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Research Article

QUALITATIVE ASSESSMENT OF ETHANOLIC EXTRACT OF *MARANTA ARUNDINACEA* L. RHIZOME USING HPTLC

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ABSTRACT

The use of plants for the treatment of various ailments has been well authenticated and practiced since age old. During last decades, many diseases such as cancer, cardiovascular diseases, have been great challenging targets in medical field. Although many synthetic drugs has been in use, their adverse side effects plays a devastating role. Herbs, an ever promising candidate in achieving their role as antioxidants, antimutagens, anticarcinogens are believed to comfort these effects and also to act as a major contributor to the health care. Hence it is obligatory to screen the secondary metabolites, the key factor in therapeutics. *Maranta arundinacea* .L is a starchy rich rhizomatous tuber. This study focuses on the determination of total phenol,flavonoid and flavonol content and qualitative analysis of the ethanolic extract of *M.arundinacea*.L rhizomes by HPTLC for analysis of flavonoids, phenolics, steroids, tannins and glycosides. HPTLC profile provides a good lead to carry out the isolation of the active principle from this plant.

Keywords: *Maranta arundinacea*.L, Total Phenolic Content, Total Flavonoid Content, High Performance Thin Layer Chromatography.

INTRODUCTION

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. For example, some studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments[1]. Research over the last decade has delivered a vast amount of data indicating possible prevention of chronic diseases by antioxidant phytochemicals in food[2].Natural antioxidants present in plants are closely related in their medicinal and beneficial properties. Thus antioxidant capacity is a widely used parameter for assessing the bioavailability of foodstuffs as medicinal plants. The antioxidant properties of plant extracts should be evaluated in a variety of model systems using several indices to ensure the effectiveness of such antioxidant materials[3]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods[4,5]. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation[6-8]. These compounds have been reported to be well correlated with antioxidant potential[9]. Hence a qualitative and quantitative assessment of the phytochemicals like phenols, flavonoids, steroids, terpenoids, alkaloids, tannins serves as an important indice in evaluating the medicinal property of a plant.

M.arundinacea L, a member of Marantacea family is a starch rich rhizomatous tuber, widely cultivated for its starch for commercial preparation of weaned foods and biscuits. The starch also is reported to have medicinal uses and is an important ingredient in the preparation of barium meals and tablets. The tubers are used in the treatment of diarrhea. The plant has been reported for its immunostimulatory activity both *in vitro* and *in vivo* in rats[10].In our previous investigations, the tubers were found to be a rich source of antioxidant[11].

In the present study, the ethanolic extract of *M.arundinacea* L tubers was screened for its total phenolic, flavonoid and flavonol content. The qualitative and quantitative analysis of bioactive phytoconstituents like flavonoids, phenolics, tannins, steroids, and glycosides in the extract were determined by high performance thin layer chromatography (HPTLC).

MATERIALS AND METHOD

Plant material

The tuberous rhizomes with leaves and flowers of *M. arundinacea* L. were collected in December from Malampuzha, Palakkad District, Kerala and authenticated by Dr.G.V.S.Murthy. A voucher specimen was deposited in the herbarium of the Botanical Survey of India, TNAU Campus, Coimbatore with herbarium code number No.BSI/SRC/5/23/10-11/Tech.Dated 19-10-2010.

Preparation of extracts

Plant materials were washed with distilled water and dried at room temperature. The dried rhizomes were manually ground to a fine powder. The coarsely powdered tuberous rhizomes of *M.arundinacea* L. were extracted sequentially with petroleum ether, ethyl acetate and ethanol by cold percolation method. The ethanolic extract was used for the study, based on the yield and the phytochemical screening.

Determination of total phenolic content

Total phenolic content (TPC) were determined by Folin-Ciocalteu method[12]. Briefly, an aliquot of the extract (0.1 ml) was mixed with distilled water (3 ml) and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min, 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly 1 min, then cooled and the absorbance was measured at 650 nm using spectrophotometer (Shimadzu UV 1640) against the reagent blank.TPC was expressed as mg gallic acid equivalent (GAE)/100 g sample dry weight.

