

Antibacterial effects of *Citrus limon* peel extract on human pathogenic bacteria with special reference to Urinary Tract Infection

Neha Sharma^{1*}, D.S. Rathore²

¹Department of Biotechnology, Govt. Kamla Raja Girls P.G. (Autonomous) College, Gwalior, India

²Department of Biotechnology, Govt. Kamla Raja Girls P.G. (Autonomous) College, Gwalior, India

*Corresponding author: drneha16may@gmail.com

Available online at: www.isroset.org

Received: 13/Mar/2018, Revised: 19/Mar/2018, Accepted: 16/Apr/2018, Online: 30/Apr/ 2018

Abstract- Urinary tract infections (UTI) are the most common pathogenic infections, specially found in working ladies as well as seen in men and lead to many difficult problems. As the microorganism are resistant day by day from antibiotics. The main objective of this study is evaluated the antimicrobial activity and future prophylactic potential of the *Citrus limon* peel against strains of human pathogenic bacteria. Phytochemical analysis of citrus fruits shows that they are rich source of flavanones and many polymethoxylated flavones, which are found very rare in other plants, have very strong antibacterial activity. Antimicrobial analysis was done by using agar well diffusion method. The result was found that, the peel extract of lemon were inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* as well as *Bacillus subtilis* which show the resistance against nalidixic acid, co-trimoxazole and nitrofurantoin antibiotics. On the basis of the present finding lemon peel extract might be a good candidate in the search for a natural antimicrobial agent and can be considered to be as equally potent as the antibiotics, such as, ciprofloxacin.

Keywords: *Citrus limon*, Human pathogens, UTI, antimicrobial agent.

I. Introduction

In women urinary tract infections (UTI) are the second most common type of infection in body. Anatomical reasons of women made more susceptible to UTIs compare to men. It could even affect the kidneys, ureters, bladder or urethra and considered as one of the most common infections in humans especially in women.¹ For women, the lifetime risk of having a UTI is greater 50 % than male.²

It is reported that about 35% of healthy women suffer symptoms of Urinary tract infection and about 5% of women each year suffer with the problem of painful urination (dysuria) and frequency.³ Many potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria has made therapy of UTI more difficult. Bacteria have the genetic ability to transmit and acquire resistance to drugs.⁴ Any plants which have substances that can be used for therapeutic purpose or which are used as a precursor of chemo-pharmaceuticals semi synthetic new drugs is referred as medicinal plant⁵, this is based on world health organization (WHO).

Medicinal plant would be the best source to obtain a variety of drugs as the phytochemical. Phytochemicals have structural diversity & biological functionality, offer unique platform for for drug discovery. Lemon (*Citrus limon*)

belongs to *Rutaceae* family, and it originated from South East Asia, probably in India or Southern China. Lemon is a pale yellow, elliptically shaped berry fruit. Citrus fruits are rich with sugar, polysaccharide, organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. Lemon is a good source of potassium, calcium & vitamin C. *Limon* or juice of lemon have been reported to exhibit antimicrobial activity against *Vibrio cholera* ⁶.

The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of microorganisms⁷. Commonly citrus fruit products are known to potent antimicrobial agents like, bacteria, fungus⁸.

Different parts of plants are good source of potent and powerful drugs and are used in medicine in different countries. Their extracts are used as raw medicine and believed to be the important source of new chemical substances with potential therapeutic properties. Phytochemicals produced by plants could be used directly for the production of new drugs. The phytomedicines are believed to be more acceptable by the human body, as compared to modern synthetic drugs ⁹. Pharmacologically, *Citrus limon* is a good source and primarily important for

vitamin C & potassium content and some studies also showed that it also possesses antioxidant activity but clinical trials are lacking due to insufficient information related to antibacterial activity specially to urinary tract infection.

II. Material and Method:

Collection of urine sample: Urine samples were collected from clinical laboratories of Gwalior (M.P.) for isolation of the affecting agents, from 50 subjects who were clinically suspected to suffer from urinary tract infection.

