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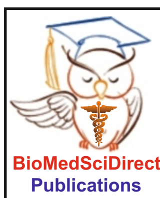

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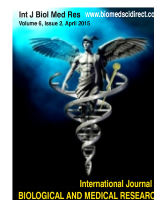

Original article

SCREENING AND EVALUATION OF PHYTOCHEMICALS FROM MARANTA ARUNDINACEA L.

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ABSTRACT

Objective: The present study was undertaken for preliminary screening of phytochemicals as well as quantification of phenol, flavonoid and tannin by using different solvent extracts of leaf and rhizome of Maranta arundinacea L. Methods: The leaf and rhizome were extracted by using different solvent systems like aqueous, methanol, ethanol and hexane. Phytochemical screening and its quantification were characterized by using standard protocols. Results: Both leaf and rhizome extracts showed the presence of phenols, flavonoids, tannins, alkaloids, steroids, terpenoids and glycosides. The ethanolic extracts showed the maximum content of phenolics, flavonoids and tannins followed by methanolic, aqueous and hexane extracts. The total phenolic content varies from 05.35±0.15 to 20.10±0.22 mg GAE/ g whereas, flavonoid is 01.01±0.05 to 04.36±0.14 mg RE/ g and tannin content is 08.08±0.19 to 50.12±0.24 mg GAE/ g of dried samples of leaf and rhizome with incorporation of various solvent extracts. Conclusion: From the results of the present study, it is concluded that the ethanolic extracts of rhizomes and leaves of M. arundinacea are the best sources of phytomedicine.

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Introduction

Nature has bestowed us by providing a rich botanical wealth and large number of diverse types of plants growing in different parts of the earth. India is the largest producer of medicinal herbs and appropriately called Botanical Garden of the world. In India almost 95 % of the prescriptions have been reported to be plant based traditional system of Unani, Aurveda, Homeopathy and Siddha. According to World health organization medicinal plants would be the greatest source to obtain an array of drugs. Thus such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness [1]. In other hand, medicinal plants are considered to be chemical factories as they contain multitude of chemical compounds such as alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactone and oils etc. [2]. The different physiological action of organic compounds is due to their different formulation of these bioactive substances [3]. Moreover, compounds are chemically and taxonomically extremely diverse with abscure function. They are widely used in human therapy, verterinary, agriculture, scientifically research and countless other areas [4]. Extraction and characterization of several phytocompounds from green factory (plants) have given birth to some high active profile drugs [5]. It is believed that crude extract from medicinal plants are more biologically active than isolated compound due to their synergistic effects [6]. However, phytochemical screening is essential to detect the various active compounds which could be used as the base of modern drugs for curing various ailments.

Maranta arundinacea which belongs to family Marantaceae, commonly known as arrowroot and is considered as an economically important plant. The plant is native to South America, West Indies, Mexico and Central America and for its starchy rhizome; it is widely cultivated in India, China, Sri Lanka and Philippines. The rhizome of this plant is able to decrease diarrhoea and relieve abdominal pain in those, suffering due to irritable bowel syndromes. It relieves acidity, indigestion and different types of chronic abdominal pain and irritation on gastrointestinal tract. The plant has antiseptic, anti-inflammatory, antidiarrhoeal and antioxidant activities. The mashed rhizomes are also used in septic wound, scorpion and black spider bites and draws out poison from injured areas [7, 8]. Therefore due to the above vital medicinal properties, the study was undertaken to screen and evaluate the phytochemical properties of aqueous, methanol, ethanol and hexane extracts of leaf and rhizome.

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Between 2007 and 2010, cross-resistance to DDT and pyrethroids was reported in An. gambiae s.l. through entomological surveys with strong geographical variations in a South-North transect [25-26]. Gene expression analysis with other molecular techniques revealed the over-expression of two P450 and one GST genes (CYP6M2 & CYP6P3; GSTe2) in addition to the kdr L1014F mutation potentially involved in DDT and pyrethroids resistance [6, 27]. Carbamates and organophosphates resistance due to the substitution of Glycine to Serine at position 119 in the oxyanion hole of the acetylcholinesterase enzyme [28] has also been detected in Côte d'Ivoire, Burkina-Faso, and Benin [29-31]. While the epidemiological consequences of pyrethroid resistance remain to be established, the rapid evolution of insecticide resistant alleles over the past decade is a real cause for concern for vector control management [32]. Significant advantages can be obtained for the insecticide resistance management by monitoring these markers of pyrethroid resistance. A better acknowledge of the genetic mechanisms involved in insecticide resistance and the occurrence of Plasmodium could be an important step to achieving success with insecticide resistance and malaria transmission management strategies. This study aimed to investigate the status of insecticide resistance genes and the level of Plasmodium infection in the An. gambiae population from Kpome, a rural area of tomatoes cultivation, through a combination of toxicological test and TaqMan assays.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

Rhizomes of M. arundinacea were collected from coastal fertile belt of Cuttack, Odisha during the month of February 2013 and cultivated in the garden of Post Graduate Department of Botany, Utkal University, Odisha, India. The plant specimen was identified by Dr. P.C. Panda, Principal Scientist of Regional Plant Resource Centre (RPRC), Bhubaneswar and a specimen was deposited in the herbarium of Post Graduate Department of Botany with the registration number BOTU10573.