Determination of total flavonoid content

Total flavonoid Content (TFC) was determined using a standard method of[13]. Briefly an aliquot of 0.5 ml of 2% $AlCl_3$ was added to 0.5 ml of sample solution. After 1h at room temperature, the absorbance was measured at 420 nm at the final concentration of 0.1 mg/ml. TFC was calculated as mg quercetin equivalent (QE)/100 g sample dry weight.

Determination of total flavonol content

Total flavonol in the plant extract were estimated using the method reported previously by[14]. To 2.0 mL of sample, 2.0 mL of 2% $AlCl_3$ ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/ ml. Total flavonoid content was calculated as mg quercetin equivalent (QE)/100 g sample dry weight.

HPTLC Analysis

A densitometric HPTLC analysis of *M.arundinacea* ethanolic extract was performed for the development of characteristic fingerprinting profile. The *M.arundinacea* ethanolic extract was dissolved in ethanol 100mg/1ml.The solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. The samples (2µl) were loaded as 5mm for flavonoid and 6mm for phenolics, steroids, tannins and glycosides band length in 3x10 silica gel $60F_{254}$ TLC plate, using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plates were kept in TLC twin trough developing chamber (after saturation with solvent vapour) with respective mobile phase upto 90 mm as described below. The developed plate was dried using hot air to evaporate solvents from the plate and sprayed with the respective reagents mentioned below. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254nm and UV366nm. Finally, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and the scanning was done at UV 366nm, 500nm, 500nm, UV 254nm, 500nm respectively for flavonoids, phenolics, steroids, tannins and glycosides. The peak table, display and densitogram were identified[15].

Mobile phase

Flavonoids - Toluene : Acetone : Formic acid (4.5 : 4.5 : 1)
Phenolics - Toluene : Acetone : Formic acid (4.5 : 4.5 : 1)
Steroids - Toluene : Methanol (9 : 1)
Tannins - Toluene : Ethyl acetate : Formic acid : Methanol (3 : 3 :0.8 : 0.2)
Glycosides - Chloroform : Methanol : Water (6.5 : 2.5 : 0.4)

Spray reagent

Flavonoids: 1% Ethanolic aluminium chloride reagent.
Phenolics: Folin-Ciocalteu reagent.
Steroids: Anisaldehyde sulphuric acid.
Tannins: 5% ferricchloride reagent.
Glycoside: Libermann-Burchard reagent.

Statistical Analysis

The results were expressed as mean (n = 3) ± Standard deviation (SD).

Table 1: Total Phenol, Flavonoid and Flavonol Content of the ethanolic extract of *M.arundinacea*

Total phenolic content (TPC)	390 ± 11 mg GAE/100g
Total flavonoid content (TFC)	290 ± 7mg QE/100g
Total flavonol content (TFC)	150 ± 9 mg QE/100 g

Table 2: Peak table with Rf values, height and area of Flavonoids and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.69	384.8	6998.7	Flavonoid standard
Sample A	1	0.04	12.7	234.1	Unknown
Sample A	2	0.09	10.4	222.4	Flavonoid 1
Sample A	3	0.18	15.8	473.9	Unknown
Sample A	4	0.29	15.6	435.4	Unknown
Sample A	5	0.43	19.5	531.1	Unknown
Sample A	6	0.68	119.8	4614.2	Flavonoid 2
Sample A	7	0.78	20.0	360.0	Unknown
Sample A	8	0.83	20.1	317.5	Unknown
Sample A	9	0.92	40.0	2341.3	Flavonoid 3

