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Phytochemical analysis and antimicrobial activity of galls of *Pistacia integerrima* Stew ex. Brand

Research article

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Abstract

The gall of *Karkatshringi* (*Pistacia integerrima Stew Ex. Brand)* is a well known drug used in paediatric diseases. *Sushrutacharya* has mentioned *karkatshringi* is one of the drugs in *Rakshoghna Dravyas*, used in treatment of *Grahabadha*. Symptoms of *Grahabadha* are similar to the symptoms of various infectious diseases. The galls powder of *Karkatshringi* was evaluated preliminary physico-chemically. The water extracts was prepared and performed antibacterial activity by disc diffusion method and assayed for MIC using micro dilution technique. It showed that the galls powder of *Pistacia* was sensitive against *staphylococci and E-coli* and resistant to *pseudomonas*.

Key words: *Pistacia integerrima Stew Ex. Brand,* Antibacterial activity, *Rakshoghna* , Physico-Chemical analysis.

Introduction

In day to day life infectious diseases are the major problem. The environmental factor, pollution, change in atmosphere, changed living habits and changed dietary contents are affecting the human body and its immune system, resulting in increased number of infectious disease. Also increase in number of infectious organisms.

Modern scientists have evolved various remedies such as antibacterial, antiviral, anti-fungal drugs to overcome these infections. But increasing resistance of microorganism to these drugs, their untoward effects and their cost are some of the factors. Considering all the above facts, we should find an alternative way to treat such infections. WHO is also promoting plant based drug research to overcome drug resistance in various infections.

In *Ayurveda*, there are many medicinal plants described which may prove effective in various infections due to their actions on microorganism. They are described under the headings *Rakshoghna, Krumighna, Jantughna* (antimicrobial) etc (1). *Sushrutacharya* has mentioned *Karkatshringi* (*Pistacia integerrima stew ex. brandis*), as one of the drug in *Rakshoghna dravyas* (2) which is described for the treatment of *Grahabadha*(infectious diseases). Symptoms of *Grahabadha* are similar to the symptoms of various infections seen now days(3). *Karkatshringi* is mainly used in paediatric diseases such as cough, diarrhoea etc (4). It is a good expectorant

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and *Kaphaghna*(5). As Kapha provides favourable condition for growth of bacteria. This drug may act on bacteria. Taking all the above things into consideration, it is necessary to carry out research regarding preliminary phytochemical analysis and antibacterial activity of *Karkatshringi.* In this study this is a small approach to study the *Ayurvedic* concept in view of modern science.

Materials and methods

Plant Material

The selected plant species i.e. galls of *Pistacia intergerrima* was collected from local market of Banaras and authenticated by Raw material herbarium and Museum, NISCAIR, New Delhi. The dried powder of gall is passed through 72 size sieve which is used to evaluate the preliminary phytochemical analysis and antibacterial activity.

Methods

The dried powder of galls was examined preliminarily chemical evaluation parameters likes water, alcohol, methanol, petroleum ether and diethyl ether soluble extracts, Ph values, optical density, foam index, swelling index were carried out by standard methods(6)

Determination of extractives

Determination of water Soluble Extractive**:** 2.5 g of the air-dried drug was powdered. It was dissolved in 50 ml of water in a conical flask and closed with rubber cork. Then it was placed in a electrical shaker for twenty four hours, shaking frequently during six hours and allowing standing for eighteen hours. After that it was filtered rapidly taking precaution against loss of solvent, filtrate was evaporated to dryness in tarred flat bottomed shallow dish and dried at 105 ^{0}c to constant weight. Then the weight was calculated with reference to the percentage of water soluble extractive to the air dried drug. This procedure was followed for *Pistacia integerrima Stew Ex. Brand* , same procedure was applied for determination of alcohol, methanol, diethyl ether, petroleum ether extractive.

In TLC study petroleum ether extracts of *P. intergerrima was* run on 20 cm long glass plate coated with silica-G gel by using petroleum ether : chloroform (6:3) and pure chloroform as a solvent system. The solvent were allowed to run upto 18 cm distance. Then the plates was heated at 110^0 c in oven and then plates were exposed to iodine vapours and spots resolved were noted down and Rf values were calculated. (7)

In Spectroscopy 1 gm of the above sample, was taken and extracted in 10 ml of the respective solvent i.e. water and alcohol (ethanol) for 24 hours and filtered, particles were collected from filtrate and UV-visible spectrum was recorded.(Table-4 and 5) (8)

Extraction for antibacterial activity

30 mg of dried and powdered test material was extracted with 10 ml distilled water for 24 hours and made serial diluted solution as 1:2, 1:4, 1:8 for each samples i.e. the solution of 30 mg/ml, 15 mg/ml, 0.75 mg/ml and 0.375 mg/ml were prepared.

