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ABSTRACT

The Pinus roxburghii Sarg (Pinaceae) is commonly known as chir pine. It is a large tree up to 28-55 m in height. The bark is red-brown, leaves are needle-like, in fascicles of three, very slender, distinctly yellowish green. The P. roxburghii is an economically valuable species, balancing the ecosystem of the Indian mountains. The plant have their own pharmaceutically importance. In traditional system of medicine different parts of the plant have been used for cough, cold, influenza, tuberculosis, bronchitis antiseptic, diaphoretic, diuretic, rubefacient, stimulant and vermifuge. Bark paste is used in burns. Resin is used to relieve cough and gastric troubles. Plant is used as intestinal antiseptic, hypolipidimic, antioxidant property. The present study was undertaken to assess pharmacognostic parameters and phytochemical screening of bark of Pinus roxburghii Sarg. Organoleptic evaluation of bark were done by studying organoleptic features like shape, size, colour, odour, taste and fracture. In microscopy the bark was soaking into water filled beaker so that sufficient moistening is obtained for microscopy. In powder microscopy dried powdered material was obtained by grinding bark and visual under microscope taken at 100x. To evaluate the Physico chemical property the crude dried plant material was subjected to study the various parameters such as extractive value using different solvent pet ether, chloroform, methanol and water, also calculate the percentage yield of moisture content, ash value including acid insoluble, water soluble ash, fluorescence analysis and loss on drying etc were evaluated by using standard procedures. To evaluate the phytochemical screening of extracts the different solvents were used such as Sodium hydroxide, Chloral hydrate, Ferric chloride, Sulphuric acid, Iodine, Lead acetate, Magnesium,
Potassium iodide, Picric acid, Mercuric chloride, Nitric acid and identify which chemical constituents are present in the bark of *P. roxburghii*.

**KEYWORDS:** Pinus roxburghii, extractive value, microscopy, phytochemical screening and picric acid.

**INTRODUCTION**

The genus *Pinus* is one of the most widely distributed genera of trees in the northern hemisphere, encompassing nearly 100 species of evergreen trees belonging to the family *Pinaceae* (Xu *et al.*, 2012). The species of *Pinus* are of great economic value and are the main source of timber, fuel and resins (Mirov, 1967, Dallimore and Jackson 1966, Kurose *et al.*, 2007). More than 40 taxonomic treatments have been recognized of several major divisions within the genus (Kaushik *et al.*, 2012).

**Table 1: Pharmacological activities of *Pinus roxburghii* Sarg.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Type</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. halepensis</em></td>
<td>Essential oil</td>
<td>Antibacterial</td>
<td>Abi-Ayada <em>et al.</em>, 2011</td>
</tr>
<tr>
<td><em>P. longifolia</em></td>
<td>Oil</td>
<td>Antioxidant</td>
<td>Guri <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>P. pinea, P. nigra, P. brutia, P. sylvestris</em></td>
<td>Bark</td>
<td>Larvicidal and Mosquito repellent</td>
<td>Ansari <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><em>P. pinaster, P. radiata</em></td>
<td>Bark</td>
<td>Radical scavenging and Anti-cancer</td>
<td>Yesil-Celiktas <em>et al.</em>, 2009</td>
</tr>
<tr>
<td><em>P. cembra</em> L.</td>
<td>Bark, Needles</td>
<td>Antioxidant, Antimicrobial</td>
<td>Apetrei <em>et al.</em>, 2011</td>
</tr>
</tbody>
</table>

*Pinus roxburghii* Sarg. (Pinaceae), commonly known as chir pine, is a tall tree with a spreading crown found in the Himalayan from Kashmir to Bhutan, Afghanistan and in southern Indian hills. The vernacular names of *P. roxburghii* are Bhadradaru, Manojna in Sanskrit; Chil, Chir, Salla in Hindi; Saralgachha in Bengal. (Kirtikar and Basu, 1999). The chir pine also occurs in valleys, which receive the bulk of the rainfall during the monsoon (Anonymous, 2003).

