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Evaluation of Antibacterial Activity of Lemon Plant Extract

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ABSTRACT

The anti-microbial activity of plants had received attention many years ago as one of the most effective mechanism for the control of micro-organisms. Anti-microbial are usually considered as the agents that either kill micro-organisms or inhibit their growth by interfering with the necessary mechanism of the microbe's cell. There are many natural products, which show antibacterial properties. These agents work either by the slow release of the active ingredient or by surface contact with the microbes. In the present study, the antibacterial property of the peel of lemon fruit (*Citrus limon*) was assessed, which is a rich source of flavanones and many polymethoxylated flavones, that are found very rare in other plants. The antibacterial activity of lemon peel extract was evaluated by Agar well diffusion method, against *Bacillus* spp. and *Escherichia coli*. The screening for antibacterial activity was evaluated by measuring the zone of inhibition. The lemon peel was found to have strong antibacterial property. Hence, the lemon peel waste can be utilized in an eco-friendly manner for application as antibacterial finish on textiles.

Keywords Anti-microbial, Flavanones, Agar well diffusion method, *Bacillus* spp.

INTRODUCTION

Natural products are a source of new chemical diversity and are the choice of today's world. The sources of natural product are plants, animals and micro-organisms. Among them, plant and plant products are more reliable for its renewability and therefore considered as catalyst for human welfare (Mahesh *et al.* 2011). Plants have their own self defense mechanism and protect themselves from microbes due to the presence of substances known as phytochemicals (phyto-from greek–phyto meaning 'plant') (Yalavarthi and Yhiruvngadaragan 2013). Many plant products, which exhibit anti-bacterial properties are roots, stem, leaves, flowers, fruits and seeds of diverse species of plants. Use of plant extracts not only provides protection from environmental hazards but also safeguards the environment, prevents pollution and promotes eco-friendly textiles (Jayaprakasha *et al.* 2001). Use of such products also ensures the health benefits to the individual as well as the masses. (Prashanth *et al.* 2001, deCastillo *et al.* 2001). Anti-microbials are usually considered as the agents that either kill micro-organisms or inhibit their growth by interfering with the necessary mechanism of the microbe's cell. These agents work

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either by slowing the release of the active ingredients or by surface contact with the microbes (Parthiban and Thilagavathi 2012).

The extract of plants such as neem, tulsi, karanja leaves, aloe-vera, pomegranate containing active substances can be used effectively for microbial resistance. Moreover, extract from plant source with active substances are eco-friendly, non-toxic and non-allergic.

Many researchers work on assessment of anti-microbial properties of different plants sources. Some of studied are reviewed here : Jamal (2016) showed that as the concentration of guava leaves extract increase the anti-microbial property of guava leaves extract also increased and increased the zone of inhibition. Gupta (2016) also observed that the anti-microbial activity of the treated sample increased with the increase in *S. cumini* (L.) extract concentration. In a study, Kumar *et al.* (2011) reported that the *Citrus limon* peel extract proves to be a good solvent for the extraction of antibacterial agents from both the sources as it has shown better yield as well as antibacterial activity relating that higher yield means high concentration of single or variety of phytochemicals and therefore high antibacterial activity. Naderi *et al.* (2011) tested methanolic extracts of Iranian green tea and black tea against dental caries pathogens. The mean zone of inhibition was found to be 9.5 mm and 10.9 mm respectively.

One of the plant sources is citrus fruits as these possess anti-microbial properties and proved to be an interesting field for applications in different areas. Most common citrus fruit, lemon, is the most important fruit crop in India as the demand for its consumption is very high due to the nutritional value and its availability at cheap prices. The *Citrus limon* is an important medicinal plant of the family *Rutaceae*. It is a pale yellow, elliptically shaped berry fruit. It is cultivated mainly for its alkaloids, which are having anticancer activities and the anti-microbial potential. Lemon was originated in South East Asia, probably in India or Southern China. In India, it is mainly grown in Himachal Pradesh, Rajasthan, Uttar Pradesh, Punjab and Haryana. Lemon is used as flavoring agent to prepare juices, pickles and other recipes while the peel are generally wasted. The lemon peel also contain high quality of phenolic compounds including several flavonoid compounds which are known to exhibit various biological activities such as anti-microbial and antioxidant activities.

Now-a-days, the by-products of fruits (Peel waste) represent a serious problem, as they exert an influence on environment and need to be managed and utilized to the fullest extent. To overcome these types of problems, an increased attention has been given in bringing useful products from waste materials and citrus wastes are no exceptions. Hence, the work was planned to assess the antibacterial property of extract of lemon peel, so that the peel waste can be utilized in an eco-friendly manner for application as herbal finish on textiles.

