



## **Screening of Antimicrobial Properties of *Citrus limon* L. Against Some Pathogenic Bacteria**

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### **ABSTRACT**

Botanicals of plant origin appear to be most favorite alternatives for control of antibiotic resistant human pathogens. Over the past few decades interest in investigating natural substances for novel antibacterial agents has increased tremendously. In the present study antibacterial activity of Leaves, fruit peel and fruit juice of *Citrus limon* L. (Lemon) was tested against some human pathogenic bacteria viz. *E. coli*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas syringae* and *Agrobacterium tumefaciens* procured from IMTECH, Chandigarh. Disc diffusion method was used to determine the antimicrobial activity of different plant parts extracts (Aqueous, Ethanol, Chloroform, Acetone and Petroleum Ether). Ciprofloxacin was used as positive control whereas DMSO and water as negative controls. All the extracts exhibited significant antibacterial activity and highest activity was observed in petroleum ether extract of lemon peel against *E. coli*. Similarly petroleum ether extract of leaves was found highly effective against *P. putida*. Crude Lemon juice extract was effective against all the tested bacteria. This study has helped to understand the use of Lemon plant parts as traditional medicine and as an economic and safe alternative to treat diseases.

**Key Words:** *Citrus limon*, Lemon, peel, Antibacterial, Kirby-bauer, *Pseudomonas*

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### **INTRODUCTION**

From many centuries, different communities all over the globe have been extensively using plants to treat various ailments. Medicinal plants are thought to be of great source for getting variety of drugs, as antibiotics are showing fewer efficacies in treating human and animal diseases and must be replaced with some herbal product. Such plants are needed to be investigated for exploring their properties, safety and efficiency in combating diseases. Traditional health care systems around the world, which utilize herbal remedies, forms an important basis for discovery of novel herbal antibiotics and some traditional remedies have already proved effective against disease causing pathogens [1]. The growing demand of medicinal plants is due to many factors including cultural acceptability, easily accessible, affordable and less or reduced toxicity [2]. From ancient period, it is well accepted fact that compounds of plant origin have been used to cure various diseases and microbial infections; and with increasing resistance of microbes towards traditional antibiotics, needs urgent attention to develop new medicines for human health care [3]. Due to genetic modifications in pathogens, the effect of general antibiotics may be lost with in a period of 5-6 years, leading to very serious situation related to community health [4]. It is well documented that various plants and plant products have antibacterial, antifungal and antiprotozoal effect and are preferred in large part of world due to their potent therapeutic effects, low toxicity and economic viability as compared to modern day synthetic medicines [5]. Antimicrobial potential of ethnomedicinally important plants, used by local populations, against some pathogenic bacteria was evaluated by Singh *et al* (2021) [6].

*Citrus limon* (L.) belongs to family Rutaceae and is commonly known as *Nimbuin* hindi and Lemon in English language. In family Rutaceae, the genus *Citrus* is most significant operational herb in traditional health care systems. *Citrus limon* is a small thorny, frost sensitive plant/ tree, with evergreen leaves and prolate yellow (berry) fruit. It is the third most important species of citrus fruits cultivated all over the world [7]. Lemon fruit is rich source of nutrients and a key to a healthy diet. The lemon fruit juice is

common food ingredient in both commercial and home food preparations, and is valued for its tart, fresh and tangy flavor. The peculiar fragrant rind or fruit peel is a major source of essential oil and aroma compounds [8]. The plant is popular for its medicinal properties among local populations and specifically lemon fruit and fruit juice are known for its high nutritional, therapeutic and cosmetic properties [9]. The plant, particularly lemon juice, is rich in vitamin C and is being used to cure fevers, suppressing cough, scurvy, sore throat, high blood pressure and irregular menstruation [10]. *Citrus limon* L. represent considerable therapeutic potential and is very rich in phytochemicals such as citric acid, ascorbic acid, essential oils, minerals and flavonoids leading to its high antibacterial, antifungal, anti-inflammatory, anticancer and hepatoprotective characteristics [11][12]. The anticancer and antibacterial potential of *Citrus limon* L. is due to alkaloid content present in different plant parts such as leaves, stem, root and flowers [13]. Lemon is a common constituent of local's kitchen and is used as food, flavoring agent, aids in digestion, increase urination reducing fluid retention and even used to cure kidney stones [14]. Considering the significant medicinal value of *Citrus limon* plant, the present study is an attempt to evaluate antibacterial potential of different plant parts of Lemon tree against some human and plant pathogenic bacteria.

## MATERIAL AND METHOD

**Plant Collection:** In the present research work leaves and fruits of *Citrus limon* were collected from residential teachers quarter of JNV University, Jodhpur and their botanical identity was determined and authenticated from literature available in Department of Botany, and the voucher specimens were deposited in herbaria of Department of Botany, J.N.V. University, Jodhpur (Raj.), India.

**Plant Preparation:** The selected plant parts leaves and fruits peel were thoroughly washed and then dried under shade at  $28 \pm 2^\circ\text{C}$  for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried powder of leaves and fruit peel was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were petroleum ether, chloroform, acetone, ethanol and water as solvent and the extracts were dried. The dried extracts were stored in a refrigerator at  $4^\circ\text{C}$ . For determining antibacterial activity of lemon fruit juice, fresh fruits were washed 3-4 times with tap water and distilled water and then surface sterilized with 90% alcohol and cut open with a sterile laboratory knife and the juice pressed out into a sterile universal container separately and then filtered to remove the seeds and other tissues and used freshly as crude without refrigeration.

