

FULL LENGTH RESEARCH ARTICLE

PHYTOCHEMICAL PROFILE OF METHANOLIC LEAF EXTRACT OF *BREYNIAVITIS-IDAEA* (BURM.F.) C.E.C. FISCH (*EUPHORBIACEAE*) BY GC-MS ANALYSIS FROM AUTHUKURICHI SACRED GROVE, TAMILNADU

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ABSTRACT

Breynia is a plant genus of the family *Euphorbiaceae*. *Breynia* have high medicinal importance and the selected species *vitis-idaea* is used traditionally to treat various diseases. The crude methanolic leaf extract of *B. vitis-idaea* was subjected to phytochemical screening for the presence of various phytochemicals. The present study was undertaken to analyze the chemical composition of methanolic crude extract from the leaves of *B. vitis-idaea* by using GC-MS. About 28 different compounds were reported. Also two important components Breynin A and *Breyniaionoside* A identified.

Key words: *Breyniavitis-idaea*, Phytochemicals, GC-MS.

INTRODUCTION

Phytochemicals are responsible for medicinal activities of the plants. Based on this fundamental knowledge several pharmaceutical industries are established (Joy *et al.*, 1998). Plants derived natural products such as alkaloids, flavonoids, terpenoids have received considerable attention in recent years due to their diverse pharmacological properties including antimicrobial, antioxidant and anticancer activities (Verma and Singh 2008). Plant crude extracts were containing large number of natural antioxidants, which are used as traditional medicines. *B. vitis-idaea* belonging to the family *Euphorbiaceae*. It is an evergreen tall glabrous erect shrub with horizontal branches. Bark is yellowish grey; flowers are small, greenish yellow or pink. Leaves are elliptic to elliptic-ovate, exchange dark brown or black when dry. The fruits are thickset, pink to red which turns black when grown. The seeds are black and have a very hard seed coat (Pullaiah 2002; Chandrashekar *et al.*, 2011; Yoganarasimhan 2000). The present study gives a clue towards the immense medicinal properties of this plant. The decoction of the root is employed as mouthwash for toothache. Leaves applied as poultice to hasten suppuration. The juice of the stem is used in conjunctivitis. The leaf juice given after parturition to prevent the hemorrhage.

Dried leaves are smoked like tobacco to relieve in tonsillitis. Astringent bark used to guard against hemorrhage (Pullaiah and Moulali, 1997). The plant showed larvicidal (Jeyasankar, & Ramar 2014) antibacterial (Venkatesh *et al.*, 2015).

MATERIALS AND METHODS

Collection of plant materials and preparation of the extract: The fresh leaves of *Breyniavitis-idaea* were collected from the sacred grove of Authukurichi (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. The specimen was botanically identified and confirmed by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The preserved plant specimens were submitted to the Department of Botany, Annamalai University, Annamalai nagar, Tamilnadu for further reference. The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizer. The powdered leaf were then subjected to successive extraction with organic solvents such as hexane chloroform and ethanol by Soxhlet method (Ingle *et al.*, 2017). The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in vacuum and stored at 4°C. They were used for GC-MS analysis.

Preliminary Phytochemical screening

Phytochemical Screening: The chemical tests were performed on the n-hexane, chloroform, ethyl acetate and methanolic leaf extracts of *B. vitisidaea* using standard procedure to identify

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the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).



Fig. 1. *Breyniavitis-idaea*. Flowering and fruiting twig

Alkaloids: About 0.2 g of each of extract was warmed with 2% H_2SO_4 , for two minutes. Then they were filtered and a few drops of Dragendroff's reagent were added to each filtrate. No orange red precipitate indicated the absence of alkaloids.

Tannins: A small quantity of each extract was mixed with water and heated on water bath and then filtered. A few drops of ferric chloride were added to each of the filtrates. A dark green solution indicated the presence of tannins.

Anthraquinones: About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath, filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrates. Few drops of 10% ammonia was added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthraquinones.

Glycosides: The extracts (0.5 g) were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. No red precipitate indicated the absence of glycosides.

Reducing Sugars: The extracts were shaken with distilled water, filtered and boiled with few drops of Fehling's solution A and B for few minutes. No orange/red color indicates the absence of reducing sugars.

Saponins: About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boiling. No frothing (appearance of creamy mass of small bubbles) shows the absence of saponins.

Flavonides: Extracts of about 0.2g were dissolved in diluted NaOH and HCl was added. No yellow solution that turns colorless indicates the absence of flavonoids.

Steroids: Each extract (0.5 g) was dissolved in 2 ml of acetic anhydride and added 2 ml of conc. sulphuric acid. The color change from violet to blue or green in some samples indicated the presence of steroids.

