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Original Research Paper

IN-VITRO ANTIMICROBIAL ACTIVITY OF *PISTACIA INTEGERRIMA* LEAF GALL EXTRACTS

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ABSTRACT

The ethanol and aqueous fractions of leaf galls of *Pistacia integerrima* were evaluated for antibacterial activity using the agar-well diffusion method. All the strains showed concentration dependent susceptibility towards both the extract (25, 50, 100, 250, 500 µg/100µL). The antibacterial activity was more pronounced against *Gram positive* bacteria, while it showed moderate activity on *Gram negative* bacteria strains studied. The ethanol extract was found to show better activity profile than the aqueous extract. The inhibitory effect of the extracts was compared with standard antibiotic Ciprofloxacin.

Keywords: *Pistacia*, *Anacardiacea*, Karkatshringi, Antibacterial.

INTRODUCTION

Since ancient times, plants have been a veritable source of drugs; man tends to ignore the importance of herbal medicine.[1] To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified manner.[2,3,4] Over the past few years, many efforts have been made to discover new antimicrobial compounds from various kinds of natural sources such as microorganisms, animals and plants. In this regard several Indian medicinal plants have been evaluated, a fair number of which possess potential antimicrobial activity[5] and few natural products have been approved as new antibacterial drugs.[6,7] However, the increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics

may render the current antimicrobial agents insufficient to control some bacterial diseases and hence research for identifying novel substances that are active against human pathogens is an urgent need.[8]

Pistacia integerrima (Anacardiacea) leaf galls, commonly known as Karkatshringi in Sanskrit are one of the appendages of plant formed due to the invasion of insect *Dasia acidofactor*. Karkatshringi is used in indigenous system of medicine (Ayurveda, Unani and Siddha) as a remedy for cough, asthma, fever, respiratory and in liver disorders.[9,10] Karkatshringi also finds usage in the treatment of children's ear infections, suppress *haemorrhage* from gums and used to suppress bleeding from nose.[11,12] Hakims consider galls useful in pulmonary infections, *diarrhoea* and *vomiting*.[13]

The present study was undertaken to investigate the antibacterial activity of *Pistacia integerrima* leaf galls, against some enteric pathogens.

MATERIALS AND METHODS

Plant material

Pistacia integerrima leaf galls were purchased from local market of Pune, India and authenticated at Regional Research Institute (Ayurveda), Pune.

Preparation of extract

Ten grams of pulverized plant part (leaf galls) were separately soaked in 100 ml of distilled water and ethanol (LR grade, Merck, India) and kept on a rotary shaker for 24 h. Each extract was filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds had been extracted. Extraction was considered to be complete when the filtrate had a faint colour. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland). A portion of the residue was used for the antibacterial assay.

Microorganisms

The bacterial strains used for studied are *Bacillus cereus*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Escherichia coli*; *Klebsiella pneumoniae*. The organisms were maintained on nutrient agar slope at 4^{o}C. For the experiments, stock culture was prepared by inoculating each culture from slants to flask in sterile nutrient broth and incubated at 37^{o}C for 24 h. The stock culture was serially diluted by ten fold with sterile peptone water and 0.1ml from each dilution was spread over nutrient agar plates and incubated at 37^{o}C for 24 h. The number of colony forming units (CFU) was counted from plates of each dilution and there by the total CFU was calculated in the stock culture. For antimicrobial screening the stock cultures of 1x10^{5} CFU per ml were used.

Antibacterial assay

The selected strains of bacteria, grown on nutrient broth were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter.[14,15] Using a micropipette, 100 micro litres of different concentrations of gall extracts (500, 250, 100, 50, and 25 µg) were added to different wells in the plate. Pure DMSO was taken as the negative control and 100 µg/ 100µl Ciprofloxacin as the positive control. The plates were incubated in an upright position at 37ºC for 24 hours.[16,17] The diameter of inhibition zones was measured in mm and the results were recorded.

