCH, DW, KMW and AR conceived and designed the study. TF executed the experiment. WB, FMZ and ML analyzed the data. All authors have interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

Abbas A, Abbas RZ, Khan MK, *et al*., 2019. Anticoccidial effects of Trachyspermum ammi (Ajwain) in broiler chickens. Pak Vet J, 39:301-4.

Abbas A, Iqbal Z, Abbas RZ, *et al*., 2017. Immunomodulatory effects of *Camellia sinensis* against coccidiosis in chickens. J Anim Plant Sci 21:415-21.

Arshad MS, Hussain I, Mahmood MS *et al*., 2017. Evaluation of anti-microbial potential of *Acacia nilotica* (Kikar) against oral pathogens associated with caries and Periodontitis. Pak J Agri Sci 54:423-30.

Aslam A, Mahmood MS, Hussain I *et al*., 2014. Evaluation of antiviral effect of epigallocatechin gallate, epigallocatechin, epicatechin gallate and green tea extract against fowl adenovirus-4. Pak J Zool 46:1283-94.

Baratta MT, Dorman HD, Deans SG, *et al*., 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flav Frag J 13:235-44.

Buckova M, Puskarova A, Kalaszova V, *et al*., 2018. Essential oils against multidrug resistant gram-negative bacteria. Biologia (Bratisl) 73:803-8.

Cimanga K, Kambu K, Tona L, *et al*., 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the democratic republic of congo. J Ethnopharmacol 79:213-20.

Cui P, Niu H, Shi W *et al*., 2016. Disruption of membrane by colistin kills uropathogenic *Escherichia coli* persisters and enhances killing of other antibiotics. Antimicrob Agents Chem 60:6867-71.

Duraipandiyan V, Ayyanar M and Ignacimuthu S, 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complem Altern Med 6:35.

Fatima N, Mahmood MS, Hussain I, *et al*., 2016. Transesterification of oil extracted from fresh water algae for biodeisal production. Energy Sources, Part A: Recovery Utiliz Environ Eff 38:2306-11.

Fayyaz MR, Abbas RZ, Abbas A, *et al*., 2019. Potential of botanical driven essential oils against *Haemonchus contortus* in small ruminants. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromatica 18:533-43.

Hindumathy CK, 2011. *In-vitro* study of antibacterial activity of *Cymbopogon citratus*. World Academy Sci Eng Technol, 5.

Hung CS, Dodson KW and Hultgren SJ, 2009. A murine model of urinary tract infection. Nature Protocols 4:1230.

Jafari B, Branch A and Mohammadi-aghdam B, 2012. Antibacterial activitiesof Lemon grass methanol extract and essence on pathogenic bacteria. Eurasian J Agri Env Sci 12:1042-6.

Khan MM, Iqbal M, Hanif MA, *et al*., 2012. Antioxidant and antipathogenic activities of Citrus peel oils. J Essential Oil Bear Plants 15:972-9.

Mahmood MS, Ahmad MF, Hussain I, *et al*., 2014. Growth promoting effect of *Pimpinella anisum* (Aniseed) in broiler chickens. Boletín Latinoamericano Caribe Plantas Med Arom 13:278-84.

Mahmood MS, Rafique A, Younas W *et al*., 2019. *Momordica charantia* L. (Bitter gourd) as a candidate for the control of bacterial and fungal growth. Pak J Agri Sci 56:1031-6.

Mothana RA, Al-Rehaily AJ and Schultze W, 2010. Chemical analysis and biological activity of the essential oils of two endemic *Soqotri Commiphora* species. Molecules 15:689-98.

Naik MI, Fomda BA, Jaykumar E *et al*., 2010. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacteria. Asian Pac J Trop Med 3:535-8.

Naveed R, Hussain I, Tawab A, *et al*., 2013. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against Salmonella and other multi-drug resistant bacteria. BMC Complement Altern Med 13:265.

Onawunmi GO, Yisak WA and Ogunlana EO, 1984. Antibacterial constituents in the essential oil of *Cymbopogon citratus*. J Ethnopharmacol 12:279-86.

Patel A, 2007. Mangement of urinary tract infections in womens. US Pharm, 32:26-33.

Pereira RS, Sumita TC, Furlan MR, *et al*., 2004. Antibacterial activity of essential oils on microorganisms isolated from urinary tract infection. Revista de Saude Publica 38:326-8.

Redfern J, Kinninmonth M, Burdass D, *et al*., 2014. Tips & tools using Soxhlet ethanol extraction to produce and test plant. J Microbiol Biol Edu 15:45-6.

Ronald A, 2002. The etiology of urinary tract infection: traditional and emerging pathogens. Am J Med 113:14-9.

Sienkiewicz M, Łysakowska M, Denys P, *et al*., 2012. The antimicrobial activity of thyme essential oil against multidrug resistant clinical bacterial strains. Microb Drug Resist 18:137-48.

Yasmin S, Nawaz M, Anjum AA, *et al*., 2020. Phytochemical analysis and In Vitro activity of essential oils of selected plants against Salmonella enteritidis and Salmonella gallinarum of poultry origin. Pak Vet J 40:139-44.

Younas M, ur Rahman S, Shams S, *et al*., 2019. Multidrug resistant carbapenemase-producing Escherichia coli from chicken meat reveals diversity and co-existence of carbapenemase encoding genes. Pak Vet J 39:241-5.

Zhang K, Li X, Na C, *et al*., 2020. Anticoccidial effects of Camellia sinensis (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. Pak Vet J 40:77-80.