PREPARATION OF VARIOUS SOLVENT EXTRACTS

The healthy leaves and rhizomes were collected and washed under running tap water and finally with distilled water. After washing, both the parts were cut in to small pieces and allowed to air dry and grinded in to a coarse power. The finely powdered samples were extracted with aqueous (distilled water), methanol, ethanol and hexane in a Soxhlet apparatus for 48 h. Extracts were concentrated with the help of rotary evaporator and stored at -4 °C for analysis of different phytochemicals.

SCREENING OF PHYTOCHEMICALS

The residues, obtained after evaporation were subjected to preliminary screening of important phytochemicals like phenols (ferric chloride test) [9], flavonoids (alkaline reagent test) [10], tannins (ferric chloride test) [11], alkaloids (Wagners test) [12], glycosides (Keller killani test) [13], terpenoids (Salkowshi test) [14] and Steroids (Salkowshi test) [15] using respective standard procedures.

Evaluation of Phytochemicals

DETERMINATION OF TOTAL PHENOL

Total phenol content in the extracts was determined using the method of Folin-Ciocalteu reagent method [16-19]. 1 ml of sample (1mg/ ml) was mixed with a solution of 1 ml of Folin-Ciocalteu reagent to which, 10 ml of 7 % sodium carbonate solution was added and mixed thoroughly. Then 13 ml of distilled water was added and mixed and the mixture was allowed to retain under dark for 90 min at 23 °C for colour development. The blue colour developed was read at 760 nm against blank and the total phenol content was determined from standard calibration curve which was made by preparing gallic acid solution. Total phenol content was expressed in mg of gallic acid equivalents (GAE) per g of dried sample.COLLECTION OF PLANT MATERIAL

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DETERMINATION OF TOTAL FLAVONOID

Total flavonoid content was estimated by using the method of Quettier et al., [20]. Where, 1 ml of 2 % AlCl3 solution was added to 1 ml of different solvent extract sample (1 mg/ ml) and incubated for 1 hr at room temperature to complete the reaction. Thereafter, absorbance was taken at 415 nm and for standard rutin was taken with increasing concentrations. The flavonoid content was measured and expressed in mg of rutin equivalents per g of dried sample.

DETERMINATION OF TOTAL TANNIN

The total tannin content was determined using the method of Folin-Ciocalteu method [21-24]. In a reaction mixture, 0.1 ml of the extracts was added to 7500µl of distilled water, 500 µL Folin-Ciocateu's phenol reagent and 1000 µl of 35 % sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was incubated for 30 minutes at room temperature and measured against a blank at 725 nm. The total tannin content was expressed in terms of mg of GAE/ g of dried sample.

STATISTICAL ANALYSIS

All results are the mean of three independent experimental replicates (n = 6) and data is reported as mean ± standard error.

Results and Discussion

PHYTOCHEMICAL SCREENING

The results of phytochemicals screening of aqueous, methanol, ethanol and hexane extracts from leaf and rhizome samples are presented in the table 1. The both leaves and rhizomes of M. arundinacea exhibited the presence of phenols, flavonoids, tannins, alkaloids, steroids, terpenoid and glycosides. However, strong responses are come towards phenols, flavonoids and tannins in leaf samples whereas, rhizomes are stronger in alkaloids, terpenoid and steroids including phenols, flavonoids and tannins. Moreover, the study revealed that among various extracts, the aqueous, methanol and ethanolic extract were the best for tested secondary metabolites. But different qualitative analysis indicates the presence of bioactive compounds which have pharmaceutical value. Secondary metabolites like alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc. are the natural compounds from plant sources, which are being utilized from ancient era to heal various alignments. In the present study, qualitative phytochemical screening evidenced that the leaf and rhizome possess valuable phyto-constituents. These secondary metabolites are reported to possess biological activities such as antimicrobial [25, 26], anti-inflammatory [27] and antioxidant [28] activities.

DETERMINATION OF PHENOL

Total phenolic contents of different solvent extracts of leaf and rhizome of M. arundinacea were determined and compared with standard gallic acid. In rhizome, ethanolic extract yielded highest quantity of phenol (20.10±0.22 mg GAE/ g) followed by methanol (13.27±0.14 mg GAE/ g), aqueous (11.07±0.19 mg GAE/ g) and hexane extract (6.25±0.16 mg GAE/ g) [Table 2]. Similarly in leaves, the highest amount of phenol also noticed in ethanol extract (13.28±0.20 mg GAE/ g) followed by methanol (7.16±0.17mg GAE/ g), aqueous (10.07±0.18 mg GAE/ g) and the lowest amount was found in hexane extracts (5.35±0.15 mg GAE/ g) [Table 3]. The searching of phenolic compounds is highly needful as they are one of the largest and most ubiquitous groups of plant metabolites which possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [29, 30].

