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Research Article

QUALITATIVE AND QUANTITATIVE SCREENING OF PHYTOCHEMICALS OF *MELIOSOMMA PINNATA* (DERMI), A FOREST BASED VEGETABLE PLANT TRADITIONALLY USED BY MISING COMMUNITY OF ASSAM, INDIA

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ABSTRACT

Aim: current study was carried out for the qualitative and quantitative screening of phytochemicals in the leaves of *Meliosomma pinnata* (Dermi).

Materials and methods: Alkaloids were extracted from the leaves of *Meliosomma pinnata*, with the help of soxhlet apparatus, using methanol, ethanol, petroleum ether, ethyl acetate, benzene and chloroform as solvents.

Results: The study reveals the presence of protein carbohydrate, amino acid, phenolic, alkaloid, flavonoid, saponins and tannin content of 8.85±0.005, 76.87± 10.65, 11.87± 0.4,27.53±0.07, 11.0±0.67, 3.78±0.015, 5.98±0.12 and 7.18±0.016 mg/g respectively and vitamin C (ascorbic acid) concentration was found to be 210.03± 0.008 mg/g.

Conclusion: The significance of the study is the successful qualitative and quantitative screening of phytochemical present in the plant which supports the traditional knowledge of the Mising community of Assam on the use of the plant as a food additive for better digestion of pork meat.

Keywords: *Meliosomma pinnata*, Traditional knowledge, Vitamin C (ascorbic acid), Flavonoids, Phenolics.

INTRODUCTION

Plants have potent biochemical factors and phytochemicals which have been used by tribal communities from the very beginning. About 60% of the total global population remains dependent on traditional medicines for their healthcare system [1]. In India thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [2]. North-East India is regarded as a hot spot of biodiversity and hence different types of herbal and medicinal plants are available there. These medicinal plants have been used traditionally by its local people for a long time to cure diseases. A knowledge of the chemical constituent is desirable for the discovery of therapeutic agents as well as discovering new source of economic materials, such as herbals, tannins, oil, gums and precursors for the synthesis of complex substances. Medicinal plants will continuously provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto-medicine for the treatment of diseases [3]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [5]. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds etc. [6]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [7]. According to many report, phytochemical constituents are found to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. The present work was concerned with qualitative and quantitative estimation of phytochemicals present in the leaf extract of *Meliosomma pinnata,* (Dermi). *Meliosomma pinnata,* (Dermi) *a* forest based vegetable plant of Assam. This plant is used as a food plant by the Missing tribe of Assam. They use the leaves of this plant along with pork meat. This plant is available in the plain districts of The Borak and Brahmaputra valley of Assam. It is also known as a Banposola or Mamoi or Hengunia in Assamese and in Mising language it is called as Dermi. It is a medium sized plant, height ranges from 30-50m. The tender leaves of this plant are also used as fish spices. Mising people also used its leaves in their traditional functions. Till date, no report published on the screening of important phytochemicals from *Meliosomma pinnata* (Dermi).

MATERIAL AND METHODS

Chemicals and reagents

All the chemicals used in the study were procured from Merck India Pvt. Ltd.

Collection of leaf samples

The leaves of *Meliosomma pinnata* were collected from different areas of Dibrugarh town of Assam (India). Leaves were washed, air dried and homogenized in to fine powder and stored in air tight containers. Fresh leaves were kept in refrigerator for analysis primary metabolites.

Preparation of extracts

Ethanol, methanol, chloroform, ethyl acetate, Petroleum ether and benzene extracts of leaves of *Meliosomma pinnata* were prepared in 0.5 g/mL. The solvents of organic extracts were dried at 60^{o}C protected from light. The residue was weighted and dissolved in a known volume of dimethyl sulphoxide (DMSO). DMSO was maintained at a minimum concentration to avoid DMSO- induced events. These extracts were used for the detection of phytochemical analysis.

Screening procedure

The extract was tested for the presence of bioactive compounds by using standard methods [8, 9].

Test for primary metabolites

The presence of protein in the leaf extracts of the plant was determined by using Millon's test and Ninhydrin test. The presence of carbohydrate was determined by using Molisch's test, Fehling's test and Iodine test.

