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Research Article

Phytochemical Investigation of Water Soluble Phytoconstituents of *Leptadenia reticulata* (Retz.) Wight & Arn

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ABSTRACT

Leptadenia reticulata (Retz.) Wight & Arn, referred to as 'Jivanti' in ayurvedic texts, is held in high esteem in terms of its medicinal value. Apart from terpenoids and sterols, very few of the phytochemicals are reported in literature, especially water soluble constituents. In the present work, three flavonoids apart from those previously reported, have been identified occurring as O-glycosides. A flavone occurring as C-glycoside has also been identified in the plant. The analysis for flavonoids was done for leaf, young stem, mature stem and root individually using separation by paper chromatography and identification of the separated components using UV spectroscopy. Phenolic acid analysis involved a 2-D separation technique on paper, followed by derivatization using diazotizing reagents. Ferulic acid was the only phenolic acid reported earlier for this plant. The present study has unearthed six more phenolic acids. The amino acid content for the leaf, young stem, mature stem and root have been analyzed using co-chromatography of plant extracts, with 21 authentic amino acid standards, using paper chromatography.

Keywords: *Leptadenia reticulata;* flavonoids; phenolic acids; amino acids.

INTRODUCTION

Leptadenia reticulata (Retz.) Wight & Arn is a twiner found in Gujarat, sub-Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India, ascending up to an altitude of 900m. This plant, belonging to the Asclepiadaceae family, is considered to be a *Rasayana* (tonic) drug and is thus used to vitalize, nourish and rejuvenate the body (M Daniel, 2006). The plant is referred to as 'Jivanti' in ayurvedic texts. The major therapeutic claim is its galactogogue action, which has been proved in rats (Anjaria *et al*, 1975), cows (Anjaria *et al*, 1974) as well as humans (Patel *et al*, 1982). Aqueous extract of the stem demonstrated negative, chronotropic and prolonged hypotensive effect in dogs (Agrawal *et al*, 1960). The antibacterial (Patel *et al*, 1986) and antifungal (Patel *et al*, 1958) activities have been proved. Aqueous extracts are safely tolerable up to a dose of 3.125g/kg (Anjaria *et al*, 1970) .Previously reported chemical constituents of *Leptadenia reticulata* are α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentriacontanol (Krishna *et al*, 1976), a triterpene alcohol simiarenol (Subramanian *et al*, 1977) and apigenin (Sastry *et al*, 1985). Pregnane glycosides reticulatin, deniculatin and leptaculatin have also been isolated from the aerial parts (Srivastav *et al*, 1995).

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MATERIALS AND METHODS

The plant material was collected from Vadodara (Gujarat).The specimen was identified and authenticated at Botanical Survey of India, Pune (No.BSI/WC/ Tech/ 2007/734). The voucher specimen of this plant is deposited at the Herbarium, B.S.I., Pune. The leaves, young stem, mature stem and root were separated for their individual analysis. The plant materials were washed, shade dried for a day and then dried completely in an oven at 38^{o}C. The plant materials were coarsely powdered using a rotary grinder and stored in airtight plastic containers. This powder was used for all phytochemical analysis.

Fifty grams of plant powder was extracted in a Soxhlet's apparatus with methanol for 48 hrs till the plant material became colourless. The methanolic extract was concentrated to dryness in a water bath. 25-30 cm^{3}of water was added to the dry residue and the water soluble components like phenolic glycosides, sugars and amino acids were filtered out. About 5 cm^3 of this aqueous filtrate was directly used for amino acid analysis (Fraction A). Rest of the filtrate was hydrolyzed in a water-bath for one hour using 7% HCl. This hydrolysate was extracted with diethyl ether, whereby the aglycones got separated into ether fraction (Fraction B).

The ether fraction was used for analysis of O-glycosides of flavonoids and phenolic acids. For analysis of C-glycosides of flavonoids (glycoflavones), the aqueous fraction remaining after the separation of aglycones was neutralized by the addition of anhydrous $BaCO_3$ and concentrated to dryness. When $BaCO_3$ was used, barium chloride got precipitated and it was filtered out. This filtrate was concentrated to dryness. To this dried residue, ethanol was added to dissolve the glycoflavones. The alcoholic filtrate was concentrated and used for analysis of C-glycosides (Fraction C).