Terpenoids Test: 0.2g of each extract was mixed with 2ml of chloroform and concentrated H_2SO_4 , (3 ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicated positive results for the presence of terpenoids.

Gas chromatography- mass spectrometry (GC-MS) analysis: GC-MS analysis was performed with GC-MS Clarus 500 Perkin Elmer Equipment.

Compounds were separated on Elite-5 capillary column (Crossbond 5% Phenyl 95% dimethylpolysiloxane) Oven temperature was programmed as follows: isothermal temperature at 60°C then increased to 200°C at the rate of 10°C/min., then increased up to 280°C at the rate of 5°C/min. held for 9 min. Ionization of the sample components was performed in the Electron energy (70 eV). The helium was used as gas carrier (1ml/min.), and 1.0 μ L of sample was injected. The detector was Mass detector Turbomass gold Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2.0 was used in this GC-MS study.

Identification of compounds: All the compounds were identified from methanol extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, National Institute Standard and technology (NIST) (Version year 2005).

RESULTS AND DISCUSSION

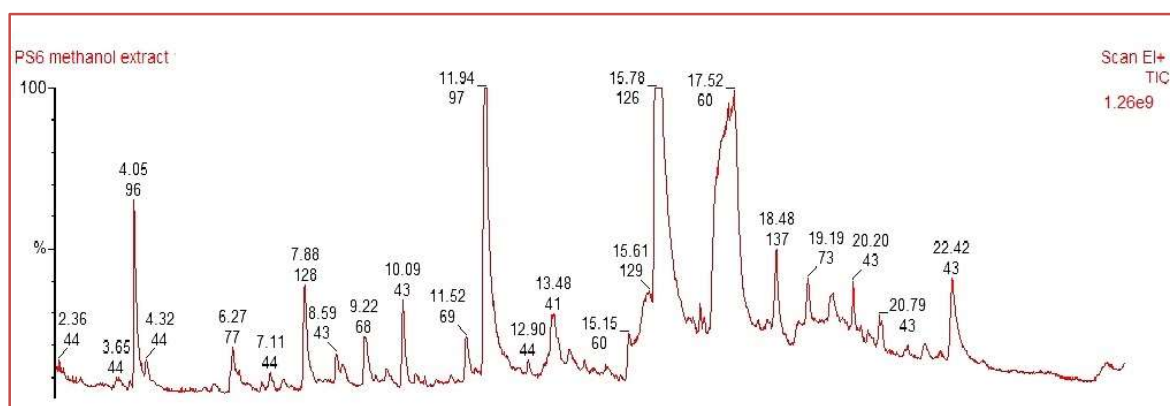
The results for preliminary phytochemical investigation of *B. vitis-idaea* leaves extracts are shown in Table 1. The result of phytochemical investigation shows that *B. vitis-idaea* leaves contain a number of active principles like alkaloids, flavonoids, carbohydrates, glycosides, protein, amino acid, steroids, saponin, terpenoids, tannins and phytosterol. The presence of these phytoconstituents reveals medicinal importance of the leaves of this plant. The chemical constituents identified by the GC-MS analysis on various extracts of the leaves of *B. vitis-idaea* were enumerated along with Molecular Formula (MF), Molecular Weight (MW), Retention Time (RT), and Peak area and Peak area (%) is presented in Table-2.

Table 1. Phytochemical screening of the leaf extracts of *B. vitis-idaea*

Chemical components	Extracts			
	n-hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	-	-	-
Steroids	+	+	-	+
Terpenoids	-	-	+	+
Flavonoids	-	-	-	-
Anthraquinones	-	-	-	-
Tannins	-	-	+	+
Saponins	--	--	-	-
Glycosides	-	-	-	+
Reducing sugars	-	-	-	+

