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Quantitative Phytochemical Constituents, Antioxidant activity and HPTLC analysis of Tuber part of Ipomoea mauritiana Jacq.

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Abstract

Ipomoea mauritiana is a much branched glabrous twining perennial shrub with large tuberous roots used in the treatment of anorexia, fever, inflammation and burning sensation .Its used asappetizer, galactogogue, stimulant, carminative, and tonic. The aim of the present study was to quantitatively analyze the phytochemical constituents like, phenols, tannins and flavonoids in different fractions of *Ipomoea mauritiana* root tubers. It was found that higher amounts of all these phytoconstituents were found in acetone extract(Phenolics (285.05mg GAE/g extract), tannins (190.02mgGAE/g extract) and flavonoids (174.44mgRE/g extract).HPTLC profiling carried out in acetone extract revealed the presence of 10peaks, the maximum peak area was found to be 27.17 and the corresponding Rf value is 0.71.The extracts were subjected to assess their antioxidant potential using various *invitro*systems such asFRAP, Phosphomolybdenum assay, Metal Chelating activity,ABTS and DPPH.The study revealed that the acetone extract possessed highest antioxidant potential.This potent antioxidant activity may be attributed to its high phenolic and flavonoid contents.

Introduction

Indian traditional medicines is one of the richest medicinal system those available around the world. The phytochemicals identified from traditional medicinal plants are providing an excellent opportunity for the development of new types of therapeutics. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. These phytochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. Fingerprint analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents profile of the plants with botanical identity and for estimation of chemical and

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biochemical markers(Manikandan et al.,2010) Antioxidants prevent free radical induced tissue damage by preventing the formation of free radicals, scavenging them or promoting their decomposition. Reactive oxygen species like hydroxylradical, superoxide anion, hydrogen peroxide can cause injuries which may cause DNA and protein damage and also oxidation of enzymes in human body. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Plants therefore constitute the main source of natural antioxidant molecules which have the capacity to eliminate or neutralize the deleterious reactive oxygen species. The importance of medicinal plants to prevent or control diseases has been attributed to the antioxidant properties of their constituents, commonly associated with a large number of molecules such as phenols and flavonoids(Atawodi.2005). *Ipomoea mauritiana* is one such plant belonging to family convolvulaceae, which is used in ayurvedic medicine commonly called as Kshiravidari. It is used as aphrodisiac, cardiotonic, demulcent, diuretic, refrigerant, galactogogue and tonic (Chopra *et al*., 1992). Based on the traditional knowledge of medicinal system, the present study was carried out to quantify the phytochemical constituents and evaluate the antioxidant activity of different solvent and extracts of tubers of *Ipomoea mauritiana*.

Materials and methods

Preparation of extracts

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

Quantification assay

Measurement of total phenolics, tannins and Flavonoids

Total phenolics concentration was measured by Folin-ciocalteu assay (siddhuraju and Becker 2003).The total phenolics contain tannin and non tanninphenolics. The same extracts

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were used in tannin estimation using polyvinylpyrrolidine (PVPP) (Siddhuraju and Manian, 2007).The total flavonoid content was determined by the method described previously by Zhishen*etal.*, 1999. The experiments were done in triplicates and the results were expressed as Rutin equivalents (RE).

HPTLC finger printing analysis

HPTLC technique was carried out using the method of Harbone (1998).The acetone tuber extract was dissolved in 1ml of acetone and centrifuged for about 5 minutes at 3000rpm, and this solution was utilized as test solution for HPTLC analysis.Mobile phase used was ethyl acetate - 80%, water-10%, acetic acid- 5% and formic acid- 5%.1µl of test solution was spotted on the form of band of 8mm length using Hamilton syringe on silica gel $60F_{254}$ (precoated on aluminium plate 10x10 cm) with the help of CAMAG LINOMAT 5 applicator which was programmed through WINCATS software.The chromatogram was developed in ascending order with CAMAG twin trough glass chamber (20x10cm)which was pre saturated with mobile phase for 15min.The length of each run is cm. The TLC run was performed under laboratory conditions of temperature 25±2 and humidity 60±5^{o}C.The plates were air dried by hot air to evaporate solvents.The Reprostar 3(CAMAG, Switzerland) was used for documenting and evaluating the planar chromatograms at UV366nm,UV 254nm and in white light.After derivatization the plate was fixed in CAMAG TLC scanner 3 and scanning was done at UV254nm.The peak numbers with their height and area, peak display and peak densitogram and Rf values were programmed through WINCATS software 1.3.4 version.