Table 3: Peak table with Rf values, height and area of Phenolics and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.53	489.2	21733.2	Phenolic standard
Sample A	1	0.04	131.7	3272.1	Phenolic 1
Sample A	2	0.10	138.5	3251.8	Phenolic 2
Sample A	3	0.54	12.1	249.7	Unknown
Sample A	4	0.60	12.7	203.6	Unknown
Sample A	5	0.69	212.2	6263.3	Phenolic 3
Sample A	6	0.74	131.6	4844.0	Phenolic 4
Sample A	7	0.89	211.1	8470.0	Phenolic 5
Sample A	8	0.96	82.5	3464.4	Unknown

Table 4: Peak table with Rf values, height and area of steroids and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.45	133.4	10466.3	Steroid standard
Sample A	1	0.03	11.3	68.0	Unknown
Sample A	2	0.04	10.8	88.6	Unknown
Sample A	3	0.17	17.8	601.2	Unknown
Sample A	4	0.32	58.2	1573.6	Steroid 1
Sample A	5	0.36	41.2	1553.2	Unknown
Sample A	6	0.44	112.1	3841.2	Steroid 2
Sample A	7	0.48	92.8	5465.6	Unknown
Sample A	8	0.58	30.6	991.4	Unknown
Sample A	9	0.98	19.4	309.8	Unknown

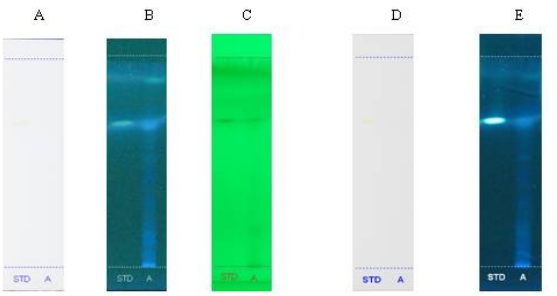
Table 5: Peak table with Rf values, height and area of tannins and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.48	348.6	13880.4	Tannin standard
Sample A	1	0.01	77.9	589.5	Unknown
Sample A	2	0.04	17.3	212.9	Unknown
Sample A	3	0.08	23.1	355.8	Unknown
Sample A	4	0.12	12.8	215.6	Unknown
Sample A	5	0.16	21.7	531.3	Unknown
Sample A	6	0.24	30.8	598.8	Unknown
Sample A	7	0.29	15.9	323.8	Unknown
Sample A	8	0.49	28.5	549.4	Tannin 1
Sample A	9	0.58	17.2	382.7	Unknown
Sample A	10	0.62	36.2	999.0	Unknown
Sample A	11	0.69	60.9	1927.4	Unknown
Sample A	12	0.88	103.5	6676.5	Tannin 2
Sample A	13	0.93	195.4	10502.3	Unknown

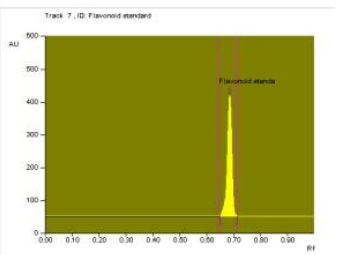
Table 6: Peak table with Rf values, height and area of glycosides and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.77	58.2	5372.0	Glycoside standard
Sample A	1	0.02	150.6	2411.1	Glycoside 1
Sample A	2	0.06	127.1	3187.5	Glycoside 2
Sample A	3	0.14	448.6	24505.8	Glycoside 3
Sample A	4	0.31	120.3	7713.9	Glycoside 4
Sample A	5	0.40	101.5	5164.8	Glycoside 5
Sample A	6	0.56	12.1	126.9	Unknown
Sample A	7	0.59	14.0	181.8	Unknown
Sample A	8	0.97	61.3	1261.9	Unknown

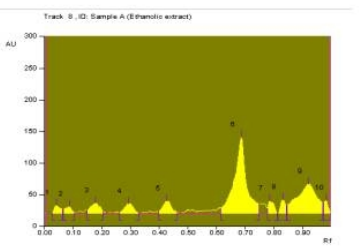
Fig: 1 Chromatograms of extract in HPTLC analysis for Flavonoids. (A) Before derivatization under day light, (B) Under UV 366 nm (C) Under UV 254 nm, (D) After derivatization under day light, (E) Under UV 366nm.



