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Volatile oil composition and antimicrobial activity of the galls of *Pistacia integerrima* Stewart ex Brandis

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ABSTRACT

Pistacia integerrima Stewart ex Brandis (Pistaceaceae) is a moderate-sized deciduous tree, found in the Himalayas from Indus to Kumaon. Its galls are aromatic, astringent and expectorant and prescribed to treat asthma, phthisis, ailments of the respiratory tract, dysentery, vomiting of children, skin diseases, nose-bleed, snake-bite, scorpion sting, psoriasis, fever, to increase appetite and to remove bed humors. The volatile oil of the galls is composed mainly of benzyl benzoate (52.1%), α-pinene (35.9%), β-caryophyllene (4.9%) and n-tetradecanoic acid (2.3%). Among twelve monoterpenes, there are nine monoterpene hydrocarbons (39.1%) and two monoterpene alcohols (0.3%). There were two sesquiterpenes (6.1%) and one fatty acid (2.3%). The volatile oil and ethanolic extract of the galls showed significant antimicrobial activity.

KEYWORDS

Pistacia integerrima;
Pistaceaceae;
Galls;
Volatile oil composition;
Antimicrobial activity.

INTRODUCTION

Pistacia integerrima Stewart ex Brandis (Pistaceaceae), commonly known as kakra shingi, is a moderate-sized deciduous tree, found in the Himalayas from Indus to Kumaon[1]. Pale greenish-brown, hard, horn-shaped, rugose, hollow galls like excrescences are formed on the leaves and petioles of the plant which are produced by an insect of *Pemphigus* species[2]. The galls are aromatic, astringent and expectorant and are prescribed to treat asthma, phthisis, ailments of the respiratory tract, dysentery, chronic bronchitis, hiccough, vomiting of children, skin diseases, nose-bleed, snake-bite, scorpion sting, psoriasis, fever, to increase appetite and to remove bed humors[3]. *P.integerrima* has depressant action on the central nervous system[4], analgesic and anti-inflammatory activities[5-8]. Monoterpenes[4,9], triterpenoids[8-15], sterols[13,14,16], dihydromalvalic acid[17], aliphatic and phenolic constituents[14,16,19] and flavonoids[16,20] have been reported from *Pistacia* species. This paper describes the isolation and characterization of volatile oil from the galls of *P.integerrima* and antimicrobial activity of the volatile oil and ethanolic extract of the galls.

MATERIALS AND METHODS

Plant material

The galls of *P.integerrima* (3 kg) were obtained from Khari Baoli market, Delhi and identified by

Full Paper

Dr.M.P.Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen PRL/JH/09/18 is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi.

Preparation of ethanolic extract

Air-dried galls of *P.integerrima* (50 g) were coarsely powdered, defatted with petroleum ether (60-80ºC) and then extracted with ethyl alcohol (95%) for 48 hrs in a Soxhlet apparatus. The extract on removal of the solvent yielded a dark reddish brown viscous mass (2.9 g).

Extraction of volatile oil

The galls of *P.integerrima* (1 kg) were powdered and hydrodistilled using an all glass Clavenger apparatus. A colourless essential oil (1.1 %) was obtained. It was dried over anhydrous sodium sulfate and stored at 4°C in the dark.

GC analysis

The gas chromatographic analysis of the volatile oil was carried out on Shimadzu 2010 Gas Chromatograph (Japan) equipped with a flame ionization detector (FID) and ULBON HR-1 fused silica capillary column (60 m x 0.25 mm x 0.25 μm). The injector and detector (FID) temperatures were maintained at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 mL/min with column pressure of 155.1 kPa. The sample (0.2μl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230 °C at a rate of 3° C/min and then held at 230° C for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC-MS analysis

The GC–MS analysis of the volatile constituents were performed on a silicon DB-1 fused silica column directly coupled to the MS. The carrier gas was helium with a flow rate of 1.21 mL/min. Oven temperature was programmed as 50°C for 1 min and subsequently held isothermal for 2 min., injector port: 250°C, detector: 280°C, split ratio 1:50, volume injected: 1μL of the oil. The recording was performed at 70 eV, scan time 1.5 s; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station.

Identification of compounds

The individual compounds were identified by comparing their retention indices (RI) of the peaks on ULBON HR-1 fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature[21-24]. Relative amounts of identical components were based on peak areas obtained without FID response factor correction. The components of the oil, the percentage of each constituent and their RI values are summarized in TABLE-1. The constituents were arranged in order of GLC and GC-MS elution on silicon DB-1 and ULBON HR-1 fused silica column, respectively.