Ferric Reducing Oxidant Assay (FRAP)

The antioxidant capacities of different extracts of sample were estimated according to the procedure described by Pulido *et al.* (2000).The absorbance of the reaction mixture was read at 593 nm. The values are expressed as mmol Fe (II)/g extract.

Phosphomolybdenum assay

The antioxidant capacities of different extracts of sample were estimated according to the procedure described by Prieto *et al*. (1999). The absorbance of the mixture was measured at 695 nm. The results reported are mean values expressed as grams of ascorbic acid equivalents (AAE) per 100 g extract.

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Metal Chelating activity

The chelation of ferrous ions by various extracts was estimated by the method described by Dinis*et al.* (1994). Absorbance of the solution was measured spectrophotometrically at 562 nm. The results were expressed as mg ethylenediaminetetraacetic acid (EDTA) equivalent/g extract.

Antioxidant activity by ABTS assay

Radical scavenging activity of extracts was assessed spectrophotometrically by [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] . ABTS radical- scavenging activities of the extracts were determined according to Re *et al*. (1999). Triplicate determinations were made at each dilution of the standard and percentage inhibition was calculated against the blank (ethanol) absorbance at 734nm and then was plotted as a function of Trolox concentration. The unit of antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmoles/g sample extract.

DPPH radical scavenging activity

The antioxidant activity of different extracts were determined in terms of hydrogen donating and radical scavenging ability using the stable radical DPPH according to the method described by Blois(1958).The absorbance of the samples was measured at 517 nm.Radical scavenging activity of the samples was expressed as IC_{50}, which is the concentration of the sample required to inhibit 50% DPPH concentration.

Statistical analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) test using a statistical package program (SPSS 10.0) and the significance of the difference between means was determined by Duncan's Multiple Range Test (DMRT) at $p<0.05$ significant level. Triplicates were maintained and represented as mean±SD(Standard Deviation) (Gomez and Gomez, 1976).

Results and Discussion

Determination of total phenolics, tannins andflavonoid contents

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The amount of total phenolics was analyzed and shown in Table 1 .The total phenolics content of *Ipomoea mauritiana*was found to be high in acetone extract (258.05mg GAE/g extract) and low in petroleum ether fraction (24.56 mg GAE/g extract). In the study species, highertannin content was detected in acetone extract (190.02 mg GAE/g extract) followed by methanol extract(114.33 mg GAE/g extract) (Table 1).Flavonoid content was registered high in acetone extract (174.44 mg RE/g extract), and followed by methanol and chloroform extract (97.50 and 40.83 mg RE/g extract). Phenolic compounds such as flavonoids, phenolics acid, and tannins possess diverse biological activities including anti-inflammatory, anti-carcinogenic, and antiatherosclerotic activities. These activities might be related to their antioxidant activity (Chung et al., 1998).Phenolics are powerful antioxidants which act in a structure-dependent mannerand can scavenge reactive oxygen species (ROS), and chelate transition metals which play vital roles in the initiation of deleterious free radical reactions (Fresco *et al*.,2006)

Table 1. Total contents of phenolics, tannins and flavonoids in various alcoholic and aqueous extracts of root tubers of *Ipomoea mauritiana*

Sl.No.	Sample	Phenols (mg GAE/g extract)	Tannins(mg GAE/g extract)	Flavonoids (mg RE/g extract)
1.	Petroleum ether	24.56±3.11[a]	21.10±0.74[a]	10.83±0.83[a]
2.	Chloroform	80.43± 0.97[c]	53.14±3.86[c]	40.83±2.50[c]
3.	Acetone	258.05± 2.91[e]	190.02±5.43[e]	174.44±5.85[e]
4.	Methanol	157.73±2.56[d]	114.33±4.36[d]	97.50±3.82[d]
5.	Hot Water	43.40±2.28[b]	35.75±1.01[b]	27.22±2.93[b]

GAE - Gallic Acid Equivalent, RE - Rutin Equivalent, Values are performed in triplicates and represented as mean ± SD (standard deviation).Mean values followed by different superscripts in a column are significantly different ($p<0.05$)

High Performance Thin Layer Chromatography – HPTLC

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The acetone root tuber extract of *Ipomoea mauritiana*was subjected to High Performance Thin Layer Chromatography to analyse the fingerprint profile of secondary metabolites.It was found that there were 10 peaks (Table 2 and Fig.2). The maximum peak area was found to be 27.17 and

thecorresponding Rf value is 0.71. The photo documentation observed at 254nm, 366nm and white light are given (Fig.3.). The 3D densitogram is given in Fig.1.

