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Preliminary Phytochemistry and Antimicrobial Studies of an Endangered Madicinal Herb *Exacum Bicolor* Roxb

Paulsamy S* and Jeeshna MV

Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, India

ABSTRACT

Exacum bicolor, a traditional medicinal herb distributed in northern Kerala, India with four different leaf shape variants such as linear-lanceolate, ovate-elliptic, oblanceolate and ovate have been of keen interest in phytochemical composition and antimicrobial research as it is an important source of secondary metabolites, notably flavonoid derivatives. Study revealed that ovate-elliptic leaf variant is a better potential source of phytochemical compounds than the other variants. The analysis by TLC reports the presence of alkaloids, flavonoids, saponins, tannins, steroids and phenolics in this species. Antimicrobial study of the plant extracts by using the alcoholic solvents *viz*., petroleum ether, chloroform, acetone and methanol against ten bacteria and ten fungi showed better inhibitory activity of the species. *Salmonella paratyhi*-A and *Cladosporium* sp. were determined to be the most sensitive bacterium and fungus respectively to the methanol extract of *E.bicolor*. The antimicrobial activity further confirms the traditional therapeutic usage of *E. bicolor* in northern Kerala.

Key words: *Exacum bicolor*, leaf shape variants, preliminary phytochemistry, antimicrobial activity.

Corresponding author
Email: paulsami@yahoo.com

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INTRODUCTION

A variety of materials derived from plants have been used for the prevention and treatment of diseases virtually in all the cultures, because it contains many bioactive chemical substances that produce definite physiological and biochemical actions in the human body. These bioactive constituents are alkaloids, tannins, flavonoids, phenolic compounds etc [1, 2]. *Exacum bicolor* Roxb. [Gentianaceae] is one such plant being used in traditional health care systems in northern Kerala to treat the infectious diseases related to eye and skin [3]. In addition, it is also used for the treatment of malaria, fever and urinary disorders, and the whole plant is prescribed as tonic [4, 5]. However no scientific reports are made for this species on these medicinal uses. Hence, the present study was undertaken to know the phytochemical profile and inhibitory activity against certain selective bacteria and fungi.

MATERIALS AND METHODS

Plant material

The four leaf shape variants [linear-lanciolate, ovate-elliptic, oblanceolate and ovate] collected from the grasslands of Taliparamba [Kannur district] and Thirunelli [Wayanad district] of Kerala, India. Then they were air dried separately for 20 days to avoid denaturation of active compounds and then powdered well for further analysis.

Extraction

100 g powdered whole plant materials of each leaf shape variant of *E. bicolor* were exhaustively extracted using soxhlet apparatus for 24 hr in order to get maximum yield of soluble compounds [6]. The powdered parts were sequentially extracted with petroleum ether, hexane, chloroform and finally with methanol according to increasing polarities, starting with the least polar solvent. After successive and exhaustive extraction, the crude extracts were filtered and concentrated under vacuum and controlled temperature with a rotary evaporator and residues were freeze dried. All extracts were stored at -8°C in deep freezer until further use.

Preliminary phytochemical analysis

The extracts of all the four leaf shape variants were analyzed separately for knowing the presence of secondary metabolites by following the standard methods as described under: alkaloids, steroids and resins [7], flavonoids [8], glycosides [9], saponins [10], tannins [11, 7] and phenols [12].

Thin layer chromatography [TLC]

The TLC studies were performed by using silica gel-G as stationary phase in the chromatographic plates of 15x5 cm with 3 mm thickness to confirm the presence of secondary metabolites identified already.

For the separation of phytochemical compounds, chloroform and methanol extracts of E. bicolor were spotted manually using capillary tube. The spotted plates were put in a solvent chamber which contained various solvent systems to detect the suitable mobile phase [13]. After the separation of phytochemicals, various spray reagents such as Dragondorff's, Vanillin sulphuric, Vanillin HCl and Kedde's reagents were used to identify the compounds. The colour of the spots was noted.

