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Estimation of total phenolic content and total flavonol content in leaves and stem extracts of *Ventilago maderaspatana* Gaertn. and leaves, stem and stem bark extracts of *Ziziphus xylopyrus* (RETZ) Willd

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Abstract

Aqueous and ethanolic extract were prepared from the leaf, stem of *Ventilago maderaspatana* and leaf, stem, stem bark of *Ziziphus xylopyrus*. Preliminary phytochemical analysis was carried out for all extracts. Both plants were found to contain different phytoconstituents such as alkaloids, glycosides, steriods and sterols, saponins, flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds. Total phenol and flavonoid content were evaluated for all the extracts of both plants. Both plants showed good amount of phenol and flavonoids.

Keywords: Estimation, phenolic and flavonol content *V. maderaspatana*, leaf, stem, and *Ziziphus xylopyrus*, leaf, stem, and stem bark extract

Introduction

V. maderaspatana (Rhamnaceae) is commonly known as Red creeper in English, Raktavalli in Sanskrit and pittu in Hindi [1]. It is climbing shrub and identified by its dark grey bark branchlets with brownish pubescent. It flowers in winter with an offensive odour [2, 3]. It is found in Indonesia, Malaysia, Sri Lanka, Bhutan and throughout India [4, 5]. The powdered root bark is carminative, stomachic, tonic and stimulant; useful in atonic dyspepsia, debility and slight cases of fever. The powdered bark (mixed with gingelly oil) is used in South India as external application for itch and other skin diseases [6]. The root bark is a valuable source of reddish dye (Ventilagin), used for colouring mordanted cotton, wool and *tasar* silk. In combination with the root of *Hedyotis umbellate*, the root bark yields a beautiful chocolate colour. The bark also yields fibres, used for cordage. The pale yellow wood may be used as fuel. The long climbing stems are sometimes used by fishermen as substitute for ropes. The seeds are eaten when cooked, and the oil from them is used for cooking [4]. Five isofuranonaphthoquinones have been isolated from the root bark [7]. Two new naphthalene derivatives and three naphthoquinones from the root bark have been isolated [8]. Antibacterial, Nitric Oxide *In-Vitro* and *Ex-Vivo* scavenging, anti-denaturation property and anti-oxidant activity of stem-bark of *V. maderaspatana* was reported [9, 10, 11]. However, a pharmacognostic study on leaf of *V. maderaspatana* was reported [12].

Ziziphus xylopyrus family Rhamnaceae is commonly known as Jujab in English [13]. It is a large, straggling shrub or a small tree, armed with spines, up to 4 m in height. Fruits are globose, 3- rarely 2 or 4-celled, with usually a seed in each cell, very hard and woody. It is found in Pakistan and China, North-Western India, Uttar Pradesh, Bihar, Central and South India [14]. The fruit decoction of this plant is used in birth control in some parts of Rajasthan, India [15]. This plant is widely used in Turkish medicine as a potent sedative. [16] Bark and leaf powder paste are applied externally for chest pain arising from cough. In stomach ache and indigestion, fruit powder (3-4 g) is administered with a pinch of ginger powder thrice in a day. The leaves contain quercetin and quercitrin. The bark contains tannins (7.2%) 7, 3, 4-trihydroxy flavan-3, 4-diol and oleic acid [17]. *Z. xylopyrus* is used in Pyorrhoea and to check oogenesis [18]. The bark is used for its astringent activity and as dental sticks for teeth cleaning. In different parts of India this plant is also used in the treatment of diarrhea [19].

Leaf paste is applied on pimples and it is ground along with latex of *Ipomea carnea* and applied on boils^[20]. One to two inches of the fresh stem bark of this species are chewed with 1-2 peppers (*Piper nigrum*) and the sap swallowed once a day for 5 days in the treatment of cough.^[21] Xylopyrine-A and Xylopyrine-B, the two new 13-membered cyclopeptide alkaloids are also present in *Ziziphus* species i.e. *Z. xylopyra*^[22]. Pharmacognostic and phytochemical study on leaves of *Ziziphus xylopyrus*^[23].

