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Full Length Research Paper

Pharmacognostic and phytochemical investigation of the stem bark of *Pistacia integerrima* Stew ex Brandis

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Pistacia integerrima, **is a well known plant among Pakistani indigenous medicinal plants and used very commonly in the management of various diseases. In this research work the pharmacognostic profile, phytochemical and physicochemical parameters of the *P. integerrima* bark was carried out for standardization, quality, purity and sample identification. Macroscopic and microscopic characteristics as well as transverse section of the bark were studied. The bark contains various secondary metabolites such as alkaloids, tannins, flavonoids etc. The crude methanolic extract and its subsequent solvent fractions were tested for their phytochemical contents and physicochemical parameters such as ash values, moisture contents and extractive values. These pharmacognostic findings will be helpful for establishing parameters for the standardization, prevention from adulteration and identification of *P. integerrima* Stew ex Brandis bark used commonly in traditional medicine.**

Key words: *Pistacia Integerrima* bark, pharmacognostic, phytochemical contents, physicochemical parameters and microscopy.

INTRODUCTION

Plants have provided man with all his needs in terms of shelter, clothing, food, flavours, fragrances, as well as medicines. Plants have formed the basis for sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies (Ameenah, 2006). The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value (Nisar et al., 2007; Asakawa, 2007). The use of medicinal plants has a long history throughout the world and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries. Ayurveda, Unani, Kampo, and traditional Chinese medicine have flourished as systems of medicine in use for thousands of years (Ameenah, 2006). The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000 (Farnsworth, 1990), with a lower level at 215,000 and an upper level as high as 500,000 (Schultes, 1972). Of these, only about 6% have been screened for biological activity, and 15% have been evaluated phytochemically (Schultes, 1972). World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth et al., 1985).

In Pakistan a huge population is using the phytomedicines for various aliments like diabetes, fever, arthritis, wound healing etc., such treatments are provided by a large number of local practitioners in the form of hakims. Most of the local hakims have no idea in identification of plants in the same genus. As in the same genus a large number of plants are present with little differences in their morphology. Pharmacognostic studies help us in establishing such parameters which are suitable for the identification and standardization of plants. *P. integerrima* Stew ex Brandis belongs to family

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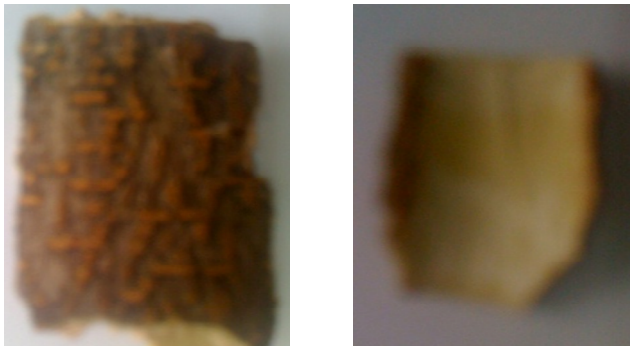

External view Internal view

Figure 1. Macroscopic study of bark.

Anacardiaceae, The plant is up to 25 m tall, single stem, deeply roots, leaves are 25 cm long pinnate bearing 2 to 6 pairs of lanceolate and long leaflets. Flowers are small and red in color. Fruits are globular having diameter of 5 to 6 mm blue or purplish when matured. The bark is of light brown color. It is widely distributed in the sub-alpine regions of Himalaya ranging from Indus to Kumaun at an altitude of 350 to 400 m (Ahmad et al., 2010) also found in Afghanistan and in various localities of Pakistan (Ahmad et al., 2006). The plant is used in traditional medicine for the treatment of fever, cough, wound infections, Rheumatic pain, asthma, diarrhea, and jaundice (Matin et al., 2001; Jain and Puri, 1984). The phytochemical constituents of leaves of *P. integerrima* Stew ex Brandis have been reported (Ahmad et al., 2008). Triterpenoids from galls of *P. integerrima* Stew ex Brandis has been isolated (Ansari and Ali, 2006). There is no published pharmacognostic study on the bark of *P. integerrima*; therefore, this research work will provide such studies for the first time.

