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RESEARCH ARTICLE

Antimicrobial and Antioxidant Activity of Fruit Pulp of *Livistonia Chinensis*

Tanu P[*1], Thakur K[2], Kaur G[3]

[*1]*Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur (Punjab) -148101, India.*

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ABSTRACT

The present study reveals the *in-vitro* antimicrobial activity and antioxidant activity of ethanolic extract of fruit pulp of plant of *Livistonia chinensis* has been evaluated using disc diffusion method against bacterial strains of *Bacillus subtilis, Staphylococcus aureus, Shigella dysenteriae, Salmonella enteritidis* and fungal strain of *Candida albicans* using specific standard Ciprofloxacin and Fluconazole respectively and DPPH method for antioxidant activity.

KEYWORDS

Livistonia chinensis, Arecaceae, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Since ancient times, mankind all over the world mainly depended upon plant kingdom to meet their all needs of medicines, for alleviating ailments, search for eternal health, longevity and to seek remedy to relieve pain and discomfort, fragrance, flavours and foods.

Medicinal plants play important role in emerging and developing countries of Asia, both in preventive and curative treatments, despite advances in modern western medicine. There are several reports on antimicrobial activity of crude extracts prepared from plants that inhibit various bacterial pathogens, but a limited numbers of *in vitro* studies on herb preparations have been published. It is need of the hour to identify antibacterial potential of herbal products based on diseases for which no medicine or only palliative therapy is available. Hence an attempt was made to screen the antibacterial potential of herbal preparations in the control prevention of enteric bacterial infection.[1, 2]

Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethnomedicinal plants in India. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and glycosides etc., which have been found *in vitro* to have antimicrobial properties.[3] *Livistonia* is one such genus with no. of species like *Livistonia rotundifolia, L. papuana, L. merrillii, L. endauensis, L. speciosa, L. carinensis, L. muelleri, L. humilis and L. lorophylla*.

***Address for Correspondence:**
Tanu priya
Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur (Punjab) -148101, India.
E-Mail Id: tanu16priya@gmail.com

Livistonia chinensis also known as Chinese Fan Palm. It is single stemmed fan palm native to Japan and china that is cultivated worldwide in tropical and temperate climates. It is native to Asia and common in West Indies, Bermuda, Africa, Pacific Islands, and Australia. It is able to reach 50 feet in height but usually seen at 30 feet with a 10 to 12 feet spread. Leaves are palmate, petioles upto 1.8 m, with green or black colour, recurved spines along with margins, spines denser proximally, fewer distally on petioles. Inflorescences 1–1.2 m, branched to 3 orders, flowers hermaphroditic, borne in clusters of 4–7, white or yellow in colour, 2-2.5 mm in diameter. Fruits are green or blue-green in colour, globose to ellipsoid or pear-shaped, 1.5-2.6 × 0.9-1.8 cm. In traditional medicine practices, it is an important medicine used as analgesic, as anti cancer agent, as anti sagging in cosmetics, as haemostatic, anti-nasopharyngeal carcinoma, anti-choroid carcinoma, anti-oesophageal cancer, and anti-leukaemia.[4,5] The fruits showed significant anti proliferative effects against human myeloid leukaemia cell (L1210, P388, HL-60), gastric cancer cell (SGC7901), cervical cancer cell (HeLa), human liver cancer cell (HepG2, Hele7404), melanoma cell (B16), colon cancer cell (HT-29), and bladder cancer cell (T24).[6,7]

MATERIALS AND METHODS

Plant Material

The fruits of *Livistonia chinensis* were collected from the surroundings of Mastuana Sahib, Sangrur. The specimen was further authenticated by Dr. N. S. Attri, Department of Botany, Punjabi University, Patiala (Punjab) under accession no. 56568 on dated 13-12-2011. In the present study, the fruits were carefully selected, pulp was removed, and then it is dried under shade. The dried material were reduced to coarse powder in a mechanical grinder and passed through a sieve no. 60 to obtain a desired particle size.

Preparation of Plant Extract

About 200 gm of powdered material was subjected to successive extraction with various organic solvents with increasing degree of polarity like petroleum ether (40^0-60^0), acetone, chloroform, and ethanol. The material was first extracted with two litre of petroleum ether (40^0-60^0) in a Soxhlet extractor at 40^0 C for about 4 h in each batch. The said temperature was maintained on an electric heating mantle with thermostat control. The filtered solvent was concentrated at 40^0 C to $1/10^{th}$ volume. The concentrated extract was then reduced to a thick mass by allowing the solvent to evaporate at room temperature. The thick mass was then placed in a desiccator to remove traces of the solvent. The weight of this extract was recorded.[8]

Microorganisms

The two positive bacterial strains *Bacillus subtilis*, *Staphylococcus aureus*, and two negative bacterial strains *Shigella dysenteriae*, *Salmonella enteritidis* including one fungal strain *Candida albicans* are collected for their antimicrobial testing from ITC (International Testing Centre) Mohali.

