



Qualitative Analysis and Determination of Total Phenolic And Total Flavonoid Content Of Needles Of *Pinus roxburghii* From Srinagar Garhwal

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Abstract: *Pinus roxburghii* is a gymnosperm tree species, which is abundantly found in the north western Himalayan region and possess medicinal properties. In this study, extracts of *Pinus roxburghii* needles were made in different solvents which were used for phytochemical screening. The extracts were used to carry out the study of the total phenolic content and the total flavonoid content using Folin-Ciocalteu method and aluminium chloride method respectively. Methanolic extract of *Pinus roxburghii* needles showed the maximum amount of total phenolic content (TPC) and total flavonoid content (TFC) that were 306.7053 ± 0.88711 GAE/g and 188.1283 ± 0.09029 RE/g respectively. It was concluded that the presence of secondary metabolites can be further investigated for medicinal purposes.

Keywords: - *Pinus roxburghii*, Needles extract, Phytochemical Screening, Total Flavonoid Content, Total Phenolic Content.

Introduction

India is rich in floristic diversity which constitute of vast range of herbal plants that are used traditionally as well as commercially for their unique property to cure various diseases and physiological conditions. Many ethnic groups use these plants for treating various ailments like minor infections, skin diseases, dysentery, malaria, asthma etc. (Kumar et al., 2006). The main reason for these medicinal properties is presence of phytoconstituents or secondary metabolites such as quinones, carotenoids, sterols, tannins, terpenoids, alkaloid, flavonoids, phenol, glycosides etc. (Devi et al., 2021). *Pinus roxburghii* (Pinaceae family), commonly known as “Chir pine” is a gymnosperm tree species that is found in abundance in the North western Himalayan region (Hounsoume et al., 2010). It is distributed around temperate region (northern hemisphere) of South Asia which covers north India, northern Pakistan, Nepal and Bhutan. In

India, it is spread from Kashmir covering Jammu, Punjab, Himanchal Pradesh, Uttarakhand and Sikkim (Price et al., 1998; Arya et al., 2000). The different species of *Pinus* cover about one-third area of Uttarakhand Himalaya and out of that maximum abundancy is of *Pinus roxburghii* (Tewari, 1994). It is a common indigenous species of Himalayan region found at an elevation ranging from 450 m to 2300 m (Sharma et al., 2000). *Pinus roxburghii* is reported to be used as intestinal antiseptic, spasmolytic, antidiyslipidemic, antimicrobial and antioxidant (Puri et al., 2011). It is also used traditionally as medicine to cure diseases related to ears, eyes, blood and skin, throat, inflammations, diaphoresis, ulcer and itching as the whole plant body constitute of chemical elements which shows vast range of activity (Abbasi et al., 2010). In Ayurveda, *Pinus* is used to treat foul smell because of over sweating known as “svedadaurgandhya” and fever known as “jvara” and little number of doses is used as expectorant



and stimulants. It is also used in minor hemorrhage, chronic bronchitis, typhoid and constipation (Sharma et al., 2015; Nirala et al., 2020). The major phytochemical constituents reported from *Pinus roxburghii* in earlier studies from India and outside of India includes phenol, flavonoid, terpenoids, xanthenes, tannins (Willför et al., 2009; Khan et al., 2012). The present study was taken up to detect the presence of metabolites by phytochemical screening and to determine the total phenolic and the total flavonoid content in the needles of *Pinus roxburghii* from Srinagar Garhwal region of Uttarakhand.

Materials and Methods

Chemicals and Reagents: Chloroform, Methanol, Wagner's reagent, Mayer's reagent, Ninhydrin, Nitric acid, Sodium hydroxide, Sodium bicarbonate, Ferric chloride, Lead acetate, Glacial acetic acid, Copper sulphate, Ethanol, Potassium hydroxide, Benedict's reagent, α -naphthol, Gelatin, Sodium Chloride, Ferric Chloride, Sulphuric acid, Rutin, Gallic acid, Folin-Ciocalteu reagent, Sodium carbonate, Aluminium chloride, Sodium nitrite. All were of analytical grade.

Collection of plant material: Needles of *Pinus roxburghii* were collected from Srinagar, Pauri Garhwal, Uttarakhand. The sample was cleaned with running water and then air dried in shade. It was then crushed with the help of grinder and stored at 4°C for further analysis.

Preparation of extracts: Extracts were prepared by Soxhlet extraction method using three solvents (chloroform, methanol, distilled water). 50 gm of sample was used for extraction. Extracts were dried in vacuum rotary evaporator and stored at 4° C (Banu et al., 2015).

