

ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS FROM LEAVES OF *PISTACIA INTEGERRIMA* (ANACARDIACEAE) STEW EX BRAND

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ABSTRACT

Objective: The ethanol extract of leaves of *Pistacia integerrima* (EPI) was evaluated for antibacterial activity.

Methods: The leaves of *P. integerrima* were collected from wild source and authenticated by Dr. Manoj Joshi, botanist environmental education expert, Una, HP, India. Antimicrobial activity was performed using the Mueller–Hinton (MH) agar using disk diffusion method. In the MH agar media 38 g after dissolving in 1000 ml of distilled water (pH 7.3±0.2), the extract was incorporated such that concentration per ml will be 66.67 µg, 133.33 and, 166.66.

Results: The minimum inhibitory concentration recorded was 133.33 for *Pseudomonas aeruginosa*, *Salmonella* Typhi, and *Klebsiella pneumonia*, whereas 166.6 for *Staphylococcus aureus*, Coagulase-negative *Staphylococcus*, and *Escherichia coli*. The maximum zone of inhibition was found 19 mm and 18 mm for *S. aureus* and *E. coli*, respectively. *S. aureus* have been reported for skin pneumonia, heart valve, and bone infections whereas *E. coli* causes diarrhea and many other infections in children.

Conclusion: After reporting the better zone of inhibition for these two bacteria, EPI can be used to formulate better herbal remedy against them.

Keywords: Ethanol extract, *Pistacia integerrima*, Antibacterial, Mueller Hinton agar, Dilution and zone of inhibition.

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INTRODUCTION

The Ayurvedic system of medicine includes number of plants which should be investigated to determine the hidden potential like using the modern methodology. Pharmacological activities for several plants with antimicrobial activity of *Marsilea minuta* [1], antimicrobial and antioxidant activity of *Glycyrrhiza glabra*, *Eclipta alba*, and *Matricaria chamomilla* [2], and antibacterial activity of *Vigna unguiculata* [3]. The goal of pharmacognosist should be searching for drugs of plant origin with minimum side effects and maximum benefits. The plant *Pistacia integerrima* (stew ex brand) is an indigenous tree which was chosen for this study. The plant belongs to the family Anacardiaceae. The scanty availability of information on this plant facilitates the study on it. Since ages various parts of this plant are being used for their medicinal use. The researchers have reported for its medicinal potential several time [4,5]. Reported pharmacognostical and phytochemical activity of *P. integerrima* shows its physicochemical parameters and preliminary phytoconstituents [6,7]. Similarly microscopic studies on leaves, galls, and stem show cells responsible for chemical constituents [5]. Three new phytoconstituents such as n-decan-3'-ol-yl-n-eicosanoate, n-octadecan-9-11-diol-7-on, and 3-oxo-9β-lanost-1,20(22)-dien-26-oic acid along with the known compound beta sitosterol [8]. The antibacterial activity of *P. integerrima* galls has been reported by several researchers using different extracts. The *in vitro* antimicrobial activity of ethanolic extract of the galls of *P. integerrima* has reported on gram positive bacteria whereas the moderate activity on gram negative [9]. For the ethanolic and chloroform extract, anticancer and antifungal activity has been reported of the plant [10]. Powder from its galls has also been reported for the antimicrobial activity [11,12]. Here, the attempt is made to study the antimicrobial activities of the leaves of the plant. The antibacterial activity of two Gram-positive and total six Gram-negative bacteria was evaluated using the ethanolic extract of *P. integerrima* (EEP) stew ex brand. All the bacterial strains were grown in Mueller–Hinton (MH) agar media.

Antibacterial studies

The ethanol extract of leaves of *Pistacia integerrima* (EPI) was screened for antibacterial activity against Gram-positive and Gram-negative organisms. The organisms used were:

Gram-positive organisms

- Coagulase-negative *Staphylococcus* and *Staphylococcus aureus*.

Gram-negative organisms

- *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* Typhi.

MATERIALS AND METHODS**Minimum inhibitory concentration (MIC)***Preparation of medium*

The media used for the growth of bacteria was Mueller–Hinton agar (MH). Agar (38 g) was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.3±0.2, sterilized by autoclaving at 121°C at 151b pressure for 15 min and used for the sensitivity tests.

Preparation of the agar plates

The extracts were incorporated into MH agar such that the final concentration of extract being 1000 µg/15 ml, 2000 µg/15 ml, and 3000 µg/15 ml in the plates so that the concentration per ml is 66.67 µg, 133.33µg, and 166.66 µg.