Peak densitogram display of Flavonoids

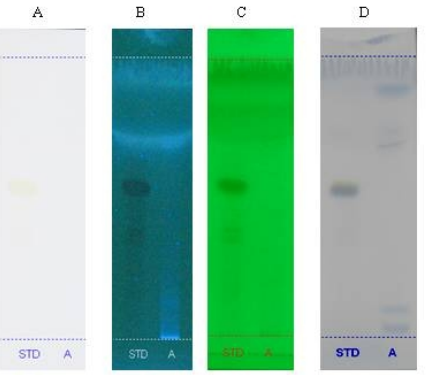


Rutin

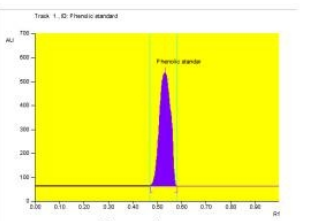


Ethanolic extract of *M.arundinacea*.L

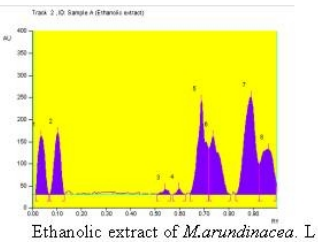
Fig: 2 Chromatograms of extract in HPTLC analysis for Phenolics. (A) Before derivatization under day light, (B) Under UV 366 nm (C) Under UV 254 nm, (D) After derivatization under day light.



Peak densitogram display of Phenolics

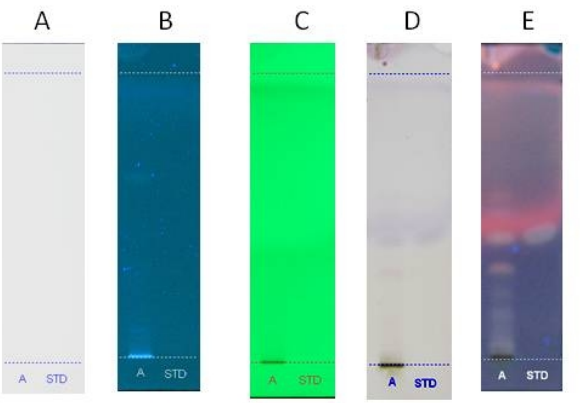


Quercetin

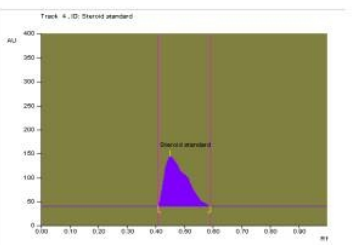


Ethanolic extract of *M.arundinacea*. L

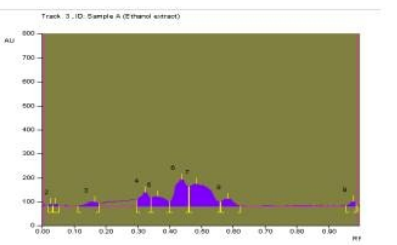
Fig: 3 Chromatograms of extract in HPTLC analysis for Steroids. (A) Before derivatization under day light, (B) Under UV 366 nm (C) Under UV 254 nm, (D) After derivatization under day light, (E) Under UV 366nm.