TABLE 1 : Chemical composition of the volatile oil of *P. integerrima* galls

S. No.	Components	RI	Percentage
1.	α-Pinene	928	35.9
2.	Camphene	940	0.3
3.	Sabinene	961	0.5
4.	β-Pinene	967	0.6
5.	Myrcene	974	0.3
6.	α-Terpinene	1001	0.2
7.	*p*-Cymene	1004	0.3
8.	Limonene	1018	0.7
9.	γ-Terpinene	1037	0.3
10.	*trans*-Verbenol	1120	0.1
11.	Borneol	1147	0.1
12.	α-Terpineol	1194	0.2
13.	β-Caryophyllene	1403	4.9
14.	Caryophyllene oxide	1551	1.2
15.	n-Tetradecanoic acid	1741	2.3
16.	Benzyl benzoate	1866	52.1

Antimicrobial activity

Test organisms and inoculums

Pure cultures of *Escherichia coli* (NCTC-6571) and *Staphylococcus aureus* (NCTC-10418) were ob-

tained from the Biotechnology Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Antimicrobial standard

Aimikacin discs with specific activity of 30 μg was obtained from the Department of Microbiology, Majeedia Hospital, New Delhi.

Media

Dehydrated nutrient agar media was prepared in distilled deionized water. The media (g/100 ml) was composed of peptone (5.1 g), sodium chloride (5.0 g), beef extract (1.5 g), yeast extract (1.5 g) and agar (1.5 g).

Preparation of media

Dehydrated nutrient agar medium (28 g) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

Sterilization of media

The conical flask containing the nutrient agar medium was plugged with the help of a non-absorbent cotton plug. The mouth of the conical flask and the cotton bung were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15-lbs/in^2 pressure for 20 minutes.

Preparation of test organisms

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37^{0}C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hours at 37+2^{0}C. The growth from the nutrient surface was washed using 50 ml of distilled water. A dilution factor was determined which gave 25 % light transmission at 530 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Temperature control

Thermostatic control is required in several stages of a microbial assay when culturing a micro-organism and preparing its inoculums and during inoculation in a plate assay.

Cup-plate method

A previously liquefied and sterilized medium was poured into plastic Petri-plates of 100 mm size. Sixteen plates in duplicate were prepared and kept for solidifying. Four holes were made in each plate with a stainless steel borer having 6 mm internal diameter. Different dilutions of the alcoholic extract and volatile oils of *P.integerrima* galls were made having concentration of 200 μg, 100 μg and 50 μg / 0.1 ml of solution. Aimikacin discs of 30 μg concentration was used as standard (S). The plates were labelled as Co (control), S (standard), A (200 μg / 0.1 ml), B (100 μg / 0.1 ml) and C (50 μg / 0.1 ml) corresponding to different holes. The plates were divided into four groups (I, II, III and IV) comprising four plates in each group. In group-I, *P.integerrima* extract was used as test solution. Each group contained 2 plates each for *E. coli* and *S. aureus*. The test solutions were made in DMSO (dimethyl sulphoxide) solvent which was used as control. Micropipette was used to deliver the solutions into the holes. The volume of solution added to each hole was kept uniform (0.1 ml in each hole). One strip of Aimikacin (standard) was placed aseptically to the centre of each plate. One hole was kept for blank (Co). The plates were then left for standing for 1 hour for proper diffusion of the drug solutions. They were incubated for about 24 hours at 32 ± 2^{o}C. After 24 hours the plates were examined and the diameters of zones of inhibition were accurately measured.

RESULTS AND DISCUSSION

Hydrodistilled volatile oil obtained from the galls of *P.integerrima* was analyzed by GC and GC-MS. The oil composition is summarized in TABLE 1. The components are arranged in order of GC elution on silicon DB column. About sixteen constituents are detected in the volatile oil. It was characterized by a large number of monoterpenes. Among twelve monoterpenes, there were nine monoterpene hydrocarbons (39.1%) and two monoterpene alcohols (0.3%). There were two sesquiterpenes (6.1%) and one fatty acid (2.3%). Benzyl benzoate was the only aromatic constituent occurring

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predominantly in 52.1%. The other prominent constituents were α-pinene (35.9%) and β-caryophyllene (4.9%). *n*-Tetradecanoic acid (2.3%) was the only aliphatic compound present in the oil. An earlier report of the volatile constituents of the galls and stem bark of *P.integerrima* indicated the presence of 38 components in the galls of which 91% were found to be monoterpenes. The bark oil was found to contain 22 constituents of which 82.3% were the monoterpenes[25].