RESULTS AND DISCUSSION

To determine antimicrobial activity, *P. integerrima* leaf gall extracts were tested against *Gram positive* and *Gram negative* bacteria. The result of the antimicrobial activity of aqueous and ethanolic extract is shown in Table 1. Among the bacteria selected, both aqueous and ethanolic extract inhibited the *Gram positive* bacteria better

then the *Gram negative* bacteria. In the *Gram positive* bacteria *B. cereus* was found to be more susceptible. Among *Gram negative* bacteria, *K. pneumonia* is found to be less susceptible than the other bacteria. The results of the previous study on the antimicrobial activity of extracts of galls of *Q. infectoria, P. integerrima* and *R. succedanea* have also shown that *Gram positive* bacteria were more susceptible than the *Gram negative* bacteria.[18, 19,20] But contrary to the report of Parmar[21], that *P. aerugenosa* is not susceptible to aqueous extract of galls of *P. integerrima*, our study shows that *P. aerugenosa* is susceptible to both aqueous and ethanolic extract of galls of *P. integerrima*. Ethanolic extract appears to be more potent in antibacterial activity than the aqueous extract.

These observations may be attributed to two reasons; firstly, due to the nature of biologically active components (alkaloids, flavonoids, sterols, quinine, tannins, phenols etc.) which might be enhanced in the presence of ethanol.[22] It has been documented that alkaloids, flavonoids, tannins and phenols are plants metabolites well known for their antimicrobial activity.[18,23,24] Secondly, the stronger extraction capacity of ethanol could have produced a greater number of active constituents responsible for antibacterial activity. Ciprofloxacin, which was used as a positive experimental control against all bacterial strains assayed, produced a zone of inhibition of 18.00±0.58 to 25.53±0.29 while no inhibitory effect could be observed for DMSO used as negative control.

CONCLUSION

The present results offer a scientific basis for the therapeutic potency of *P. integerrima* galls used as a source of drug karkatasringi, which is widely used in many preparations of Ayurveda and Siddha systems of medicine to treat various diseases. However, the activity level of the extracts may be more accurately evaluated in terms of MIC values as the zone of inhibition might be influenced by solubility and diffusion rate of the phytocompounds. In addition, *in vivo* studies are necessary to determine the toxicity of the active constituents, their side effects, circulating levels, pharmacokinetic properties and diffusion in different body sites. The antimicrobial activities could be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in curbing administration of inappropriate concentration, a common practice among many traditional medical practitioners in India.

Table 1: Antibacterial activity of *P. integerrima* leaf gall extracts by agar well diffusion method.

Bacterial strains	Diameter of inhibition zone (in mm) against various concentrations(µg)										
	Aqueous extract					Ethanolic extract					Ciprofloxacin
	500	250	100	50	25	500	250	100	50	25	
Bc	20.03±0.15	18.47±0.29	15.97±0.15	13.03±0.52	10.77±0.39	24.73±0.37	21.70±0.35	18.83±0.44	15.43±0.30	12.13±0.24	25.53±0.29
Sa	16.60±0.31	13.50±0.29	12.87±0.47	12.33±0.20	10.50±0.25	21.33±0.20	20.20±0.12	18.60±0.31	14.53±0.29	11.83±0.12	24.73±0.37
Pa	14.60±0.31	12.53±0.29	11.83±0.27	10.50±0.25	8.93±0.52	20.80±0.12	18.27±0.18	17.47±0.26	14.27±0.15	10.37±0.20	23.00±0.12
Ec	11.60±0.23	9.90±0.38	9.73±0.18	8.60±0.23	8.43±0.30	16.60±0.31	14.93±0.18	13.07±0.18	10.40±0.31	9.77±0.15	22.53±0.29
Kp	6.60±0.31	5.47±0.26	0.00±0.00	0.00±0.00	0.00±0.00	8.60±0.31	7.93±0.18	0.00±0.00	0.00±0.00	0.00±0.00	18.00±0.58

The values are the mean of three experiments ± S.E.

Abbreviations: Bc, *Bacillus cereus*; Sa, *Staphylococcus aureus*; Pa, *Pseudomonas aeruginosa*; Ec, *Escherichia coli*; Kp, *Klebsiella pneumoniae*.

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