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QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ESTIMATION OF TOTAL PHENOLS AND FLAVONOIDS IN LEAF EXTRACT OF *SARCOCHLAMYS PULCHERRIMA*

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ABSTRACT
The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritive components are called phytochemicals. The qualitative analysis as well as quantification of phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research.*Sarcochlamyspulcherrima*belongs to family Urticaceae, leaves which has long been used by some tribal people of Assam as food and to treat several diseases. Young shoots, leaves and fruits are eaten as vegetable especially by theMishing tribe with pork; based on the strong believe that it kills the tap worms.Leaves are used for diarrhea and dysentery, as carminative and digestive and there is no scientific evidence for above said activities. The present study was carried out to test the presence of various phytochemicals in the leaf extract of the plant and also to estimate the total phenol and flavanoids in the leaf extract.Phytochemical analysis of the leaf extract of *Sarcochlamyspulcherrima* revealed the presence most of the biochemicals tested for such as carbohydrate**,**protein, alkaloid, tannin, flavanoid, Steroids, terpenoids**,**phenol, saponin and glycoside. The total phenolic content of the methanolic leaf extract is found to be 29.42 $\pm$ 0.006 mg/g and the total flavonoid contents of the leaf extract is found to be 1.091$\pm$ 0.008 mg/g. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

KEY WORDS:*Sarcochlamyspulcherrima*, Phytochemical analysis,Total Phenols and Flavonoids, Dibrugarh

INTRODUCTION

Medicinal plants are being used by human since immemorial time. The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These are non-nutritive chemicals that have protective or preventive power against diseases. The phytochemicals are grouped into two main categories[1], primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc. (Krishnaiah*et al.,* 2009 and Edeoga*et al.,* 2005) .The qualitative analysis as well as quantification of phytochemical constituents of a medicinal plant is considered to be a vital step in any kind of medicinal plant research. *Sarcochlamyspulcherrima*belongs to family Urticaceae, leaves which has long been used by some tribal people of Assam as food and to treat several diseases. Young shoots, leaves and fruits are eaten as vegetables especially by theMishing tribe with pork based on the strong believe that it kills the tap worms.Leaves are used for diarrhea and dysentery, they are carminative and digestive and there is no scientific evidence for above said activities. The present study was carried out to test the presence of various phytochemicals in the leaf extract of the plant and also to estimate the total phenol and flavanoids in the leaf extract.

MATERIALS &METHODS

1. Collection of Plant Material

Fresh leaves of *Sarcochlamyspulcherrima*were collected from different localities of Dibrugarh, Assam and washed properly for removal of dust particles. The leaves were shade dried and crushed using blender. The dry powder thus obtained was stored in air tight container.

2. Preparation of Extract

Crude leaf extract was prepared by using Soxhlet apparatus. About 30g of finely powdered leaves were filled in a thimble and were extracted using different solvents (250 ml) such as Methanol, Ethanol and Petroleum ether for about 48 hours. The extracts were concentrated by heating on a hot plate at about 30º-40ºC and preserved at 4^0 C until use. The aqueous extract was prepared by boiling 10g of dried leaf powder in 300 ml of water for about 30 minutes over a hot pan at 30-40^0 C. The extract was filtered, concentrated and then stored at 4^0 C for further use.

3. Phytochemical Tests

Phytochemical analysis was done following standard methods(Sadasivam and Manickam 1996, Tyler 1994 and Harborne 1973).

a) Test for Carbohydrate

Molisch's Test: To 2ml of extract, 3-4 drops of Molisch's reagent was added and mixed properly. To this concentrated Sulphuric acid was added by the walls of the

test tube. Appearance of a purple or blue ring in between the two layers indicates the presence of carbohydrate.

b)Test for Protein

Biuret Test: 2ml of biuret reagent (mixture of 2ml of 10% NaOH and 2-3 drops of 0.5% $CuSO_{4)}$ was added to the crude extract and heated. Appearance of purple/blue colour confirms the presence of proteins.

***Ninhydrin Test*:** 2 ml of extract was treated with 0.2 % Ninhydrin and heated for 5-10 minutes. Blue colour indicates the presence of proteins.

c) Test for Alkaloid

Mayer's Test:The crude extracts were evaporated to dryness and residues were heated with 2% Hydrochloric acid on a boiling water bath. The extract were cooled, filtered and treated with the Mayer's reagent. Presence of yellow precipitation or turbidity shows the presence of alkaloids.

d)Test for Phenol

***Ferric chloride test*:**To 2ml of plant extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenol.

e) Test for Saponin

Foam Test:2 ml of extract was taken in a test tube and 10 ml of distilled water was added and shaken vigorously. Formation of foams confirms the presence of saponin.

f) Test for Tannins

***Gelatin Test*:** Crude plant extracts were treated with 5 ml of 1% gelatin solution containing NaCl and observed for the occurrence of white precipitate.