Quantitative determination of the chemical constituency

The quantitative phytochemical study was carried out for the six important secondary metabolites viz., alkaloids, flavonoids, saponins, tannins, steroids and phenolics. Phenolics and steroids were estimated respectively by the standard methods [14, 15]. The alkaloids and saponins were estimated by following the methods [16, 17]. Flavonoids and tannins were quantified as per the methods prescribed by [18, 19] respectively.

Antimicrobial analysis

Collection and maintenance of test organisms

Clinical isolates of bacteria viz., *Bacillus subtilis, B. thuriengensis Enterococcus faecalis, Strephylcoccus faecalis, S. pyogenes* [Gram positive bacteria], *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyhi-A* [all Gram negative bacteria] and fungi viz., *Aspergillus flavus, A.niger, Candida albicans, Cladosporium sp., Fusarium oxysporum, F. solani, F. sp., Mucor sp., Pencillium sp. and Rhizopus sp*. were collected from the Microbiology Laboratory, Tamil Nadu Agriculture University, Coimbatore. They were collected in culture bottles containing nutrient agar and PDA slants and stored at 4°C in labelled bottles until required. Further, subcultures were carried out at 2-week intervals to maintain the viability of these organisms.

Antimicrobial screening

Antimicrobial activity of the organic extracts of the plant sample was evaluated by the paper disc diffusion method. The different extracts of four leaf variants of *E. bicolor* at two different concentrations viz., 50 and 100 mg/100 ml were used for antimicrobial activity. The antibiotic discs tetracycline [30 µg] and ampicillin [10 µg] were taken as positive control for bacteria and fungi respectively. Nutrient agar and PDA medium were used for the inoculation of bacteria and fungi respectively by spreading the microbes on the medium. Sterile filter paper

discs impregnated with 50 and 100mg/100ml extracts and antibiotics were applied over the culture plates. Bacteria were incubated at 37^{o} C for 18 hr and fungal cultures were incubated at room temperature [30-32^{o} C] for 48 hr. Antimicrobial activity was determined by measuring the zone of inhibition around each paper disc. For each extract three replicates were maintained.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening in the four ecological leaf shape variants [linear-lanceolate, ovate-elliptic, oblanceolate and ovate] of the species, *E. bicolor* using different alcoholic solvents such as petroleum ether, chloroform, acetone and methanol are given in Table.1. The study revealed that all the whole plant extracts of four ecological leaf variants contained the secondary metabolites viz., alkaloids, flavonoids, glycosides, saponins, tannins, steroids, resins and phenols with different degrees. However, the petroleum ether and hexane extracts showed the presence of fewer amounts of these secondary metabolites. On the other hand, methanol extracts showed the presence of more varieties of secondary metabolites with high degree followed by chloroform extracts. It may be attributed to variation in polarity of these two solvents [20]. Further it was determined that the ovate-elliptic leaf variant registered more varieties of secondary metabolites with high quantities. It may be explained that the ecotypic differentiation [21] in this species could cause variations in the production of secondary metabolites. It was already reported the variations in phenophases and the content of secondary metabolites in the medicinal shrub, Gaultheria fragrantissima as influenced by ecotypes [22].

The TLC studies showed that generally the chloroform and methanol extracts of the species, E. bicolor contained high degree of secondary metabolites of medicinal importance viz., alkaloids, flavonoids, saponins and glycosides in different degree across the variants studied [Table 2]. Polarity level of solvents perhaps be a reason for this fact [8]. It has been reported the similar trend of higher content of secondary metabolites respectively in the medicinal plant species, Acalypha fruticosa and Acacia caesia in chloroform and methanol extracts [23 and 24].

