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Characterization of Flavonols Present in Barks and Needles of *Pinus wallichiana* and *Pinus roxburghii*

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Flavonoids are a diverse group of natural products found in all plants. In present study five flavonols namely quecetin, kampherol, rhamnetin, isorhamnetin and myrcetin were identified and estimated in *Pinus roxburghii* and *Pinus wallichiana* bark and needles extracts in different solvents. Study was carried out on acid hydrolyzed methanolic extracts which was further fractionated into diethyl ether, *n*-butanol, ethyl acetate and water extracts. Quercetin was found to be the most abundant flavonol present in *Pinus wallichiana* and *Pinus roxburghii* barks and needles. Myrcetin was not present in *Pinus wallichiana* needles while. rhamnetin was only present in *Pinus wallichiana* bark.

Key Words: Antioxidants, Flavonoids, *Pinus roxburghii*, *Pinus wallichiana*.

INTRODUCTION

Plants have the ability to produce a large variety of secondary metabolites, such as terpenoids, phenylpropanoids, flavonoids and alkaloids, which together account for over 200,000 compounds[1]. The National Cancer Institute has identified a host of compounds found in foods and plants that possess cancer preventing properties. Among these are antioxidants, phytosterols, carotenoids, triterpenes, saponins, tannins and flavonoids. These phytochemicals may augment immune function, inhibit the formation of cancer-causing nitrosamines, hinder hormonal activity, as well as induce Phase I or Phase 2 detoxification enzymes, thus protecting the body against chronic diseases, such as cancer. Even so, a substantial amount of additional research is needed in order to obtain a better understanding of the role these agents play in cancer chemoprevention. Flavonoids, including the anthocyanins, flavonols and flavones, are among the most intensely studied secondary products with over 6,000 known compounds[2]. Many of them play important roles as flower and fruit pigments, UV protectants, signaling molecules between plants and microbes and regulators of auxin transport[3,4]. The flavonoids are also thought to have antioxidant, antiallergenic and antiinflammatory effects, thus contributing to human health[5,6].

HPLC is gaining increasing importance for the analysis of plant extracts. The qualitative analysis which produces a "fingerprint" chromatogram obtained under standard conditions can be very useful for quality control of phytochemicals. Although TLC is also a powerful and simple technique used for this purpose, there are situations

in which it can produce doubtful results. HPLC can also be a useful tool in chemosystematics helping, for example, to characterize species on the basis of their secondary metabolite contents.

Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants. In one study it was used to distinguish species based on the quantitative variation of flavonoids among them.

EXPERIMENTAL

All reagents were of analytical grade. Quercetin (3,3′,4,5,7-tetrahydroxyflavonol), myricetin (3,3′,4,5,5′,7-hexahydroxyflavone), kampherol, rhamnetin and isorhamnetin were purchased from Sigma Aldrich.

Extraction method: The needles and bark of *Pinus roxburghii* and *Pinus wallichiana* were collected from Kuldana, Murree in Sep. 2006 and a voucher specimen was deposited at Lahore College for Women University Herbarium. The plant material (1.00 kg each) were dried away from the sunlight, powdered and exhaustively extracted with methanol using Soxhlet extraction method to give solvent free crude methanolic extract (Table-1). These methanolic extracts were then acid hydrolyzed and tested for flavonoid contents using standard myrcetin, kampharol, rhamnetin, isorhamnetin and quercetin HPLC. The methanol extracts were then fractionated using diethyl ether, *n*-butanol, ethyl acetate and water to evaluate the most suitable solvent for separation.

TABLE-1
PERCENTAGE OF METHANOL EXTRACTS OF EXPERIMENTAL PLANTS

Names of Plants	Code	Weight of fresh plant (Kg)	Percentage concentration of MeOH extract
Pinus wallichiana bark	PWB	1	7.056
Pinus roxburghii bark	PRB	1	6.966
Pinuswallichiana needles	PWN	1	4.066
Pinus roxburghii needles	PRN	1	3.169

Acid hydrolysis: Controlled acid hydrolysis was carried out with 10 % acetic acid under reflux for 3.5 h. These fractionated samples were then analyzed by HPLC without any further separation[7].

HPLC analysis: The HPLC system (Waters) consisted of a pump 1500 series) and a UV detector (2487). Column was a C18, (250 × 4.6 mm, 5 mm particle sizes). Acetonitrile was purchased from Merck. Water was HPLC grade and acidified with 1 % acetic acid. Qualitative analysis was made with samples, in isocratic mode, with acetonotrile:water 1:1 at a flow-rate of 1 mL min^{-1}. The injection volume was 10 µL and the elute was monitored at 254 nm. The filtered methanol extracts (0.5 µ) of needles and bark of *Pinus wallichiana* and *Pinus roxburghii* and their fractions were injected under these conditions and compared with authentic standards of myrcetin, quercetin, kampherol, rhamnetin and isorhamnetin injected under similar conditions.