MATERIALS AND METHODS

Plant materials

The bark of *P. integerrima* bark was collected in April 2010 from district Buner, Khybar Pukhtonkhuwa,Pakistan. The plant was authenticated by Chairman, Department of Botany Prof. Dr. Muhammad Ibrar University of Peshawar. A voucher specimen (No.10420Bot) was kept in the herbarium at Botany Department, University of Peshawar for future reference.

Preparation of different solvent extracts

P. integerrima bark was air dried and ground into powder. The powder (4.5 kg) was extracted with methanol by maceration. The methanolic extract was concentrated to dryness in vacuo. The methanol extract thus obtained was further fractionated using the solvent-solvent extraction method (Saeed et al., 2010; Ruffa et al., 2002). Thus n-hexane, chloroform, ethyl acetate, butanol, and aqueous fractions were obtained.

Morphological features

Macroscopic features

Macroscopic appearance of fresh bark and microscopy of dried powered bark was carried out. The color, shape, size, surface, odor and taste of the drug were studied.

Microscopic features

Thin hand Transverse section of the bark was prepared. The material was mounted in center of potato pith and a transverse cut were made across the material with the help of a sharp razor and was kept moist in water. The staining was carried out by putting the section in safranin for 3-4 minutes. The section was then dehydrated in 10%, 30%, 50%, and 90% of alcohols. The dehydrated section was then put into a drop of methylene green and dehydrated again in absolute alcohol for 2-3 minutes. Finally the section the section was mounted on Canada balsam to make them permanent and examine under Olympus Digital microscope (MIC-D). The powder drug was separately treated on glass slide, mounted in Canada balsam and was subjected to microscopic examinations (Akcin et al., 2010; Evans, 2002).

Qualitative phytochemical tests

Different qualitative chemical tests of the n-hexane, chloroform, ethyl acetate, butanol, methanol and aqueous fractions were performed for the determination of alkaloids, tannins, phenolic compounds, flavonoids, saponins, sterols and carbohydrates, following the recommended standard methods (Shome et al., 1984).

Physiochemical parameters

Total ash, water soluble ash, acid insoluble ash, acid soluble ash, percentage of loss on drying, moisture contents and extractive values were determined, following the well established procedures (Evans, 2002; Shome et al., 1984).

RESULTS AND DISCUSSION

Macroscopic features

The bark is grayish-brown in color, thick and hard. The external surface has striations, fractures and is rough. The pieces are 2 to 3 cm long 0.5 to 1.2 cm broad and 2 to 4 mm thick.

The inner surface is slightly smooth, sticky and yellowish in color, attached with the wood as shown in Figure 1. The dried powder is light brown in color and rough, having bitter taste and pungent odor.

Transverse section

The transverse section shows that stem bark consists of lenticels, cork cells, phellogen, phelloderm, cortex, phloem, oil cells and medullary rays. The phloem consists of sieve tubes, fibers and is transverse by the medullary rays. Pith is also present in the bark. The transverse section is shown in Figure 2.