The results of the gravimetric and spectrophotometer studies also confirmed the presence of varied quantity of secondary metabolites [alkaloids, flavonoids, saponins, tannins, steroids and phenolics] across the four ecological leaf variants of *E. bicolor* studied [Table. 3]. The whole plant part of these ecological variants contained higher phenolics [ovate-elliptic - 10.12 mg/g, oblanceolate - 7.63 mg/g, linear-lanceolate - 6.40 mg/g and ovate- 5.05mg/g] than the other secondary metabolites estimated. Next to phenolics, the other secondary metabolites such as alkaloids, flavonoids, steroids and saponins are determind to be higher in all the four variants. It is explained that the species of higher phenolic content generally have antibacterial and antiviral activities [25]. Flavonoids, on the other hand are potent water-soluble antioxidant and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity [26]. Saponins have the property of precipitating and coagulating red blood cells [27

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Table 1. Yield and results of phytochemical screening of four leaf shape variants of *Exacum bicolor*.

Sl. No	Extract	Leaf shape variants	Percentage yield	Secondary metabolites							
				Alkaloids	Flavonoids	Glycosides	Saponins	Tannins	Steroids	Resins	Phenols
1.	Petroleum ether	Linear-lanceolate	5.21	-	+	-	-	-	+	+	-
		Ovate-elliptic	5.02	-	+	-	-	-	+	-	+
		Oblanceolate	5.34	-	+	-	+	-	+	-	+
		Ovate	5.07	+	+	+	+	+	-	+	-
2.	Hexane	Linear-lanceolate	6.42	++	++	-	+	+	+	+	++
		Ovate-elliptic	6.31	++	++	+	++	++	++	_	+
		Oblanceolate	6.52	++	++	+	+	-	+	-	+
		Ovate	6.30	++	+++	+	+	++	-	+	-
3.	Chloroform	Linear-lanceolate	11.31	+++	+++	++	+	++	+++	-	++
		Ovate-elliptic	11.81	+++	+++	+++	+++	++	++	++	++
		Oblanceolate	12.74	++	+++	+++	+	+	++	-	++
		Ovate	11.20	++	+++	++	++	+	+	+	++
4.	Methanol	Linear-lanceolate	28.42	+++	+++	++	+	+++	+++	++	++
		Ovate-elliptic	26.9	+++	+++	+++	+++	+++	++	++	+++
		Oblanceolate	29.03	+++	+++	+++	++	+++	++	+	++
		Ovate	25.04	+++	+++	+++	++	++	++	+	++

+++ indicate high degree of presence, ++ moderated degree of presence, + low degree of presence and - indicate the absence

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Table 2. Determination of phytochemicals in the four leaf shape variants of *Exacum bicolor* with suitable mobile phases through thin layer chromatography

Mobile phase	Spray reagent	Leaf shape variants	Colour of the spot/band	R_f value (%)		Compound
				Chloroform extract	Methanol extract	
Ethyl acetate: methanol: water 100:10:10	Dragendorff reagent	Linear-lanceolate Ovate-elliptic Oblanceolate Ovate	Orange Orange Orange Orange	0.62 0.64 0.61 0.59	0.77 0.79 0.76 0.74	Alkaloids
Chloroform: methanol 16:8	Vanillin HCL reagent	Linear-lanceolate Ovate-elliptic Oblanceolate Ovate	Red Red Red Red	0.36 0.39 0.35 0.37	0.17 0.19 0.15 0.12	Flavonoids
Chloroform: methanol 95:15	Vanillin sulphuric acid reagent	Linear-lanceolate Ovate-elliptic Oblanceolate Ovate	Blue - violet Blue - violet Blue - violet Blue - violet	0.50 0.51 0.53 0.50	0.61 0.59 0.56 0.58	Saponins
Ethyl acetate: methanol : water 20 : 10: 10				0.86 0.84 0.87 0.85	0.92 0.90 0.89 0.87	
Ethyl acetate : Methanol: water 100: 13: 10	Kedde's reagent	Linear-lanceolate Ovate-elliptic Oblanceolate Ovate	Pink Pink Pink Pink	0.80 0.87 0.82 0.85	0.84 0.86 0.83 0.81	Glycosides

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Table 3. Variation in phytochemical contents of four leaf shape variants of *E. bicolor*.