**Taxonomy**

Kingdom: Plantae  
Division: Pinophyta  
Class: Pinopsida  
Order: Pinales  
Family: Pinaceae  
Genus: *Pinus*
Species: roxburghii

Binomial name: Pinus roxburghii Sarg. It is used in preparation of ointments and plasters and in many products such as chewing gum, polishes, and varnishes, but is a common cause of contact allergy. The resin is applied to cure boils (Rajbhandari, 2001) and administered orally to combat gastric troubles (Manandhar, 2002). The resin is useful in adhesives, printing ink, electric isolation, paper, soldering flux, varnish and matches. In printing ink industry resin gives adhesive Physicians in colonial America also recommended tar water, or ground pine resin mixed with water, as a remedy for ulcers, smallpox, and syphilis (Langenheim, 2003). Different parts of the plant are prescribed to treat cough, influenza, tuberculosis, bronchitis, as antiseptic, diaphoretic, diuretic, rubefacient, stimulant and febrifuge (Chopra et al., 1986; Puri et al., 2011). The gum has shown good effect in diseases of the vagina and uterus (Kirtikar and Basu, 1999; Anonymous, 2003). The plant is used to treat diabetes in India and Africa, and it is known to be a rich source of terpenoids, flavanoids and there is interest among the scientist to use this for therapeutic purposes. Almost all the parts of the plant (bark, leaves and root) are found to contain active principles19-22 Plant is used as intestinal antiseptic, antidyslipidemic and antioxidant (Puri et al., 2010) P. roxburghii has a long history of numerous traditional and ethnobotanical application due to many active constituents present in it. (Kunwar et al., 2009). Two new xanthone identified as 1,5-dihydroxy-3,6,7-trimethoxy-8-dimethylallyloxy-xanthone and 1-hydroxy-3,6-dimethoxy-2-β-Dglucopyranoxanthone have been isolated from the methanolic extract of the bark of Pinus roxburghii (Rawat et al., 2006). The presence of a -carene, -longifolene and longicyclone has also been reported. The bark contains 7-10 % of tannins (Anonymous, 2003). Ferulic acid, p-coumaric acid and pinoresinol were isolated and their structures were established (El-Shaer, 2002). Abietic acid contains a tricyclic perhydro phenanthrene (Kuchimanchi et al., 2002). Three of the diarylamines with lower oxidation potential proved to be as active as isopropylidiphenylamine (IPPD) and superior to tert-butylhydroxytoluene (BHT), both commercially available synthetic antioxidants (Esteves et al., 2001). A hydroperoxide, 15-hydroperoxydehydroabietic acid (15-HPDA), with contact allergenic properties has been detected in rosin obtained from Pinus species (Shao et al., 1995). Longifolene, an important member of the sesquiterpene class of the major mevalonoid group of natural products, was first isolated from Indian turpentine oil from Chir pine (Jadhav and Nayak, 1980). The maximum (29.67 %) content of -pinene was found in the oil obtained from the trees of Rawalpindi seed etc are present (Verma and Suri, 1978). All these problems can be solved by
pharmacognostic studies of medicinal plants. The present study is based on pharmacognostic parameters and phytochemical screening of *P. roxburghii* plant.

**MATERIALS AND METHODS**

**Plant material**
The plant material was collected from Gagret, Distt Una Himachal Pradesh, and India in the month of October. It was authenticated in the herbarium of the same institute.

**Pharmacognostic evaluation**

**Macroscopy**
Organoleptic evaluation of bark were done by studying organoleptic features like shape, size, colour, odour, taste and fracture.

