

Full Length Research Paper

Chemical composition and biological screening of essential oils from *Pistacia integerrima*

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The chemical composition and biological screening of the essential oils of the gall of *Pistacia integerrima* was performed. Volatile chemical constituents were obtained by hydrodistillation followed by liquid-liquid extraction. The oil was found rich in 1-Tepinen-4-ol (28.82%), p-meth-1-en-8-ol, (43.38%), n-Octyl acetate (19.91%), and beta-Farnesene (7.88%). The concentration of α -terpinolene, limonene and α -thujene were less than 1%. The essential oils at the tested concentration (10, 100 and 1000 $\mu\text{g/ml}$) showed moderate to maximum phytotoxic effect. The maximum effect was observed with 1000 $\mu\text{g/ml}$ (80%) followed by 100 $\mu\text{g/ml}$ (60%) and 10 $\mu\text{g/ml}$ (50%). The tested oils exhibited promising antibacterial activities. The zone on inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Straptodirimu*, *Bacillus stearothermophilus* and *Salmonella typhimurium* was 16, 18, 26, 22, 18 and 20 mm, respectively. In case of antioxidant activity, the oils exhibited a concentration dependent free radical scavenging effect.

Key words: *Pistacia integerrima*, essential oil, gas chromatography-mass spectrometry (GC-MS), antibacterial, antioxidant and phytotoxic.

INTRODUCTION

Pistacia integerrima Stewart is known as kakarsinghi. It is 18 m moderate sized deciduous tree found in the Himalayas from Indus to Kumaon (Uddin et al., 2011). An insect of *Pemphigus* species forms hard, horn shaped, rugose, hollow galls like excrescences on the leaves and petioles of the plant (Chopra et al., 1982). Dry crushed galls have a very sharp and to some extent bitter taste and terebinthine odour. The galls are aromatic, astringent and expectorant and highly valued in Indian medicine as a medication for asthma, phthisis and other disorders for the respiratory tract; they are also useful in dysentery, chronic bronchitis, hiccough, vomiting of children, skin diseases, psoriasis, fever, to increase hunger and to

remove bed humors (Chopra et al., 1965). Sushruta used the galls in combination with other drugs for the treatment of snake bite and scorpion sting (Uddin et al., 2011). On the central nervous system *P. integerrima* has depressant action (Ansari et al., 1993), it has analgesic and anti-inflammatory activities (Ansari et al., 1996) and hyperuricemia effect (Ahmad et al., 2008), monoterpenes (Ahmad et al., 2010), triterpenoids (Monaco et al., 1975), sterols, (Uddin et al., 2011), dihydromalvalic acid (Vickery, 1981) and flavonoids (Kalidhar and Sharma, 1985) have been reported from the different parts of *Pistacia* species. The literature review revealed that no remarkable work has been carried out for the determination of essential

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oil in *P. integerrima* galls oil which is needed in order to explore its pharmacological importance. The objective of this study was to analyse the essential oils of the galls of *P. integerrima* and evaluate their antioxidant, antibacterial and phytotoxic profile.

MATERIALS AND METHODS

Plant specimens

P. integerrima galls were collected from Toormang, Razagram Khall area of district Dir, Khyber PukhtunKhawa province of Pakistan in the month of February, 2010. The plant material was identified by plant taxonomist, at the Department of Botany, University of Peshawar, Pakistan. A voucher specimen (No. RF-895) was deposited in the herbarium of the said department.

Essential oil extraction

A modified Clevenger type Dean Stock apparatus were used for the extraction of essential oil from the galls of *P. integerrima* through hydrodistillation. The galls were shade dried, grind and subjected to hydro-steam distillation for about 5 h. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated (liquid-liquid extraction) out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labeled (RF2) and stored in light resistant vials at 4 to 6°C for further use (Essien et al., 2008).

Preparation of sample

Approximately 40 mg of oil sample that weighed accurately up to 0.1 mg, together with 2 ml of dichloromethane, were filtered through 0.45 µm-membrane filter and were injected (1 µl) to GC-MS using auto injector system.

Analysis of the essential oil

The essential oil from the galls of *P. integerrima* were analyzed by Gas Chromatograph (Shimadzu) hyphenated to a Mass Spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. Helium was used as the carrier gas. All chromatographic separations were performed on a capillary column (TRB-FFAP; Technokroma) having specifications: length, 30 m; id., 0.25 mm; thickness, 0.25 µm; treated with polyethylene glycol. Other GC-MS conditions are: ion source temperature (EI): 240°C; interface temperature: 240°C; pressure: 80 KPa; solvent cut time, 1.5 min. 1 µl of the sample and standard were injected into the GC column. Injector was operated in a split mode with a split ratio 1:50. Injection temperature was 240°C. The column temperature program started at 40°C for 3 min and changed to 90°C at the rate of 15°C min⁻¹. The temperature was raised to 240°C at the rate of 2.5°C min⁻¹ and was held for 5 min. Then the temperature was increased to 220°C at the rate of 10°C min⁻¹ and was kept constant for 5 min. Total elution time was 15 min. MS scanning was performed from *m/z* 40 to 500. GC-MS solutions software provided by the supplier was used to control the system and to acquire the data. Oil sample was prepared and injected to GC-MS

for further operation (Cunniff, 1985).