Materials and methods

Plant material

The leaf and stem of *V. maderaspatana* and the leaf, stem and stem bark of *Z. xylopyrus* were collected from Tirupati district and were authenticated by the botanist, Dr. K. Madhvachetty, Professor, Department of Botany, Sri Venkateshwara University, Tirupati, India. Voucher No S.V.U./SC/10/26/10-11, S.V.U./SC/11/27/10-11 and the specimen were deposited at the department of Pharmacognosy, JSSCP, Ooty. The plant material was subjected for processing which included initially, removal of other than the plant parts (foreign matters) such as grass, sand and mud to ensure the plant quality^[24].

Extraction of selected plant materials

The coarse powdered materials of the plant parts were subjected to the cold maceration. 500 gm. each of the leaf and stem of *V. maderaspatana* and the leaf, stem and stem bark of *Z. xylopyrus* parts were extracted by cold maceration process. For extraction, two solvents were used, ethanol and distilled water. The nature and yield of the extracts were noted. The total of ten extracts, two extracts each of different parts of both plants, were stored in a refrigerator at 4 °C until further used and the extracts were labelled as *V. maderaspatana* leaf ethanolic (VMLE), *V. maderaspatana* leaf aqueous (VMLA), *V. maderaspatana* stem ethanolic (VMSE), *V. maderaspatana* stem aqueous (VMSA), *Z. xylopyrus* leaf ethanolic (ZILE), *Z. xylopyrus* leaf aqueous (ZILA), *Z. xylopyrus* stem ethanolic (ZISE), *Z. xylopyrus* stem aqueous (ZISA), *Z. xylopyrus* stem bark ethanolic (ZIBE) and *Z. xylopyrus* stem bark aqueous (ZIBA), respectively, for the purpose of convenient identification. Preliminary phytochemical analysis was carried out for all extracts^[25, 26, 27]. Both plants were found to contain different phytoconstituents such as alkaloids, glycosides, steroids and sterols, saponins, Flavonoids, carbohydrates, and triterpenoids and showed the absence of amino acids, proteins and acidic compounds, Results were mentioned in table 1.

Estimation of total phenolic content^[28]

Principle: Total phenol was determined in extracts by using the Folin-Ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation, a green blue complex formed was measurable at 750 nm. The total phenol content of a tested material was related to the antioxidant activity showed by it.

Chemicals and reagents

- All reagents and chemicals used during the study were of analytical grade.
- **Folin-Ciocalteu Reagent:** Folin-Ciocalteu reagent was diluted (1:10) with distilled water and used.

- **Sodium carbonate:** 20.25 g of sodium carbonate (Na₂CO₃.10 H₂O) was dissolved in 100 mL of distilled water and used.
- **Methanol:** Distilled.

Preparation of test solutions

10 mg each of the extracts were separately dissolved in 10 mL of methanol to get 1 mg/mL solution. These solutions were diluted with methanol to obtain solution ranging from 1000 µg/ml to 15.62 µg/mL.

Preparation of standard solution

Gallic acid: 100 mg of gallic acid monohydrate was dissolved in 100 mL of distilled water to get 1000 µg/mL solution. It was serially diluted with distilled water to obtain solutions ranging from 1000 µg/ml to 15.62 µg/mL.

Procedure

400 µl of the extracts (1 mg/mL) were separately mixed with 2 mL of Folin-Ciocalteu reagent and 1.6 mL of sodium carbonate. After shaking, it was kept for 2 h reaction time. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 1000 µg/mL to 15.62 µg/mL. Using the standard curve the total phenolic content of extracts were obtained. The total phenol content was expressed as gallic acid equivalent in mg/g or % w/w of the extracts.

Estimation of total flavonol content^[29]

Flavonoids are water-soluble polyphenolic compounds, which are extremely common and wide spread in the plant kingdom as their glycosides.