Apart from extraction of components in methanol using a Soxhlet extractor, about 10 grams each of leaf, young stem, mature stem and root powders were separately refluxed in 500 cm^3 distilled water for 3 hours. The slurry was filtered and the aqueous filtrate was subjected to hydrolysis using 7% HCl. The further procedure of obtaining Fractions A, B and C remained the same as mentioned earlier for the methanolic Soxhlet extract. This dual mode of extraction was done to compare the difference in phytoconstituents in the methanolic and the water extracts.

1. Amino acids: The Fraction A was concentrated *in vacuo* and the concentrated extract was spotted on Whatmann No.1 paper and chromatographed along with 21 authentic amino acid standards. The solvent system employed was n-butanol:glacial acetic acid:water (4:1:5 v/v/v). Post-run, the papers were air-dried. The papers were dipped in 1% ninhydrin in acetone and heated at 60°C. The amino acids were identified on comparison of R_f values and colour with those of the authentic samples.

2. Flavonoids: The Fraction B was concentrated *in vacuo* and was banded on Whatman No. 1 paper. The solvent system employed was 30% glacial acetic acid. The developed chromatograms were dried in air, observed under ultra-violet light (360 nm) and the bands were marked. The marked bands were cut out from the papers and the compounds were eluted using spectroscopic grade methanol. The UV absorption spectra of these compounds were recorded in methanol using Perkin- Elmer Lambda 25 UV/Vis spectrophotometer. The observed lambda maxima values were compared with those reported in literature and the flavonoids were identified.

The presence of the flavonoids was further confirmed by co-chromatography with authentic samples.

Fraction C was banded on Whatman No.1 paper and the chromatogram was developed using water as mobile phase. Glycoflavones were visualized by their colour in UV & with 10% aqueous Na_2CO_3 spray. Further analysis and identification were done by measuring the λ max and by co-chromatography with authentic samples.

3. Phenolic acids: Analysis of phenolic acids was carried out by two-dimensional ascending paper chromatography, for which Fraction B was spotted on Whatmann No.1 paper. Toluene: acetic acid: water (6:7:3, upper organic layer) in the first direction and sodium formate: formic acid: water (10:1:200 w/v/v) in the second direction were used as irrigating solvents. The reagents used to locate the compounds on the chromatograms were diazotized *p*-nitroaniline and diazotized sulphanilic acid, both followed by a 10% Na_2CO_3 overspray.

The various phenolic acids presents in the extract were identified based on the specific colours they produce on reaction with the spray reagents and the relative R_f values when run in the different solvent systems as well as co-chromatography with authentic samples.

RESULTS AND DISCUSSION

The analysis of the water soluble constituents of the individual parts of *Leptadenia reticulata* revealed differences in the phytoconstituents. The amino acid content in all the four different parts was different. The flavonoids showed significant variation in methanolic as well as aqueous extracts.

L.reticulata shows the presence of 13 amino acids [Table 1], out of which 8 are essential amino acids. Leaf contains 9 amino acids whereas young stem contains 8 amino acids, out of which the number of essential amino acids are 5 each. Root and mature stem show identical presence of 10 amino acids, out of which 6 are essential amino acids. Leucine, valine and glutamic acid were restricted only to leaf while isoleucine and methionine was found in the remaining parts. Phenyl alanine, tryptophan, arginine, alanine, tyrosine and proline are found in all parts of the plant. The amino acids restricted only to the mature stem and root are lysine and serine. Thus the presence of these amino acids *L.reticulata* increases the nutritive value of the plant as a whole.

The phytochemical screening of the methanolic extracts for flavonoids [Table 2] occuring as O-glycosides revealed the presence of diosmetin in leaf and young stem, apigenin in leaf and both young and mature stems, acacetin in young and mature stems, while 3',4'-dimethoxy luteolin was found to be restricted only to the leaf. Acacetin was found to be the only flavones occurring as C-glycoside in the leaf, young stem and mature stem.

The analysis of the O-glycosides of flavonoids extracted in boiling water showed the presence of diosmetin in leaf and young stem while 3',4'-Dimethoxy luteolin was found in young stem. Chrysoeriol was found to be present in leaf. This is the first report of this flavonoid in *L. reticulata*. The water extract of mature stem was devoid of flavonoids. Root of the plant showed complete absence of flavonoids occurring as either O- or C-glycosides.