The plates were prepared using agar and extract of various dilutions, allowed to solidify. Then, a loop full of the cultures was inoculated at the labeled spots. The plates were then incubated at 37°C for 24 h. The presence or absence of growth of the organism was noted and the results were tabulated in Table 1.

Table 1: Effect of alcoholic extract of *Pistacia integerrima* on growth of various bacteria

Extract	Conc. µg/15 ml	Conc. µg/ml	1	2	3	4	5	6
Alcoholic	1000	66.66	+	+	+	+	+	+
	1500	100	+	+	+	+	+	+
	2000	133.33	+	+	+	-	-	-
	2500	166.66	-	-	-	-	-	-

Table 2: Minimum inhibitory concentration of ethanolic extract of leaves of *Pistacia integerrima*

Alcoholic extract	Minimum inhibitory concentration (µg/ml)					
	<i>Staphylococcus aureus</i>	Coagulase-negative <i>Staphylococcus</i>	<i>Escherichia Coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella Typhi</i>	<i>Klebsiella pneumonia</i>
	166.6	166.6	166.6	133.3	133.3	133.3

Table 3: Zone of inhibition (mm) of the alcoholic extract of the leaves of *Pistacia integerrima*

Organisms	Ciprofloxacin	EEPI
<i>Staphylococcus aureus</i>	26	19
Coagulase-negative <i>Staphylococcus</i>	32	16
<i>Escherichia coli</i>	25	18
<i>Pseudomonas aeruginosa</i>	37	17
<i>Salmonella Typhi</i>	33	18
<i>Klebsiella pneumonia</i>	33	16

Antibiotic disk diffusion method

Preparation of disks

The disks of 6 mm diameter were prepared from Whatman filter paper number 1 and were sterilized in hot air over at 160°C for 1 h. The disks were then impregnated with the EEPI. Ciprofloxacin disks were prepared such that each disk contains 5 mg. The seeding of organisms was done on MH agar medium with the help of a sterile swab. MIC of the EEPI, ciprofloxacin (std) were observed and tabulated in Table 2.

Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry. The EEPI and ciprofloxacin (std) disks were placed on the seeded medium plates were kept at 40°C for 30 min for prediffusion. The plates were then incubated at 37°C for 24 h. The zone of inhibition around the disks was measured and the results were shown in Table 3.

RESULTS

1. Coagulase-negative *Staphylococcus*, 2. *Staphylococcus aureus*, 3. *Escherichia coli*, 4. *Klebsiella pneumonia*, 5 *Pseudomonas aeruginosa*, and 6. *Salmonella Typhi*. (+ = growth, - = no growth)

DISCUSSION

Total four Gram-negative strains used were *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *S. Typhi*, whereas two Gram-positive strains were studied here, namely Coagulase-negative *Staphylococcus* and *S. aureus*. Total inhibitory concentrations were recorded by growing bacteria in Mueller-Hinton agar at the concentration 133.33µg/ml for *K. pneumonia*, *P. aeruginosa*, and *S. Typhi* (Tables 1 and 2) and 166.66 µg/ml for *S. aureus*, Coagulase-negative *Staphylococcus*, and *E. coli*, as these concentrations of PIEE were inhibiting the growth of inoculated bacteria. In a similar antimicrobial study on *Pistacia* galls using a well diffusion method, here, the researcher experimented both aqueous and ethanolic extract at the concentrations of 25, 50, 100, 250, and 500 µg/100µL against Gram-positive and Gram-negative bacteria [11]. The disk diffusion comparison between PIEE extract and ciprofloxacin showed better

result against *S. aureus* and *E. coli*. Both the bacteria showed zone of inhibition of 19 mm and 18 mm (Table 3). The zone of inhibition for *S. Typhi* was observed at 18 mm, whereas the same was recorded 17 mm for *Pseudomonas aeruginosa*. The minimum zone of inhibition at 16 mm was recorded for coagulase-negative *Staphylococcus* and *Klebsiella pneumoniae*.

CONCLUSION

Staphylococcus aureus is a Gram-positive bacterium commonly present on skin and nose. The bacteria mainly responsible for skin infections, pneumonia, heart valve infection, and bone infections. From nose it gets spread to other body parts [12]. The virulent strain of *E. coli* mainly presents in children and is responsible for most diarrheal infections, meningitis, septicemia, and urinary tract infection [13,14]. As the EEPI give effect against these two bacteria, this extract hence can be used to formulate an effective antiseptic and antibacterial remedy against *S. aureus* and *E. coli*.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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