INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL SCREENING OF *ARDISIA BLATTERI* GAMBLE: AN ENDEMIC PLANT OF SOUTHERN WESTERN GHATS, TAMIL NADU, INDIA

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Article Received on: 23/12/15 Revised on: 13/01/16 Approved for publication: 21/01/16

DOI: 10.7897/2230-8407.07216

ABSTRACT

The genus *Ardisia* Sw (coralberry or marlberry). is the largest genus in the family Myrsinaceae and comprises approximately 500 species distributed throughout subtropical and tropical regions of the world. This genus is a good source of health promoting compounds and potent phytopharmaceuticals. In India, 13 species are enumerated. Within this, seven species are endemic to India and nine species are recorded in Tamil Nadu. *Ardisia blatteri* is an evergreen small tree, endemic to southern Western Ghats. The Preliminary phytochemical screening of plant revealed the presence of various classes of compounds such as alkaloids, flavonoids, saponins, reducing sugars, tannins, and terpenoids. GC-MS analysis has investigated on the ethanol extract of *Ardisia blatteri,* resulted with 40 known compounds in various degrees of distribution. Hexadecanoic acid (21.74%), Octamethyl (24.73%) are the major constituents. About 8 constituents are minor and 30 constituents are trace amount in the plant extract.

Keywords: *Ardisia blatteri*, Myrsinaceae, phytochemical screening, GC-MS analysis.

INTRODUCTION

In India, medicinal plants are widely used by the population with an estimated 8500 species of higher plants. Between 60 to 70% of populations in developing countries, living in agricultural and forest areas, they collected various plant parts and foods from the forest species such as roots, leaves, fruits and nuts which forms an integral part of their daily diets[1]. In India, Western Ghats area is one of the world's ten "Hottest biodiversity hotspots" and has over 5000 species of flowering plants, 139 mammal species, 508 bird species and 179 amphibian species, many undiscovered species lives in the Western Ghats. Plants produce phytochemicals to protect themself and that phytochemicals can also protect human beings against diseases. The most important such bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds[2].

The genus *Ardisia*, a Myrsinaceae (primulaceae) family, consists of more than 200 species growing in the warm climates of tropical and subtropical regions on the earth. *Ardisia* species are distributed in India, Indonesia, Malaysia, Thailand, Vietnam, China and Taiwan[3]. Traditional medicinal uses attributed to *Ardisia* include all liver cancer, rheumatism, cough, fever, diarrhea, dysmenorrhea, respiratory tract infections, inflammation, pain, snake and insect bites, birth complications and to improve general blood circulation, among others[4]. *Ardisia* species are rich in polyphenols, triterpenoid, saponins, isocoumarins, quinones and alkylphenols. The genus *Ardisia* is a good source of health promoting compounds and potent phytopharmaceuticals[5].

Ardisia crenata was reported to possess relieving cough and asthma, anti-inflammatory, antibacterial, antineoplastic, antifertility, anticoagulant, lower blood pressure, triggering uterine contractions[6]. Four components have been isolated from the root of *Ardisia crenata* and identified as bergenin, friedelin, β-sitosterol and rapanone. Friedelin was isolated for the first time from the genus of *Ardisia*[7]. Preliminary phytochemical screening of both the leaf and bark extract of *Ardisia colorata* revealed the presence of various classes of compounds such as saponins, reducing sugars, tannins, and terpenoids with minor presence of alkaloid and flavonoid[8]. The present investigation is aimed to screen out the phytochemicals from the leaf extract of *Ardisia blatteri.*

LIST OF *ARDISIA* SPECIES IN INDIA

Species	Endemic to India	Distribution in Tamil nadu
1.Ardisia amplexicaulis	+	+
2.Ardisia blatteri	+	+
3.Ardisia depressa	-	-
4.Ardisia elliptica	-	+
5.Ardisia missionis	-	+
6.Ardisia paniculata	+	+
7.Ardisia pauciflora	-	+
8.Ardisia rhomboidea	+	+
9.Ardisia solanaceae	-	+
10.Ardisia solanaceae var. parviflora	+	-
11.Ardisia Sonchifolia	+	+
12. Ardisia stonei	+	-
13.Ardisia zeylanica	-	-

MATERIALS AND METHODS

Ardisia blatteri is an evergreen small tree, growing widely in southern Western Ghats. The leaves were collected from megamalai wildlife sanctuaries at southern Western Ghats during May 2014. The leaves were shade dried and powdered. Now it is used for the extraction. Voucher specimen is preserved as herbarium in the department of botany, The Madura College, Madurai. The identification was done with help of BSI, Southern Circle, Coimbatore.

Classification

Domain–Eukaryota
Kingdom – Plantae
Unranked-Eudicots
Unranked–Asterids
Order – Ericales
Family- Primulaceae
Subfamily – Myrsinoideae
Genus – *Ardisia*
Species–*Blatteri*
Botanical Names – *Ardisia blatteri*

Natural habitat of *Ardisia blatteri*

Herbarium of *Ardisia blatteri*

Extraction

Leaf powder is subjected to the hot extraction with ethanol in Soxhlet's apparatus in continuous 8 hrs reflux. After the extraction, solvent is evaporated with room temperature and the extract is subjected to further analysis.