Peak densitogram display of Steroids

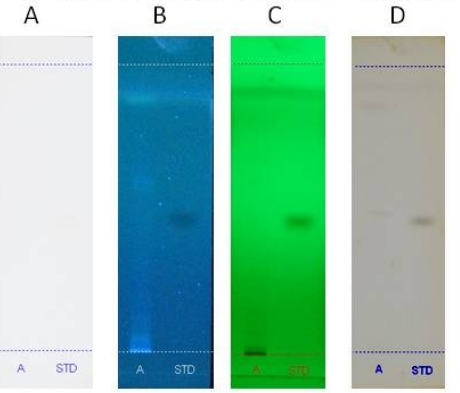


Solasodine

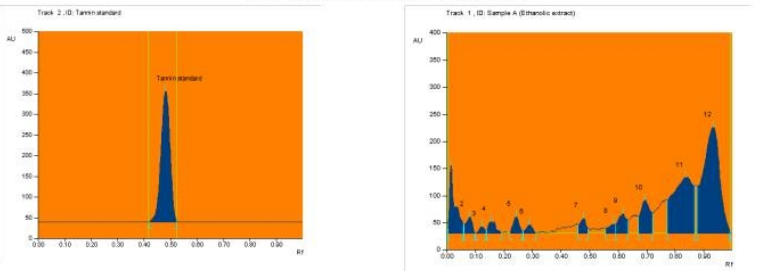


Ethanolic extract of *M.arundinacea*.L

Fig: 4 Chromatograms of extract in HPTLC analysis for Tannins. (A) Before derivatization under day light, (B) Under UV 366 nm (C) Under UV 254 nm, (D) After derivatization under day light.



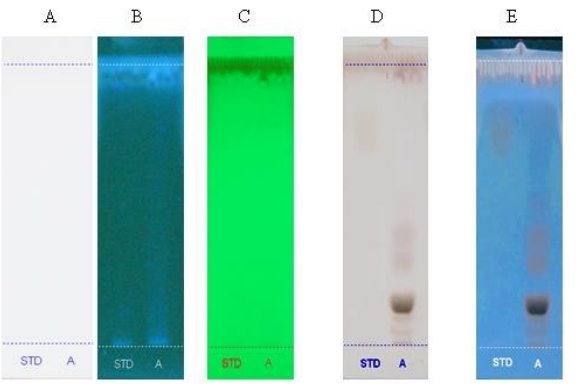
Peak densitogram display of Tannins



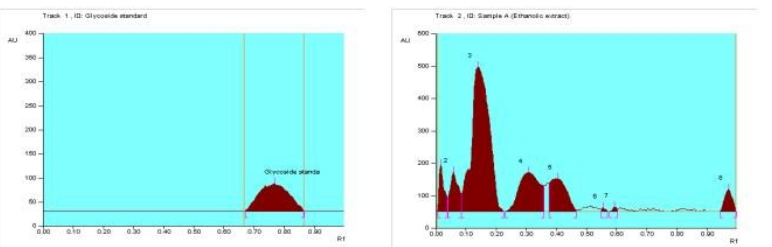
Gallic acid

Ethanolic extract of *M.arundinacea* .L

Fig: 5 Chromatograms of extract in HPTLC analysis for Glycosides. (A) Before derivatization under day light, (B) Under UV 366 nm (C) Under UV 254 nm, (D) After derivatization under day light, (E) Under UV 366 nm.



Peak densiogram display of Glycosides



Stevioside

Ethanolic extract of *M.arundinacea*.L

RESULTS

Total Phenol, Flavonoid and Flavonol Content of the extract

Total Phenol, Flavonoid and Flavonol Content of *M.arundinacea* ethanolic extract was shown in the table 1. The ethanolic extract of *M. arundinacea* exhibited a TPC of 390 ± 11 mg GAE/100g, TFC of 290 ± 7mg QE/100g and Total flavonol content of 150 ± 9 mg QE/100 g.