Table 2. GC-MS profiles of methanolic leaf extract of *B.vitis-idaea*

S.No.	Compound Name	Formula	MW	Retention Time	Peak Area	% Peak area
1.	Furfural	C ₅ H ₄ O ₂	96	4.05	5817836	5.5928
2.	2-Cyclopenten-1-one, 2-hydroxy-	C ₅ H ₆ O ₂	98	5.83	3422742	0.3231
3.	1-Benzoyl-3-amino-4-cyano-3-pyrroline	C ₁₂ H ₁₁ N ₃ O	213	6.26	9222308	0.8827
4.	2(3H)-Furanone, 3-acetyldihydro-	C ₆ H ₈ O ₃	128	6.91	2364036	0.2263
5.	Phentermin-propionyl	C ₁₃ H ₁₉ NO	205	7.11	6600468	0.6339
6.	cis-1,2-Dihydrocatechol	C ₆ H ₈ O ₂	112	7.39	4075486	0.3930
7.	1,2-Butanediol, 1-phenyl-	C ₁₀ H ₁₄ O ₂	166	7.88	4374243	4.2054
8.	Hydrouracil, 1-methyl-	C ₅ H ₈ N ₂ O ₂	128	8.58	9973383	0.9582
9.	Methyl 2-furoate	C ₆ H ₆ O ₃	126	8.73	9219279	0.8876
10.	Levoglucosonone	C ₆ H ₆ O ₃	126	9.22	2350919	2.2623
11.	1-Deoxy-d-altritol	C ₆ H ₁₄ O ₅	166	9.74	2329068	0.2230
12.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	10.09	2743836	2.6382
13.	Benzoic acid, 2-hydroxy-, methyl ester	C ₈ H ₈ O ₃	152	10.58	1108840	0.1063
14.	1,4:3,6-Dianhydro- α -D-glucopyranose	C ₆ H ₈ O ₄	144	11.52	1506280	1.4477
15.	-Furancarboxaldehyde, 5-(hydroxymethyl)-	C ₆ H ₆ O ₃	126	11.94	1402236	13.4879
16.	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	12.90	3369913	0.3239
17.	Hydroquinone	C ₆ H ₆ O ₂	110	13.82	7601301	7.3114
18.	Methyl- α -D-ribofuranoside	C ₆ H ₁₂ O ₅	164	15.15	7201260	0.6921
19.	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126	15.78	3043818	29.2783
20.	1,3-Cyclohexanediol, 4,6-dimethyl-2-nitro-, diacetate (ester), (1 α ,2 α ,3 α ,4 α ,6 α)-	C ₁₂ H ₁₉ NO ₆	273	16.76	412103	0.3959
21.	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	16.86	2974226	0.2864
22.	D-Allose	C ₆ H ₁₂ O ₆	180	17.52	1586222	15.2529
23.	Benzeneacetic acid, 4-hydroxy-3-methoxy-	C ₉ H ₁₀ O ₄	182	18.46	2831303	2.7227
24.	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	C ₁₃ H ₂₂ O ₂	210	19.19	2044102	1.9665
25.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	20.20	6831457	0.6570
26.	3,5-Dimethoxy-4-hydroxyphenylacetic acid	C ₁₀ H ₁₂ O ₅	212	21.82	7012663	0.6748
27.	4-hydroxy-4-[(E)-4-hydroxy-3,3,4,5-trihydroxy-6-(hydroxymethyl)-trimethylcyclohexan-1-one	C ₁₉ H ₃₂ O ₉	404	22.42	5372316	5.1678
28.	6-3-3,5-dihydroxy-6-(hydroxymethyl)-4-3,4,5-trihydroxy-6-methylloxan-2-4-hydroxybenzoate	C ₄₀ H ₅₆ O ₂₃	936	25.89	1124322	0.9769

Fig. 2. Chromatogram of methanolic leaf extract of *B.vitis-idaea*

Leaves of *Breynia vitis-idaea* were evaluated and preliminary screening confirms the presence of glycosides, flavonoids, saponins, terpenoids and polyphenols. Since most of the active chemical constituents were extracted in methanolic solvent it was selected for the further use. In the present study two important compounds identified from methanolic leaf extract of *B. vitis-idaea* 1. Breyniaionoside A (C₁₉H₃₂O₉) and 2. Breynin A (C₄₀H₅₆O₂₃). Presence of these phytochemicals correlated with earlier studies and confirmed the antioxidant properties. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases (Cuzzocrea *et al.*, 2001). There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary

butylhydroquinone which are commonly used in processed foods. However, there is a widespread agreement that synthetic antioxidants need to be replaced with natural antioxidants because some synthetic antioxidants have shown potential health risks and toxicity, most notably possible carcinogenic effects. Therefore, it is of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in foods and pharmaceutical preparations to replace synthetic antioxidants (Premanand *et al.*, 2010, Lee *et al.*, 2004, Mundhe *et al.*, 2011). The antioxidant capacities and total phenolic contents of *B. vitis-idaea* were evaluated for the first time and the study showed a correlation between the phenol content of the extracts with their antioxidant potential (Chandrashekar *et al.*, 2011). Also confirmed by another

CONCLUSION

The breynins have strong anti-arthritis activities, which is responsible to the anti-inflammatory effects. Strong antioxidant activities and medicinal functions of *B. vitis-idaea* makes it a promising source of natural antioxidants in food and pharmaceutical industries.

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