MATERIALS AND METHODS

To assess the antibacterial property of lemon peel extract, materials and methods followed were :

Selection of plant material

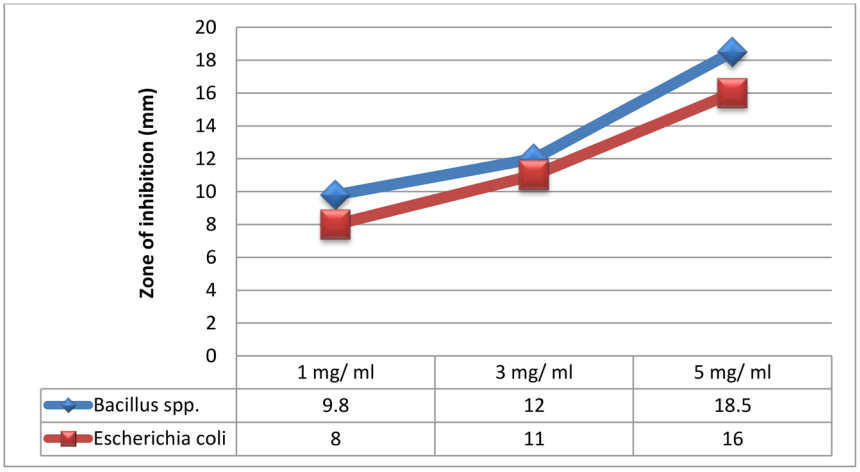
For this study, *Citrus limon* (Nimbu) were selected because of easy accessibility and more consume ability. The fresh and healthy lemon fruits obtained from University Campus (CCS HAU, Hisar).

Preparation of lemon plant extract

The collected fresh and mature lemon fruits were washed in the laboratory to remove the contaminants. They were separated by hand into peeled fruits and peel. Peel was cut into small parts with a sterile knife manually. The lemon peel was allowed to dry in shade to avoid break down of important compounds.

Dry grinding of the selected plant material was done in grinder mixer to make into a fine powder and sieved using metal sieves with mesh size of 2.5 mm to remove the dirt and unkind particles for aqueous extraction and preserved in an air tight container at dry place as a stock throughout the research.

For extraction, the powdered lemon peel (150 g) was extracted in 1000 ml distilled water and was



No activity (-mm), weak (<6mm), moderate (7-12mm) and strong activity (>12)

Fig. 1. Antibacterial property of lemon peel extract.

subjected to evaporation using soxhlet extractor. The fluids were evaporated at a temperature not exceeding the boiling point of the solvents and the process continued till 6-7 h until a solidified mass was obtained. The extract obtained was transferred to petri dishes and kept at a temperature of 40°C and the same were dissolved to prepare a stock solution for further research.

Selection of bacterium for testing the antibacterial activity of lemon plant extract

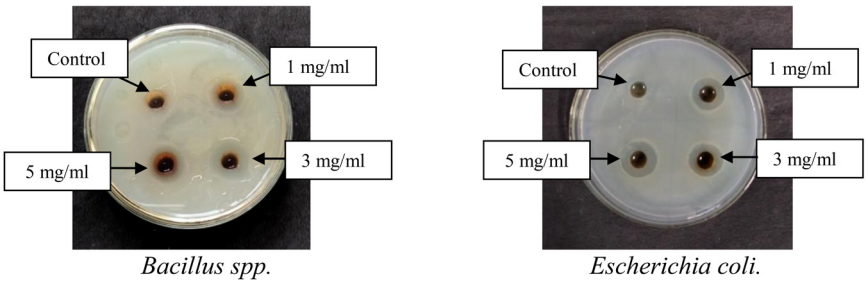
Pure cultures of two common human pathogenic bacteria viz., Gram-positive (*Bacillus* spp.) and Gram-negative (*Escherichia coli*) were obtained from the Department of Microbiology, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar. One ml of bacterial culture was aseptically transferred to inoculate 50 ml of broth medium contained in test tubes. The inoculated test tubes were incubated to rest for 24 h at 37°C at 130 rpm. After 24 h, the bacterial growth in the broth tubes was appeared in the form of turbidity and the tubes were stored at 40°C for further research work.

Preparation of nutrient agar (NA) medium

For preparation of NA medium, one liter of NA containing 5g of glucose, 5g peptone, 3g beef extract, 5g sodium chloride and 20g agar was dissolved in distilled water and the volume was made one liter in a conical flask and mixed properly to get a uniform mixture. The solution was sterilized in autoclave at 15 lbs pressure at 121°C temperature for 20 minutes for absolute suspension. The prepared medium was allowed to cool and poured into sterile petri plates and agar medium was left to solidify in Laminar Air Flow Chamber. The petri plates containing medium were kept in incubator for 24 h to check any type of microbial growth. The sterile medium petri plates were stored at 2-8°C.