Principle: Total flavonol was determined by aluminum chloride colorimetric method. The principle of this method is that aluminum chloride forms acid stable complexes with the C4 keto-group and either the C3 or C5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids.

Chemicals and reagents

- **Aluminum chloride (10%):** 10 g of aluminum chloride was dissolved in 100 mL of distilled water, filtered and used.
- **Potassium acetate (1 M):** 9.81 g of potassium acetate was dissolved in 100 mL of distilled water and used.
- **Methanol:** Distilled.

Preparation of test solutions

100 mg each of the extracts were dissolved in 10 mL of methanol to get 1 mg/mL solution. These solutions were diluted with methanol to obtain solution ranging from 1000 µg/ml to 15.62 µg/mL.

Preparation of standard solution

Rutin: 10 mg of rutin was dissolved in 10 mL of methanol to get 1000 µg/mL solutions. It was serially diluted with methanol to obtain solutions ranging from 1000 µg/mL to 15.62 µg/mL.

Procedure: 0.5 mL of the extract was separately mixed with 1.5 mL methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water.

After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Using rutin, standard curve was prepared and linearity was obtained in the range of 1000 µg/mL to 15.62 µg/mL. Using the standard curve the total flavonol content of each of the extract was obtained. The total flavonol content was expressed as rutin equivalent in mg/g or % w/w of the extracts.

Results and Discussion

Phytochemical studies

The ethanolic and aqueous crude extracts of different parts of *V. maderaspatana* and *Z. xylopyrus* were subjected to preliminary phytochemical screening for the detection of

phytoconstituents. The results obtained were given below

Abbreviations used for different prepared extracts and are as follows

VMLE - *V. maderaspatana* leaf ethanolic extract.

VMLA - *V. maderaspatana* leaf aqueous extract.

VMSE - *V. maderaspatana* stem ethanolic extract.

VMSA - *V. maderaspatana* stem aqueous extract

ZILE - *Z. xylopyrus* leaf ethanolic extract.

ZILA - *Z. xylopyrus* leaf aqueous extract.

ZISE - *Z. xylopyrus* stem ethanolic extract.

ZISA - *Z. xylopyrus* stem aqueous extract.

ZIBE - *Z. xylopyrus* stem bark ethanolic extract.

ZIBA - *Z. xylopyrus* stem bark aqueous extract.

Table 1: Preliminary phytochemical screening of the prepared extracts

Sr. No.	Phytochemical tests	<i>Ventilago maderaspatana</i>				<i>Ziziphus xylopyrus</i>					
		Leaf extract		Stem extract		Leaf extract		Stem extract		Stem bark extract	
		VMLE	VMLA	VMSE	VMSA	ZILE	ZILA	ZISE	ZISA	ZIBE	ZIBA
1.	Alkaloids	+	+	+	+	+		+	+	+	+
2.	Carbohydrates	+	+	+	+	+		+	+	+	+
3.	Steroids and sterols	+	-	+	-	+		-	+	-	+
4.	Glycosides	+	+	+	+	+		+	-	-	-
5.	Saponins	+	+	+	+	-		-	+	+	+
6.	Protein and amino acids	-	-	-	-	-		-	-	-	-
7.	Flavonoids	+	+	+	+	+		-	+	-	+
8.	Phenolic	+	+	+	+	+		+	+	+	+
9.	Acidic	-	-	-	-	-		-	-	-	-
10.	Fixed oils	-	-	-	-	+		-	+	-	+
11.	Triterpenoids	-	-	+	+	-		-	-	-	+

Estimation of total phenolic content

Among the four extracts, the alcoholic leaf extract of *V. maderaspatana* has showed high total phenol content 25.26±0.32% w/w equivalent of gallic acid. The other three extracts showed total phenol content in the range of 0.48±0.02% w/w to 11.92±0.01% w/w equivalent of gallic acid. Among the six extracts, the alcoholic leaf extract of *Z. xylopyrus* has showed high total phenol content 16.31±0.11% w/w equivalent of gallic acid. The other five extracts showed total phenol content in the range of 5.29±0.04% w/w to 12.61±0.31% w/w equivalent of gallic acid. The details of results are given in table 2.