The methanol extract of tubers from *Cyperous scariosus* exhibited a TPC of (128.83 ± 0.32 mg GAE/g extract) and TFC of (118.93 ± 0.23 mg QEE/g extract[16]. Plant phenolic compounds are secondary metabolites with interesting properties for animal or human health. The beneficial effects of those molecules are related to their antioxidant activity[17], particularly their ability to scavenge free radicals, to donate hydrogen atoms or electrons, or to chelate metal cations[(18)]. Besides, phenolic compounds contribute largely to the colour and sensory characteristics of fruits and vegetables. In addition, they take part in growth and reproduction processes, and they provide protection against pathogens and predators[18]. At the cellular level, they participate in cell protection against the harmful action of reactive oxygen species (ROS), mainly oxygen free radicals, produced in response to environmental stresses such as salinity, drought, high light intensity or mineral nutrient deficiency[19], because of the imbalance between the production and scavenging of ROS in chloroplasts[20]. These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids[21]. Accordingly, plants containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the ROS. Flavonoids are polyphenols naturally present in nearly all plant materials[22]. Flavonoids are a class of compounds that remain of great scientific and therapeutic interest, and their antioxidant activity has attracted most attention. Their high antioxidant potential is attributable to their capacity to scavenge harmful ROS and other free radicals that originate from various cellular activities and lead to oxidative stress[23].

HPTLC Finger printing Profile for Flavonoids

Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities[17]. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts[24], activate antioxidant enzymes[25], reduce alpha-tocopherol radicals[26] and inhibit oxidases[27].

HPTLC profile of ethanol extract of *M. arundinacea* rhizomatous tubers was recorded in Table 2. Yellowish, Yellowish blue coloured fluorescent zone at UV 366 nm mode after derivatization in the chromatogram. Figure 1 confirms the presence of flavonoids. The extracts were run along with the standard flavonoid compound rutin. The extract which shows the presence of flavonoids in the chromatograph as well as in UV after derivatization. The Rf value of the extract was found to be 0.04, 0.09, 0.18, 0.29, 0.43,0.68,0.78,0.83,0.92 of peak 1,2,3,4,5,6,7,8,9 respectively, where as that of standard was 0.69. Among them peaks 2, 6, 9 were found as flavonoids.

HPTLC Finger printing Profile for Phenolics

Phenolic compounds are effective hydrogen donors, and this makes them good antioxidants[28]. Phenolic compounds are constituents of both edible and nonedible parts of plants. Many have antioxidant activity, which delays the oxidation of various ‘‘important for life” compounds by inhibiting the initiation or propagation of oxidising chain reactions[29]. Findings from epidemiological studies have confirmed a positive correlation between the consumption of phenolic-rich foods and a decrease in several chronic disease states[30].

HPTLC profile of ethanol extract of *M. arundinacea* rhizomatous tubers was recorded in Table 3. Blue coloured zone at daylight after derivatization in the chromatogram. Figure 2 confirms the presence of phenolics. The extracts were run along with the standard phenolic compound quercetin. The extract which shows the presence of phenolics in the chromatograph as well as in UV after derivatization. The Rf value of the extract was found to be 0.04, 0.10, 0.54, 0.60, 0.69,0.74,0.89,0.96 of peak 1,2,3,4,5,6,7,8 respectively, where as that of standard was 0.53. Among them peaks1, 2, 5, 6, 7 were found as phenolics.

HPTLC Finger printing Profile for Steroids:

Plants elaborate a very wide array of steroidal compounds, partly as endogenous hormones (in low amounts) or as allelopathic defence compounds (in much higher concentrations). The distributions of the various classes of defence steroids vary between plant families, between species within the family, within the species (ecotypes) and within the organs of the plants which contain them. Phytosteroids possess many interesting medicinal, pharmaceutical and agrochemical activities[31]. *M.arundinacea.* L is highly resistant to many pests and diseases, indicative of the presence of allelopathic chemicals, a type of steroid in the leaves and roots.

