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RESEARCH ARTICLE

PHYTOCONSTITUENTS ANALYSIS, AND GC-MS PROFILING OF TUBERS OF *IPOMOEA MAURITIANA* JACQ (CONVOLVULACEAE)

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ABSTRACT

The aim of this study is to quantify the amount of phenols, tannins and flavonoids present in different fractions of root tubers of *Ipomoea mauritiana*, a traditional medicinal twining shrub and GC-MS profiling of acetone extract. It was found that the higher contents of phenolics (285.05mg GAE/g extract), tannins (190.02mgGAE/g extract) and flavonoids (174.44mgRE/g extract) were determined to be present in acetone fraction. Further, the GC-MS analysis of the acetone extract revealed the presence of 27major compounds. It indicates the presence of therapeutic potential of this species.

INTRODUCTION

According to WHO, 80% of the world population mainly depends on the traditional medicines. India is the country with rich biodiversity has many flourishing traditional systems of medical practice viz., ayurveda, siddha and unani. Some people of ancient period have started exploring nature and have tapped the plants for their medicinal properties and bioactive components (Boopathi and Shivakumar, 2011) Phytochemicals are bioactive compounds produced by the plants as secondary metabolites which include alkaloids, phenols, tannins, flavonoids glycosides etc.. These phytoconstituents reduces the risk of major chronic diseases (Duraipandian et.al., 2006). Knowledge of the chemical constituents of plants is desirable because such information will be more necessary for the synthesis of complex chemical substances (Parekh and Chanda, 2008).

Ipomoea mauritiana of Convolvulaceae family is a much branched glabrous twining perennial shrub with large tuberous roots. It is distributed throughout India in deciduous and evergreen forests and coastal tracts and widely naturalized in tropical parts of the world. It is a medicinal plant and is one of the source plants of 'Vidari', a most popular ayurvedic drug. 'Vidari' is a component of about 50 Ayurvedic formulations including Chyavanaprash. The Ayurvedic Pharmacopoeia of India correlates 'Vidari' to tubers of *Pueraria tuberosa* (Roxb. ex Willd.) DC (Fabaceae).

It is used as aphrodisiac, cardiogenic, demulcent, diuretic, refrigerant, galactagogue and tonic (Chopra *et al.*1992) It is used in consumption, emaciation, enteric fever and spermatorrhoea (Pandey 2004). In the present study to investigate the bioactive components present in the tubers of *Ipomea mauritiana* GC-MS was used.

MATERIALS AND METHODS

Preparation of extracts

I. mauritiana tubers were collected from Aryavaidya Pharmacy Medicinal Plant Garden, Kanjikode, Palakkad, Kerala, India. The collected tubers were cut into small pieces, shade dried, powdered and extracted with organic solvents viz., petroleum ether, chloroform, acetone and methanol and hot water in the increasing order of polarity using a soxhlet apparatus. The different solvent extracts were concentrated by rotary vacuum evaporator.

Quantitative phytochemical analysis

Determination of total phenolics

Total phenolics concentration was measured by Folin-ciocalteu assay (Siddhuraju and Becker, 2003). Fifty microlitre aliquots of the extracts were taken in test tubes and made up to 1 ml with distilled water. To this, 0.5 ml of folin-ciocalteu phenol reagent (1:1 with water) was added followed by the addition of 2.5ml of sodium carbonate solution (20%). The reaction mixture was vortexed and the test tubes were incubated in dark for 40 minutes and the absorbance was measured at 725 nm

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against the reagent blank. Analysis was performed in triplicates and the results were expressed as Tannic acid equivalents.

Determination of Tannins

The same extracts were used in tannin estimation using polyvinylpyrrolidone (PVPP) (Siddhuraju and Manian, 2007). Hundred mg of PVPP was weighed into Eppendorf tubes and to this 1mL of distilled water and then 1mL of sample extracts were added.

The content was vortexed and kept in the freezer at 4° C for 15 minutes. Then the sample was centrifuged at 4000rpm for 10 minutes and the supernatant was collected. The supernatants have simple phenolics, while tannins would have been precipitated along with PVPP. The phenolic content of the supernatant was measured and expressed as the content of non tannin phenolics on a dry matter basis.