Identification of the compounds was carried out on comparison of the retention times (RT) and mass spectra of the samples with those obtained from standards used. Relative percentage of the was calculated from the total chromatogram by computer (Cunniff, 1985).

Antioxidant assay

The antioxidant activity of the essential oil of *P. integerrima* galls was determined spectrophotometrically using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay (Uddin et al., 2012b,c) with slight modification. 25 mg of oil was taken and dissolved in distilled methanol and diluted up to 50 ml. From this stock solution, different micrograms solution of 20, 40, 50, 60, 80 and 100 µg/ml were prepared by dilution method. 5 ml of each solution was taken in a test tube and 1 ml of 0.001 M of DPPH solution was added to it. All these solutions were kept in dark for 30 min. Also 5 ml methanol was taken and 1 ml of DPPH solution was added, for control solution. At the end of incubation period, the mixtures were examined for the antioxidant activity using Optima UV-Visible spectrophotometer at wavelength of 517 nm. The experiments were performed with triplicate readings. Percent DPPH was determined using the formula as follows:

$$\text{DPPH (\%)} = \frac{\text{Control abs} - \text{Extract abs}}{\text{Control}} \times 100$$

Antibacterial activity

The antibacterial activity was performed by using the well diffusion method described earlier by Uddin et al. (2012c, d) with slight modifications. Mueller Hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 h. 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 Mueller Hinton Broth (MHB) was added. Holes were bored in to the medium using 0.2 ml of the oil. Streptomycin was the standard antimicrobial agent at a concentration of 2 mg/ml. Inoculation was done for 1 h to make possible the diffusion of the antimicrobial agent into the medium. After incubation for 24 h at 37°C, the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeter (mm). The bioassays were performed in triplicate.

Phytotoxic assay

In the present investigation, the essential oils extracted from *P. integerrima* was tested against Lemna minor (Ahn et al., 1995; Rauf et al., 2012b). In this bioassay, three conical flasks were inoculated with a sufficient stock solution of 20 mg/ml to achieve a final concentration of 500, 50, and 5 µg/ml, respectively. Each conical flask was then added a 20 ml medium of 10 plants, each one containing Rosette of three fronds. Parquet was used as a standard growth inhibitor. The whole flasks were kept in growth cabinet for incubation up to seven days. After this growth, regulation in percentage was determined with reference to the negative control.

RESULTS AND DISCUSSION

The oil was found rich in 1-Tepinen-4-ol (28.82%), p-meth-1-en-8-ol (43.38%), *n*-Octyl acetate (19.91%), and betaFarnesene (7.88%) Table 1. The concentrations of α-

Table 1. Chemical composition of essential oils of gall of *P. integerrima*.

ID No.	Name	Area	Concentration (%)	RT	SD
1	1-Tepinen-4-ol	9043	28.82	19.618	0.0001
2	p-meth-1-en-8-ol	13611	43.38	20.051	0.0001
3	n-Octyl acetate	6248	19.91	20.58	0.0001
4	beta-Farnesene	2472	7.88	24.919	0.0000
5	α -terpinolene	2566	0.163	18.81	0.0000
6	Limonene	20488	0.122	19.44	0.0002
7	α -thujene	6414	0.023	20.02	0.0003

Table 2. Antibacterial activity of essential oils from gall of *P. integerrima*.

Bacterial strain	Strain	Streptomycin	DMSO	Zone of inhibition (mm)
<i>Escherichia coli</i>	+	28	-	16
<i>Staphylococcus aureus</i>	+	30	-	18
<i>Klebsiella pneumonia</i>	-	30	-	26
<i>Straptodirimu</i>	-	30	-	22
<i>Bacillus stearothermophilus</i>	+	28	-	18
<i>Salmonella Typhimuriun</i>	+	28	-	20

+: Active; -: inactive

terpinolene, limonene and α -thujene were less than 1%. The essential oils at the tested concentration (10, 100 and 1000 $\mu\text{g/ml}$) showed moderate to maximum phytotoxic effect. The maximum effect was observed with 1000 $\mu\text{g/ml}$ (80%) followed by 100 $\mu\text{g/ml}$ (60%) and 10 $\mu\text{g/ml}$ (50%) as presented in Table 2.

In case of antibacterial assay, the tested oils showed outstanding effect against various Gram positive and Gram negative bacterial as presented in Table 3. The negative control used was DMSO and the positive control was taken as streptomycin (2 mg/ml). In comparison with negative control, almost all the tested bacteria exhibited promising antibacterial activities. The zone on inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Straptodirimu*, *Bacillus stearothermophilus* and *Salmonella typhimurium* was 16, 18, 26, 22, 18 and 20 mm, respectively.

In case of antioxidant activity, the oils were tested for their DPPH free radicals scavenging properties. The standard antioxidant drug used was quercetin. The oils exhibited a concentration dependant free radical scavenging effect as shown in Figure 1.