**Microscopy**

**Study of section**
Preliminary treatment to bark of *P. roxburghii* is given by soaking into water filled beaker so that sufficient moistening is obtained for microscopy. Thin transverse section of bark is cut by placing it into potato pith which is divided into equal halves by razor or with the help of suitable support (WHO, 1998). Collect the thin sections in a large petriplate filled with water. Then place one suitable thin section on glass slide, treated the material with chloral hydrate so that clear section is obtained and mounted with glycerine then prepared glass slide observed under microscope.

**Powder microscopy**
Dried powdered material was obtained by grinding bark in grinder. Then powdered material was cleared with chloral hydrate and mounted with glycerine. Prepared glass slide is observed under microscope for evaluation of microscopic features of drugs (Khandelwal, 2004).

**Physico-chemical evaluation**
The crude dried plant material was subjected to the physico-chemical evaluation. The various parameters such as extractive value, moisture content, ash value including acid insoluble and water soluble ash etc were evaluated by using standard procedures (Mukherjee P.K, 2002, Tatiya A et al., 2007).
Ash values
Ash content of the crude drug is generally taken to be the residue remaining after incineration. It represents the inorganic salts naturally occurring in the drug adhering to it but it may also include inorganic matter added for the purpose of adulteration.

Total ash is the residue remaining afterward ignition. Acid insoluble ash is the fragment of the total ash which is insoluble in dilute hydrochloric acid. Water soluble ash is the part of total ash, which is soluble in hot water (Khandelwal, 2004).

Determination of total ash
Air dried powdered (5 gm) was weighed precisely in a silica pot and was burned at a temperature not exceeding 450°C until free from carbon, then cooled and weighed. On the off chance that a carbon free fiery debris can't be acquired along these lines, burned mass was depleted with heated water, buildup was gathered on an ashless channel paper, the deposit and channel paper was burned until the point when the powder was white or about thus, filtrate was included, dissipated to dryness and lighted at a temperature not exceeding 450°C. The percentage of ash with reference to the air-dried drug was calculated. (Khandelwal, 2004).

Determination of acid insoluble ash
Ash attained from the technique mentioned above (Determination of total ash) was boiled with 25 ml of 2 M hydrochloric acid for 5 min, insoluble matter was collected in a silica crucible or on an ash less filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated (Khandelwal, 2004).

Determination of water soluble ash
To the crucible containing the total ash, add 25ml of water and boil for 5 minutes. Collect the insoluble matter in a silica crucible or on ash less filter paper, washed with hot water and ignited.

Extractive values
Varieties of chemical compounds are available in crudes drugs having variable properties. Various solvents are used for extraction of desired chemical compound in an appropriate
value (Joseph and George, 2011). The extracts achieved by depleting crude drugs are demonstrative of estimated methods of their chemical.

**Ether soluble extractive**

4 g powdered drug was macerated with 100 ml of petroleum ether in a closed flask for 24 hrs, with frequent shaking during the first 6 hrs, the flask was allowed to stand for 18 hrs, the extract was filtered and filtrate was evaporated to dryness in a shallow dish and weighed. The percentage of ether soluble extractive with reference to air dried drug was calculated (WHO guidelines, 1998).

**Chloroform soluble extractive**

4 g powdered drug was macerated with 100 ml of chloroform in a closed flask for 24 hrs, with frequent shaking during the first 6 hrs, the flask was allowed to stand for 18 hrs, the extract was filtered and filtrate was evaporated to dryness in a shallow dish and weighed. The percentage of chloroform soluble extractive with reference to air dried drug was calculated (WHO guidelines, 1998).

**Alcohol soluble extractive**

4 g powdered drug was macerated with 100 ml of methanol in a closed flask for 24 hrs, with frequent shaking during the first 6 hrs, the flask was allowed to stand for 18 hrs, the extract was filtered and filtrate was evaporated to dryness in a shallow dish and weighed. The percentage of Alcohol soluble extractive with reference to air dried drug was calculated (WHO guidelines, 1998).

