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Qualitative analysis and determination of total phenolic and total flavonoid content of different parts of *Pinus roxburghii* from Dwarahat, Almora

Preeti Nirala[1], Seema Devi[1], Asha[2]
[1] Research Scholar, Department of Botany and Microbiology, HNB Garhwal University, Srinagar Garhwal, Uttarakhand, India
[2] Assistant Professor, Department of Botany, Lucknow University, Lucknow, Uttar Pradesh, India

Abstract
The gymnosperm tree species *Pinus roxburghii* is widely distributed in the northwest Himalayan region and is known for its therapeutic qualities. *Pinus roxburghii* extracts of different parts were prepared in various solvents for phytochemical screening. The total phenolic content and total flavonoid content were investigated using the Folin-Ciocalteu and aluminum chloride methods, respectively. The maximum amounts of total phenolic content (TPC) and total flavonoid content (TFC) were found in the methanolic extract of *Pinus roxburghii* needles and stem, which were 396.12±1.65 mg GAE/g DW and 500.67±1.37 mg RE/g DW, respectively.

Keywords: *Pinus roxburghii*, extracts, phytochemical screening, total flavonoid content, total phenolic content

Introduction
India boasts a high floristic diversity, which includes a wide variety of traditionally and commercially utilized herbal plants valued for their special ability to treat a wide range of illnesses and physiological problems. These herbs are used by many ethnic groups to cure a wide range of illnesses, including mild infections, skin conditions, diarrhea, malaria, asthma, and other conditions [1]. The presence of phytoconstituents or secondary metabolites, such as quinones, carotenoids, sterols, tannins, terpenoids, alkaloids, flavonoids, phenol, glycosides, etc., is primarily responsible for these therapeutic effects [2]. The gymnosperm tree species *Pinus roxburghii* (Pinaceae family), also referred to as "Chir pine," is widely distributed in the northwestern Himalayan region [3]. It is found in the temperate zone of South Asia's northern hemisphere, which includes Nepal, Bhutan, northern Pakistan, and north India. According to previous literature, it originated in Kashmir and later expanded to Jammu, Punjab, Himanchal Pradesh, Uttarakhand, and Sikkim in India [4, 5]. About one-third of the Uttarakhand Himalaya is covered by *Pinus* species, with *Pinus roxburghii* having the highest abundance [6]. This common native species is found in the Himalayan region at elevations between 450 and 2300 meters [7]. According to reports, *Pinus roxburghii* is utilized as an antioxidant, antibacterial, spasmolytic, intestinal antiseptic, and antidyslipidemic [8]. Due to the fact that the entire plant is made up of chemical components with a wide spectrum of activity, Additionally, historically, it has been used as medication to treat ailments of the throat, blood and skin, eyes, ears, diaphoresis, ulcers, and itching [9]. A small number of dosages are also used as stimulants and expectorants. Additionally, it is used to treat constipation, typhoid, chronic bronchitis, and small hemorrhages [10]. According to previous research conducted in India and abroad, the main phytochemical components of *Pinus roxburghii* are phenol, flavonoids, terpenoids, xanthones, and tannins [11, 12]. The goal of the current study is to identify the metabolites and ascertain the total phenolic and total flavonoid content in different parts (needles, bark, female cone and stem) of *Pinus roxburghii* using three different solvents namely; chloroform, methanol and distilled water from the Dwarahat region of Almora, Uttarakhand.

Materials and Methods
Chemical and reagents
Wagner's reagent, methanol, chloroform, Benedict's reagent, ferric chloride, sulphuric acid, rutin, gallic acid, lead acetate, glacial acetic acid, hydrogen chloride, ferric sulphate, sulphuric acid, sodium hydroxide, aluminum chloride, sodium nitrite, sodium carbonate, and Folin-Ciocalteu reagent. Each one was of analytical grade.

Collection of plant material
Pinus roxburghii parts (needles, bark, female cone and stem) were gathered from the Dwarahat region of Almora, Uttarakhand (1510m asl). After washing the sample under running water, it was allowed to air dry in the shade. After being ground with a grinder, it was kept at 4ºC for further examination.

Preparation of extract
Using distilled water, methanol, and chloroform as the three solvents, the Soxhlet extraction method was used to prepare the extracts using 50 grams of the plant material. The extracts were preserved at 4º C after being dried in a vacuum rotary evaporator [13].

Phytochemical analysis of *Pinus roxburghii* extracts of different parts
Qualitative Analysis
The tests for alkaloids, saponins, tannins, flavonoids, carbohydrates, phenol, glycosides and terpenoids were conducted using the standard methods as reported earlier [10, 13, 14, 15, 16, 17, 18, 19].