Test for secondary metabolites

Test for flavonoids by Shinoda test

Leaf extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent test

Leaf extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for glycosides

Liebermann's test

Leaf extract was mixed with each of 2ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice and then concentrated H_2SO_4 was added carefully. A color change from violet to blue to green indicates the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test

Leaf extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test

Leaf extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for saponins

The extract was diluted with 20 ml of distilled water and it was agited in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for steroids

1mL of extracts was dissolved in 10 ml of chloroform and equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer showed yellow with green fluorescence. This indicates the presence of steroids.

Test for phenols and tannins

1mL of extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins

Test for Alkaloid

10 gm of powdered dried sample is treated with petroleum ether and filtered. The residue is taken and is treated with chloroform and filtered, the obtained residue is taken and is treated with methanol and filtered. Now the filtrate is evaporated and pH is maintained at 2 and the volume is made up to 50 ml with distilled water. Then the filtrate is taken to test with Wagner`s and Mayer's alkaloid reagent.

Quantitative estimation of primary and secondary metabolites

Carbohydrate, protein and amino acid was quantified by Anthrone [10], Lowery's method [11] and Ninhydrin method [10] respectively.

Vitamin C (ascorbic acid) was quantified taking 1.5 gm of fresh leaves in 4% oxalic acid. The sample is centrifuged for 5 minutes at 3000rpm. The supernatant is pooled out and make up 20 ml by 4% oxalic acid solution. Then, from this solution 1.5 ml of aliquot is taken and 0.5 ml of distilled water was added. After then 0.2 ml of 1N Folin is added to above solution and then optical density is taken at 760nm by using spectrophotometer [12].

Quantity of tannins is determined by using spectrophotmetric method. 0.5 gm of plant sample was weighed and taken in a 50 ml plastic bottle and 50 ml of distilled water was added and stirred for 1 hour. The sample is filtered and made the volume upto 50 ml by adding distilled water. 5 ml of filtered sample is then pipette out into a fresh test tube and mixed with 20 ml of 0.1 ml $FeCl_3$ in 0.1 ml HCl and 0.008M $K_4Fe(CN)_6$ H_2O. The absorbance is measured by spectrophotometer at 395 nm wavelength within 10 minutes [13] against a suitable blank.

Saponin was quantified by grounding 20 gm of the plant sample in 100 mL of 20% C_2H_5OH and heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture is then filtered and residue re-extracted with another 20 ml of 20% C_2H_5OH. Then the extract was reduced to 40 ml over a water bath at about 90°C. The concentrated is then transferred into a 250 ml separation funnel and 20 ml of $CH_3(CH_2)O$ is added to the extract and shaken vigorously. The aqueous layer was recovered while the $CH_3(CH_2)O$ layer was discarded and the purification process is repeated twice with 60 ml of the N-C_4H_9OH and 10 ml of 5% NaCl. The remaining solution is heated in a water bath and after evaporation the sample dried in the oven to a constant weight [13].

Estimation of total alkaloid

20 ml of finely powdered material was exhausted by alcohol in soxhlet apparatus and distilled off. Then latex was washed warm water and then diluted 0.1% H_2SO_4 and it was filtered and again washed with ether to remove the non alkaloid impurities and then ammonia solution was added. Alkaloid extracted by shocking several times with chloroform and then it was washed with little water and anhydrous sodium sulphate. The solvent was distilled off and the residue dried at 110°C and reweighted [13].

Estimation of total phenolic content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu reagent method with some modifications. 2.5 mL of 10% Folin- Ciocalteu reagent and 2ml of 2% solution of Na_2CO_3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound) [13].

Estimation of total flavonoid content

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content. 1ml of sample plant extract was mixed with 3ml of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as Quercetin equivalent (mg/g of extracted compound [14].

RESULTS

The phytochemical screening of the organic extracts of the leave of the plant *Meliosomma pinnata* shows the presence of primary metabolites and secondary metabolites. The leaf extracts contain protein, carbohydrate, amino acid, alkaloid, flavonoid, glycosides, saponins, steroid and tannin.