Qualitative analysis: The method developed for HPLC fingerprinting provided a quick analysis of the methanolic extract and fractions obtained after fractionation. The conditions used led to a good separation of the peaks which could be identified by comparing the chromatogram with the chromatogram of the reference compounds obtained under the same conditions.

Quantification of flavonols: Quantitative studies of flavonols were made by comparing with standard solutions of known concentration.

RESULTS AND DISCUSSION

Myrcetin, kampherol and rhamnetin were not detected in all the fractionated extracts of needles of *Pinus wallichiana*, while quercetin was the most abundant flavonoid aglycone (21.426 %) present in methanol extract, most of it dissolved into *n*-butanol extract (15.714 %) and the rest in diethyl ether extract (5.712 %). when fractionated. It was observed that isorhamnetin was present only in methanol extract of needles of *Pinus wallichiana* (2.857 %) all of which was extracted in diethyl ether, while *Pinus roxburghii* needles were devoid of it (Table-2).

TABLE-2
PERCENTAGE OF FLAVANOLS IN DIFFERENT EXTRACTS OF NEEDLES OF *Pinus wallichiana* AND *Pinus Roxburghii*

	Myrcetin (%)	Kampherol (%)	Rhamnetin (%)	Isorhamnetin (%)	Quercetin (%)
PWN-Methanol	0	0	0	2.857	21.426
PWN-Diethyl ether	0	0	0	2.857	5.712
PWN-*n*-Butanol	0	0	0	0	15.714
PWN-Ethyl acetate	0	0	0	0	0
PWN-Water	0	0	0	0	0
PRN-Methanol	1.00	0	0	0	4.290
PRN-Diethyl ether	0.08	0	0	0	2.857
PRN-*n*-Butanol	0	0	0	0	1.428
PRN-Ethyl acetate	0	0	0	0	0
PRN-Water	0	0	0	0	0

Methanol extracts of barks of *Pinus wallichiana* and *Pinus roxburghii* were also compared for their flavonoid aglycone contents and it transpired that *Pinus wallichiana* (bark) methanol extract contained all the tested flavanols in considerable amounts while bark extract of *Pinus roxburghii* was devoid of myrcetin and isorhemnetin but rich in quercetin (10.01 %) and campharol (3.04 %) comparatively (Table-3).

Conclusion

Quercetin has been reported to have interesting biological activities including the inhibition of the anticancer drug target, heat shock protein-9 (Hsp90)[8-11]. *Pinus wallichiana* needle extract in methanol presented a better source of quercetin having antihypertensive properties.

TABLE-3
PERCENTAGE OF FLAVANOLS IN DIFFERENT EXTRACTS OF BARK OF *Pinus wallichiana* AND *Pinus roxburghii*

	Kampherol (%)	Rhamnetin (%)	Myrcetin (%)	Isorhamnetin (%)	Quercetin (%)
PWB-Methanol	2.300	2.08	3.0	2.005	5.009
PWB-Diethyl ether	0	2.08	0	1.847	0
PWB-*n*-Butanol	0	0	0	0	0
PWB-Ethyl acetate	1.857	0	0	0	5.001
PWB-Water	0	0	0	0	0
PRB-Methanol	3.040	1.00	0	0	10.010
PRB-Diethyl ether	0	0	0	0	0
PRB-*n*-Butanol	0	0.90	0	0	0
PRB-Ethyl acetate	1.002	0	0	0	0
PRB-Water	0.532	0	0	0	9.573

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REFERENCES

1. R.A. Dixon and D. Strack, *Phytochemistry*, **62**, 815 (2003).
2. J.B. Harborne and C.A. Williams, *Phytochemistry*, **55**, 481 (2000).
3. H.K. Dooner, T.P. Robbins and R.A. Jorgensen, *Ann. Rev. Genet.*, **25**, 173 (1991).
4. R.A. Dixon and N.L. Paiva, *Plant Cell*, **7**, 1085 (1995).
5. A. Scalbert, I.T. Johnson and M. Saltmarsh, *Am. J. Clin. Nutr.*, **81**, 215S (2005).
6. J.A. Ross and C.M. Kasum, *Ann. Rev. Nutr.*, **22**, 19 (2002).
7. F. Imperato, *Am. Fern J.*, **74**, 14 (1984).
8. N. Nagai, A. Nakai and K. Nagata, *Biochem. Biophys. Res. Commun.*, **208**, 1099 (1995).
9. R.K. Hansen, S. Oesterreich, P. Lemieux, K.D. Sarge and S.A.W. Fuqua, *Biochem. Biophys. Res. Commun.*, **239**, 851 (1997).
10. M. Kudo, Z. Naito, M. Yokoyama and G. Asano, *Exp. Mol. Pathol.*, **66**, 66 (1999).
11. B.Y. Wu and A.C.H. Yu, *J. Neurosci. Res.*, **62**, 730 (2000).

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