HPTLC profile of ethanol extract of *M. arundinacea* rhizomatous tubers was recorded in Table 4. Blue, blue-violet coloured zone at daylight after derivatization in the chromatogram. Figure 3 confirms the presence of Steroids. The extracts were run along with the standard steroid compound solasodine. The extract which shows the presence of steroids in the chromatograph as well as in UV after derivatization. The Rf value of the extract was found to be 0.03, 0.04, 0.17, 0.32, 0.36,0.44,0.48,0.58,0.98 of peak 1,2,3,4,5,6,7,8,9 respectively, where as that of standard was 0.45. Among them peaks 4, 6 were found as steroids.

HPTLC Finger printing Profile for Tannins:

Tannins are polyphenols sometimes called plant polyphenols[32]. The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former: binding to proteins, basic compounds, pigments, large-molecular compounds and metallic ions, and also anti-oxidant activities, etc. These features of tannins lead to qualitative and quantitative analytical differences between tannins and other polyphenols[33].

HPTLC profile of ethanol extract of *M. arundinacea* rhizomatous tubers was recorded in Table 5. Blue, bluish-brown coloured zone at daylight after derivatization in the chromatogram. Figure 4 confirms the presence of tannins. The extracts were run along with the standard tannin compound gallic acid. The extract which shows the presence of tannins in the chromatograph as well as in UV after derivatization. The Rf value of the extract was found to be 0.01, 0.04, 0.07, 0.12, 0.16, 0.24, 0.29, 0.49, 0.58, 0.62, 0.69, 0.88, 0.93 of peak 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 respectively, where as that of standard was 0.48. Among the peaks 8 & 12 were found as tannins.

HPTLC Finger printing Profile for Glycosides:

Glycosides are usually compounds of plant origin. They are made up of one or more sugars combined with an alcohol, a phenol, or a complex molecule such as a steroid nucleus. In

addition to the cardiac glycosides with their cyclopentanoperhydrophenanthrene nucleus in the genin moiety there are other glycosidal plant substances such as the anthraquinone derivatives senna, rhubarb, aloe, and cascara; saponins; cyanogenetic glycosides such as amygdalin from bitter almond; terpene and sterol glycosides; glycosidal dyes and pigments; and finally gums and tannins[34].

HPTLC profile of ethanol extract of *M. arundinacea* rhizomatous tubers was recorded in Table 6.Violet-brown coloured zone at daylight after derivatization in the chromatogram. Figure 5 confirms the presence of glycosides. The extracts were run along with the standard glycoside compound stevioside. The extract which shows the presence of glycosides in the chromatograph as well as in UV after derivatization. The Rf value of the extract was found to be 0.02, 0.06, 0.14, 0.31, 0.40, 0.56, 0.59, 0.97 of peak 1, 2, 3, 4, 5, 6, 7, 8, respectively, where as that of standard was 0.77. Among the peaks 1,2,3,4 & 5 were found as glycosides.

DISCUSSION

Natural products have been our single most successful source of medicines. Each plant is like factory capable of synthesizing unlimited number of highly complex and unusual chemical substances whose structures could otherwise escape the imagination forever[35]. There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, while several other drugs are simple synthetic modifications of the natural products[36]. The Indian herbal industry is growing in a tremendous rate. More number of herbal products is arrived in the market. The safety and efficacy of herbal products are dependent upon the standardization of these herbal drugs. The traditional approach towards standardization is insufficient for current herbal market and hence there is need for more advanced techniques for standardization. There are basically two techniques used for standardization these are chromatographic fingerprinting and DNA fingerprinting. The chromatographic fingerprinting is based on the chromatographic separation and identification of marker compound from other constituents[37]. High Performance Liquid Chromatography (HPTLC) serves as a significant tool for the initial characterization of the crude plant extract. In the present study, the major bioactive compounds viz., flavonoids, phenols, steroids, tannins and glycosides were characterized from the ethanolic extract of *M.arundinacea* .L. As the information on this plant, are very rare in the literature, further studies are warranted on these compounds to identify lead compounds.

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