Phytochemical analysis of needles extracts

Qualitative Analysis:

Phytochemical screening was carried out to detect the presence of phytochemical constituents. The tests for alkaloid, amino acid, saponins, tannin, flavonoid, protein, carbohydrates, phenol, glycosides, gums and mucilage, terpenoid were done using the standard methods as reported earlier (Sharma et al., 2015; Banu et al., 2015; Geetha et al., 2014; Purohit et al., 2003; Semwal et al., 2014; Kokate, 1999; Yasuma et al., 1953; Whistler et al., 1993).

Quantitative analysis

Total phenolic content: In *Pinus roxburghii*, the total phenolic content was determined by Folin-Ciocalteu method using gallic acid as a standard (Makkar, 2003). The gallic acid solution was made in methanol ranging from 0-1 mg/ml concentration. The absorbance was measured at 725nm via UV- Vis spectrophotometer and a calibration curve was made. The determination of the result was done from the regression equation of calibration curve and was expressed as milligram gallic acid equivalents per gram sample. Calculation was done with the standard curve of gallic acid as seen in Fig.1 [Regression equation of the calibration curve: $y=1.6736x + 0.0024$; Correlation coefficient (R^2) = 0.9988].

Total flavonoid content: The aluminium chloride assay method was used to determine the total flavonoid content (Zhishen et al., 1999). Methanol was used to prepare the rutin solution (ranging from 0-1 mg/ml) which was used as a standard. The absorbance was measured at 510nm via UV- Vis spectrophotometer. Calculation of the total flavonoid content was done using the calibration curve of rutin. The result was expressed as milligram rutin equivalents per gram sample with the standard curve of rutin as seen in Fig.2 ($y= 0.6347x + 0.0041$; $R^2 = 0.9996$).

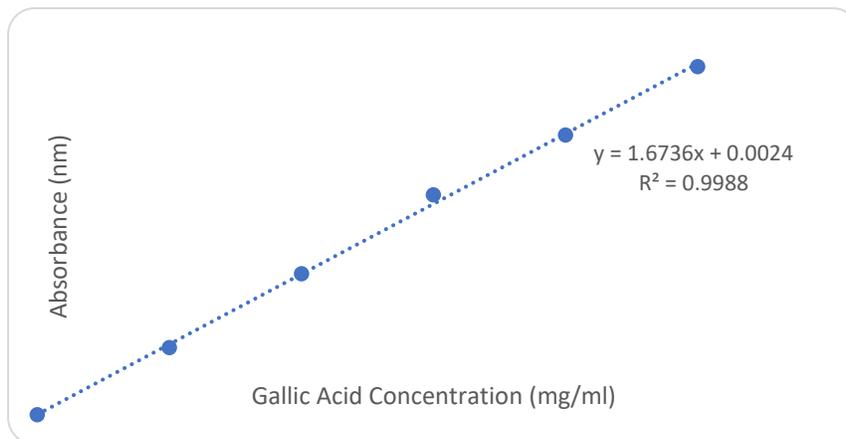


Fig 1: Standard calibration curve of Gallic acid

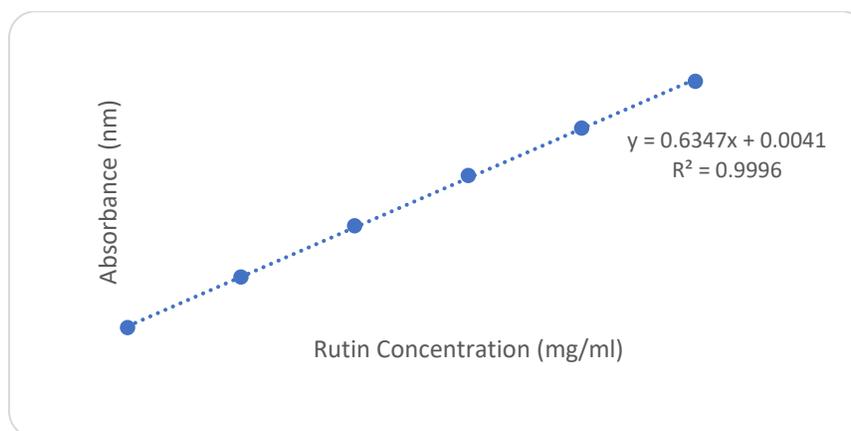


Fig 2: Standard calibration curve of Rutin

Results and Discussion

Phytochemical Screening of needles extract:

The result of qualitative analysis is shown in Table.1. It indicates that the extracts in all the 3 solvents shows the presence of phytochemicals such as alkaloid, saponin, tannin, flavonoid, carbohydrates, phenol, glycosides and terpenoid (Table.1) (Sharma et al., 2015; Chaudhary et al., 2012; Thapa et al., 2018; Maimoona et al., 2011).