Estimation of total flavonol content

Among the four extracts, the alcoholic leaf extract of *V. maderaspatana* has showed high total flavonol content 65.29±0.21 mg/g. The other three extracts showed total flavonol content in the range of 6.39±0.02 mg/g to 50.04±0.07 mg/g.

Among the six extracts, the alcoholic leaf extract of *Z. xylopyrus* has showed high total flavonol content 27.39±0.32 mg/g. The other five extracts showed total flavonol content in the range of 4.62±0.06 mg/g to 18.26±0.16 mg/g. The details of results are given in table 2.

Table 2: Estimation of total phenolic and total flavonoid content of various extracts of *V. maderaspatana* and *Z. xylopyrus*

Sr. No.	Extract	Total phenoli content (GAmg/gm of extract)	Total flavonoid content (RA mg/gm of extract)
1.	VMLE	25.26±0.32	65.29±0.21
2.	VMLA	7.98±0.03	50.04±0.07
3.	VMSE	11.92±0.01	30.12±0.04
4.	VMSA	0.48±0.02	6.39±0.02
5.	ZILE	16.31±0.11	27.39±0.32
6.	ZILA	10.59±0.40	35.45±0.26
7.	ZISE	7.45±0.32	19.34±0.27
8.	ZISA	5.29±0.04	4.62±0.06
9.	ZIBE	12.61±0.31	18.26±0.16
10.	ZIBA	10.89±0.19	30.76±0.17

- **TPC*:** Total phenolic contents (% w/w equivalent of Gallic acid), Results are mean (n=3)
- **TFC*:** Total flavonol contents (mg/g equivalent of Rutin); Results are mean (n=3)
- **SD#:** Standard Deviation (n=3)

Summary: Aqueous and ethanolic extract were prepared from the leaf, stem of *Ventilago maderaspatana* and leaf,

stem, stem bark of *Ziziphus xylopyrus*. Preliminary phytochemical analysis was carried out for all extracts. Both plants were found to contain different phytoconstituents such as alkaloids, glycosides, steriods and sterols, saponins, phenol, flavonoids, carbohydrates, and tri- terpenoids and showed the absence of amino acids, proteins and acidic compounds.

Conclusion

Total phenol and flavonoid content were evaluated for all the extracts of both plants. The phenolic and flavonoids estimation study revealed the presence of both these compounds in all the four extracts and is highest in the *Ventilago maderaspatana* Gaertn. alcoholic leaf extract. The phenolic and flavonoids estimation study revealed the presence of both these compounds in all the six extracts and is highest in the *Ziziphus xylopyrus* alcoholic leaf extract.