TABLE 2 : Antimicrobial activity of ethanolic extract of *P. integerrima* galls

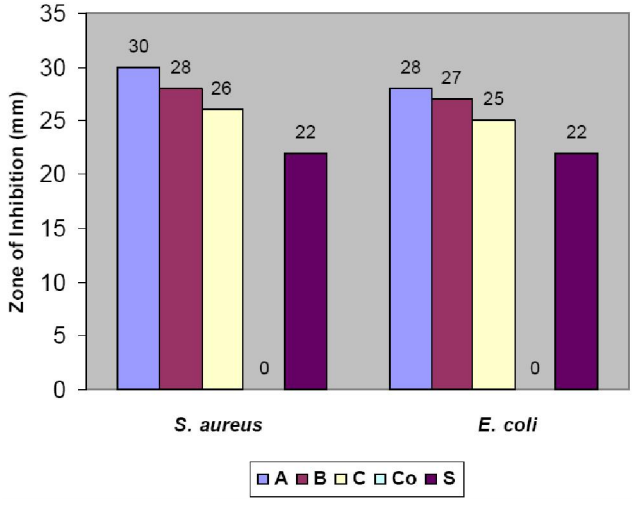
Sample code	Sampleconc. (µg)	Zone of inhibition (mm) against *S.aureus*	Zone of inhibition (mm) against *E.coli*
A	200	30	28
B	100	28	27
C	50	26	25
Co	Control	00	00

A=200 µg / 0.1 ml, B=100 µg / 0.1 ml, C =50 µg / 0.1 ml, Co =control, S=standard

TABLE 3 : Antimicrobial activity of volatile oil of *P. integerrima* galls

Sample code	Sample conc. (µg)	Zone of inhibition (mm) agains *S. aureus*	Zone of inhibition (mm) against *E. coli*
A	200	31	26
B	100	29	24
C	50	27	21
Co	Control	00	00
S	Standard	22	18

A=200 µg / 0.1 ml, B=100 µg / 0.1 ml, C =50 µg / 0.1 ml, Co =control, S=standard



A=200 µg / 0.1 ml, B=100 µg / 0.1 ml, C =50 µg / 0.1 ml, Co =control, S=standard

Figure 1 : Antimicrobial activity of ethanolic extract of *P. integerrima*.

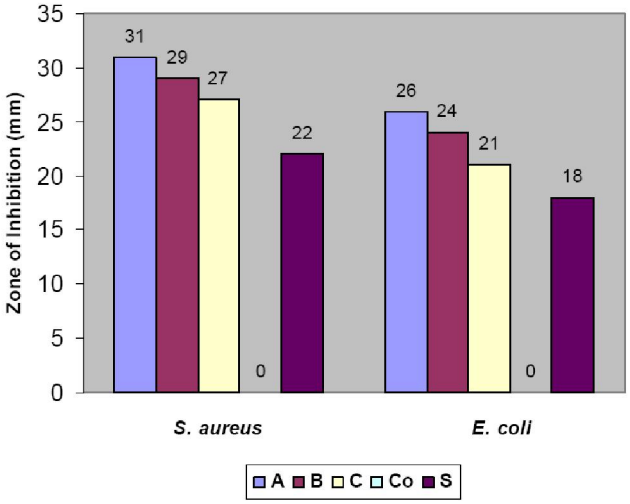


Figure 2 : Antimicrobial activity of volatile oil of *P. integerrima*

CONCLUSION

The volatile oil obtained from the galls of *Pistacia integerrima* procured from Delhi was composed mainly of benzyl benzoate (52.1%) followed by α-pinene (35.9%), β-caryophyllene (4.9%) and n-tetradecanoic acid (2.3%). The volatile oil and the ethanolic extract of the galls showed significant antimicrobial activity.

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REFERENCES

[1] Anonymous; The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products. Publication and Information Directorate, CSIR, New Delhi, **8**, 120 **(1998)**.

[2] R.N.Chopra, I.C.Chopra, K.L.Handa, L.D.Kapoor; Indigenous Drugs of India, Academic Publishers, Delhi, 377**, (1982)**.

[3] R.N.Chopra, R.K.Badhwar, S.Ghosh; Poisonous Plants of India. Indian Council of Agricultural Research, New Delhi, 270**, (1965)**.

[4] S.H.Ansari, M.Ali, J.S.Qadry; Essential oils of *Pistacia integerrima* galls and their effect on the

central nervous system. Internat. J.Pharmacognosy, **31**, 89–95 **(1993)**.