**Water soluble extractive**

4 g powdered drug was macerated with 100 ml of water in a closed flask for 24 hours, with frequent shaking during the first 6 hours, the flask was allowed to stand for 18 hours, the extract was filtered and 25 ml of filtrate was evaporated to dryness in a shallow dish. The residue was dried at 105°C and weighed. The percentage of water soluble extractive with reference to air dried drug was calculated (WHO guidelines, 1998).

**Loss on drying**

2 g of air dried powder material of bark of *P. roxburghii* and tarred weighing bottle. The sample was dried in oven at 100-105°C until two consecutive weighing were not diff by more
than 5mg. The loss of weight in mg per g dried material was calculated (WHO guidelines, 1998).

**Fluorescence analysis**
A small quantity of dried and finely powdered of *P. roxburghii* bark were placed on a grease free clean microscopic slide and added 1-2 drops of freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 m) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded (WHO guidelines, 1998).

**Phytochemical screening**
Various extract *Pinus. roxburghii* bark, were subjected to preliminary phytochemical screening using standard methods (Ambi et al., 2007). All the extracts were screened for different classes of phytoconstituents using specific standard reagents.

**Chemicals used in phytochemical screening of extracts**
Sodium hydroxide, Chloral hydrate, Ferric chloride, Sulphuric acid, Iodine, Lead acetate, Magnesium, Potassium iodide, Picric acid, Mercuric chloride, Nitric acid, were used for phytochemical screenin of the plant extracts.

**Sample preparation**
About 50 ml of different solvents in which the extract was prepared and added 2.5 g of extract and stirred with slight warming. The solution was cooled and filtered through whatman filter paper no.1 and examined under UV chamber (250 nm).

**RESULTS**

**Macroscopic evaluations of Pinus roxburghii bark**
The size of bark of *P. roxburghii* is 2-5.1 cm long, colour of bark is reddish brown, outer surface is rough and scaly, inner surface is smooth, shape is irregular and its taste is acrid.

**Microscopy**

**Transverse section of bark**
Thin transverse section of the bark is taken and observed under microscope to reveal the types of cells present in *P. roxburghii*. All the images of transverse section of bark are first observed under 100x magnification of microscope, a bright image is taken at 100x of
microscope so that perspicuous or lucid image is obtained. Thin section of the bark reveals the presence of medullary rays, lignified phloem fibres and u shaped stone cells in upper part of bark are shown in Figure 1: A, B and C. A represents the presence of medullary rays which is narrow at inner side and phloem fibres having lignified with scleride present in the lower layar of bark.

**Powder microscopy**

Powder of *P. roxburghii* when observed under microscope having magnification powere 10x of microscope, a bright image is taken at 10x of microscope so that perspicuous or lucid image is obtained, the powder of bark reveals the presence of lignified thick walled cells in A, lignified fibers in B, cuboidal calcium oxalate crystals in C and oil glands in D (figure 2).

![Microscopic images of bark sections](image1.png)

**Figure 1:** Representative photomicrographs of transverse section of *P. roxburghii* shows (A) medullary rays, phloem fibre (B) stone cells (C) cork cells (taken at 100x).
Figure 2: Representative photomicrographs of powder microscopy of *P. roxburghii* shows. (A) lignified thick walled cells (B) Fibre (C) cuboidal calcium oxalate crystals (D) oil glands (taken at 100x).

**Determination of Physical parameter**

Data obtained from physical parameter of bark of *P. roxburghii* shows the percentage yield of powder using following parameters.
Table 2: Physical parameters of *P. roxburghii*.

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Yield (% W / W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>1.6</td>
</tr>
<tr>
<td>Water-soluble ash</td>
<td>1.4</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.2</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>6.6</td>
</tr>
</tbody>
</table>

**Extractive values**

Data obtained from extractive values of bark of *P. roxburghii* shows the percentage yield of powder using different solvents.