Preliminary phytochemical screening

Test for carbohydrates
Plant Extract(PE) + 1ml of Molisch's reagent and few drops of Conc. Sulphuric acid ----Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins
PE + 2ml of 5% ferric chloride----Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins
PE + distilled water---- Formation of foam indicates the presence of saponins.

Test for flavonoids
PE + 1ml of Sodium hydroxide---- Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids
PE + Conc.Hydrochloric acid + few drops of Mayer's reagent---Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones
PE + 1ml of Conc. Sulphuric acid---- Formation of red color indicates presence of quinones.

Test for glycosides
PE + 3ml of choloroform + 10% Ammonia solution----Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides
PE + 2ml of glacial acetic acid + few drops of 5% Ferric chloride + 1 ml of Conc. Sulphuric acid---- Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids
PE + 2ml of chloroform + Conc. Sulphuric acid---- Formation of red brown color at the interface indicates presence of terpenoids.

Test for phenols
PE + 2ml of distilled water + few drops of 10% Ferric chloride-----Formation of blue or green color indicates presence of phenols.

Test for coumarins
PE + 1ml of 10% NaOH was added---- Formation of yellow color indicates presence of coumarins.

Test for proteins and aminoacid
PE + few drops of Ninhydrin reagent----Formation of blue color indicates the presence of proteins.

Steroids and Phytosteroids
PE + Chloroform + Conc. Sulphuric acid----formation of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Phlobatannins
PE + 2% HCL----Formation of red color precipitate indicates the presence of phlobatannins.

Anthraquinone
PE + drops of 10% ammonia solution ----Formation of pink color precipitate indicates the presence of anthraquinones.

Gas Chromatography

The GC-MS analysis was carried out by using GCMS-QP 2010 plus. Gas Chromatography (GC) is interfaced to Mass Spectrometry (MS) and equipped with a silica capillary coloumn [25nm×0.25nm][film thickness 0.25µm]. For GC-MS detection, an electron (e^-) ionization system with ionizing energy of 70 eV was used. Helium (He) was used as the carrier gas at constant flow rate 1.21ml/min and an injection volume of 3ml was employed, split ratio of 10.0, injector temperature 260^0C; ion source temperature 230^0C. The initial oven temperature was programmed from 100^0C (for 5 min), with an increasing

temperature of 200^0C (for 5 min) then to 300^0C (for 15 min). Mass Spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 650 m/z. Total GC-MS running was 64.99 min. The relative % of amount of each compounds were calculated by comparing its average peak area to the total areas, software adopted to handle Mass Spectra and recorded the total event.

Identification of compounds from extract

GC-MS spectra was analysed with standard library check with help of National Institute of Standard and Technology (NIST). The name, molecular weight and structure of the compounds of the sample materials was ascertained by the comparison of GC peak and estimated the molecular weight by MS.

Table 1

Phytochemical test	Inference			
	Chloroform	Petroleum Ether	Aqueous	Ethanol
Carbohydrates	–	–	–	–
Tannins test	+	–	–	–
Saponin test	–	–	+	–
Flavonoid test	+	–	–	+
Alkaloid test	+	–	+	+
Quinones	–	–	–	–
Glycosides test	–	–	–	–
Cardiac glycosides test	–	–	–	–
Terpenoids test	–	–	+	–
Phenols	–	–	+	–
Coumarins	+	+	–	–
Proteins	–	–	–	–
Steroids and Phytosteroids	–	+		–
Phlobatannins	–	–	–	–
Anthraquinones	–	–	–	+