Preparation of discs

i) 10 µl of these solutions were poured on the sterile standard filter paper discs (Whatman No. 1) used for antibacterial susceptibility test and dried in incubator under all aseptic precaution. Thus the final concentration drugs were 30 µg, 15µg, 7.5 µg, 3.75 µg per disc respectively.
ii) Disc of standard antibiotic i.e. ceftriazone 30 µg/disc is used as a control.

Selection of Bacteria:

Both gram +ve (Staphylococcus aureus) **(9)** & Gram - ve (E-coli and Psudomonas aeruginosa) (10) obtained from Vishakha Microbiology Laboratory, Nagpur, were grown in nutrient agar medium and lawn

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culture of the standard strain of these bacteria were used to access the antibacterial activity.

Result and Discussion

On exploring various markets throughout India, from Deharadun in North to Banglore in South, we observed mainly two varieties of *Karkatshringi* commonly being sold in the market. One of them is rounded irregular in shape while other resembled the description from *Ayurvedic* texts i.e. horn like cylindrical and hallow (11). The galls authenticated by Raw material herbarium and Museum, NISCAIR, New Delhi. The natural habitat of *Karkatshringi* described in Garwhal region, in the Himalaya (12), the samples in the local market usually sold as *Karkatshringi* was authenticated, collected and selected for the physicochemical standardization and antimicrobial study. We obtained different extractive values, minimum solubility in Diethyl ether and maximum solubility in methanol solvent. As per the observation the Ph value was acidic in nature. The foaming index and swelling index was < 100 and 2.5 ml which indicate high content of saponin. Optical density at 630 nm was 0.28 and at 670 nm was 0.15. While observing the Rf values we have been noticed that the maximum values were differ in different solvent system and different mobile phases, exposed to iodine vapours and under uv light. Visible spectrum of alcohol extract showed 2 peaks and uv-visible spectrum of alcohol extract showed 3 peaks. The qualitative tests for tannin, resins, saponin, glycosides, oxalic acid, iron and sulphate were positive.

While studying the antibacterial activity, bacteria causing commonest infections of respiratory tract and gastrointestinal tract were selected because *Karkatshringi* mainly acts on such type of infectious diseases like *Kasa(cough), Swas(asthama), Atisar* (diarrhoea)etc. Discs diffusion method was selected which is easy, non hazardous and reliable. Water extract of galls powder of *Pistacia integerrima Stew Ex. Brand* was used to evaluate antibacterial activity because the powder solubility of galls was quite good as compared to other solvents and water which is easiest form of consuming medicine. The disc was prepared similar to standard antibiotic disc. After stipulated time the sensitivity of drug was observed. The zone of inhibition (in mm) in staphylococci aureus and E-coli was moderate and in pseudomonas aeruginosa it was non sensitive as compared to the standard antibiotic (ceftriazone) inhibition zone. It showed that the galls powder of *Pistacia integerrima Stew Ex. Brand* was good sensitive against staphylococci and E-coli and resistant to pseudomonas.

Table 1: Extractive values in different solvents

Water Soluble Extractive%	Alcohol Soluble Extractive%	Methanol Soluble Extractive%	Diethyl ether Soluble Extractive%	Petroleum ether Soluble Extractive%
23.10	22.13	38.46	5.24	11.57

Table 2: Ph-Optical Density-foaming index and swelling index values

PH Values	**Optical Density**		**Foam Index (FI)**	**Swelling Index**
	at 630 nm	at 670 nm		
4.47	0.28	0.15	< 100	2.5 ml

Table 3: Thin layer chromatography (TLC)

Extract	Mobile phase	Spray/ Exposed	No. Of Spots	RF Values
Petroleum ether	Chloroform	Iodine vapours	7	0.07, 0.15, 0.25, 0.35, 0.51, 0.69, 0.95
Petroleum ether	Petroleum Ether : Chloroform (6:3)	Iodine vapours	7	0.03, 0.10, 0.18, 0.53, 0.56, 0.64, 0.94
Petroleum Ether	Petroleum Ether : Chloroform (6:3)	UV light at 366 nm	3	0.03, 0.07, 0.95

