SHORT COMMUNICATION

ANTIMICROBIAL ACTIVITY OF CITRUS FRUITS ON CERTAIN PATHOGENIC MICROORGANISM

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Abstract: The main objective of present study was to study the antibacterial effect of *Citrus limon* juice extract against *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes*. Extract of *Citrus limon* juice was prepared for antibacterial study and Norfloxacin was taken as control antibiotic. The antibacterial activity of *Citrus limon* juice extract was detected by using agar well diffusion method. In the present study it was observed that *Citrus limon* juice extract showed maximum antimicrobial activity against *Staphylococcus aureus* which was 115% more as compared to Norfloxacin (10mg/ml). Similar results have been observed against bacteria such as *Salmonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes*. These results confirmed that *Citrus limon* is a very important and effective medicinal plant against bacterial.

Keywords: *Citrus limon, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Streptococcus pyogenes*

INTRODUCTION

Citrus limon is a conventional fruit which belong to plant family *Rutaceae* and is commercially know as sweet orange. *Citrus sp.* is a spreading evergreen, sometimes spiny trees which could be 12m tall with oral elliptic leaves and rounded fruits that are up to 12cm in diameter (Susser, 1997). Okwu (2008) investigated that citrus trees are evergreen trees that produce fruits of different forms and sizes (from round to oblong), which are full of fragrance, flavor and juice. Chanthaphon *et al.* (2008) reported that citrus fruit belong to six genera (*Fortunella, Eremocitrus, clymendia, poncirus, Microcitrus and citrus*), which are native to the tropical and subtropical regions of Asia, but the major commercial fruits such as oranges, mandarins, lime, lemons and grape fruits. Mandalari *et al.* (2006) reported that orange constitute about 60% of the total citrus world production.

Adode (2002) observed that fruits contain 80 to 90% sugar and acids, citric acid are abundant acid in the sap. Roger (2002) reported that the internal constitutes the pulp which is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and potassium salt that gives the fruit its characteristics citrine flavor. Hasija *et al.* (2015) reported that citrus peel oil can be used as natural preservative to minimize the ill effects of these synthetic preservatives and protect consumer health.

The emergence of multidrug resistance bacterial strains are also becoming a global concern, with particular emphasis on *E. coli* (Ithete *et al*., 2013), *Salmonella* (Zaki and Karande, 2011), *Pseudomonas aeruginosa* (Hirsch and Tam, 2010), *Proteus vulgaris* (Mandal *et al*., 2015), *Staphylococcus aureus* (Neyra *et al*., 2014), *Streptococcus pyogenes* (Pieretti *et al*., 2017). The increasing occurrence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to the search for new infection-fighting and safe strategies (Janovská *et al*., 2003, Deshwal and Vig, 2011, Deshwal, 2013).

Suja *et al.* (2017) investigated that Citrus fruit are highly nutritious medicinal plant and found to be commonly in cultivation throughout the tropic. Hindi and Chabuck (2013) have demonstrated the antimicrobial effects of aqueous extracts of peel and juice from fresh and dried citrus and sweet lemon against gram-positive and gram-negative bacteria and yeast isolates, including *Staphylococcus aureus, Enterococcus faecalis, Salmonella typhi, E.coli and Candida albicans*. So aim of present study is to evaluate antimicrobial activity of citrus fruits on certain pathogenic microorganism

MATERIALS AND METHODS

Collection of test pathogenic microorganisms: Characterized *Escherichia coli, Salmonella, Pseudomonas aeruginosa, Proteus vulgaris, and Staphylococcus aureus* were collected from Microbiology department, BFIT, Dehradun and *Streptococcus pyogenes* culture was obtained from the IMTEC Chandigarh (MTCC NO. 1926).

Collection of plant materials: Freshly *Citrus limon* was purchased from the local market of Dehradun.

Preparation of extracts: The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered into another sterile container to remove the seeds and other tissues and used freshly as crude without refrigeration (Hindi and Chabuck, 2013).

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Activation of test organism: The microorganism was activated by inoculating a loop full of the strain in the nutrient broth and incubated on a rotary shaker for 24hrs at 37^{o}C.