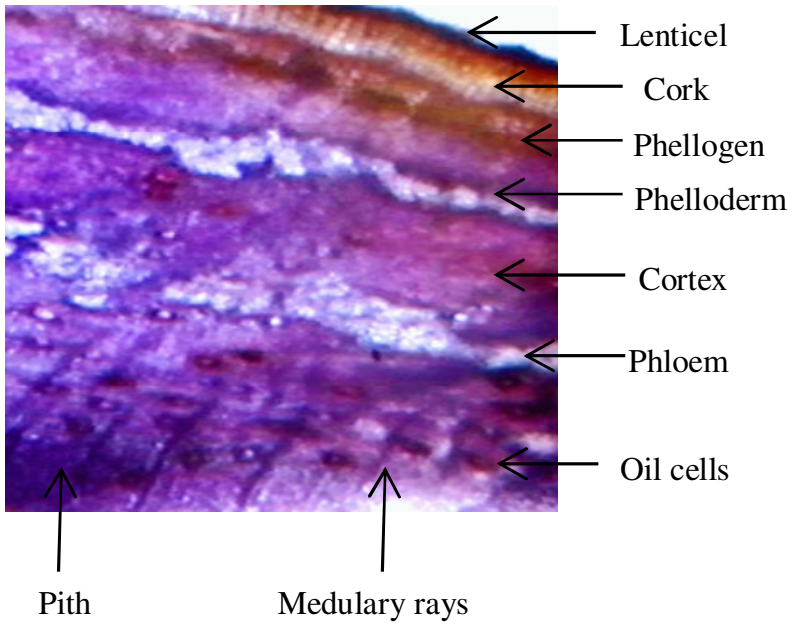

Figure 2. Microscopic study of the transverse section of the bark.

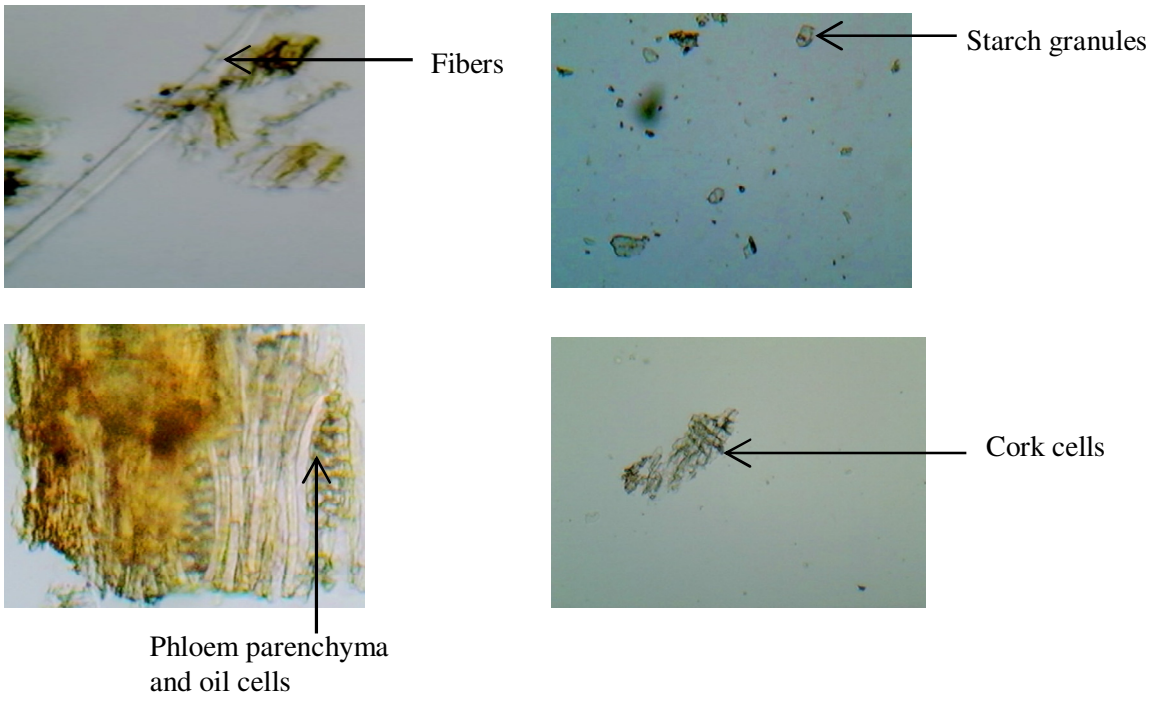

Figure 3. Microscopic study of the powered bark.

Powder microscopy

Microscopic examination of powder revealed the presence of starch granules, fibers, phloem, cork cells, parenchyma and oil cells as shown in Figure 3.

Qualitative phytochemical tests

The phytochemical test of different solvent extracts and their results are tabulated in Table 1. The crude methanolic extract showed positive results for all of the

Table 1. Preliminary phytochemical profile of *Pistacia Integerrima* bark.

