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In-vitro Antimicrobial Profile of *Pistacia integerrima* Galls Stewart

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Abstract: The objective of the present investigation was to explore the antimicrobial properties of *n*-hexane, ethyl acetate, chloroform and methanol fractions *Pistacia integerrima* and its phytochemical profiling. Phytochemical screening and antibacterial profile was performed before bulk extractions for identification of chemical constituents using standard procedures. The results showed that the various fractions of *P. integerrima* give positive results for alkaloids, terpenoids, flavonoids, reducing sugar, soluble starch, combined reducing sugars and tannins. The chloroform, ethyl acetate and methanol extract of galls showed significant activity against two Gram negative and one Gram positive bacterial stains and thus displayed highest inhibitory zone of (28:0 mm) at the tested concentration (22 mg/ml). The current investigation, suggest that *P. integerrima* used as a folk medicine for the treatment in hepatitis and liver disorders contain potential secondary metabolite which needs to explore.

Key words: Phytochemical screening · *Pistacia integerrima* · Galls · Antibacterial activity

INDRODUCTION

Pistacia integerrima (J. L. Stewart ex Brandis) belong to family anacardiacea. It is found in eastern Himalayan range from Indus to Kumaon [1] at a height of 12000 to 8000 feet. *P. integrrima* is a medium sized deciduous tree that can attain a height of forty feet. *Pistacia integrrima* is an important medicinal plant and is used as anti-inflammatory, antidiabetic agent, a blood purifier, a remedy for gastrointestinal disorders and as a expectorant. In India it is used as an herbal remedy for aliments such as cough, asthma, fever, vomiting and diarrhea [2-3]. In Pakistan, galls of *P. integrrima* are used for treatment of hepatitis and other liver disorders [4]. Galls of *P. integrrima* are used as herbal drug for diarrhea in northern India [5], infections, diabetes, pain, inflammatory conditions and fever [4, 6]. Monoterpenes triterpenoids sterols, dihydromalvalic acid and flavonoids have been isolated from the different parts of *Pistacia* species [4, 7]. In current investigation, we have made an effort to discover potential secondary metabolite responsible for its folk use in hepatitis and liver disorders.

MATERIALS AND METHODS

Collection of Plant Materials: The fresh galls of *Pistacia integrrima* galls were collected from the region of Toormang, Razagram area of district Dir, Khyber Pakhtunkhwa province of Pakistan in the month of February, 2010. The plant material was identified by Dr Abdur Rashid plant taxonomist Department of botany, university of Peshawar. A voucher specimen no (RF-895) was deposited in the herbarium of the same institution.

Extraction and Fractionation: Shade dried and crushed bark of *Pistacia integerrima* (Stewart) (14 kg) was subjected to cold extraction with MeOH. MeOH extract (600g) was suspended in water and successively partitioned with hexane, $CHCl_3$, EtOAc and Methanol as discuss earlier [8, 9].

Phytochemical Screening: The Chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of *P. integrrima* galls using standard procedures of [10-15] to identify the chemical constituents.

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Test for Alkaloids: About 0.2 g of each of the fractions was warm with 2% H_2SO_4 for few minutes .The reaction mixture were filter and added a few drops of Dragendroff,s reagent to each filtrate . Orange red precipitate indicates the presence of alkaloids moiety.

Test for Tannins: About 0.6gm of each extract was mixed with water and heated on water bath for 5 minutes and then filtered. A few drops of ferric chloride were added to each filtrate. A dark green colour formation shows the presence of tannins.

Test for Anthraquinone: About 0.5 g of each extract was boiled with 10 % HCL for few minutes on water bath. The reaction mixture was filter and allows to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10 % ammonia was added to each mixture and heated. Rose - pink color formation indicates the presence of anthraquinone.

Test for Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A Few drops of Feelings solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugar: Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Feling, s Solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Test for Saponins: 0.2 g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins Test for flavonoids: About 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCl were added. A yellow solution that turn colorless indicates the presence of flavonoids.

Test for Phlobatanins: About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Test for Steroids: Exact 2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H2SO4 (2ml). The color change from violet to blue or green in some samples indicates the presence of steroids.

Test for Terpenoids: About 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicates positive results for presence of terpenoids.

Test for Cardiac Glycoside: Exact 0.2mg of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated H_2SO_4 were added. Presence of greenish blue colour indicates the presence of cardiac glycosides.

Test for Caumarine: Exact 3 ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow colour indicates the presence of caumarine.

Test for Emodins: Exact 2ml of NH_4OH and 3ml of benzene was added to extract. Appearance of red color indicates the presence of emodins.

Test for Anthocyanin and Betacyanin: Exact 2ml of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100°C. Formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Test for Carbohydrates: Few drops of Molisch's reagent were added to each extract dissolved in distilled water; this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red/violet colour at the interphase of the two layers was a positive test for carbohydrates.

Test Monosaccharide's: About 0.25 g each portion was dissolved in distilled water and filtered. 0.5 ml of the filtrate was then mixed with 0.5 ml of Barfoed's reagent in a test tube and then heated on a water bath for a period of 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test

Test for Combined Reducing Sugars: About 0.5 g each extract was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide (NaOH) solution. To this solution few drops of Fehling's solution was added and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

Shinoda's Test for Flavonoids: About 0.5g of each extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

Lead Ethanoate Test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5 ml of each of the filtrate, 3 ml of lead ethanoate solution was then added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids.

Test for Soluble Starch: Small quantity of each portion was boiled with 1 ml of 5% KOH, cooled and acidified with H_2SO_4. A yellow coloration was taken as the presence of soluble starch.