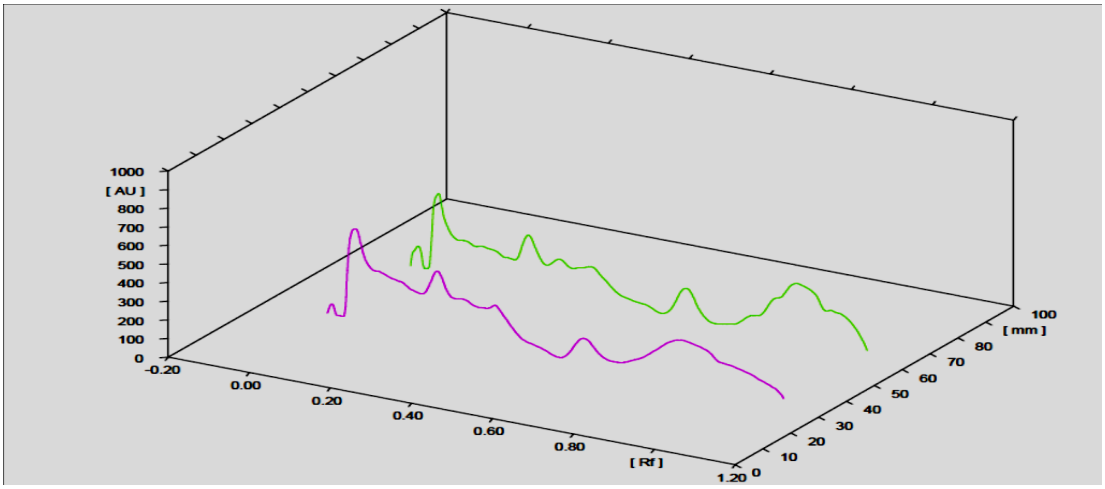

Fig.1. HPTLC densitogram and 3D display for acetone extract of tuber part of *Ipomoea mauritiana*Jacq.

Table 2. HPTLC – flavonoid profile of acetone extract of root tuber of *Ipomoea mauritiana*.

Peak	Retention factor (Rf)	Height AU	Peak area AU	Area %
1	0.04	112.8	1389.8	1.47
2	0.01	417.5	12135.1	12.85
3	0.07	189.1	4750.4	5.03
4	0.11	177.2	7601.6	8.05

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5	0.21	285.1	10270.6	10.87
6	0.29	186.6	6475.1	6.85
7	0.35	179.4	11974.0	12.67
8	0.57	160.1	5490.0	5.81
9	0.71	298.6	25665.4	27.17
10	0.98	188.5	8719.0	9.23

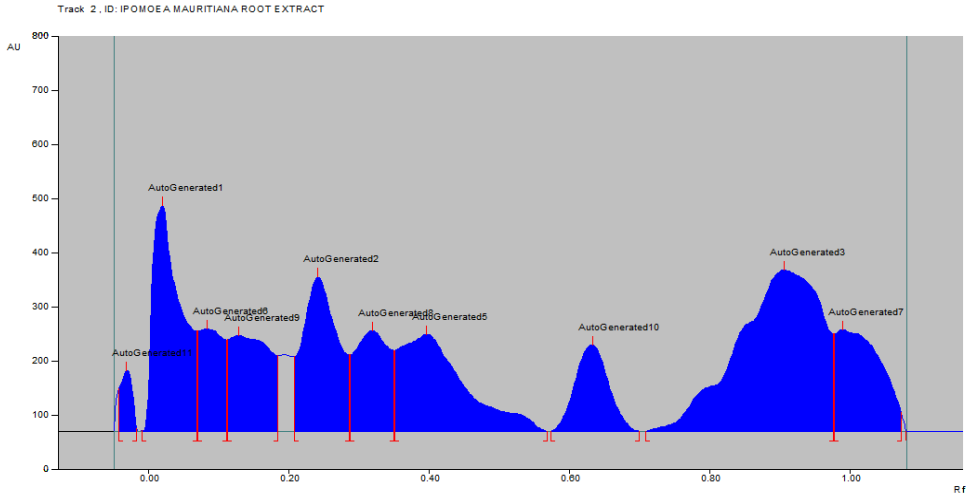

Fig.2.Chromatogram for acetone extract of *Ipomoea mauritiana* at 254nm.

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Fig.3. Photo documentation of acetone extract of*Ipomoea mauritiana* at white light, 254nm and 366 nm.