Compound (mg/g)	Leaf variant			
	Linear-lanceolate	Ovate-elliptic	Oblanceolate	Ovate
Alkaloids	3.59±0.04	4.12±0.02	3.82±0.02	3.49±0.07
Flavonoids	2.40±0.01	4.23±0.02	3.42±0.01	3.32±0.01
Saponins	1.95±0.02	2.75±0.03	2.45±0.06	1.63±0.02
Tannins	0.42±0.01	0.47±0.02	0.43±0.01	0.36±0.01
Steroids	3.16±0.08	7.33±0.06	4.08±0.01	2.23±0.09
Phenolics	6.40±0.05	10.12±0.02	7.63±0.01	5.05±0.03

and 26]. These facts further support the existing traditional medicinal value of E. bicolor in northern Kerala.

As the ovate-elliptic leaf type variant contained higher amount of almost all secondary metabolites studied, further studies on antimicrobial properties were carried out only for this variant. Results of antibacterial and antifungal activities of the various extracts of E. bicolor are shown in Tables 4 and 5 respectively. Generally, both the non-polar [petroleum ether, hexane] and polar [chloroform, methanol] solvent extracts are determined to be active against both bacteria and fungi tested. In addition, it has been noted that the Gram positive bacteria showed more susceptibility to the extract than the Gram-negative bacteria. It may be explained that the Gram-negative bacteria are known to be resistant to the action of many antimicrobial therapeutic agents including plant based extracts [28, 29 and 30]. The highest inhibition activity [diameter of zone of inhibition, 27.72mm] was observed in the methanol extract of the study species at 100mg/100ml against the Gram negative bacterium, Salmonella paratyhi-A. The highest antifungal activity [diameter of zone of inhibition, 20.18 mm] was demonstrated by methanol extracts of the plant species at 100mg/100ml concentration against the fungus, Cladosporium sp. It may be attributed to the specificity of plant extract towards the susceptibility of fungal species. Generally, the large zones of inhibition produced by the plant extracts against the test organisms confirm the potency of the active compounds of the plant against all the test organisms. The wide spectrum of secondary metabolites present in Exacum bicolor may have a synergetic effect upon the inhibitory activity on microorganisms. It has been already reported that the Gentianaceae members are showing better antimicrobial activity due to the presence of many kinds of bioactive compounds [31, 32 and 33].

CONCLUSION

This preliminary study confirms the traditional knowledge on medicinal uses of E. bicolor to treat infectious diseases. However, large data must be generated through pharmacological studies for commercializing this species.

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Table 4. Antibacterial activity of plant extract of ovate-elliptic leaf shape variant of *E. bicolor*.

Plant extract	Conce-ntration	Diameter of inhibition zone (mm)									
		Gram positive bacteria					Gram negative bacteria				
		Bacillus subtilis	*B. thuriengensis*	*Enteroco-ccus faecalis*	*Strephyl-coccus faecalis*	*S.pyogen-es*	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus mirabilis*	*Pseudm-onas aeruginosa*	*Salmonella paraty-hi-A*
Petroleum ether	C*	22.24±0.07	30.17±0.12	30.26±0.04	24.23±0.09	22.21±0.15	24.25±0.05	21.24±0.06	18.77±0.07	18.77±0.07	22.18±0.08
	50	-	8.14±0.09	-	-	-	-	-	-	-	-
	100	10.26±0.06	-	-	-	-	-	-	-	-	-
Hexane	C*	22.25±0.06	22.14±0.05	26.26±0.09	23.24±0.06	34.16±0.82	22.14±0.08	18.75±0.06	22.25±0.08	26.18±0.09	26.18±0.09
	50	-	12.23±0.09	-	8.25±0.09	10.74±0.08	-	8.22±0.09	14.35±0.06	10.43±0.08	-
	100	-	14.26±0.08	8.24±0.08	-	8.16±0.06	10.18±0.07	10.17±0.09	10.43±0.09	10.28±0.09	-
Chloroform	C*	22.15±0.12	20.32±0.09	20.16±0.03	24.65±0.08	24.16±0.07	24.23±0.07	14.73±0.08	30.53±0.09	24.27±0.09	24.16±0.09
	50	-	10.35±0.09	9.26±0.16	-	8.25±0.09	8.33±0.09	12.27±0.08	12.23±0.10	12.74±0.08	-
	100	-	12.47±0.08	10.16±0.06	-	12.35±0.06	10.35±0.08	8.23±0.07	14.36±0.09	10.16±0.09	-
Methanol	C*	22.33±0.06	20.23±0.08	22.22±0.06	24.15±0.10	25.83±0.08	26.44±0.05	14.26±0.06	24.43±0.08	20.16±0.08	26.43±0.08
	50	-	10.22±0.10	8.25±0.10	10.32±0.09	8.25±0.08	8.24±0.08	8.24±0.08	8.84±0.07	14.24±0.10	-
	100	8.32±0.09	10.18±0.10	-	-	10.24±0.08	12.33±0.06	-	-	12.22±0.10	20.72±0.08