Isolation and identification of bacteria: First, the microorganisms present in urine samples of UTI infected patients were cultured on nutrient agar medium and the morphology and colony characteristics of organisms were studied for the shape, size, odour, margin and surface characteristics.

Gram staining procedure was adopted to differentiate between gram positive and gram negative organisms. Selective agar medium like MacConkey and EMB were used broadly for further identification of *E. coli*.

After phenotypical identification of bacteria, further confirmation was done on the basis of their biochemical characterization viz amylase production, hydrolysis of gelatin, IMViC test, catalase test, skim milk test, carbohydrate fermentation test.

Collection of plant material- Plant material was collected from the garden area of Govt. Kamla Raja Girls P.G. (Autonomous) College, Gwalior, (M.P.)

Ethanol extraction of medicinal plants: The plant part viz fruits were washed with distilled water, dried in the air and pulverized. Twenty grams of pulverized material was used for extraction with 99.9% ethanol using Soxhlet's apparatus. The extract was kept overnight in oven at 50°C to evaporate excess amount of ethanol. Hundred milligram of residue of ethanolic extract was dissolved in 10 ml of ethanol to get final concentration of 1 %.

Testing of antimicrobial activities: Antimicrobial activity was studied by well agar plate diffusion method according to Pandey *et al*, (2011)¹⁰. Antimicrobial activity test of ethanolic extract of *Citrus limon* peel extract was carried out against *Escherichia coli* and *Staphylococcus aureus* along with chosen antibiotics for their comparative study.

III. Result

Results of the present study showed that the ethanolic extracts of *Citrus limon* showed notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Antibacterial activity was done by the disc diffusion method. The ethanolic extracts of *Citrus limon* showed significant

inhibitory activity against both the above microorganisms when compared to antibiotics present in Hexa UTI- 4 disc, which is mostly being used against UTI infections.

Citrus limon showed maximum zone of the inhibition against *E. coli* with 6 mm diameter as compared to Ampicillin, gentamycin, nitrofurantoin, nalidixic acid and co-trimoxazole with 4mm, 7mm, 5mm, 5mm, 6 mm zone of inhibition respectively and is next to ciprofloxacin with 15 mm diameter. It was also observed that *Citrus limon* peel extract worked against *Staphylococcus aureus*. In case of *Staphylococcus aureus*, ampicillin, gentamycin, ciprofloxacin, co-trimoxazole was showed 7 mm, 8mm, 8 mm, 10mm respectively where as *Citrus limon* showed 10 mm zone of inhibition which is greater than ampicillin, gentamycin, and also with nitrofurantoin and nalidixic acid which did not show any zone of inhibition.

Table 1. Phytochemical activity of Citrus limon

S.No.	Name of the phytochemical activity	Citrus limon activity	
		Water extract	Ethanolic extract
1.	Alkaloid test		
A.	Mayer's reagent	-ve	+ve
B.	Wagner's reagent	-ve	-ve
2.	Carbohydrate test		
A.	Molisch test	+ve	+ve
B.	Fehlings test	+ve	+ve
3.	Protein test		
A.	Biuret test	-ve	-ve
B.	Ninhydrin	-ve	-ve
4.	Phenolic compound		
A.	Ferric chloride	+ve	+ve
B.	Gelatine	-ve	-ve
5.	Flavonoides		
A.	Alkaline test	-ve	+ve
6.	Phytosterol (Saponine)	-ve	-ve
7.	Glycosides	-ve	-ve

Table 2. Biochemical activities of Escherichia Coli and Staphylococcus aureus

S.No.	Biochemical activity	Escherichia .coli	Staphylococcus aureus
1.	Amylase Production Or Starch Hydrolysis	-ve	-ve
2.	Gelatin Hydrolysis	-ve	+ve
3.	IMViC test		
A.	Indole production	+ve	-ve
B.	Methyl Red	+ve	+ve
C.	Voges- Proskauer	-ve	-ve
D.	Citrate	-ve	-ve
4.	Catalase	+ve	+ve
5.	Skim milk	-ve	-ve
6.	Carbohydrate		

	fermentation		
A.	Sucrose	A	A
B.	Dextrose	A	A
C.	Starch	-ve	A
D.	Xylose	-ve	-ve
E.	Fructose	-ve	-ve
F.	Maltose	A	A
G.	Lactose	A,G	A