***In vitro* antioxidant assays**

Ferric reducing antioxidant assay

FRAP is a simple and reliable test to measure the reducing potential of an antioxidant reacting with ferric2, 4, 6 tripyridyl S triazine (Fe (III) TPTZ) complex and producing a colouredferrous 2, 4, 6 tripyridyl S triazine (Fe (II) TPTZ) complex at low pH.Higher the absorbance higher will be the reducing power.It was found that FRAPvalues were consistently higher in acetone fraction (70.93±1.09mM Fe(II)E/1 mg), followed by methanol (51.94±1.05mM Fe (II) E/1 mg)(Table3).

Ferric reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action (Senthilkimar*et al*.,2012).

Phosphomolybdenum assay

The phosphomolybdenum assay is basedon the reduction of Mo (VI) to Mo (V) by the antioxidant compound which turns green Mo (V) complex with absorption maximum at 695nm.

Total antioxidant capacity of different extracts were analyzed and shown in Table 3. The better antioxidant capacity was shown by acetone fraction (216.80 ±5.51g AA/1g extract). This can be correlated with the free radical scavenging activity of natural antioxidant, Ascorbic acid.

Metal chelating activity

Metal chelating action is based on chelation of Fe^{2+}ions by the reagent Ferrozinewhich results in the formation of a complex with Fe^{2+} ions (Dinis*et al*.,1994).When other chelating agents are present it would decrease the formation of red coloured complex. Measurement of the rate of reduction of colour, therefore allows estimation of chelating activity. In *Ipomoea mauritiana* the metal chelation capacity of hot water extract (65.90 ± 2.55 mg EDTA/g) was higher followed by acetone extract (47.96±1.11 mg EDTA/g) (Table 3 and Fig.4). The result showed that the extracts have protective role against oxidative damage by sequestering ions that may catalysehydroperoxide decomposition reaction.The scavenging potential and metal

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chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups .The acetone extract possessed higher amount of phenolics which may interact with ferrous and ferrozine complex suggesting that they have chelating properties which may capture Fe^{2+} ions before ferrozine.

Table 3. FRAP, Phosphomolybdenum,metalion, $ABTS^{+}$, radical scavenging activity of *Ipomoea mauritiana*root tuberextract.

Sample extract	FRAP (mM Fe(II)E/1 mg)	Phosphomolybdenum (gAA/g)	Metal ion chelating (mg EDTA/g extract)	$ABTS^{+}$ (µmoles TE/g)
Petroleum Ether	10.35±0.79	11.5±2.38	8.64±0.56	25.64±6.02
Chloroform	25.82±1.30	137.01±2.39	28.68±3.68	116.19±9.60
Acetone	70.93±1.09	216.80±5.51	47.96±1.11	629.58±4.72
Methanol	51.94±1.05	172.11±6.02	31.75±1.57	568.65±6.10
Hot water	22.79±0.88	72.02±2.17	65.90±2.55	85.90±4.58

Values are mean of triplicate determination=3±standarddeviation

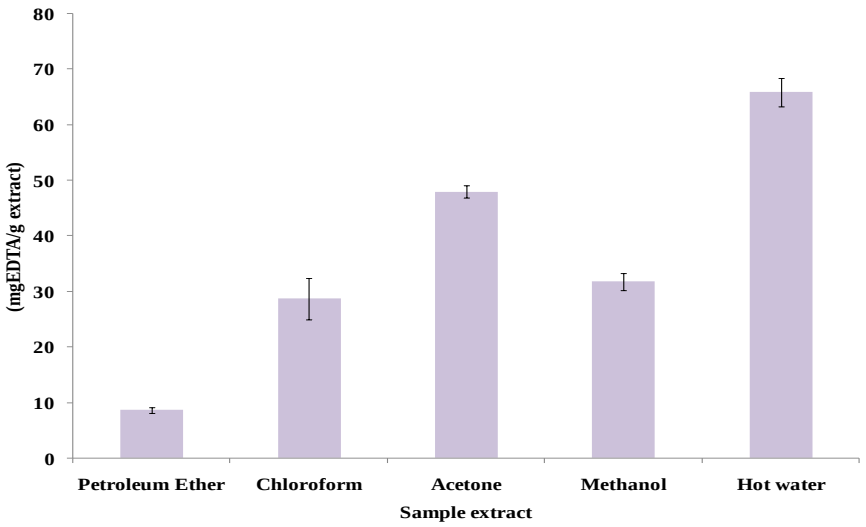

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Fig. 4. Metal ion scavenging activity of *Ipomoea mauritiana* root tuber extract.