[5] S.H.Ansari, M.Ali, J.S.Qadry, N.Siddiqui; Analgesic activity of tetracyclic triterpenoids isolated from *Pistacia integerrima* galls. Update Ayurveda-94, **73,** (1994).

[6] S.H.Ansari, M.Ali; Analgesic and anti-inflammatory activity of tetracyclic triterpenoids isolated from *Pistacia integerrima* galls. Fitoterapia, **67**, 103–105 **(1966)**.

[7] N.S.Ahmad, M.Farman, M.H.Najmi, K.B.Mian, A.Hasan; Pharmacological basis for use of *Pistacia integerrima* leaves in hyperuricemia and gout. J.Ethnopharma., **117**, 478–482 **(2008)**.

[8] N.S.Ahmad, A.Waheed, A.M.Farman, A.Qayyum; Analgesic and antiinflammatory effects of *Pistacia integerrima* extracts in mice, J.Ethnopharm., **129**, 250–253 **(2010)**.

[9] P.Monaco, L.Previtera, L.Mangoni; Terpenes in Pistacia plants: a possible defence role for monoterpenes against gall-forming aphids; Phytochemistry, **21**, 1408 **(1982)**.

[10] P.Monaco, R.Caputo, G.Palumbo, L.Mangoni; Triterpene components of galls on the leaves of *Pistacia terebinthus* produced by *Pemphigus semilenarius;* Phytochemistry, **13**, 1992 **(1974)**.

[11] R.Caputo, L.Mangoni, P.Monaco, G.Palumbo; Triterpenes from the galls of *Pistacia palestina.;* Phytochemistry, **18**, 896–898 **(1979)**.

[12] S.H.Ansari, M.Ali, J.S.Qadry; New tetracyclic triterpenoids from *Pistacia integerrima* galls; Pharmazie, **49**, 356–357 **(1994)**.

[13] R.Caputo, L.Mangoni; Triterpenic acids of *Pistacia terebinthus* galls. Gazzetta Chimica Italica, **100**, 317–325 **(1970)**.

[14] S.Ahmad, M.Ali, S.H.Ansari, F.Ahmad; Phytoconstituents from the galls of Pistacia integerrima Stewart. J.Saudi Chem.Soc., **14**, 409-412 **(2010)**.

[15] G.Uddin, A.Rauf, M.W.Arfan, I.Khan, M.Ali, M.Taimur, I.Ur-Rehman, Samiullah, Pistagremic acid, a new leishmanicidal triterpene isolated from *Pistacia integerrima* Stewart. J.Enzyme Inhib.Med.Chem., **27**, 646-648 **(2012)**.

[16] T.Hiroi, T.Takahashi, H.Imamura; Wood extractives. XV. Constituents of *Pistacia chinensis* Wood. Nippon Mokuzai Gakkaishi, **12**, 324–326 **(1966)**.

[17] J.R.Vickery; The occurrence of dihydromalavalic acid in some seed oils. J.Amer.Oil Chem.Soc., **58**, 731–732 **(1981)**.

[18] S.Ahmad, M.Ali, S.H.Ansari; Phenolic constituents from the galls of Pistacia integerrima Stewart; Indian J.Chem, **50B**, 115-118 **(2011)**.

[19] K.Mehta, S.Balwani, A.Kulshreshtha, D.Nandi, P.Jaisankar, B.Ghosh; Ethyl gallate isolated from *Pistacia integerrima* Linn. Inhibits cell adhesion molecules by blocking AP-1 transcription factor. J.Ethnopharmacol, **137**, 1345-52 **(2011)**.

[20] S.H.Kalidhar, P.Sharma; Chemical components of *Pistacia integerrima*. J.Indian Chem.Soc., **62**, 261 **(1985)**.

[21] W.S.Jennings, R.P.Adams; Identification of essential oil components by gas hromatography/Qudrupol mass spectrometry. Allured publication Corp Carol Stream, Illinois, **(2001)**.

[22] T.Shibamoto; Qualitative Analysis of Flavour and Fragrance Volatiles by Capillary Gas Chromatography. Academic Press: New York, **(1980)**.

[23] M.Ali; Techniques in Terpenoid Identification. Birla Publications, Delhi, **(2001)**.

[24] D.Joulain, W.Konig; The Atlas of spectral data of sesquiterpenes hydrocarbons, E.B.Verlag, Hamburg, Germany, **(1998)**.

[25] S.H.Ansari, M.Ali, A.Velasco-Negueruela, M.J.Perez-Alonso; Volatile constituents of the Galls and Stem bark of *Pistacia integerrima* Stew. Ex Brandis.J.Essent.Oil Res., **10**, 313-316 **(1998)**.