Antimicrobial Assay

The bacteria were maintained on nutrient broth (NB) at 37°C and fungus was maintained on Sabouraud Dextrose agar (SDA) at 28°C was tested by the disc diffusion method (Table-1).

Dried and sterilized filter paper discs (6 mm diameter) were impregnated with known amount of the test substances dissolved in methanol (30µg/ml) and water separately using micropipette and the residual solvents were completely evaporated. Discs containing the test material with different concentrations each were placed on nutrient agar medium for bacterial strains and Sabouraud Dextrose Agar (SDA) for fungal strain uniformly seeded with the test microorganisms. Negative controls were prepared using the same solvents as employed to obtain the extracts. As positive controls, Ciprofloxacin (10 µg/ml) was used for Gram-positive and Gram-negative bacteria and Fluconazole (10 µg/ml) for *Candida* spp. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and at 35°C for 48

h for fungal strain (yeast). The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter.[9]

Antioxidant Screening

In- vitro antioxidant activity studies of ethanol extract of *Livistonia chinensis* seed pulp was done by DPPH method which involves the following steps:

Preparation of Standard Solution

Ascorbic acid (10 mg) weighed and dissolved in 100 ml ethanol to get 100 µg/ ml stock solution. Lower concentrations i.e. 5, 10, 20, 30, 40, 60, 130 and 260 ppm for ascorbic acid was prepared by serial dilution with ethanol.

Preparation of Sample Dilution

Stock solution of sample was prepared by dissolving 10 mg of dried ethanolic extract in 10 ml ethanol to give concentration of 1mg/ ml.

Preparation of DPPH Solution

4.3 mg of DPPH was dissolved in 3.3 ml of ethanol. It was protected from light by covering the test tubes with the aluminum foil.

Protocol for Estimation of DPPH Scavenging Activity

150µl DPPH solution was added to 3ml ethanol and absorbance was taken immediately at 516 nm for control reading.

Different volume levels of test sample (5, 10, 20, 30, 40, 60, 130 and 260 ppm) were screened and made 260 ppm of each dose level by dilution with ethanol

Dilute with ethanol up to 3 ml.

150µl DPPH solution was added to each test tube.

Absorbance was taken at 516 nm in UV visible spectrophotometer (Shimadzu, UV1800 Japan) after 15 min using ethanol as a blank.

The percentage reduction and IC50 were calculated as follows.

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

% antiradical activity = Control absorbance-Sample absorbance/Control absorbance ×100.[10]

RESULTS AND DISCUSSION

The results of the antimicrobial screening have been represented in Table 1. The zone of inhibition of ethanol extract ranged from 8.19 mm to 33.94 mm. The highest inhibition zone 33.94 mm was formed by the ethanol extract of *Livistonia chinensis* against *Salmonella enteritidis* at the highest concentration followed by *Shigella dysenteriae, Staphylococcus aureus and Bacillus subtilis.* The ethanol extract that showed antibacterial activity against the pathogens was active in all the given concentration i.e. 32000, 16000, 8000, 4000, 2000 and 1000 µg/ml. Ethanol extract of *Livistonia chinensis* also showed a significant zone of inhibition against a fungal species, *Candida albicans* at all the given concentrations (Table 1) *In vitro* antibacterial activity of *Livistonia chinensis* against Gram positive *Staphylococcus aureus, Bacillus subtilis,* Gram negative *Shigella dysenetriae, Salmonilla enteritidis ,* and fungal species *Candida albicans* was carried out and ethanol extract of *Livistonia chinensis* showed significant results against pathogens.

The results of this investigation should be helpful in the further experiments on antimicrobial activity of *Livistonia chinensis*. These studies confirms the potential of this plant but further more mechanistic work is essential to prove it as one of the specific antimicrobial plant.

DPPH is nitrogen centered free radical that show strong absorbance at 516 nm. DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical .the free stable radical us reduced to the corresponding hydrazine when it react with hydrogen donors; this ability was evaluated by

Table 1: Antimicrobial activity of ethanol extract of *Livistonia chinensis* fruit pulp

S. No.	Name of Bacteria	Microbial activity/ zone reading (mm)						
		10(std sol.)	1000µg /ml	2000µg/ ml	4000µg/ ml	8000µg/ ml	16000µg/ ml	32000µg/ ml
1.	*B.subtilis*	35.25	7.63	7.79	10.68	12.09	24.86	30.91
2.	*S.aureus*	33.68	Nil	Nil	Nil	13.30	18.19	27.12
3.	*S.dysenteriaeS*	37	6.44	7.42	7.54	10.10	22.13	31.89
4.	*S.enteritidis*	40.23	8.19	8.49	8.74	9.04	26.12	33.94
5.	*C.albicans*	12.84	Nil	7.21	7.74	8.44	9.11	9.63

more frequently used discolouration assay, which evaluate the absorbance decrease at 516 nm produced by the addition of the antioxidant to a DPPH solution in the ethanol .this method is widely used to check the free radical scavenging antioxidants.to evaluate the antioxidant activity of the specific compounds or extract (the antioxidant) were allowed to react with a stable radical DPPH* in ethanol solution. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC50 values.