Infectious diseases are one of the biggest problems of this modern world. The spread of infectious diseases is on its full swing; this has not been seen before. The situation has become worse because of certain reasons which include some problems that human beings are facing such as high growth rate of population, deterioration of environment by fast industrialization, travelling long distances, irrational use of antibiotics and last but

not the least, the resistance developed by microorganisms against the available antibiotic. The 20th century has seen a rise in deaths due to microbial infectious diseases (Gregory et al., 1999). This microbial infections are not only the problem of third world or other developing countries, but many very developed countries, where there is greater understanding of the microorganism and their control, also face this problem (Wu et al., 1999).

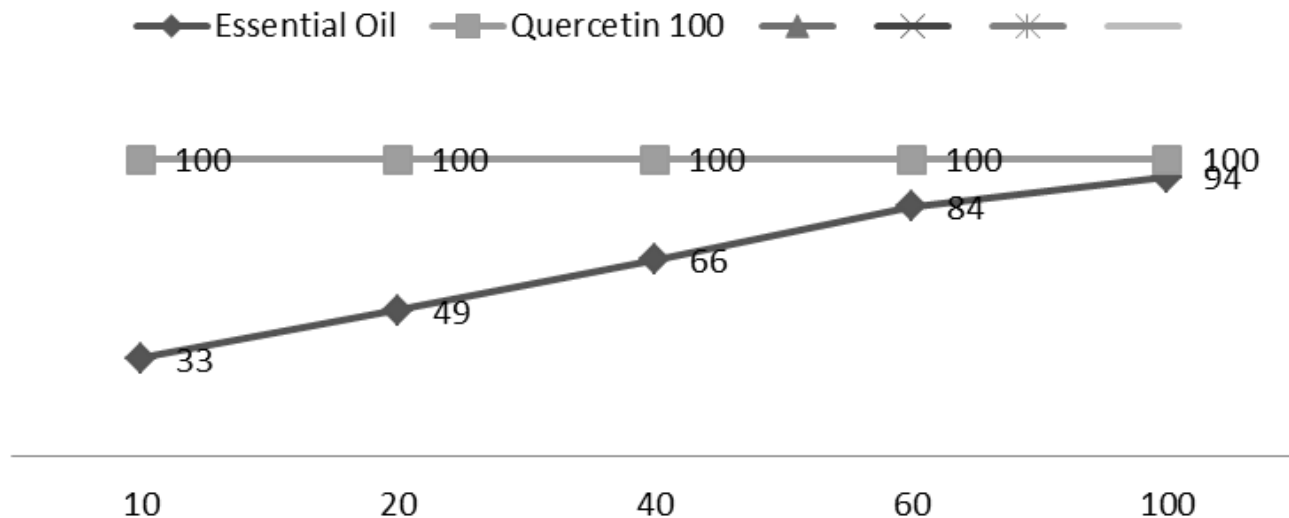
Various antibiotic resistant strains of bacteria have been found. The epidemics due to the resistant strains have been declared as medical disaster (Butler and Antony, 2006); the problem of resistant is growing day by day since the penicillin resistant bacteria have been found. *S. aureus* and *Mycobacterium tuberculosis* are the two examples (Muhammad and Saeed, 2011). This situation has turned into havoc when today almost every antibiotic has resistant strains against them. Vancomycin was left, but in recent years it has been reported that vancomycin has also developed resistant strains. Major contributor to the death all over the world is *Streptococcus pneumoniae* and penicillin-resistant *S. aureus* (Wu et al., 1999). In these connections, the essential oils of our tested plant are the best tool to further work on the isolation of pure chemical moieties.

There are number of plants, plant products and various isolated compounds that have been proven to show phytotoxicity to other plants, so that they will become a good candidate for being a herbicide (Barkatullah and Ali, 2012). Due to these certain advantages, compound like

Table 3. Phytotoxic assay of essential oil of *P. integerrima* galls.

Oil	Concentration ($\mu\text{g/ml}$) of sample	Fronds survived	Fronds died	Growth regulation (%)
Essential oil	10	10	10	50
	100	8	12	60
	1000	4	16	80

Total no. of fronds: 20; Concentration of standard drug: 0.015 $\mu\text{g/ml}$.

**Figure 1.** DPPH radical scavenging activities of essential oil of gall *P. integerrima*.

pyrethrum and rotenone were being imported from all over the world.

Herbicides play a key role in weeds management system, because they not only help the farmers to increase yield but also decrease labor for them. Due to the frequent use of herbicides, a problem of resistance has arisen against these weedicides. The species have a tendency to transform into some other species that are very much similar to the useful plants. This indiscriminate use also produces many health and environmental pollution problems. According to Molisch (1937), allelopathy is an emerging branch of applied sciences which studies any process primarily involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological and agricultural systems, including positive and negative effects. So there is need to discover new natural drugs which can help farmers to kill weeds.

Conclusion

The isolated crude essential oils are good antioxidant as well as having broad spectrum antibacterial properties. Therefore, the present research work strongly recommends

the use of these oils as antioxidant and antibacterial for various bacterial infections.

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