Ferric chloride Test:2 ml of 5 % $FeCl_3$ solution was added to 2 ml of plant extract. Appearance of dark blue of greenish black colour indicated the presence of tannins.

g)Test for Flavanoid

4 ml of extract was taken and about 2ml of50% methanol was added. The solution was warmed and metal magnesium was added. This was followed by addition of 5 to 6 drops of concentrated hydrochloric acid. Red coloration confirms the presence of flavanoids.

h)Test for Glycosides

Libermann's Test:To the crude extract, 2ml of chloroform and 2 ml of acetic acid was added. The solution was ice cooled followed by addition of conc. H_2SO_4. Colour change from blue to green indicates the presence of glycosides.

Salkowski's Test:Crude extract was dissolved in 2 ml of chloroform. To this conc. H_2SO_4 was added and the mixture was shaken. Formation of reddish brown colour indicates the presence of glycosides.

Keller-kilani Test:To the crude extract was added 2ml of acetic acid and few drops of 2 % $FeCl_3$ solution. The entire mixture was then poured in a test tube containing 2 ml of conc. H_2SO_4. A brown ring at the junction indicates the presence of glycoside.

i) Test for Steroids

Sulphuric acid Test: To the plant extracts 2 ml of chloroform was added. 2 ml of conc. H_2SO_4 was added by the sides of the test tubeand observed for red colour at the lower chloroform layer.

j) Test for Terpenoids

Sulphuric acid Test:Crude plant extract was dissolved in 3ml of chloroform. This was than evaporated to dryness and 2ml of conc. H_2SO_4 was added and heated for about 3minutes. A grayish colour indicated the presence of terpenoids.

4. Estimation of Total Phenol

Phenol content in the plant extract was determined by Folin-Ciocalteu reagent method with slight modificationsAdedapo*et al.,* 2009; Koncic*et al.,* 2001; McDonald et al., 2001 and Nabavi*et al.* 2008). One gram of the sample was extracted with 10 ml of 80% methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and evaporated to dryness. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$.

A standard curve was prepared using gallic acid. Several dilutions of gallic acid in 80% methanol were prepared viz. 20, 40, 60, 80, 100µg/ml(Lin. and Tang, 2005). One ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water. After this 2.5 ml Folin-Ciocalteu's reagent was added. This was followed by the addition of 2.5 ml of 7.5 % $NaHCO_3$ in each test tube. The resulting mixture was left to stand for 30 minutes at roomtemperature. Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer against blank.

Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Total phenol content = GAE x V x D /m, where GAE is the gallic acid equivalence (mg/mL); V is the volume extract (mL), D is dilution factor and m is the weight (g) of the pure plant extract.

5. Estimation of Total Flavonoid

Flavonoid content was determined by spectrophotometric method (Quettier et al., 2000). To 1 ml of methanol solution of the extract (concentration of 1 mg/ml) was added 1 ml of 2% $AlCl_3$ solution (prepared in methanol). The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at 415 nm.

The same procedure was repeated for the standard solution of Quercetin of different concentration and the standard curve was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in termsquercetin equivalent (mg ofquercetin /g of extract).

Flavonoids content = QE×V×D/W, where QE - quercetin equivalent (µg/ml), V - total volume of sample (ml), D - dilution factor, W - sample weight (g).

RESULTS & DISCUSSION

Phytochemical analysis of the leaf extract of *Sarcochlamyspulcherrima* revealed the presence most of the biochemicals tested for (Table 1).

TABLE I: Phytochemical analysis of the leaf extract of *Sarcochlamyspulcherrima*

Sl No.	Phytocompound	Methanolic Extract	Ethanolic Extract	Petroleum Ether Extract	Aqueous Extract
1	Carbohydrate	+	+	-	+
2	Protein	+	+	-	+
3	Alkaloid	+	+	-	+
4	Phenol	+	+	-	-
5	Saponin	+	+	-	-
6	Tannin	+	+	+	+
7	Flavanoid	+	-	+	-
8	Glycoside	+	-	-	-
9	Steroids	+	+	-	-
10	Terpenoids	+	+	-	-