Table 4: Visible Spectrum of Alcohol Extract

PEAKS		VALLEY	
□□	Absorbance	□	Absorbance
666.0	0.024	766.0	0.002
304.0	2.231	633.0	0.010

Table 5: UV-Visible Spectrum of Water Extract

PEAKS		VALLEY	
□□	Absorbance	□	Absorbance
743.0	0.036	777.0	0.028
730.5	0.039	739.0	0.034
666.0	0.307	727.5	0.038
		635.0	0.126

Table 6: Analytical data of qualitative tests of galls powder

Components	Tests	Result
Tannin	Ferric Chloride Test, Lead Acetate Test	Positive
Resins	few mg of each extract was dissolved in a little alcohol + few drops of distilled water were added = turbidity	Positive
Saponin	few mg of each extract + few sodium bicarbonate + little water = formation of froth	Positive
Glycosides		Positive
Oxalic acid	Benedict's Reagent	Positive
Iron	Test Solution-Dilute NH4OH+Water extract of drug, add few drops of 5% lead acetate	Positive
Sulphate	5% ammonium thiocyanate	Positive
	lead acetate	

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Table 7: Antibacterial activity

Sr. No	Test organism	Activity	Water Extract				Ceftriazone
			Concentration of *Pistacia intergerrima* galls extract				
			30mg/ disc	15mg/ disc	7.5mg/ disc	0.375 mg/disc	30mg/ disc
1.	*Staphylococcus aureus*	I Z	15 mm	12 mm	10 mm	10 mm	27 mm
2.	*Escherichia coli*	I Z	14 mm	12 mm	10 mm	10mm	20 mm
3.	*Pseudomonas aeruginosa*	I Z	nil	nil	nil	nil	nil

I Z= Inhibition Zone in mm

Fig.1 TLC spots of Petroleum ether in chloroform mobile phase exposed to iodine vapours

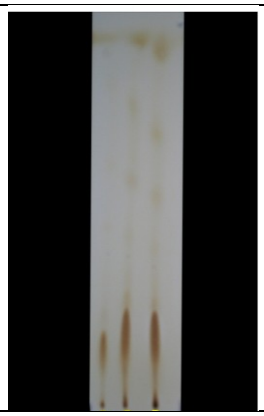

Fig.2 TLC spots of Petroleum ether extract in Petroleum Ether: Chloroform (6:3) mobile phase exposed to iodine vapours

Fig.3 TLC spots of Petroleum ether extract in Petroleum Ether: Chloroform (6:3) mobile phase Exposed to UV light at 366 nm

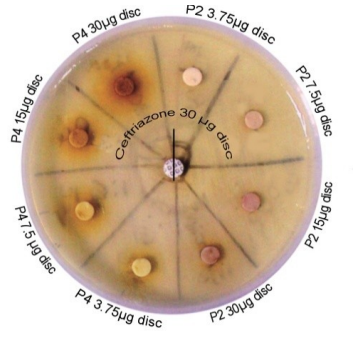

Fig.4 Area of zone of inhibition on Pseudomonas aeruginosa

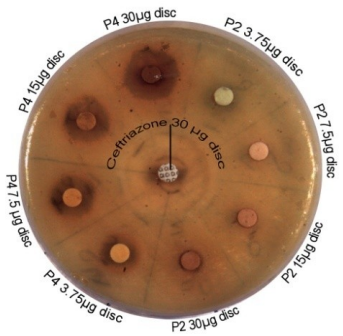

Fig.5 Area of zone of inhibition on Escherichia coli

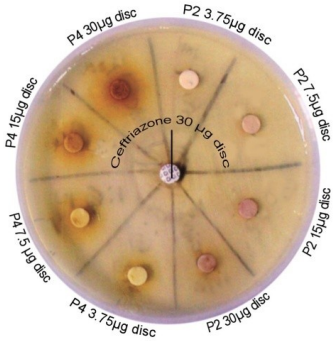

Fig. 6 Area of zone of inhibition on Staphylococcus aureus

P4 = Pistacia intergerrima

Conclusion:

On the global level there is an increasing demand to the *Ayurvedic* medicine and other traditional medicine. These medicines are now well recognized by International Community but there is a hesitation to accept well known *Ayurvedic* drugs because of the fact that the drugs are not standardize scientifically. These studies, which are based on preliminary phytochemical basis help to standardize the drug *Karkatshringi*. A detailed phytochemical analysis is necessary to understand the typical group of active components and clinical trial on such infectious diseases.

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