Table 1 shows the presence of phytochemical constituents in the leaf extract of plant that are prepared in different organic solvent. Carbohydrate, protein and amino acid are present in all solvents.

Carbohydrate, protein and amino acid were detected in methanol, ethanol, chloroform, ethyl acetate, petroleum ether and benzene extracts. Vitamin C (ascorbic acid) was found in the alcoholic and chloroform extracts. Whereas in case of secondary metabolites, flavonoid, steroid and glycosides are found in the alcoholic and chloroform extracts and tannin and saponins is found in only alcoholic solvents.

Table 2 shows the amount of phytochemical present in mg/gm and percentage of amount of phytochemicals present in the extracts of the plant.

Table 1: Qualitative screening of phytochemicals in different organic solvents

Phytochemicals	Methanol	Ethanol	Chloroform	Ethyl acetate	Petroleum ether	Benzene
Carbohydrate	+	+	+	+	+	+
Protein	+	+	+	+	+	+
Amino acid	+	+	+	+	+	+
Vitamin C (ascorbic acid)	+	+	+	_	_	_
Phenol	+	+	+	+	_	_
Alkaloid	+	+	+	_	_	+
Flavonoid	+	+	+	+	_	_
Glycosides	+	+	+	_	_	_
Saponins	+	+	+	_	_	_
Tannin	+	+	+	_	_	_
steroid	+	+	+	_	_	_

Table 2: Quantitative estimation of phytochemicals present in the leaf extract

Phytochemical constituent	Amount present mg/gm	Percentage of amount present
Protein	8.85± 0.005	7%
Phenol	76.87± 10.65	9.12%
Amino Acid	11.87± 0.4	5.6%
Vitamin C	210.12± 0.008	18.53%
Alkaloid	11.09± 0.67	13%
phenol	27.53± 0.07	8%
Flavonoid	3.78± 0.015	11%
Tannin	7.18±0.016	5%

DISCUSSION

Phytochemical analysis conducted on the plant extract revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [8]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, and alkaloids and a high amount of Vitamin C or ascorbic acid. Vitamin C or ascorbic promotes healthy teeth and gums, helps absorption of iron, aids in maintenance of normal connective tissue, promotes wound healing, and helps boost the immune system apart from the fat neutralizing capacity. Lacking of Vitamin C in body may cause scurvy disease. Vitamin C also affect on skin and skin aging. Vitamin C is also helpful against infection. The phenolic compounds are the largest groups of plant constituent [15]. They 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 [16]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [17]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [18,19]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are important constituent in regulating control of growth in some plant and their adversely affect on insect feeding [20]. The ability of flavonoids is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [21]. They also are effective antioxidant and show strong anticancer activities [22]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [23]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [24]. Steroids have been reported to have antibacterial properties [25] and they are very important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [26]. Several workers have reported the analgesic [27] antispasmodic and antibacterial [28, 29] properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [30]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION

The results revealed the presence of medicinally important constituents in the plant considered in the current study. The study reveals the presence of protein carbohydrate, amino acid, phenolic, alkaloid, flavonoid, saponins and tannin content of 8.85±0.005, 76.87± 10.65, 11.87± 0.4,27.53±0.07, 11.0±0.67, 3.78±0.015, 5.98±0.12 and 7.18±0.016 mg/g respectively and vitamin C (ascorbic acid) concentration was found to be 210.03± 0.008 mg/g. These findings scientifically confirms the traditional knowledge of the Mising tribe of Assam on the use of this plant as a food additive. Though vitamin C is an antioxidant, it affects skin aging and teeth and gum heath, this plant may be useful to treat skin and teeth and gum related problems. Therefore, extracts from this plant could be seen as a good source for useful drugs. A high amount of vitamin C found in the leave extract of this plant, which indicates that it has capacity to neutralize fat and hence can be useful to control body weight and other obesity related problems. Even though this plant has been used traditionally but still proper scientific data is not found. So it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of this plants. Also additional work is encouraged to elucidate the possible mechanism of action of this extracts.

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