Analysis of phenolic acids in the methanolic as well as aqueous extracts of *L.reticulata* showed differences

Table 1: Amino acids present in various parts of *L. reticulata*

Amino acid	Leaf	Young stem	Mature stem	Root
L-Phenyl alanine*	+	+	+	+
L-Valine*	+	-	-	-
L-Tryptophan*	+	+	+	+
L-Threonine*	-	-	-	-
L-Isoleucine*	-	+	+	+
L-Methionine*	-	+	+	+
L-Histidine*	-	-	-	-
L-Arginine*	+	+	+	+
L-Leucine*	+	-	-	-
L-Lysine*	-	-	+	+
L-Alanine	+	+	+	+
L-Tyrosine	+	+	+	+
L-Aspargine	-	-	-	-
L-Proline	+	+	+	+
L-Aspartic acid	-	-	-	-
L-Glutamine	+	-	-	-
L-Glutamic acid	-	-	-	-
L-Serine	-	-	+	+
L-Cysteine	-	-	-	-
L-Cystine	-	-	-	-
Glycine	-	-	-	-

*Essential amino acids

Table 2: Flavonoids present in various parts of *L. reticulata*

Part of plant used	Flavonoids occuring as O-glycosides (from Methanolic extract)	Flavonoids occuring as C-glycosides (from Methanolic extract)	Flavonoids occuring as O-glycosides (from Aqueous extract)
Leaf	Apigenin, Diosmetin, 3',4'-Dimethoxy luteolin	6C-Glucosyl acacetin	Chrysoeriol, Diosmetin
Young stem	Apigenin, Acacetin, Diosmetin	6C-Glucosyl acacetin	3',4'-Dimethoxy luteolin, Diosmetin
Mature stem	Apigenin, Acacetin,	6C-Glucosyl acacetin	(absent)
Root	(absent)	(absent)	(absent)

Table 3: Flavonoids present in various parts of L. reticulata

Part of plant used	Phenolic acids (from Methanolic extract)	Phenolic acids (from Aqueous extract)
Leaf	Vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxy benzoic acid	Vanillic acid, syringic acid
Young stem	Vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid	Vanillic acid, syringic acid, melilotic acid
Mature stem	Vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxy benzoic acid	Vanillic acid, syringic acid
Root	Vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid	Vanillic acid, syringic acid, ferulic acid, *p*-coumaric acid

when analyzed individually for leaf, young stem, mature stem and root [Table 3]. The analysis of phenolic acids in the methanolic extracts revealed the presence of vanillic acid, syringic acid, *p*-coumaric acid (*cis* and *trans* isomers) and ferulic acid (*cis* and *trans* isomers) in all the different parts of the plant. Only the leaf and mature stem showed the presence of p-hydroxy benzoic acid also, apart from the above mentioned phenolic acids.

Aqueous extracts of the individual parts of the plant showed vanillic acid and syringic acid to be present in all four parts. Melilotic acid was found to be confined only to the young stem. The aqueous extract of root showed the presence of *p*-coumaric acid (*cis* and *trans*

isomers) and ferulic acid (*cis* and *trans* isomers), in addition to vanillic acid and syringic acid.

Most flavonoids and phenolic acids are known to be antioxidant in nature, according to Dr.Dukes Phytochemical and Ethnobotanical Database. Chrysoeriol and diosmetin are cancer preventive, radical scavenger and antiviral in nature. Luteolin is antiallergic, antibacterial, anti-inflammatory, antipolio and antiherpetic. Apigenin is antiaflatoxin, antileukemic and antimelanomic. Acacetin is antiaflatoxin, antimalarial, hepatoprotective and antihistaminic.

Among phenolic acids *p*-hydroxy benzoic acid is antibacterial, antisickling and fungistat. Ferulic acid is analgesic, antiallergic, anti-inflammatory hepatoprotective and antihepatotoxic. *P*-coumaric acid is antibacterial, antiseptic and antitumour. Melilotic acid is antiulcerogenic. Vanillic acid is antileukemic, antioxidant, antiseptic, antibacterial and anthelmintic. Syringic acid is antioxidant, antiradicular and allelopathic (Dukes, 1997).

CONCLUSION

Previous reports only mention the presence of one phenolic acid in *L. reticulata* i.e. ferulic acid, whereas the present study has identified five more phenolic acids. Apart from flavonoids such as apigenin, luteolin, rutin and diosmetin which have been previously identified in the plant, the present study has revealed the presence of chrysoeriol, which is a structural isomer of diosmetin. This is the first report of the chrysoeriol in *L.reticulata*. The study has also identified acacetin, an important flavonoid occurring as C-glycoside, which is a new report for the plant. Amino acid analysis indicated that the plant contains 13 amino acids, out of which 8 are essential amino acids. This is an important result as far as the nutritive value of the plant is concerned.

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