-ve= Negative +ve = Positive A=Acid production
G=Gas production

Table 3: Activity of *Escherichia Coli* and *Staphylococcus aureus* against different antibiotics and leaf extract of *Citrus limon*

S.No.	Name of antibiotics and plant material	Zone of Inhibition (mm)	
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1.	Ampicilin (AMP)	4 mm	7 mm
2.	Gentamicin (GEN)	7 mm	8 mm
3.	Nitrofurantoin (NIT)	5 mm	0 mm
4.	Ciproflaxacin (CIP)	15 mm	8 mm
5.	Nalidixic acid (NA)	5 mm	0 mm
6.	Co-trimoxazole (COT)	6 mm	10 mm
7.	Citrus limon	6 mm	10 mm

IV. Discussion

Recently many scientists have showed their interest in the use of different plant parts as alternative agents to control the pathogenic and antibiotic resistant microorganisms^{11,12}. The increasing drug resistance of many pathogens is a serious problem in developing countries like India¹³. Many reports are available on plants and plants products that they showed antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti inflammatory properties¹⁴. Urinary tract infections (UTI) are common condition in the world and the agents of UTI showed day by day increasing resistance against available drugs. We conducted this work to isolate different agents of UTI from several patients and assessed their antibiotic susceptibilities. *E. coli* was the most predominant bacteria involved in UTI. There are earlier studies which are in agreement with the present findings. *Escherichia coli* is recognized as most important agent followed by *Staphylococcus saprophyticus* and *Klebsiella pneumoniae*. Many reports are also support the findings detection of UTI agents^{15,16,17}.

Normally Citrus fruits contain sugar, polysaccharide, organic-acid, lipids, carotenoids, vitamins, minerals, flavonoids, bitter lemonoids and volatile compounds. It is a good source of potassium, calcium and vitamin C. It was also reported that *limon* or lime juice exhibit antimicrobial

activity against *Vibrio cholera*¹⁸. Consequently, this study was aimed at comparatively investigating the antimicrobial potentials of extracts of *Citrus lemon* peel and to screen for the phytochemical composition responsible for their antimicrobial potentials.

The qualitative phytochemical analysis of the peel of *Citrus lemon* showed the presence of saponin, tannin, flavonoid, glycoside and steroid. These phytochemical compounds are believed to be the bioactive ingredients of *Citrus lemon* responsible for its antibacterial activity.

V. Conclusion

From the above results we can conclude that extracts of plants origin has remarkable antimicrobial activity as compare to antibiotic activity. Even though pharmacological industries have produced a number of new antibiotics in the last three decades as well as resistance to these drugs by microorganisms has increased.

The present study was undertaken to identify effective herbal medicines to control UTI caused by bacterial organisms. Results shown that the extract of *Citrus limon* plants origin has remarkable antibacterial activity as compared to antibiotics therefore can be used by all human beings as these are easily available in our environment, less expensive as well as safe. It is also envisaged that further work should be done in this direction. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties and can considered being as equally potent as the antibiotics.

VI. Acknowledgement:

Authors are thankful to the Principal, Government Kamla Raja Girls P.G. (Autonomous) College, Gwalior, M.P., for providing laboratory facilities and giving us permission to undertake the above work.