Evalution of Antimicrobial activity using Agar well diffusion method:

The screening of antimicrobial activities of juice extract against test microorganism was determined on Muller-Hinton agar media, by agar well diffusion method. Sterilized Muller-Hinton agar media was poured into sterilized petriplate. After solidification of medium, 0.5ml (10^6 bacteria/ml) bacterial culture was spreaded on Muller-Hinton agar media. Wells of 7mm depth were made on the solid agar using a sterile borer. About 100µl of *Citrus limon* juice extract and Norfloxacin was transferred into the wells separately by using sterile pipette. The plates were allowed to stand for one hour for a pre-diffusion of extracts and were incubated at 37^{o}C for 24 hrs. After incubation, the plates were collected and the zones of Inhibition were measured.

Table 1. Antimicrobial activity of *Citrus limon* juice against certain test organisms

Test organism	Zone of Inhibition (mm)				
	Citrus limon juice				Norfloxacin (10mg/ml)
	I	II	III	Mean ± S.D	
Escherichia coli	14	14	13.5	13.8±0.28	08.00
Salmonella	21	21	21	21.0±0.00	13.60
Pseudomonas aeruginosa	17	17	16	16.6±0.57	13.30
Proteus vulgaris	22	18	20	20.0±2.00	13.00
Staphylococcus aureus	29	28	29	28.6±0.57	13.30
Streptococcus pyogenes	23	22	22	22.3±0.57	15.00

RESULTS AND DISCUSSION

Present study shows that *Citrus limon* juice extract significantly inhibited the growth of Gram positive and Gram negative bacteria. *C. limon* juice extract showed maximum antimicrobial activity against *Staphylococcus aureus* which was 115% more as compared to Norfloxacin (10mg/ml). Similarly, *C. limon* juice extract showed 72.5, 54.4, 24.8, 53.8, 48.6 % more inhibition zone in *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus pyogenes* respectively as compared to Norfloxacin (10mg/ml). All these results confirmed that *Citrus limon* juice extract effectively inhibited growth of pathogenic *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes* (Table 1). Medicinal plants are good alternative of chemical antibiotics. In present study showed that *Citrus limon* juice effectively control growth of various pathogens such as *Escherichia coli*, *almonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes*. Similar research studies have shown that several medicinal plants inhibited growth of bacterial pathogens (Deshwal and Vig, 2011a, Deshwal and Vig, 2011b, Deshwal, 2012).

CONCLUSION

Present study showed that *Citrus limon* juice extract significantly inhibited the growth of various Gram positive and Gram negative bacteria. Use of antibiotic has side effect to human and medicinal plants are good alternative of chemical antibiotics.

REFERENCES

Deshwal, V.K. and Vig, K. (2011a). Screening for Antibacterial activity of seeds of *Tribulus terrestris* L. growing in Uttarakhand (INDIA). *International Journal of Pharmaceutical Invention*, **1(1)**: 42-46.

Susser, G.O. (1997). The Great Citrus Book. A Guide with recipes. Ten Speed Printing Press.

Okwu, D.E. (2008). Citrus Fruits: A rich source of Phytochemicals and their roles in Human Health. *International Journal of Chemical Science*, **6(2)**: 451-471.

Chanthaphon, A., Chanthachum, S. and Hongpattarakere, T. (2008). Antimicrobial activities of essential oils and crude extracts from tropical *Citrus spp.* against food- related microorganism. *Songklanakarin Journal of Scienece and Technology*, **30(1)**:125-131.

Mandalari, G., Bennett, R.N., Bisignano, G., Saija, A., Dugo, G., Faulds, C.B. and Waldron, K.W. (2006). Characterization of flavonoids and pectin from bergamot (*Citrus bergamia Risso*) peel, a major byproduct of essential oil extraction. *Journal of Agriculture And Food Chemistry*. **54**:197-203.

Adode, A. (2002). Nature Power: Revised Edition. Don Bosco Training Centre, Akure: 1-98.

Roger, G.D.P. (2002). Encyclopedia of Medicinal Plant, Education and Health Library Editorial Safeliz S.L. Spsin, **265(1)**:153-154.

Hasija, S., Ibrahim, G. and Wadia, A. (2015). Antimicrobial Activity of *Citrus sinensis* (orange),

Citrus limetta (Sweet Lime) and *Citrus limon* (lemon) Peel oil on Selected Food Borne Pathogens. *International Journal of life Science Research*, **3(3)**: 35-39.