Table 3: Extractive values of *P. roxburghii*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield (% W / W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>1.2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.92</td>
</tr>
<tr>
<td>Methanol</td>
<td>12.5</td>
</tr>
<tr>
<td>Aqueous</td>
<td>5</td>
</tr>
</tbody>
</table>

**Florescence analysis**

Data obtained from florescence analysis of bark of *P. roxburghii* shows the different colour of powder in different solvents using UV chamber.

Table 4: Florescence analysis of *P. roxburghii*.

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Colour under visible light</th>
<th>Colour under long UV (365 nm)</th>
<th>Colour under short UV (254 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet ether</td>
<td>Brown</td>
<td>Purple brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Concentrated ammonia</td>
<td>Dark brown</td>
<td>Black</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Concentrated hydrochloric acid</td>
<td>Black</td>
<td>Black</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Concentrated sulphuric acid</td>
<td>Black</td>
<td>Dark purple</td>
<td>Black</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Light brown</td>
<td>Light brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Iodine</td>
<td>Black</td>
<td>Black</td>
<td>Black</td>
</tr>
</tbody>
</table>

**Extraction**

Data obtained from extraction of bark of *P. roxburghii* shows the percentage yield of powder using different solvents.

Table 5: Percentage yield of extracts of *P. roxburghii*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Color</th>
<th>Yield (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>Light yellow</td>
<td>7.55</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Pale yellow</td>
<td>0.98</td>
</tr>
<tr>
<td>Methanol</td>
<td>Blood red</td>
<td>27.5</td>
</tr>
<tr>
<td>Aqueous</td>
<td>Reddish brown</td>
<td>6.43</td>
</tr>
</tbody>
</table>
Phytochemical analysis

Data obtained from the phytochemical analysis of the bark of *Pinus roxburghii* showed the presence of tannin, saponin, cardiac glycosides, flavonoids, reducing sugars and triterpene steroids.

Table 6: Phytochemical screening extracts of *P. roxburghii*.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Phytochemical constituents</th>
<th>Pet ether extract</th>
<th>Methanol extract</th>
<th>Chloroform extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Mayer’s reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Hager’s reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Wagner’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Phenolic compounds and Tanins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Fecl$_3$</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Lead acetate test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Bromine water test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Frothing test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Molisch test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Fehling’s solution A</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Fehling’s solution B</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Protein and Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Millon’s test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Ninhydrin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Glycosides test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Borntrager’s test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Kill</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Raymond test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Alkaline reagent test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Shinoda test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Phytosterols test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Liebermann’s test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Libermann Burchard test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION

The present work was designed for the pharmacognostic and antianxiety evaluation of *Pinus roxburghii* bark. The present study reveals that the *P. roxburghii* bark is irregular in shape, reddish brown color, 2-5cm long, and has acrid taste. Microscopy of transverse section of bark shows the presence of medullary rays, phloem fiber and u shaped stone cells. Powder Microscopy reveals the presence of lignified fibre, lignified thick walled cells, cuboidal...
calcium oxalate crystals and oil glands. Bark was also subjected to physiochemical evaluation and parameters like, ash values, extractive values, loss on drying, fluorescent analysis are determined following the standard pharmacopoeial procedure. Determination of ash value is useful for detecting adulteration with spurious, exhausted drugs, and excess of sandy and earthy matter. The acid-insoluble ash is determined to remove all the variable constituents of the ash using dilute hydrochloric acid. The water soluble ash is used to detect the presence of material exhausted by water. Total ash (1.6%), acid insoluble ash (1.2%), and water soluble ash (1.4%), loss on drying (6.6%), pet ether extractive value (1.2%), chloroform extractive value (0.9%), alcohol extractive value (12.5%) and water extractive values (5%) were calculated. Preliminary phytochemical analysis shows the presence of alkaloids, glycosides, saponins, flavonoids, phenolic compounds and carbohydrates present in petroleum ether, chloroform, methanol and water extracts.

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