References

- [1]. N. Sharma, A.M.Jana, N. Pathak, C.Singh and P.Singh, "A preliminary study on antimicrobial effects of ethanolic extract of *Psidium guajava* leaves on bacteria isolated from urinary tract infection with special reference to *Escherichia coli* and *Staphylococcus aureus*" International journal of biotechnology and biochemistry, Vol 13, Number 2, pp.183-189.(2017)
- [2]. A. Kumar A, N. Jhadwal, M. Lal, M. Singh, "Antibacterial activity of some Medicinal Plants used against UTI causing Pathogens". Int. Jr. of Drug Development & Research, Vol. 4 Issue 2.(2012)
- [3]. T.M. Hootan, "Urinary tract infection in adults" In: Johnson R.J., Feehally J, (Eds). Comprehensive clinical nephrology, 2nd ed, London: Mosby, , 731-744. (2003).
- [4]. D. Srinivasan, N.Sangeethan, T. Suresh, P. Lakshmana and Permalsamy "Antibacterial activity of Neem (*AzadirachTA INDICA*) and Tamarind (*Tamarindus Indica*) leaves. Asian Jr. of

- Microbiol Biotech & Env. SC. Vol.3, No. (1-2):2001:67-73.3(2001).
- [5]. H. Salih, A.M. Abass, "Study of the fruit peels of *Citrus sinensis* & *Punica granatum*". Journal of Babylon university, 243-342.(2003)
- [6]. T. Hiroyuki., K. Tetsuro K, Masayuki, K. Afework, O.Fusao. "Antimicrobial activity of citrus fruits juice against vibrio species". 52(2): 157-160.(2006)
- [7]. A. Mathur, S.Verma, R.Purohit, V. Gupta, V.K. Prasad, D. Mathur, S.K. Singh and S. Singh, "Evaluation of in vitro antimicrobial & antioxidant activity of peels & pulp of some citrus species". International journal of biotechnology & biotherapeutics, (1) 2:2229-2278.(2011).
- [8]. M. Chandra, "Antimicrobial Activity of Medicinal Plants against Human Pathogenic Bacteria", Int. J. Biotechnol. Bioengg. Res., 4(7), pp. 653-658.(2013).
- [9]. M.A.K. Saadi, H. Salih, A.M.Abbas, "Study of the fruit peels of *Citrus sinensis* & *Punica granatum*" . journal of Babylon university, 3(9):243-342.(2003).
- [10]. A. Pandey, I. Ali, K.S. Butola, T. Chatterji, "Isolation and characterization of actinomycetes against pathogen", Inter. J. appl. Biol. Pharma. Technol., 2(4), pp. 384-392. (2011)
- [11]. F. Aqil, M.S. Khan, M. Owais, I. Ahmad, "Effects of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*", J. Basic Microbiol. 45, pp. 106-114.(2005).
- [12]. A. Nostro, L.Cellini, S. Bartolomeo, "Effects of combining extracts (from propolis or *Zingiber officinale*) with clarithromycin on *Helicobacter pylori*", *Phytotherapy Res.* 20(3), pp. 187-190.(2006).
- [13]. S. Gopalkrishnan, S. George, P.J. Benny, "Antimicrobial effect of *Punica granatum* on pyogenic bacteria" J. Pharma. Biomed. Sci. 3(6).(2010).
- [14]. B.Mahesh and S. Satish, "Antimicrobial activity of some important medicinal plant extract against plant and human pathogens", World J. of Agri. Sci. 4(5), pp. 839-843.(2008)
- [15]. T.S. David and M.S. Vrahas, "Perioperative lower urinary tract infections and deep sepsis in patients undergoing total joint arthroplasty", J. Am. Acad. Ortho. Sur., 8, pp. 66-74. (2000).
- [16]. A. Ronald, "The etiology of urinary tract infection: traditional and emerging pathogens", Dis. Mon., 49, pp. 71-82. (2003).
- [17]. A.C.Gales, R.N.Jones K.A. Gordon, H.S.Sader, W.W. Wilke, M.L. Beach, M.A. Pfaller, G.V. Doern, "Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the sentry antimicrobial surveillance program" J. Antimicrobial Chemo., 45, pp. 295-303.(2002).
- [18]. Hiroyuki, (2006). "Antimicrobial activity of citrus fruit juice against *Vibrio* species". IJCSNS. 52(2):157 – 160.