\+ Present, – Absent

Table 2: GC-MS analysis of ethanolic leaf extract of *Ardisia blatteri*

Peak	R.time	Area%	Name
1	5.176	0.31	Dideoxyhexo-3-diulose
2	5.893	0.18	Dodecane
3	9.946	0.14	1,3-Dimethyltricyclo dec-3-ene
4	10.268	0.22	Hexadecane
5	11.000	0.57	Trimethyl bicyclo -4-ene,
6	11.650	0.17	3-Ethyl octene,
7	12.562	0.64	Decahydro-4a-methyl naphthalene
8	12.756	0.78	Selinene
9	14.304	0.18	Dodecanoic acid
10	14.813	0.34	Trimethyl-5- oxatricyclododecane
11	17.339	0.21	Hexahydro methanonaphthalene
12	17.972	0.20	2-Methyl cyclohexanol
13	18.640	1.48	Tetradecanoic acid
14	22.740	21.74	Hexadecanoic acid
15	24.521	0.28	Heptadecanoic acid
16	25.106	0.33	Octadecanoic acid
17	26.001	4.54	Heptadecene
18	26.084	1.58	Octadec-9-enoic acid
19	26.413	10.73	Octadecanoic acid
20	28.555	0.25	2-Hydroxy-1-hexadecanoic acid
21	29.743	0.31	Eicosanoic acid
22	31.333	0.93	1-Acetyl-5-benzoyl-L-arabinofuranose
23	31.528	0.17	12,15-Octadecadienoic acid
24	31.633	0.26	2-Hydroxy hexadecanoic acid,
25	31.851	0.19	2-Hydroxy-3-propanediyl
26	32.641	0.73	1,2-Benzenedicarboxylic acid
27	37.526	0.60	9-Octadecenamide
28	38.218	0.60	5-Pentadecyl benzenediol
29	38.361	2.41	Squalene
30	40.100	0.29	Geranyl linalool isomer B
31	40.401	0.48	2,8-Dimethyl-2-trimethyltridecyl-6-Chrom
32	41.139	5.48	5-Pentadecyl benzenediol,
33	41.908	0.27	4-Hydroxy-3,5,5-trimethyl-4-
34	41.991	0.31	Dimethylcyclopent-1- acetic acid,
35	42.094	0.69	2,7,8-Trimethyl-2-(4,8,12-trimethyltridecyl)-6-chroman
36	42.813	0.81	Heptafluorobutanoic acid
37	43.569	1.86	Vitamin E
38	47.283	0.87	1-Triacontanol
39	50.195	24.73	Octamethyl-1
40	52.591	8.83	7-Dihydro cyclopropa-3-1-cholestan

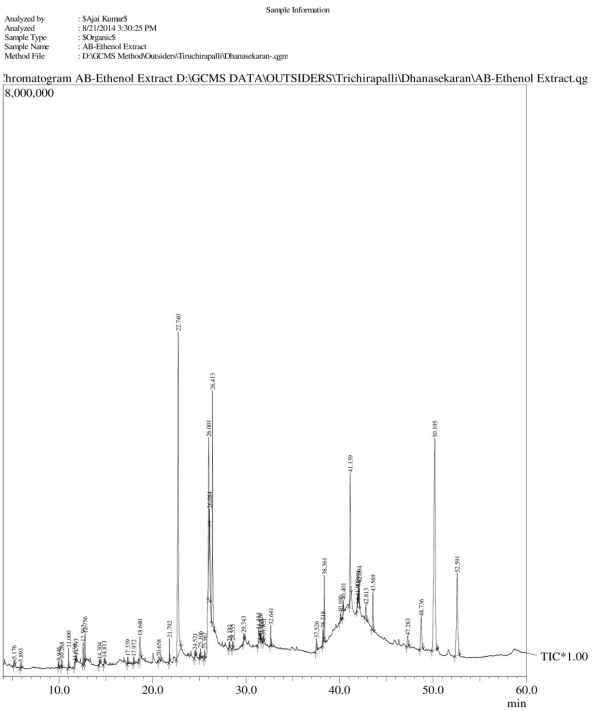

Figure 1

RESULT

Phytochemical screening showed that the leaves of *Ardisia blatteri* are rich in chemical constituents. Such as Alkaloids, tannins, saponins, anthraquinones, phenols, terpenoids and flavonoids have been documented in this study (Table.1). The compounds present in the ethanol extract of *Ardisia blatteri* are identified by GC-MS analysis (Fig.1). The active principles with a retention time (RT), area concentration, molecular name (MN) in the ethanol extract of *Ardisia blatteri* are presented. Forty compounds are identified in ethanol extract by GC-MS. In which two are major components present in the leaf extract of *Ardisia blatteri*. They are Hexadecanoic acid (21.74%), Octamethyl-1(24.73%). About 8 constituents are minor and 30 constituents are trace amount in the ethanol extract of the *Ardisia blatteri* leaf (Table 2).

DISCUSSION

Phytochemical constituents such as carbohydrate, sugars, proteins, amino acids and starch are in the form of reserved food. Medicinal significance is due to the presence of secondary metabolites such as glycosides, alkaloids tannins and phenolics. Both aqueous and ethanolic extracts of *Madhuca indica* showed effective antibacterial activity[9]

Noor et al reported that methanol extract showed the presence of carbohydrates, glycosides, saponins, alkaloids and flavonoids compounds. Gallic acid and rutin were the major phytochemicals in *Ardisia paniculata*[10]. Ma et al reported that ardicrenin sufficiently extracted by countercurrent extraction from *Ardisia crenata*[11].

Based on ethnobotanical reviews, particularly in this genus of *Ardisia*, several species have traditional therapeutic significances. The present study based on the report of preliminary phytochemical and GC-MS analysis of crude extract from the leaves of the *Ardisia blatteri* showed that the presence of phytoconstituents like tannins, flavonoids, alkaloids and coumarins.

The traditional medicine practice is strongly recommended for this plant as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents that are responsible for the activity of the whole (or) part of the plant. Additional work will encourage to find out the pharmacological properties.

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Cite this article as:

M.Dhanasekaran. Phytochemical screening of *Ardisia blatteri* gamble: An endemic plant of Southern Western Ghats, Tamilnadu, India. Int. Res. J. Pharm. 2016;7(2):31-35 http://dx.doi.org/10.7897/2230-8407.07216

Source of support: Nil, Conflict of interest: None Declared