Quantitative analysis
Total phenolic content
Gallic acid was used as a standard in the Folin-Ciocalteu method to assess the total phenolic content in different part of *Pinus roxburghii* extracts [20]. Gallic acid solution of concentration 0-1mg/ml was prepared in methanol. Using a

UV-Vis spectrophotometer, the absorbance was measured at 725 nm, and a calibration curve was created. The result was calculated using the calibration curve regression equation and was given as milligram gallic acid equivalents (GAE) per gram of dry weight of the material.

Total Flavonoid content

The total flavonoid content was ascertained using the aluminum chloride test. The rutin solution (varying from 0–1 mg/ml) that served as the standard was made in methanol. The absorbance was determined using a UV-Vis spectrophotometer at 510nm.The rutin calibration curve was used to calculate the total flavonoid content and the result was expressed as milligram rutin equivalents (RE) per gram of dry weight of the material.

Statistical analysis

Statistical analysis was evaluated using the IBM SPSS software trail version 29.0.0.0(241). With a significance level of $p<0.05$, one-way ANOVA was used to assess the significance of the results between various parts in different solvents.

Results and discussion

Phytochemical Screening of *Pinus roxburghii* extract of different parts

The outcome of the qualitative examination is displayed in Table.1. It reveals that phytochemicals such as flavonoids, carbohydrates, phenols and terpenoids are present in all solvents' extracts of different plant parts. Alkaloids and saponin are present in all extracts of bark whereas only present in the chloroform extract of needles and stem. Saponins are present in the aqueous and methanolic extract of needles and stem but only in the aqueous extract of female cone. Tannins are present in the chloroform and methanolic extract of needles and stem and only methanolic extract of bark. Glycosides are only present in the methanolic extract of bark and chloroform extract of female cone [10, 22, 23, 24].

Table 1: Qualitative Screening of *Pinus roxburghii* parts in different solvent

S. No.	Phytochemicals	Tests	Chloroform Extract				Aqueous Extract				Methanol Extract			
			N	B	C	S	N	B	C	S	N	B	C	S
1	Alkaloids	Wagner's Test	+	+	-	+	-	+	-	-	-	+	-	-
2	Saponins	Foam Test	-	+	-	-	+	+	+	+	+	+	-	+
3	Tannins	5% $FeCl_3$ Test	+	-	-	+	-	-	-	-	+	+	-	+
4	Flavonoids	Alkaline Reagent Test	+	+	+	+	+	+	+	+	+	+	+	+
5	Carbohydrates	Benedict's Test	+	+	+	+	+	+	+	+	+	+	+	+
6	Phenols	1% Lead acetateTest	+	+	+	+	+	+	+	+	+	+	+	+
7	Glycosides	Keller-Killani's Test	-	-	+	-	-	-	-	-	-	+	-	-
8	Terpenoids	Salkowski's Test	+	+	+	+	+	+	+	+	+	+	+	+

[(+ = present, - = absent); (N–needles; B–bark, C–female cone, S–stem)]

Quantitative analysis

Total phenolic content

In comparison to the other two solvents, methanolic extract had the highest phenolic concentration when compared within same part of three different solvents (Table.2, Fig.1). The total phenolic content was determined to be maximum in methanolic needles extract 396.12 ± 1.65 mg GAE/g DW and minimum was 22.52 ± 0.76 mg GAE/g DW in the chloroform extract of stem.

Table 2: Total Phenolic content of *Pinus roxburghii* parts in different solvent

S. No.	Extracts	Needles	Bark	Female Cone	Stem
1	Chloroform	69.70±0.69	76.40±1.22	60.10±0.89	22.52±0.76
2	Aqueous	274.24±1.21	231.71±0.36	69.65±0.81	231.68±0.58
3	Methanol	396.12±1.65	266.46±0.55	173.74±0.61	243.16±1.01

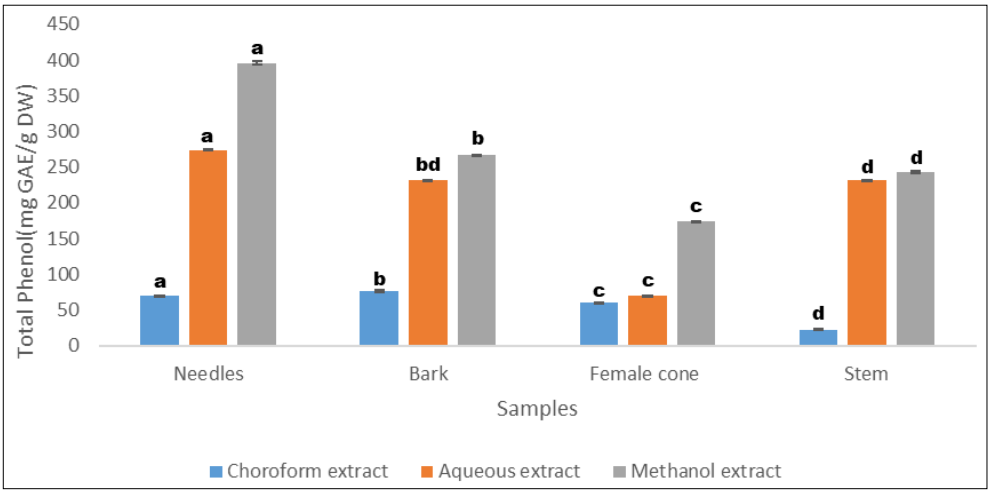

Fig 1: Total phenolic content in different parts of *Pinus roxburghii*. [Bars (with same colour) having different letters are statistically significant at $p<0.05$]