Micro-Organism Collection and Maintenance: The following microbes were used for the antimicrobial studies: *Staphylococcus aureus*, *Strap Epidermis, Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* obtained from Centre for Phytomedicine and Medicinal Organic Chemistry, Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan. These organisms were kept in muller-hinton agar in the refrigerator at 4°C, prior to subculture.

Antimicrobial Assay: The antibacterial activity was done using modified agar well diffusion method according to standard protocol [16-17]. The muller-hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 hours. The broth culture (0.6 ml) of the test organism was placed in a sterile Petri-dish to which 20 ml of the sterile molten MHA was added. Holes were bored in to the medium using 0.2ml of the extract. Streptomycin was the standard antimicrobial agent at concentration of 2 mg /ml. Inoculation was done for 1 hour to make possible the diffusion of the antimicrobial agent into the medium. Incubation was done at 37°C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

RESULTS AND DISCUSSION

The phytochemical screening of the various solvent extracted fraction of *P. integerrima*, are listed in Table 2. The preliminary phytochemical screening of *P. integerrima* exposed the presence of bioactive phytochemicals such as alkaloids, terpenoids, flavonoids, reducing sugar and tannins. These bioactive secondary metabolites are responsible for the therapeutic potential of *P. integerrima*. The polar chemical constituents were detected in polar fractions and non-polar in non-polar fraction. Further it is clear from the results achieved that the number of phytochemical identified increases as polarity increases i.e. methanol showed the presence of maximum numbers of secondly metabolites while hexane and chloroform showed only few classes of natural products. The antibacterial assay of various fractions of *P. integerrima* is presented in Table 2. Different solvent soluble fractions such as *n*-hexane, chloroform, ethyl acetate and methanol of *P. integerrima* were screened for antibacterial assay. All the tested samples were showed significant activity is given in Table 1.

The result of the whole plant extracts of galls of *Pistacia integrrima* showed that ethyl acetate fraction contains a greater proportion of the chemical constituents. The medicinal value of the title plant can be associated to the presence of various bioactive phytoconsitutants present in it. The *n*-hexane, chloroform, ethyl acetate and methanol fractions of the plant showed the presence of terpenoids which exhibits antiviral and antibacterial [18] activities. The ethyl acetate and methanol extract showed the presence of alkaloids which exhibited various activities correlated to central nervous system [19], antihelmintic activity, aphrodisiac activity, antibiotic activity, treatment of venereal diseases and anti-malarial activity [9]. Our findings correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plants showed activity against Gram-positive strains only (few are active against Gram-negative bacteria).

The pharmacological activity of this plant can be confirmed from the antimicrobial activity of various fractions. The ethyl acetate fraction possessed activity against *Klebsiella pneumonia, Straptodirimu* and *B. stearothermophihus* with a zone of inhibition ranging from 10-24mm, showing its medicinal importance in the treatment of gastroenteritis and pneumonia. The methanolic extract was active against *Staphylococcus aureus, Klebsiella pneumonia Straptodirimu and B. stearothermophihus* with a zone of inhibition ranging from 14-28mm. The hexane extracts showing activity against, *Klebsiella pneumonia*, *Straptodirimu and B. stearothermophihus* with a zone of inhibition ranging from 10-16mm while chloroform extract is active against, *Klebsiella pneumonia Straptodirimu* and *B. stearothermophihus* with a zone of inhibition ranging from 18-24mm. With regard to the components responsible for the antimicrobial activity shown, several

Table 1: Antimicrobial activity (zone of inhibition in mm) of *Pistacia integrrima* galls

Bacterial strain	Gram	Streptomycin (2mg/ml)	n-Hexane	$CHCl_3$	EtOAc	MeOH
Escherichia coli	+	30	0	0	0	0
Staphylococcus aureus	+	32	0	0	10	14
Klebsiella pneumonia	-	30	16	22	24	28
Straptodirimu	-	32	15	24	24	28
B. stearothermophihus	+	30	10	18	20	24

Key words: 0 – not active; well size: 4mm

Table 2: Phytochemical test of n-hexane, chloroform, ethyl acetate and methanolic fractions of *P. integerrima* galls

Constituents	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	+	+
Steroids	+	-	-	+
Terpenoids	-	+	+	+
Flavonoids	+	+	+	-
Anthraquinone	-	-	-	+
Tannins	-	+	+	-
Phlobatanins	-	-	-	+
Saponins	-	-	-	-
Glycoside	-	-	-	+
Reducing sugars	-	-	+	-
Carbohydrates	-	+	+	+
Anthocyanin/Betacyanin	-	+	+	+
Caumarine	-	-	-	-
Emodins	-	-	-	-
Monosaccharide	-	-	-	-
Combined Reducing sugars	-	+	+	+
Soluble starch	-	+	+	+

Key words: – = absent, + = present, Frt = fraction

compounds of distinct nature must be acting as antimicrobial agents in these plants. This is not unexpected, since in the majority of Plant species studied so far there are a large variety of active principles, including naphthodianthrones, alkaloids, terpenoids, flavonoids, xanthones, tannins, essential oils and phloroglucinols [9]. Since the antimicrobial activity in other species of this genus has been found to be closely related to the levels of alkaloids and alkaloids derivatives [9], it is reasonable to assume that these compounds are responsible for the antimicrobial activities. Our results suggest that further work is needed to locate the active principles from the various extracts and that such efforts could result in the discovery of new compounds possessing a wide range of bioactivity.

CONCLUSIONS

Results of preliminary studies showed that *P. integerrima* has variety of bioactive secondary metabolite which needs to explore. Interesting results have been obtained during biological screening i.e. antibacterial assay of various solvent extracted fractions. In addition to this, present studies showed that the title plant requires extensive phytochemical and pharmacological profiling.

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