References

1. Anonymous. The wealth of India: raw materials series. Vol. 11 (X-Z). New Delhi: NISCAIR, CSIR; c1976. p. 123-4.
2. Hanumaiah T, Rao GSR, Rao CP, Rao KVJ, Heather JC, Philip JC, Howe RA, David SM, Thomson RH. Isofuranonaphthoquinones from *Ventilago maderaspatana*: Crystal structure of ventilone-c. *Tetrahedron*. 1985;41(3):635-642.
3. Hanumaiah T, Rao GSR, Rao CP, Rao KVJ, Heather JC, Philip JC, Howe RA, David SM, Thomson RH. Naphthalenes and naphthoquinones from *Ventilago* species. *Phytochemistry*. 1985;24(8):1811-5.
4. Kirtikar KR, Basu BD. Indian medicinal plants. Vol 1. 2nd ed. Dehradun: International Book Distributors; c1987. p. 585.
5. Kokate CK, Purohit AP, Gokhale SB. The textbook of pharmacognosy. 3rd ed. New Delhi: Vallabh Prakashan; c1991. p. 606-611.
6. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. New Delhi: Wiley Eastern Limited; c1992. p. 187.
7. Chaudhary RD. Herbal drug industry. 1st ed. New Delhi: Eastern Publication; c1996. p. 373-375, 473.
8. Khandelwal KR. Practical pharmacognosy. Pune: Nirali Prakashan; c1998. p. 149-56.
9. Chang M, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*. 2002;10:178-182.
10. Jain A, Katewa SS, Chaudhary BL, Galav P. Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. *J Ethnopharmacol*. 2004;90:171-177.
11. Subhalakshmi B, Abhijit G, Banasri H. Evaluation of the antibacterial activity of *Ventilago maderaspatana* Gaertn, *Rubia cordifolia* Linn, and *Lantana camara* Linn: isolation of emodin and physcion as active antibacterial agents. *Phytother Res*. 2005;19:888-894.
12. Subhalakshmi B, Banasri H. Evaluation of nitric oxide scavenging activity, *in vitro* and *ex vivo*, of selected medicinal plants traditionally used in inflammatory diseases. *Phytother Res*. 2006;20:896-900.
13. Varier PS. Indian medicinal plants. 2006;5:352.
14. Pullaiah T. Encyclopaedia of world medicinal plants. Regency; c2006. p. 2023-2024.
15. Department of AYUSH. Ayurvedic pharmacopoeia of India. Part 1, Vol. 5. Delhi: Govt. of India, Department of ISM and H; c2006. p. 47.
16. Jagtap SD, Deokule SS, Bhosle SV. Some unique ethnomedicinal uses of plants used by the Korku tribe of Amravati district of Maharashtra, India. *J Ethnopharmacol*. 2006;107:463-469.
17. Dash SK, Padhy S. Review on ethnomedicines for diarrhoea diseases from Orissa prevalence versus culture. *J Hum Ecol*. 2006;20:59-64.
18. Reddy KN, Reddy CS, Trimurthulu G. Ethnobotanical survey on respiratory disorders in Eastern Ghats of Andhra Pradesh, India. *Ethnobotanical Leaflets*. 2006;10:139-148.
19. Raaman N. Qualitative phytochemical screening. In: *Phytochemical techniques*. New Delhi: New India Publishing Agency; c2006. p. 19-24.
20. Singh AK, Pandey MB, Singh VP, Pandey VB. Xylopyrine-A and xylopyrine-B, two new peptide alkaloids from *Ziziphus xylopyrus*. *Nat Prod Res*. 2007;21:1114-1120.
21. Anonymous. The wealth of India: Raw materials. Vol X. New Delhi: NISCAIR, CSIR; c2009. p. 442.
22. RDA-Gene Bank, Republic of Korea; c2010.
23. Dinesavalli. Ayurvedic medicinal plants; c2010.
24. Duganath N, Kumar SR, Kumanan R, Jayaveera KN. Evaluation of anti-denaturation property and antioxidant activity of traditionally used medicinal plants. *Int. J Pharm Bio Sci*. 2010;1(2):1-7.
25. Syeda S, Sandhya S, Vinod KR, David B, Rao KNV, Narender PD, *et al*. Pharmacognostic studies on the leaf of *Ventilago maderaspatana* Gaertn. *Int J Pharm Clin Res*. 2010;2(1):51-53.
26. Naidu KA, Khasim SM. Contribution to the floristic diversity and ethnobotany of Eastern Ghats in Andhra Pradesh, India. *Ethnobotanical Leaflets*. 2010;14:920-941.
27. Meena AK, Rao MM. Folk herbal medicines used by the Meena community in Rajasthan. *Asian J Tradit Med*. 2010;5:19-31.
28. Yadav M, Meena AK, Rao MM, Kapil P, Panda P, Chahal J, *et al*. Review on *Ziziphus xylopyrus*: A potential traditional drug. *J Pharm Res*. 2011;4:922-923.
29. Gandagule UB, Duraiswamy B, Zalke AS, Qureshi MA. Pharmacognostical and phytochemical evaluation of the leaves of *Ziziphus xylopyrus* (Retz) Willd. *Anc. Sci. Life*. 2013;32:245-249.