ABTS radical scavenging activity

The TEAC(Trolox Equivalent Antioxidant Capacity) was measured using improved $ABTS^{+}$ radical decolouration assay. This measures the ability of a compound to scavenge $ABTS^{+}$radical (Hagerman *et al*., 1998). The results were expressed as µmoles Trolox/g dry weight of plant material. The results of$ABTS^{+}$cation radical scavenging activity of different solvent extracts of *Ipomoea mauritiana*was shownin Table 3 and Fig.5. Higher scavenging activity of thetuber wasobserved in acetone extract (629.58±4.72µmolesTE/Gextract).Hagerman *et al*., 1998 reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\bullet}$+).

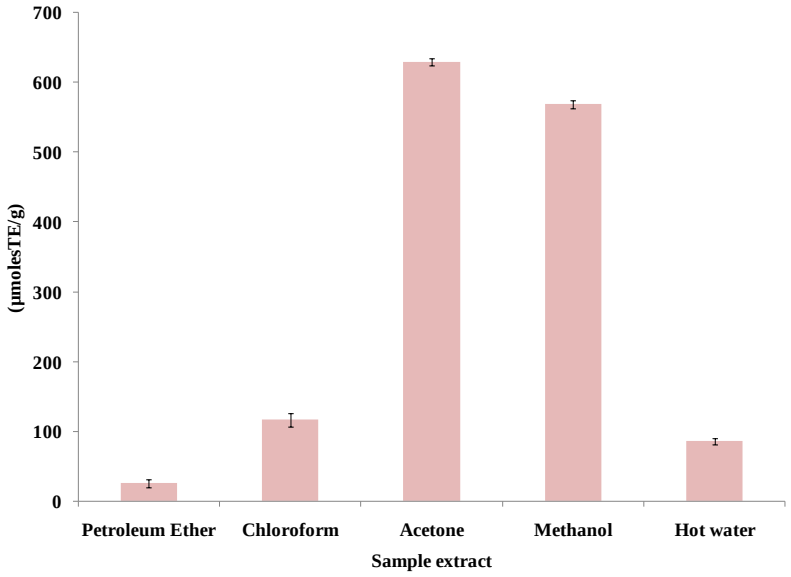

Fig. 5. $ABTS^{+}$ activity of *Ipomoea mauritiana* root tuber extract.

DPPH radical scavenging activity

The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Extracts reduce the colour of DPPH due to the power of

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hydrogen donating ability(Blois, 1958). DPPH is one of the compounds that possess a proton free radical with a characteristic absorption which decreases significantly on exposure to proton radical scavengers(Yamaguchi *et al*., 1998). Antioxidants may guard against reactive oxygen species(ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules.Importantly IC_{50} value of the extracts was also calculated to determine the amount of extract needed to quench 50% of radicals. The results of DPPH were expressed inIC_{50} value. Lower theIC_{50} value, higher will be the antioxidant activity. The free radical scavenging activity of the extracts were estimated by comparing with standards such as BHT, BHA, quercetin and rutin and the result were shown in (Table 4). In the present study the acetone fraction of *Ipomoea mauritiana* was found to be more potent free radical scavenger, with an IC_{50} value of 22.13µg/mL compared to other extracts (Table 4).The overall results showed that the antioxidant activities were enhanced with the increase in the concentration of acetone extract. IC_{50} values clearly indicate that the species have significant antioxidant activity.

Table 4. DPPH radical scavenging activity of various solvent extract of two study species

Extract	***Ipomoea mauritiana***
	IC_{50} (µg/mL)
Petroleum ether	257.75±4.26
Chloroform	134.84±5.14
Acetone	22.13±1.89
Methanol	40.59±2.62
Hot water	76.68±2.91
BHA(Synthetic antioxidant)	9.27±0.53
BHT(Synthetic antioxidant)	15.36±0.66

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Quercetin(Natural antioxidant)	5.32 ±0.38
Rutin(Natural antioxidant)	11.48 ± 0.49

Conclusion

In the present study it was found that the root tubers of *Ipomoea mauritiana*showed higher amount of phenolics and flavonoids which may be responsible for higher antioxidant activity.The HPTLC fingerprinting profile revealed the presence of ten peaks,further the quantification assays revealed the presence of higher phenolic and flavonoid contents,so the antioxidant activity shown by the study material might be due to the composite action of these phytochemicals.Thus, these extracts can be considered as new sources of natural antioxidants. Our findings now provide a basis for developing a valuable food additive to enhance human nutrition via their phenolic composition and antioxidant activity.

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