*- Tetracycline: 30 µg
Each value represented by Mean ± Standard Deviation

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Table 5 . Antifungal activity of plant extract of ovate-elliptic leaf shape variant of *E. bicolor*.

Plant extract	Conce-ntration	Diameter of inhibition zone (mm)									
		Aspergillus flavus	*A.niger*	*Candida albicans*	*Cladosporium* sp.	*Fusarium oxysporum*	*F. solani*	*Fusarium*.sp.	*Mucor* sp.	*Pencillium* sp.	*Rhizopus* sp.
Petroleum ether	C*	30.73±0.09	36.45±0.06	14.27±0.04	20.26±0.09	30.73±0.09	40.27±0.08	30.73±0.09	30.51±0.21	30.72±0.09	34.19±0.06
	50	-	-	-	-	-	-	-	-	6.20±0.11	-
	100	-	-	-	-	-	-	-	-	8.73±0.11	-
Hexane	C*	32.38±0.09	30.72±0.09	14.32±0.04	20.73±0.09	32.71±0.08	40.28±0.08	34.25±0.06	30.36±0.08	36.21±0.05	34.22±0.05
	50	-	-	-	10.36±0.05	-	18.40±0.08	14.34±0.11	-	10.71±0.08	-
	100	-	-	-	14.72±0.09	-	12.42±0.09	16.27±0.08	10.26±0.07	12.82±0.08	-
Chloroform	C*	30.73±0.08	30.26±0.07	14.27±0.08	26.42±0.13	32.44±0.08	40.29±0.08	28.27±0.07	30.42±0.11	36.29±0.09	32.33±0.07
	50	8.61±0.09	-	8.47±0.08	8.27±0.06	8.26±0.08	10.82±0.07	8.36±0.07	-	7.27±0.48	8.26±0.07
	100	8.35±0.11	-	-	12.24±0.12	10.27±0.07	8.29±0.03	10.39±0.11	-	12.24±0.12	10.74±0.08
Methanol	C*	30.33±0.04	30.75±0.08	14.18±0.08	26.37±0.08	30.23±0.09	40.22±0.06	30.65±0.11	30.24±0.05	36.25±0.07	32.4±0.16
	50	-	10.66±0.20	10.16±0.09	10.72±0.09	8.26±0.10	10.72±0.07	8.60±0.05	8.22±0.09	8.36±0.08	-
	100	10.80±0.12	10.21±0.10	8.25±0.07	20.18±0.09	12.34±0.04	12.36±0.07	10.16±0.06	12.74±0.07	14.36±0.08	12.30±0.03