The tannin content of the sample was calculated as

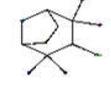
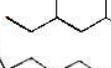
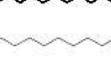
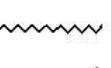
$$\text{Tannins (\%)} = \text{Total phenolics (\%)} - \text{Non tannin phenolics (\%)}$$

Table 1. Determination of total tannins and flavonoids of *I. mauritiana* root extracts

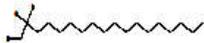
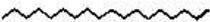
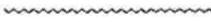
Solvent extracts	Tannins(mgGAE/g extract)	Flavonoids(mgRE/g extract)
Petroleum ether	21.10 ± 0.74	10.83 ± 0.83
Chloroform	53.14 ± 3.86	40.83 ± 2.50
Acetone	190.02 ± 5.43	174.44 ± 5.85
Methanol	114.33 ± 4.36	97.50 ± 3.82
Hot water	35.75 ± 1.01	27.22 ± 2.93

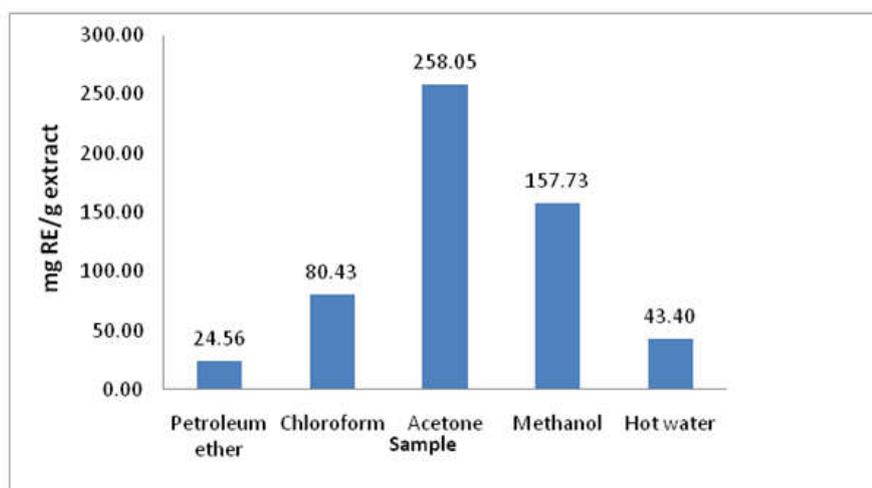
Values are expressed as mean (n=3) ± Standard Deviation (SD).

Table 2. Bioactive compounds identified in the acetone extract of tuber part of *Ipomoea mauritiana* by GC-MS technique

No.	Name of compound	RT	Molecular formula	Structure	Molecular weight (Dalton)	Nature of compound	Activity	Peak area%
1.	6,8 Dioxabicyclo (3.2.1) octan 3a-OL-2,2,4,4,-D4	4.97	C ₈ H ₈ D ₄ O ₃		130	-	-	0.42
2.	4-Acetylbutyric acid	7.36	C ₈ H ₁₀ O ₃		130	-	-	0.45
3.	2-Methyl-4,5-dihydroxy benzaldehyde	10.89	C ₈ H ₈ O ₃		152	-	-	0.38
4.	Thiosulphuric acid(H2S2O3),S-(2-ammoethyl ester)	12.24	C ₂ H ₇ NO ₃ S ₂		157	Sulphur oxoacid	-	0.39
5.	Dodecanoic acid	14.08	C ₁₂ H ₂₄ O ₂		200	Lauric acid	-	0.84
6.	Chloroacetic acid,4-hexadecylester	14.71	C ₁₈ H ₃₅ ClO ₂		318	-	-	1.44
7.	Tetradecanal	15.20	C ₁₄ H ₂₈ O		212	Myristyl aldehyde	Antimicrobial activity	0.99
8.	Tetradecanoic acid	18.31	C ₁₄ H ₂₈ O ₂		228	Myristic acid	-	0.43
9.	E-15-Heptadecenal	19.01	C ₁₇ H ₃₂ O		252	aldehyde	antibacterial activity	3.61
10.	Isopropyl Myristate	19.62	C ₁₇ H ₃₄ O ₂		270	Ester	-	0.40
11.	Ethyl 3-(trimethylsilyloxy)azabicyclo(4.2.1)oct-2-ene-8 carboxylate	20.38	C ₁₃ H ₂₃ NO ₃ Si		269	-	-	1.26
12.	Hexadecen-1-ol trans-9	20.80	C ₁₆ H ₃₂ O		240	-	Anti-inflammatory properties	4.20
13.	Hexadecanoic acid	22.47	C ₁₆ H ₃₂ O ₂		256	-	Anti-inflammatory activity	14.22
14.	1- Octadecene	23.02	C ₁₈ H ₃₆		252	Alkene	-	4.90
15.	9-Octadecene 1-ol(z)	24.27	C ₁₈ H ₃₆ O		268	Oleoalcohol	-	10.24