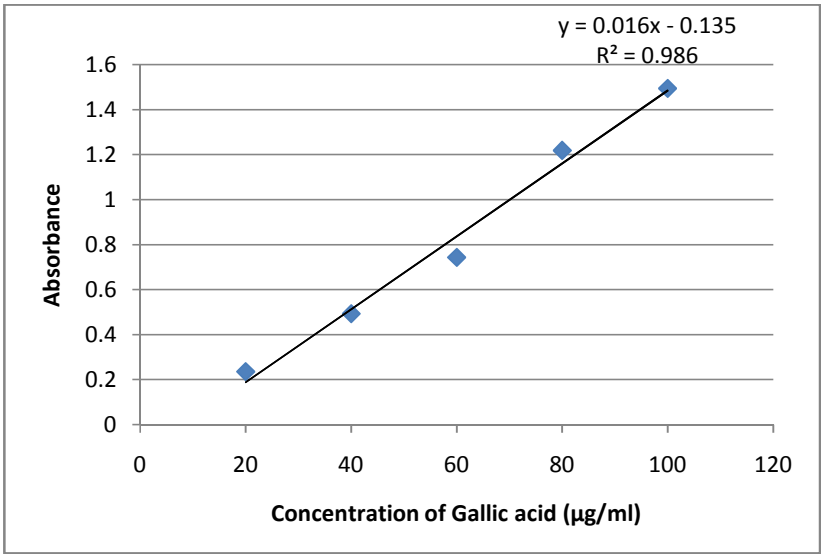

FIGURE 1: Standard curve of gallic acid.

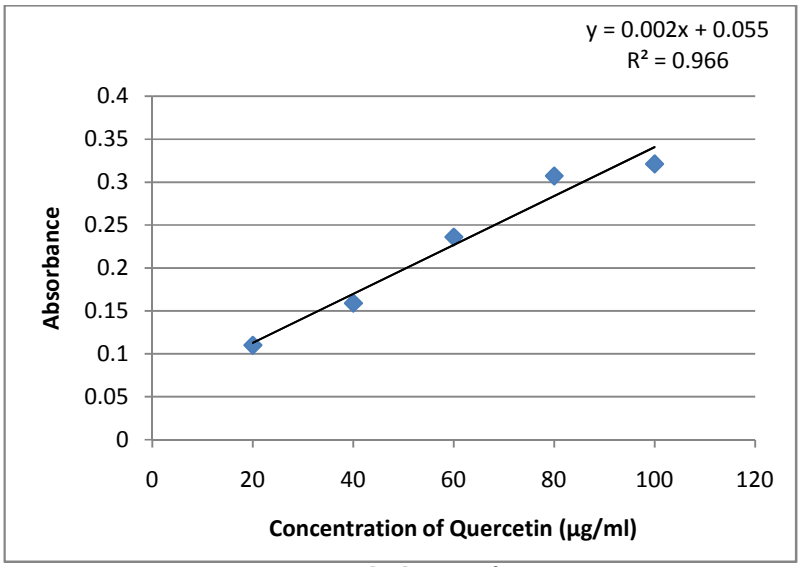

FIGURE 2: Standard curve of Quercetin

For the gallic acid, the curve of absorbance versus concentration was described by the equation y = 0.016x - 0.135 (R^2 = 0.986), where, y = absorbance and x = concentration. (Fig 1)

For flavonoids, the curve of absorbance versus concentration was described by the equation y= 0.002x + 0.055, R^2 = 0.966, where, y = absorbance and x = concentration. (Fig 2).

The values obtained for the concentration of total phenols and flavanoid are expressed in terms of gallic acid equivalent (mg of GA/g of extract) and quercetin equivalent (mg ofquercetin /g of extract) respectively. The

total phenolic content of the methanolic leaf extract is 29.42 ± 0.006 mg/g. The total flavonoid contents of the leaf extract is found to be 1.091± 0.008 mg/g.Medicinal plants are long being used as remedies for various diseases in human. The use of medicinal plants in the industrialized society has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Shrikumar&Ravi, 2007). The phytochemicals present in the plants endow them with medicinal properties. The antioxidant properties of many plants are mainly contributed by the phenolic compounds present in them (Brown and Rice-Evans, 1998 and Krings and Berger, 2001). Phenols and flavanoids are active antioxidant compounds showing many other medicinal properties. Most of the phytochemicals are known to have therapeutic properties such as insecticidals (Kambu*et al*., 1982), antibacterial, antifungal (Lemos et al., 1990) and anticonstipative (Ferdous*et al*., 1992) activities etc. The plants thus find their medicinal values due to the presence of respective phytochemical constituents. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

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