Quantitative analysis

Total phenolic content: The maximum phenolic content was found in methanolic extract as compared to the other two solvents. The phenolic content in the needle extracts was found to be in decreasing order as follows: methanol extract > distilled water extract > chloroform extract (Table.2). The total amount of phenol was found to be maximum in methanol extract that is 306.7053 ± 0.88711 GAE/g and minimum in chloroform extract i.e., 55.1354 ± 0.79835 GAE/g.



Table 1: - Qualitative Screening of *Pinus roxburghii* needles

S.No.	Phytochemicals	Tests	Chloroform	Methanol	Distilled Water
1	Alkaloid	i)Wagner's Test	+	-	-
		ii) Mayer's Test	-	+	-
2	Amino Acid	i)Ninhydrin Test	-	-	-
		ii)Xanthoproteic Test	-	-	-
3	Saponin	i)Honey comb Test	+	-	-
		ii)Foam Test	-	+	+
4	Tannin	i) 5% FeCl ₃ Test	+	+	-
5	Flavonoid	i)10% Lead acetate	+	+	+
		ii)Alkaline Reagent	+	+	+
6	Protein	i)Nitric Acid Test	-	-	-
		ii)Biuret Test	-	-	-
7	Carbohydrates	i)Benedict's Test	+	+	+
		ii)Molisch Test	-	-	+
8	Phenol	i)1% Lead acetate Test	+	+	+
		ii)Gelatin Test	-	-	-
9	Glycosides	i)NaOH Test	+	-	+
		ii)Keller-Killani's Test	-	-	-
10	Terpenoid	i)Salkowski's Test	+	+	+

[(+) = Present; (-) = Absent]

Table 2: - Total phenolic content of *Pinus roxburghii* needles in different extract

S.No.	Extract	Total phenol
1	Chloroform	55.1354 ± 0.79835
2	Methanol	306.7053 ± 0.88711
3	Distilled water	187.9950 ± 0.75685

Total flavonoid content: The total flavonoid content in methanol extract was found to be maximum as compared to the other two solvents. The flavonoid content of the extracts was in the following decreasing order in different solvents: methanol extract > chloroform extract > distilled water extract (Table. 3). The total amount of flavonoid was found maximum that is 188.1283 ± 0.09029 RE/g in the methanolic extract and the minimum was shown in aqueous extract which was 99.1494 ± 0.20191 RE/g.

Table 3: - Total flavonoid content of *Pinus roxburghii* needles in different extract

S.No.	Extract	Total Flavonoid
1	Chloroform	157.4049 ± 0.35282
2	Methanol	188.1283 ± 0.09029
3	Distilled water	99.1494 ± 0.20191

The above results show that phenolic content in methanol extract and distilled water extract is more than flavonoid content in methanol and distilled water extract (Thapa et al., 2018; Maimoona et al., 2011). As in the earlier studies similar type of results were seen where phenolic content was comparatively higher than the flavonoid content in *Pinus* species (Karapandzova et al., 2019; Ustun et al., 2012). It is known that phenolic compound is bactericidal and fungicidal in nature and flavonoid show antibacterial activities hence can be used as an antimicrobial agent (Cowan et al., 1999; Górniak et al., 2019; Cushnie et al., 2006). Phenol and flavonoid also possess antioxidant components which shows free radical scavenging (Amarowicz et al., 2004). Therefore, needles of *Pinus roxburghii* from Srinagar Garhwal region can be used for further analysis of antimicrobial and antioxidant activities.

Conclusion

It is evident from this study that *Pinus roxburghii* possesses phytochemicals such as phenol, flavonoids, tannin, terpenes which act as natural curing agent. This plant has notable amount of phenolic and flavonoid content in it which can be



used to treat various health conditions. Further studies need to be carried out to explore antimicrobial and antioxidant properties. Due to abundance of this species in Srinagar Garhwal region of Himalaya, this plant can be used to explore its potential for developing novel therapeutics to mankind that can be beneficial for pharmaceutical industry.

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Conflict of interests

The authors hereby declare no such conflicts of interest.

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