Continue

16.	1-Octadecanol	24.74	C ₁₈ H ₃₈ O		270	Stearyl alcohol	-	10.31
17.	2,2DIDEUTRO OCTADECANAL	25.20	C ₁₈ H ₃₄ D ₂ O		268	-	-	0.50
18.	9,12,Octadecadienoic acid(Z,Z)	25.64	C ₁₈ H ₃₂ O ₂		280	-	Biosynthesis of prostaglandins and cell membranes	12.14
19.	Octadecanoic acid	26.21	C ₁₈ H ₃₆ O ₂		284	-	Antibacterial activity	1.67
20.	1-Doco sanol methyl ether	26.83	C ₂₂ H ₄₄ O		340	Alcohol	Docosanol inhibits the in vitro replication of many lipid enveloped viruses, including HSV (herpes simplex virus); ₅	4.55
21.	Hexatricosane	28.57	C ₃₆ H ₇₄		506	-	-	1.50
22.	4-(3'4'(Methylenedioxy)6'formyl phenyl)6-fluro coumarin	29.42	C ₁₇ H ₉ FO ₃		312	-	-	0.67
23.	n-Tetracosanol-1	29.81	C ₂₄ H ₅₀ O		354	-	-	4.51
24.	HAHNFETT	31.86	N/A		-	-	-	0.51
25.	Octacosane	32.31	C ₂₈ H ₅₈		394	-	-	4.86
26.	Nonacosane	35.05	C ₂₉ H ₆₀		408	-	-	0.53
27.	Tetratetracontane	38.20	C ₄₄ H ₉₀		618	Alkane	-	2.29



Values are expressed as mean (n=3) ± Standard Deviation (SD). GAE – Gallic Acid Equivalents

Fig. 1.Total phenolic content in the root extract of *I. mauritiana*.

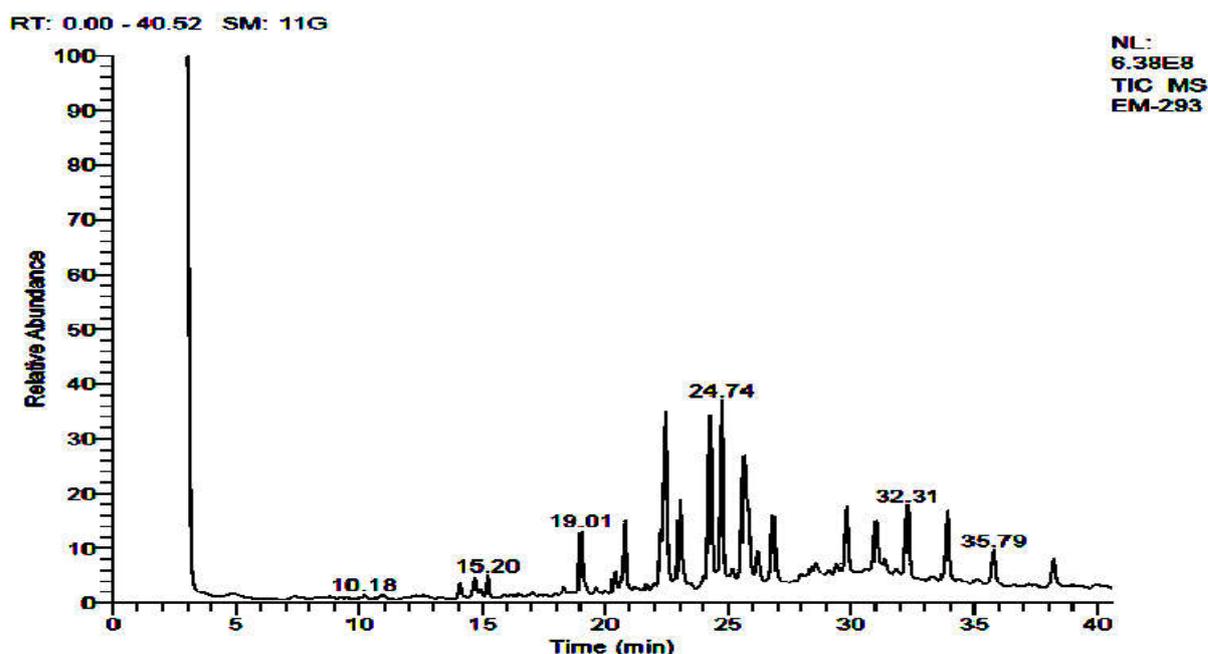


Fig. 2. GC-MS Chromatogram of the acetone extract of *I. mauritiana* root tubers

Determination of flavonoids

Flavonoids content of the extracts were quantified according to Zhishen *et al.* (1999). About 500 μ L of the plant extract was taken in different test tubes and to this 2mL of distilled water was added. Blank was prepared by taking 2.5 ml of distilled water. Then, 150 μ L of NaNO₂ as added to all the test tubes and incubated at room temperature for 6 minutes. After incubation 150 μ L of AlCl₃ was added to all the test tubes including blank. All the test tubes were incubated again for 6 minutes at room temperature.