Total flavonoid content

In comparison to the other two solvents, it was discovered that the methanol extract had the highest total flavonoid content when compared within same part of three different solvents (Table.3, Fig.2). The methanolic stem extract has the highest total flavonoid content, measuring 500.67±1.37 mg RE/g DW, whereas the chloroform extract of needles has the lowest amount, measuring 164.03±0.46 mg RE/g DW.

Table 3: Total Flavonoid content of *Pinus roxburghii* parts in different solvent

S.No.	Extracts	Needles	Bark	Female Cone	Stem
1	Chloroform	164.03±0.46	166.02±0.37	264.07±0.86	189.67±0.76
2	Aqueous	209.17±1.44	364.27±1.07	264.90±0.84	405.54±0.71
3	Methanol	253.89±0.59	476.17±0.74	409.80±0.97	500.67±1.37

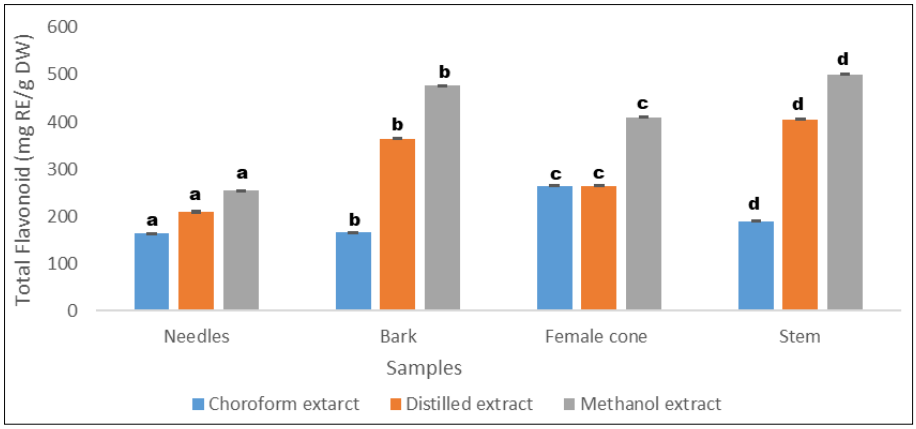

Fig 2: Total flavonoid content in different parts of *Pinus roxburghii*. [Bars (with same colour) having different letters are statistically significant at p<0.05]

The methanolic extracts have higher phenolic content in all the sample parts [22, 23]. Similar results were observed in previous studies; as in methanolic and chloroform extracts, except for needles, the flavonoid content was relatively higher than the phenolic content whereas on aqueous extracts flavonoid content was greater than the phenolic content in all the parts [25, 26]. Since flavonoids exhibit antibacterial properties and phenolic compounds are known to be bactericidal and fungicidal in nature, they can be utilized as antimicrobial agents [27, 28, 29]. Additionally, antioxidant components found in phenol and flavonoids demonstrate the ability to scavenge free radicals [30].

Conclusion

This study clearly shows that *Pinus roxburghii* from Dwarahat region of Almora district contains natural curing agents in the form of phytochemicals as phenol, flavonoids, tannin, and terpenes. This plant's significant phenolic and flavonoid content makes it useful for treating a range of illnesses. To learn more about the antibacterial and antioxidant qualities, more research needs to be carried out. Owing to the species' abundance in the Himalayan region, this plant can be exploited to investigate its potential for creating cutting-edge treatments for humans that could be advantageous for the pharmaceutical sector.

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Conflict of interests

The authors hereby declare no such conflicts of interest.