Preparation of nutrient agar (NA) broth

For preparation of NA broth, one liter of broth containing 5g of glucose, 5g peptone, 3g beef extract and 5g sodium chloride was taken. The powder was suspended in one liter distilled water in a conical flask and stirred to make a clear solution. The solution was sterilized by autoclaving at 121°C temperature at 15 lbs pressure for 20 minutes. The prepared medium was cooled and placed in incubator at 37°C for 24 h to check the contamination. After 24 h the clear solution, without any growth, was used for further experiments. The prepared medium was stored at 2-8°C.



Bacillus spp. *Escherichia coli.*

Fig. 2. Zone of inhibition produced by different concentration of lemon peel extract.

Analysis of antibacterial activity of lemon plant extract

The antibacterial efficacy of the prepared extract was assessed against bacterial growth using Agar Well Diffusion Method. The experiment was conducted in Laminar Air Flow chamber under controlled conditions.

Agar well diffusion method

There are various methods involved in testing the anti-microbial activity of test extracts, from which the agar well diffusion method (Dey *et al.* 2010) with slight modification was employed to test the antibacterial activity of aqueous extract of lemon peel. To test the antibacterial potency of the prepared extract, 0.1 ml of bacterial inoculum was taken from cultures and poured on the test petri plate of Nutrient agar and evenly spread with the help of *L spreader.* The cultured petri plates were then set aside in an incubator at 30±20°C for 24–48 h and the bacterial cultures were allowed to grow.

After a uniform and required growth of the bacteria on petri plates, a uniform well was created in the center of the plates with the help of a sterilized cork borer of 5 mm diameter and the aqueous extract was poured into the well using a pipette (Mishra 2018). Different concentrations 1 mg/ml, 3 mg/ml and 5 mg/ml of the extract were added to the grooves and one control (sterilized distilled aqueous) was added to the groove on agar plate. The plates were then rested for about 24 h in incubator at 37°C in inverted position to analyze the bacterial growth. After 24 h, the zone of clearance was observed around the well and was considered for the antibacterial activity of the extract. The amount of resistance shown by the extract toward the bacterial growth was determined by measuring the diameter of the zone of inhibition formed around the wells. The diameters of the zone of inhibition were measured by measuring scale in millimeter (mm) (Hindi and Chabuck 2013).

RESULTS AND DISCUSSION

Antibacterial activity of lemon plant extract against selected bacteria

The antibacterial activities of lemon peel extract at different concentrations were tested using agar well diffusion method against common human pathogenic bacteria i.e. *Bacillus* spp. and *Escherichia coli*. The concentrations taken were 1 mg/ml, 3 mg/ml and 5 mg/ml. The results attained were calculated in terms of zone of inhibition. No activity (-mm), weak (<6mm), moderate (7–12 mm) and strong activity (>12)

The data in Figs. 1-2 revealed that sterilized distilled aqueous kept as control had no inhibition. The concentrations of 1 mg/ml and 3 mg/ml exhibited moderate zones of inhibition i.e. 9.8 mm and 12 mm, respectively while 5 mg/ml concentration had a strong zone of inhibition i.e. 18.5 mm against *Bacillus* spp.

On the other hand, the extract from peel of lemon shows moderate zones of inhibition i.e. 8 mm and 11 mm at concentrations of 1 mg/ml and 3 mg/ml, respectively while 5 mg/ml concentration

had a strong zone of inhibition i.e. 16 mm against *Escherichia coli*.

Thus, it was found that with an increase in the concentration of lemon plant extract, the zone of inhibition indicating anti-bacterial activity against *Bacillus* spp. and *Escherichia coli* increased.

It was observed that, with an increase in concentration of lemon peel extract, the antibacterial property of extract also increased. The increase in antibacterial property with increase in concentration may be because of presence of more phytochemicals and more extract gets dissolved in higher concentration as compared to lower concentration. It is desirable as more the concentration, more phytochemicals are probably to get thawed in the solution. The finding was in harmony with the results of Mishra (2018) who indicated that with increase in concentration of extract (20, 30 and 40 mg/ml), the zone of inhibition against the micro organisms also increased. The results of *Krishnaveni* (2018) also confirm the findings of the study as it reports that increase in zone of inhibition for increased concentrations (8% and 16%) of ethanolic extract of *Tridax procumbens*.

CONCLUSION

The study shows that the peel of lemon is a good antibacterial agent. The result of antibacterial susceptibility assay showed promising evidence for the antibacterial effects of lemon fruit peel. The antibacterial activity of lemon peel extract was tested at three different concentrations by using agar well diffusion method. It was noted that with an increase in concentration of the extract, the antibacterial activities of extract also increased. Strong antibacterial activities were found at 20 mg/ml concentration with a zone of inhibition of 18.00 mm against *Bacillus* spp. and 16.00 mm against *Escherichia coli*. Hence, the peel of fruits of lemon which are generally treated as wastes can serve as an effective and economical antibacterial agent as they are available for no cost, have no side effects.

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