Then 2ml of 4% NaOH was added to all the test tubes which were then made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and allowed to stand for 15 minutes. Presence of pink color was due to the flavonoids. The absorbance was measured at 510 nm. Rutin was used as standard. The experiments were done in triplicate and the results were expressed as Rutin equivalents (RE).

GC-MS analysis

GC-MS is technique which is most commonly used for the identification of unknown organic compounds in a mixture and determined by comparing it with the referral spectra. The phytochemical investigation of acetone extract was performed using equipment (Thermo Scientific Co.), thermo GC-TRACE ultra ver.: 5.0, Thermo MS DQ II.

The column used was DB 5 MS capillary standard non polar column, the dimension are 30Mts, ID: 0.25 mm, Film thickness: 0.25 μ m, flow rate of the mobile phase gas carrier helium was set at 1.0ml/min. The oven temperature was programmed initially at 70°C, then raised to 260°C at 6°C/min. one μ l sample injection volume was utilized. Samples were dissolved in acetone and were run fully. The results were compared with the database of spectrum stored in GC-MS library.

RESULTS AND DISCUSSION

Total phenolics

The amount of total phenolics estimated in the tuber sample was varied much across the solvents used (Fig.1). It was higher in acetone extract (285.05mg GAE/g extract), followed by methanolic extract (157.73 mg GAE/g extract). As the phenolic are a class of antioxidants which can scavenge free radicals (Kessler *et al.*, 2003), their presence in high amount in *I. mauritiana* may be the possible factor for its antioxidant activity reported elsewhere (Karuppusamy and Parimelazhagan, 2013). Presence of phenolics observed in the acetone extract is an indication that the tubers may play an important role as dietary antioxidants.

Tannins

The root extracts of *I. mauritiana* was analyzed for its tannin content (Table.1). Tannins are metal chelators and the chelated metal ions are not bioavailable (Karamac 2009). The total tannins were found to be higher in acetone extract (190.02mgGAE/g extract). Tannins, poly phenols including flavonoids have been reported to exhibit a wide range of activity and prevent the attack of free radicals in human body (Narasimhan *et al.*, 2006). Therefore, higher the content of tannins greater will be the capacity to quench free radicals. Tannins inhibit the absorption of iron which may lead to anemia (Brune *et al.*, 1989).

Flavonoids

Flavonoids contents in tubers of the species, *I. mauritiana* were much varied across the solvents used (Table 1). The acetone extract of tubers possessed high flavonoids (174.44mgRE/g extract) followed by methanol (97.50 mg RE/g extract). Flavonoids are group of natural phenolics which generate H₂O₂, that can scavenge free radicals, possess the capability of chelating metal ions and inhibition of enzymes like NADPH (Benavente *et al.*, 1997).

GC-MS Analysis

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. The GC-MS identification of the chemical constituents was based on comparison of their mass spectra with NIST and WILEY libraries. Structures were defined by per cent similarity values, and they are confirmed by the peak, retention time and molecular weight. GC-MS analysis was carried out in the acetone extract of the species, *I. mauritiana*. The results revealed the presence of 27 major phytochemical components with different therapeutic properties (Table 2 and Fig. 2). The compounds identified in the tuber of *I. mauritiana* possessed various biological activities like, hexadecanoic acid – Palmitic acid(14.22%) has antioxidant and anti-inflammatory activity (Aparna et.al., 2012) and selective cytotoxicity against human leukemic cells hypocholesterolemic, nematocide, pesticide, lubricant activities (Harada et.al., 2002). The present study makes way for the identification of novel compounds that might cure many diseases, but further clinical studies have to be done to purify the active compounds responsible for therapeutic activity.

Conclusion

The present study concluded that the extraction capacity of acetone is stronger and it yielded many active constituents, which can be utilized for the development of novel compounds. The study suggests that the tubers of *I. mauritiana* may act as a potent therapeutic agent.

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