DETERMINATION OF FLAVONOID

Total flavonoid contents were maximum in ethanolic extracts of rhizome (4.36±0.14 mg RE/ g) then the values are decreased in methanol (3.56±0.12 mg RE/ g), aqueous (2.32±0.10 mg RE/ g) and hexane extracts (1.81±0.06 mg RE/ g). But in leaves same trends are observed but it varies from 1.01± 0.05 mg RE/ g to 3.81±0.13 mg RE/ g (Tables 2 and 3). Flavonoids are the largest group of phytonutrients, with more than 6,000 types which are classified as flavonol, flavanone, isoflavone, flavone, flavan-3-ols and anthocyanin. Chemically flavonoids have the general structure of a 15-carbon skeleton, which consisting of two phenyl rings (A and B) and heterocyclic ring (C). It is also experimented that flavonoids are mainly responsible for antioxidative, coronary heart prevention, hepatoprotective, antiinflamatory and antiviral activities [31, 32].

DETERMINATION OF TANNIN

Total tannin contents of different solvent extracts of rhizomes and leaves are provided in Table 2 and 3. In rhizomes, total tannin content was maximum in ethanolic extracts (50.12±0.24 mg GAE/ g) and then in methanol (48.12±0.24mg GAE/ g) and the maximum lowest was seen in hexane extracts (10.07±0.19 mg GAE/ g). In

leaves, maximum amount (45.12±0.23 mg GAE/ g) was found in ethanolic extacts and the lowest amount was detected in hexane extracts (08.08±0.19 mg GAE/ g). Tannins commonly referred to as tannic acid which is a water-soluble polyphenols and present in many plant species. Tannins are large polyphenolic compound containing sufficient hydroxyls and other suitable groups like carboxyl to form strong complexes with various macromolecules. Typically, tannin molecules require at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders and are best compound to decrease the bacterial proliferation by blocking key enzymes at microbial metabolism [33].

Table 1: Phytochemical screening of leaves and rhizomes of M. arundinacea

Phytochemicals	Leaf				Rhizome			
	Aqueous	Methanol	Ethanol	Hexane	Aqueous	Methanol	Ethanol	Hexane
Phenols	+++	+++	+++	++	+++	+	+	−
Flavonoids	+++	++	++	+	+++	+++	+++	−
Tannins	+++	+++	+++	+	+++	−	−	−
Alkaloids	+	++	++	+	+	+++	+++	++
Glycosides	+	+	+	+	++	++	++	−
Steroids	+	+	+	−	++	++	+++	++
Terpenoids	+	−	+	+	+	+++	+++	+

+++: Strongest response; ++: Strong response; +: Positive response; −: Negative response

Table 2. Quantitative determination of total phenol, flavonoid and tannins in rhizome of M. arundinacea L.

Solvents	Total phenol (mg GAE/ g)	Total flavonoid (mg RE/ g)	Total tannin (mg GAE/ g)
Aqueous	11.07±0.19	2.32±0.10	16.10±0.21
Methanol	13.27±0.14	3.56±0.12	48.12±0.24
Ethanol	20.10±0.22	4.36±0.14	50.12±0.24
Hexane	6.25±0.16	1.81±0.06	10.07±0.19

GAE: Gallic acid equivalent; RE: Rutin equivalent
The data in the table represent mean ± SE of replicates (n = 6).

Table 3. Quantitative determination of total phenol, flavonoid and tannins in leaf of M. arundinacea L.

Solvents	Total phenol (mg GAE/ g)	Total flavonoid (mg RE/ g)	Total tannin (mg GAE/ g)
Aqueous	10.07±0.18	2.12±0.09	15.29±0.21
Methanol	7.16±0.17	2.33±0.11	39.54±0.23
Ethanol	13.28±0.20	3.81±0.13	45.12±0.23
Hexane	5.35±0.15	1.01±0.05	8.08±0.19

GAE: Gallic acid equivalent; RE: Rutin equivalent
The data in the table represent mean ± SE of replicates (n = 6).

Conclusion

The present study indicates that the both rhizomes and leaves of M. aundinacea have qualitative phytochemicals like phenol, flavonoid, tannin, alkaloids, steroids, glycosides and terpenoids during initial screening. Among four extracts (aqueous, methanol, ethanol and hexane), ethanolic extracts shows best results towards phenolic, flavonoid and tannin content. These different types of phytochemicals have a great importance in the field of drug research. This study thus indicates that the extracts obtained from leaves and rhizomes of M. arundinacea can be used for development of clinically important natural drugs. The present study also provides evidence that solvent extracts contains medicinally important bioactive compounds which justifies the use of plant species as traditional

medicine for treatment of various diseases. Further purification, identification and characterization of these bioactive compounds are essential to find out the individual compounds for treatment of specific diseases.

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