**Antimicrobial Susceptibility Test:** All the plant part extracts were screened against eight pathogenic bacterial strains. The tested organisms were *E. coli*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas syringae* and *Agrobacterium tumefaciens* obtained from IMTECH, Chandigarh, India. The Disc Diffusion method (Bauer *et al*, 1966) was used to test the antibacterial activity of the plant extracts [15]. 20ml of sterilized nutrient agar medium were poured into each sterile petridish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) soaked in .1 ml of the plant extract (In case of fresh fruit juice) or loaded with 5 mg/ disc, of dry extract (Leaves and fruit peel) and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at  $37^\circ\text{C}$  for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

**Phytochemical screening:** The extracts were subjected to preliminary phytochemical screening [16][17].

## RESULTS AND DISCUSSION

Prior to the introduction of modern allopathic medicines, disease treatment was entirely administrated by herbal remedies. In developing countries, the use of plant products to treat various infections is an age old practice and there is dependence on traditional medicines for different common diseases [18]. Specifically in the last decade, emphasis is given to natural therapies and plants have been a valuable source of natural products for maintaining human health. The problem of microbial resistance is increasing and the ultimate aim is to offer appropriate and efficient antimicrobial medicines to the patients [19]. Lemons are very versatile and nutritious with medicinal significance. In the present investigation antibacterial potential and phytochemical analysis of leaves, fruit peel and fruit juice of lemon was done. The antimicrobial action of extracts was observed against some pathogenic bacteria

viz. *E. coli*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas syringae* and *Agrobacterium tumefaciens*. The leaves extract (Acetone, Petroleum ether) were found effective against all the tested bacteria and highest activity was exhibited by petroleum ether extract against *P. putida* followed by acetone extract against *B. subtilis* and petroleum ether extract against *E. aerogenes*. Similarly antimicrobial potential of leaves of some religious plants was done by Bissa [20]. The antibacterial capacity of different plant extracts against *E. aerogenes* was done by Bissa and Bohra [21]. Sah *et al* (2011) studied the antibacterial effect of leaves of *Citrus medica* against *B. subtilis*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*; and antifungal effect against *Aspergillus flavus* and *A. niger* [22]. In current research work, different solvent extracts of fruit peel of *Citrus limon* were tested against pathogenic bacteria and most significant antimicrobial activity was recorded in petroleum ether extract against *E. coli* and *E. aerogenes*. Similarly antimicrobial activity of lemon peel extract against six gram positive and eight gram negative bacteria including *S. aureus* and *E. coli* were evaluated by various researchers [23],[24]. In present study crude fruit juice of *Citrus limon* was found effective against all the tested microbes and highest antibacterial potential was exhibited against *Streptococcus mutans* and *Staphylococcus aureus*, followed by *Pseudomonas syringae* and *Agrobacterium tumefaciens*. Bissa *et al* (2007) evaluated the antibacterial potential of leaf juice of *Piper betle* against oral microflora [25]. Similarly, Lemon fruit juice is found highly effective against *Salmonella typhi*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* [26].

The phytochemical analysis of all the plant part tested revealed the presence of Flavonoids, alkaloids, saponins, glycosides, phenols and tannins in all parts. Similarly Singh *et al* (2020) studied the antimicrobial action of phytochemicals from Lemon juice [27]. Gupta *et al* (2021) also confirmed the presence of flavonoids, alkaloids, saponins, glycosides, phenols and tannins in different species of *Citrus* fruit peels [28]. The present research exhibits that plant parts of *Citrus limon* plant have high antibacterial properties and contain many phytochemicals with significant potential for use in drug production.

**Table 1. Antibacterial Activities of *Citrus limon* L. against Pathogenic Bacteria**

Plant Part	Plant Extract	Zone of Inhibition (mm)							
		EC	EA	SM	SA	BS	PP	PS	AT
Leaves	Aqueous	6	7	5	-	-	7	8	-
	Alcoholic	8	10	6	7	9	7	10	5
	Chloroform	9	9	7	-	11	10	10	8
	Acetone	10	9	11	9	12	11	8	9
	Petroleum Ether	11	12	10	11	8	14	8	6
Fruit Peel	Aqueous	5	7	7	9	5	-	6	-
	Alcoholic	10	11	7	6	5	9	9	7
	Chloroform	8	9	11	12	8	6	6	5
	Acetone	12	11	11	12	9	10	9	7
	Petroleum Ether	14	13	11	11	12	10	8	10
Fruit Juice	Aqueous	11	10	13	13	11	10	12	12

EC: *E. coli*, EA: *Enterobacter aerogenes*, SM: *Streptococcus mutans*, SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, PP: *Pseudomonas putida*, PS: *Pseudomonas syringae* and AT: *Agrobacterium tumefaciens*

**Table 2. Phytochemical Analysis plant part extracts of *Citrus limon* L.**

Phytochemical Component	Leaves	Fruit Peel	Fruit Juice
Alkaloids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenols	+	+	+

## CONCLUSION

It is estimated that among all the available options plant derived compounds have displayed potential applications in combating diseases and in overcoming antibiotic resistance in bacterial pathogens. Based on the above study, it can be concluded that tested plant part extracts of *Citrus limon* had good potential for therapeutic uses against human and plant pathogenic bacteria. The results of present studies suggest that the different plant extracts has a significant scope to develop novel broad spectrum of antibacterial

herbal formulations. Further research may be interesting to investigate the active principles and mode of action of plant extracts against resistant clinical bacterial strains.

### CONFLICT OF INTEREST

Author have no conflict of interest to declare.

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