Ithete, N.L., Stoffberg, S., Corman, V.M., Cottontail, V.M., Richards, L.R., Schoeman, M.C., Drosten, C., Drexler, J.F. and Preiser, W. (2013). Multidrug-Resistant *Escherichia coli* Bacteremia. *Emerging Infectious Diseases*, **19**:1699-1701.

Zaki, S.A. and Karande, S. (2011). Multidrug-resistant typhoid fever: A review. *The Journal of Infection in Developing countries*, **5(5)**: 324-337.

Hirsch, E.B. and Tam, V.H. (2010). Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. *Expert Review of Pharmacoeconomics Outcomes Research*, **10(4)**: 441-451.

Mandal, D., Dash, S.K., Das, B., Sengupta, M., Kundu, P.K. and Roym, S. (2015). Isolation and Characterization of multi-drug resistance *Proteus vulgaris* from clinical samples of UTI infected patients from midnapore, West Bengal. *International Journal of Life Science and Pharma Research*, **5(2)**: l32-l45.

Neyra, R.C., Frisancho, J.A., Rinsky, J.L., Resnick, C., Carroll, K.C., Rule, A.M., Ross T., You, Y., Price, L.B. and Silbergeld, E.K. (2014). Multidrug-Resistant and M ethicillin-Resistant *Staphylococcus aureus* (MRSA) in Hog Slaughter and Processing Plant Workers and Their Community in North Carolina (USA). *Environmental Health Perspectives* (http://dx.doi.org/10.1289/ehp.1306741). : 1-32.

Pieretti, B., Canovari, B., Moretti, M., Pieretti, C. and Pazzaglia, E. (2017). Drug-resistant *Streptococcus pyogenes*: a case report of pyoderma and Cellulitis. *Microbiologia Medica*, **32**: 112-113

Janovská, D., Kubīková, K. and Kokośka, L. (2003). Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine, *Czech Journal of Food Science*. **21**, 107-110.

Deshwal, V.K. and Vig, K. (2011a), Screening for Antibacterial activity of seeds of *Tribulus terrestris* L. growing in Uttarakhand (INDIA), *International Journal of Pharmaceutical Inventation*, **1(1)**: 42-46.

Deshwal, V.K. (2013). Antibacterial investigation of black pepper against *Shigella dysenteriae*. *Journal of Plant Development Sciences*, **5(1)**: 89-90

Suja, D., Bupesh, G., Rajendiran, N., Mohan, V., Ramasamy, P., Muthiah, N.S, Elizabeth, A.A., Meenakumari, K. and Prabu, K. (2017). Phytochemical Screening, Antioxidant, Antibacterial Activities of *Citrus Limon* and Citrus Sinensis Peel Extracts. *International Journal of Pharmacognosy Chinese Medicine*, **1(2)**:108.

Hindi, N.K.K. and Chabuck, Z.A.G. (2013). Antibacterial activity of different aqueous Lemon extracts. *Journal of Applied Pharmaceutical Science*, **3(06)**:074-078.

Deshwal, V.K. (2013). Antimicrobial investigation of *Piper nigrum* L. against *Salmonella typhi*. *Journal of Drug Delivery and therapeutics* (JDDT). **3(3)**: 100-103.

Deshwal, V.K. and Siddiqui, M.M.M. (2013). Screening and evaluation of anti-microbial activity in *Tylophora indica*. *Journal of Plant Development Sciences*. 5(2): 223-225.

Deshwal, V.K., Vig, K., Singh, S.B. and Devi, P.D. (2012). Evaluation of the Antibacterial Activity of bark of *Litchi chinensis* against *Escherichia coli*, a UTI causing Organism. *Journal of plant development sciences*, **4(1)**: 101-103.

Deshwal, V.K. and Vig, K. (2011a). Screening for Antibacterial activity of seeds of *Tribulus terrestris* L. growing in Uttarakhand (INDIA). *International Journal of Pharmaceutical Invention*, **1(1)**: 42-46.

Deshwal, V.K. and Vig, K. (2011b). Isolation and characterization of Urinary tract infection (UTI) causing pathogens and their comparative study in different genders. *Development Microbiology and Molecular Biology*, **2(2)**: 113-116.

Deshwal, V.K. (2012b). Antibacterial activity of *Piper nigrum* Linn. against *E. coli* causing Urinary tract infection. *International Journal of Pharmaceutical Invention*, **2(2)**: 1-7.