Ascorbic acid was used as standard controls for antioxidant study using DPPH method. Ascorbic acid exhibited attained significant antioxidant activity by inhibiting DPPH radical 73.27% at 10 ppm concentration and.at higher concentration, 20, 30, 40 ppm, a slight increase in inhibition of DPPH radical (antioxidant activity) was observed. The test sample ethanol extract showed significant antioxidant activity at concentration 40ppm and 60ppm respectively. A slight increase in activity was observed at higher concentration of ethanol extract.

Table 2: Antioxidant activity of ethanol extract of *L.Chinensis* fruit pulp

S. No.	Standard / Sample Conc. (ppm)	%FRSA (Standard)	%FRSA (Sample)
1	5.00	56.96	11.81
2	10.00	73.27	23.80
3	20.00	73.60	16.53
4	30.00	72.60	21.24
5	40.00	75.15	27.51
6	60.00	77.48	33.61
7	130.00	-	55.13
8	260.00	-	80.87

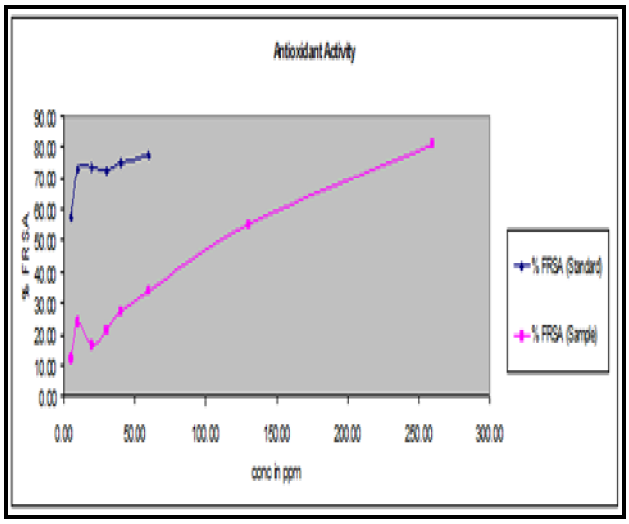

Figure 1: Preparation of standard and sample curve

CONCLUSION

The test samples ethanol extract showed significant antioxidant activity and concentration 40 ppm and 60 ppm respectively. The ethanol extract of *Livistonia chinensis* fruit pulp was evaluated for in vitro antibacterial activity against *Bacillus subtilis*, *Staphylococus. aureus*, *Shigella dysenteriae* and *Salmonella enteritidis* and antifungal activity against *Candida albicans* by disc diffusion method .The ethanol extract possesses antibacterial activity against *Staphylococus aureus*, *Bacillus subtilis*, *Shigella dysenteriae* and *Salmonella enteritidis* and antifungal activity *Candida albicans* as evident from zone of inhibition observed.

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REFERENCES

1. Kalia AN. "Text Book of Industrial Pharmacognosy" First Edition, New Delhi India: CBS Publisher, 2005, 1-3.

2. Tambekar DH and Dahikar SB "Antibacterial activity of some Indian Ayurvedic preparations against enteric bacteria pathogens", J Adv Pharm Technol Res, 2011, 24-29.

3. Bhalodia NR, Nariya PB, Shukla VJ, "Antibacterial and Antifungal activity from Flower Extract of Cassia Fistula L", International Journal of Pharm Tech Research, 2011, 3, 160-163.

4. Healthy Ministry of Guangzhou Force Logistics, "Common Chinese Herbal Medicine Handbook", Beijing: People's Health Publishing House, 1969, 772–775.

5. Zhao GP, Dai S, Chen ES, "Dictionary of Traditional Chinese Medicine", Shanghai Science and Technology Press, 2001, 2459–60.

6. Wang H, Li A, Dong XP, Xu XY, Chin J. Med Mat 2008, 31, 718–722.

7. Huang WC, Hsu RM, Chi LM, Leu YL, Chang YS, Yu JS. Cancer Lett 2007, 248,137–146.

8. Pietta PG, "Flavanoids as antioxidants", Journal of Natural Products 2000, 63, 1035-1042.

9. Jehan Bakht et al, "Antimicrobial potentials of Eclipta alba by disc diffusion method", African Journal of Biotechnology 2011, 10(39), 7658-7667.

10. Sanja's D, Shtchnr, Patel NK. "Characterization and evaluate Antioxidant activity of Porpulaca oleraceae" 2009.