*- Ampicilin: 10 µg

Each value represented by Mean ± Standard Deviation

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REFERENCES

[1] Cho E, Seddom J, Ronser B, Willet W, Hankinson S. Arch. Opthalmol 2004; 122: 883-892.
[2] Edeoga HO, Okwu DE, Mbacble BO. Afr J Biotechnol 2005; 4[7]: 685-688.
[3] Alikhan I, Khanum A. Herbal remedies for diseases. Ukay Publications, Hyderabad, 2005; 67-134.
[4] Rao MR. Flowering plants of Travancore, Bishen Singh Mahendra Pal Singh, DehraDun, 1914; 268-271.
[5] The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products- Raw Materials Series, Publications and Information Directorate, CSIR, New Delhi, Vol.III, 1952; 234.
[6] Castillo JI, Rivero CF, Celis H, Romero I. J Ethnopharmacol 2007; 114: 72-77.
[7] Ciulci I. Methodology for the analysis of vegetable drugs. Chemical industries Branch, Division of Industrial Operations. UNIDO, Romania,1994; 24:26 - 67.
[8] Sofowora LA. Medicinal plants and traditional medicine in Africa. Ibaban: Spectrum Books Ltd., Ibadan, Nigeria, 1993; 289.
[9] Gokhale SB, Kokate CK, Purohit AP. A text book of pharmacognosy, Nirali Prakashan, Maharashtra, India, 2008; 889.
[10] Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Wright Scientechica, Bristol 1975; 57-58.
[11] Mace, Gorbach SL. Anaerobic bacteriology for clinical laboraties pharmacognosy. 1963; 23: 89-91.
[12] Krishnamoorthy KV. Botanical Histochemistry, Viswanathan Publishers, Chennai, 1988.
[13] Wagner H, Bladt S, Zgainski EM, Plant drug analysis Thin layer chromatography Atlas. Springer –Verlag, Berlin, Heidelberg, New York, 1984; 1- 320.
[14] Singleton VL, Rossi JrJA. Am J Enol Viticult 1965; 16: 44-158.
[15] Zak B. Clin Chem 1977; 23: 1201.
[16] Harbone JB. Phytochemical methods, London. Chapman and Hall Ltd 1973; 49-188.
[17] Obdoni BO, Ochuko PO. Global J Pure Appl Sci 2001; 8b: 203:208.
[18] Boham BA, Kocipai-Abyazan R. Pacific Sci 1974; 48:458-463.
[19] Van- Burden TP, Robinson WC. J Agric Food Chem 1981; 1: 77.
[20] Harborne JB. Phytochemical Methods: A guide to Modern Technique of Plant Analysis. [3rd Edn.].Chapman and Hall, London, 1988; 1-138.
[21] Sreelatha U, Baburaj TS, Narayan Kutty C, Nazeem PA and Jyothi Bhaskar Ntural product Radiance 2007; 6[5]:402-404.
[22] Vijayakumar, K. K. and S. Paulsamy. J of Life Sciences 2010; 2[1]: 21-25.
[23] Anandkumar AM, Paulsamy S, Sathish kumar P, and Senthilkumar P. J of Applied and Natural Science, 2009; 1[1]: 41-43.
[24] Sathish kumar P, Paulsamy S Ananda kumar AM and Senthilkumar P. Advances in Plant Sciences, 2009; 22[II]:451-453.
[25] Ofokansi KC, Esinmone CO, Anele CK. Plant prod Res J 2005; 9: 23-27.
[26] Okwu DE. J Sustain Agric Eviron 2004; 6[1]: 30-37.
[27] Sodipo OA, Akiniyi JA, Ogunbamosu JU. Global J Pure Appl Sci 2000; 6:83-87.

[28] Kambezi L, Afolayan AJ. Afr J Biotechnol 2008; 7[1]: 012-015.
[29] El-Mahmood AM. J Med Plants Res 2009; 3[7]: 498-505.
[30] Adeshina GO, Onaolapo JA, Ehinmid JO, Odama LE. J Med Plants Res 2010; 4[8]: 649-658.
[31] Aswal BS, Bhakuni DS, Goel AK, Kar K. Mehrotra BN. I J Exp Bio 1984; 22:487-504.
[32] Devkota KP, Acharya R, Baral MP, Adhikari RP. Proceedings of the Third National Conference on Science and Technology. Nepal Academy of Science and Technology, Khumaltar, Nepal. Antimicrobial activities of some herbal plants used in traditional medicine in Nepal 1999; 1311-1317.
[33] Mothana RAA, Lindequist U. J Ethnopharmacol 2005; 96:171-181.