References

1. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology,2006:107(2):182-188.
2. Nirala P, Asha. "*Pinus roxburghii*: A Review on Medicinal and Economic Properties". In: Gautam AS (Ed.) Recent Advancements in Sciences with Special Reference to Himalaya, Ancient Publishing House, Delhi, India, 2020, 230- 239.
3. Hounsome N, Hounsome B. Biochemistry of vegetables: major classes of primary (carbohydrates, amino acids, fatty acids, vitamins, and organic acids) and secondary metabolites (terpenoids, phenolics, alkaloids, and sulfur-containing compounds) in vegetables. Handbook of vegetables and vegetable processing. Wiley-Blackwell, Hoboken, New Jersey, United States, 2011, 23-58.
4. Price RA, Liston A, Strauss SH. Phylogeny and systematics of *Pinus*. In: Ricardson DM (Ed.) Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge, United Kingdom, 1998, 49-68.
5. Arya S, Kalia RK, Arya ID. Induction of somatic embryogenesis in *Pinus roxburghii* Sarg. Plant Cell Reports,2004:19(8):775-780.
6. Tewari DN. A monograph on Chir pine (*Pinus roxburghii* Sarg.) International Book Distributors. Dehradun, India, 1994, 311.
7. Sharma CM, Baduni NP. Structural attributes and growing stock variations on different aspects of high Himalayan and Siwalik chir *Pine* forests. Journal of tropical forest science,2000:12(3):482-492.
8. Puri A, Srivastava AK, Singhal B, Mishra SK, Srivastava S, Lakshmi V. Antidyslipidemic and antioxidant activity of *Pinus roxburghii* needles. Medicinal Chemistry Research,2011:20(9):1589-1593.

9. Abbasi AM, Khan MA, Ahmad M, Zafar M, Jahan S, Sultana S. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. Journal of Ethnopharmacology,2010:128(2):322-335.
10. Sharma A, Goyal R, Sharma L. Potential biological efficacy of *Pinus* plant species against oxidative, inflammatory and microbial disorders. BMC complementary and alternative medicine,2015:16(1):1-11.
11. Willför S, Ali M, Karonen M, Reunanen M, Arfan M, Harlamow R. Extractives in bark of different conifer species growing in Pakistan. Wood Research and Technology Holzforschung,2009:63:551-558.
12. Khan I, Singh V, Chaudhary AK. Hepatoprotective activity of *Pinus roxburghii* Sarg. wood oil against carbon tetrachloride-and ethanol-induced hepatotoxicity. Bangladesh Journal of Pharmacology,2012:7(2):94-99.
13. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science,2015:2(4):25-32.
14. Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogan citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of PharmTech Research,2014:6(2):521-529.
15. Purohit AP, Kokate CK, Ghokale SB. Text book of Pharmacognosy. Nirali prakashan, Pune, India, 2003, 330.
16. Semwal P, Anthwal P, Kapoor T, Thapliyal A. Preliminary investigation of phytochemicals of *Saussurea obvallata* (Brahm Kamal) and *Pittosporum eriocarpum* (Agni): two endangered medicinal plant species of Uttarakhand. International Journal of Pharmacognosy,2014:1(4):266-269.
17. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan Publication. New Delhi, India, 1999, 115.
18. Yasuma A, Ichikawa T. NinhydrinSchiff and alloxan-Schiff staining: a new histochemical staining method for protein. The Journal of Laboratory and Clinical Medicine,1953:41(2):296-299.
19. Whistler RL, BeMiller JN. Industrial Gums [recurso Electrónico]: Polysaccharides and Their Derivatives. Academic Press, Cambridge, Massachusetts, United States, 1993, 642.
20. Makkar HPS. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Kluwer Academic Publishers. Dordrecht, The Netherlands, 2003, 102.
21. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry,1999:64(4):555-559.
22. Maimoona A, Naeem I, Saddiqe Z, Ali N. Analysis of total flavonoids and phenolics in different fractions of bark and needle extracts of *Pinus roxburghii* and *Pinus wallichiana*. Journal of Medicinal Plants Research,2011:5(21):5216- 5220.
23. Thapa R, Upreti A, Pandey BP. Chemical profiling and biological activity analysis of cone, bark, and needle of *Pinus roxburghii* collected from Nepal. Journal of Intercullectual Ethnopharmcology,2018:1(1):66-75.
24. Chaudhary AK, Ahmad S, Mazumder A. Study of antibacterial and antifungal activity of traditional *Cedrus deodara* and *Pinus roxburghii* Sarg. CELL MED,2012:2(4):37-1.
25. Karapandzova M, Stefkov G, Karanfilova IC, Panovska TK, Stanoeva JP, Stefova M, *et al*. Chemical characterization and antioxidant activity of Mountain *Pine* (*Pinus mugo* Turra, Pinaceae) from Republic of Macedonia. Records of Natural Products, 13, 50-63.
26. Ustun O, Senol FS, Kurkcuoglu M, Orhan IE, Kartal M, Baser KH. Investigation on chemical composition, anticholinesterase and antioxidant activities of extracts and essential oils of Turkish *Pinus* species and pycnogenol. Industrial crops and products,2012:38:115-23.
27. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews,1999:12(4):564-582.
28. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Reviews,2019:18(1):241-272.
29. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents,2005:26(5):343-356.
30. Amarowicz R, Pegg RB, Rahimi Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food chemistry,2004:84(4):551-562.