S/N	Phytochemical constituents	Tests	ME	HE	CHE	EE	BE	AQE
1.	Alkaloids	Mayer's test	+	-	+	+	-	-
		Wagner's test	+	-	+	+	-	-
		Hager's test	+	-	+	+	-	-
2.	Carbohydrates	Molisch's test	+	-	-	-	-	+
		Benedict's test	+	-	-	-	-	+
		Bromine water test	+	-	-	-	-	+
3.	Tannins	Ferric chloride test	+	-	+	+	+	+
		Alkaline reagent test	+	-	+	+	+	+
4.	Flavonoids	Ferric chloride test	+	+	+	+	+	+
		Sodium hydroxide test	+	-	+	+	+	+
5.	Sterols	Liebermann-Burchard test	+	-	+	+	+	+
		Salkowski's test	+	-	+	+	+	+
6.	Saponins	Frothing test	+	-	-	+	+	+
7.	Terpenoids	Liebermann-Burchard test	+	-	-	-	-	+

Where + = Positive and - = Negative, *HE = n-hexane extract, *CHE = Chloroform extract, *EE = Ethyl acetate extract, *BE = Butanol extract, *ME = Methanol extract, *AQE = Aqueous extract.

tested phytochemicals, the alkaloids, tannins, flavonoids and sterols were observed in chloroform and ethyl acetate fractions. Tannins, flavonoids, sterols and saponins were observed in butanol fraction while aqueous fraction give positive results for the presence of carbohydrates, tannins, flavonoids, sterols and saponins. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the folklore of *P. integerrima* bark. For example, some alkaloids have been reported to have anticancer and antiviral activity, saponins have been reported to be cardiotonics, while flavonoids have anti-inflammatory activity (Evans, 2002; Manthey et al., 2001; Haslam, 1996). The presence of tannins may be responsible for the ability of *P. integerrima* bark to cure diseases such as diabetes, diarrhea, and dysentery. The presence of flavonoids in *P. integerrima* bark may be responsible for its uses to cure cancer, inflammations and allergies.

Physico-chemical parameters

The physico-chemical parameters are shown in Table 2. These parameters are usually helpful in prevention of adulteration and in authentication of crude drug. The moisture content is very important factor for the stability of crude drug because moisture enhances the fungal and bacterial growth. The longer shelf life can be achieved only by reducing the moisture content. The moisture contents of *P. integerrima* bark was 10.2% indicating their moderate shelf life. The total ash value is 10% which indicates the presence of earthy matter, inorganic components and other impurities in the crude drug. Acid insoluble ash value of 2.1% indicates high digestibility when the plant is consumed. Water soluble ash value is 3.8%. The extractive values as given in Table 2 are primarily useful for the determination of exhausted or adulterated drugs.

Percent yield

The percentage yield and color of the successive solvent fractions are tabulated in Table 3. The highest yield was observed for methanol (37%) followed by ethyl acetate (31%) and water (25%).

Conclusion

The present research work was under taken with a view to lay down standards which could be useful in authenticating this valuable medicinal plant.

The microscopic and physico-chemical standards of Pistacia integerrima bark will be helpful in establishing parameters for standardization and sample identification.

Table 2. Physico-chemical parameters of *Pistacia Integerrima* bark.

S/N	Physiochemical parameters	Percentage value (w/w %)
1.	Moisture content	10.2
	Ash values	
	Total ash	10
2.	Water soluble ash	3.8
	Acid insoluble ash	2.1
	Acid soluble ash	2.9
	Extractive values	
3.	Ethanol soluble	24
	Water soluble	21

Table 3. Percent yield and color of successive solvent fractions.

S/N	Extract	Color of the extract	Percentage(w/w %) yield
1.	Methanol	Dark reddish	37
2.	n-hexane	Light brown	19
3.	Chloroform	Brownish	17
4.	Ethyl acetate	Light reddish	31
5.	Butanol	Light reddish	18
6.	Aqueous	Brown reddish	25

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