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Phytochemical and trace element analysis of *Hibiscus rosa sinensis* Linn and *Hibiscus syriacus* Linn flowers

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ABSTRACT

Herbal medicines have unique therapeutic properties and therefore, used in rural areas to cure different diseases. The crude phytochemicals (total phenols, flavonoids, tannins, carbohydrates and protein), vitamins (thiamine, niacin, riboflavin and ascorbic acid) and trace elements (calcium, phosphorus and iron) were determined quantitatively from the flowers of *Hibiscus rosa sinensis* and *Hibiscus syriacus*. The secondary plant products such as phenols, tannins and flavonoids were found maximum in *Hibiscus syriacus* whereas the primary metabolite namely carbohydrates and protein were higher in *Hibiscus rosa sinensis.* All the vitamins were higher in *Hibiscus rosa sinensis* than *Hibiscus syriacus*. The trace elements such as calcium and phosphorus were greater in *Hibiscus syriacus* whereas the content of iron was higher in *Hibiscus rosa sinensis.*

KEYWORDS

Hibiscus rosa sinensis;
Hibiscus syriacus;
Flowers;
Phytochemicals;
Trace elements.

INTRODUCTION

Hibiscus rosa sinensis (Family: Malvaceae) is a shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers most commonly known as the "shoe flower" is a native of Asia, specifically China, India and the Pacific islands[1]. Many chemical constituents such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acid[2] have been isolated from flower part of this plant. The flowers have been reported to possess anti-implantation[3], antispermatogenic[4], hypoglycaemic[5], antioxidant[6], anticonvulsant[7], antiestrogenic[8] and hypotensive activity[9]. The flowers are also good for healing ulcers and promoting growth and colour of hair[10].

Hibiscus syriacus (Family: Malvaceae) commonly known as rose of Sharon or shrub althea is an important ornamental shrub in horticulture and it is a national flower of Korea[11]. The flowers are used for anthelmintic, urethritis, headache, tooth ache, ear ache, asthma, boils, burns, cough, fever, analgesic, anti inflammatory, laxative, litholytic, menstrual irregularity and prostate disorders. The flowers of *Hibiscus syriacus* were found to contain flavonoids such as apigenidine, palargonidine, cyanidin, quercetin, crisantemin and anthocyanin. kaempferol, camphoral, citric acid, oxalic acid, and tartaric acid were also present. The juice of flower contains glycosides, triterpenoids, lipids, terpines, beta-sitosterol, teraxeril and cyanidic glycosides. Miscellaneous substances such as sucrose, fructose glucose were also present[12]. The objective of the present study is to

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evaluate the phytochemical constituents, vitamins and trace elements present in *Hibiscus rosa sinensis* Linn and *Hibiscus syriacus* Linn flowers grown in Tamilnadu.

MATERIALS AND METHODS

Plant material

Flowers of *Hibiscus rosa sinensis* and *Hibiscus syriacus* were collected from Kundrathur, Chennai in the month of July 2011. They were identified and authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre (PARC), Chennai. Herbarium specimens of those flowers (APCP/ 156/2011 and APCP/157/ 2011) were kept in Department of Pharmacognosy, Adhiparasakthi College of Pharmacy, Melmaruvathur for future reference. The collected material was shade dried, chopped and ground to coarse powder with the help of electrical grinder and stored in air tight packets until further analysis.

Estimation of phytochemical

(a) Determination of total phenols

To determine the total phenols, 5 g of each flower powder was weighed into a 250 ml titration flask and 100 ml n-hexane was added twice for 4 hours each; the filtrates were discarded for fat free sample preparation. Then, 50 ml diethyl ether was added twice, was heated for 15 minutes each, was cooled up to room temperature and was filtered into a separating funnel. About 50 ml of the 10% sodium hydroxide solution was added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 25 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% hydrochloric acid solution and 50 ml dichloro methane (DCM) twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed[13].

(b) Determination of flavonoids

To determine flavonoids, 5 g of each flower powder was weighed in a 250 ml titration flask, and 100 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 hours in an electric shaker. The entire solution was filtered through Whatman filter paper No. 42 (125 mm) and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed[14].

(c) Determination of tannins

500 mg powdered sample material was transferred to 250 ml conical flask containing 75 ml of distilled water. The contents in the flask were boiled for 30 minutes, centrifuged for 2000 rpm for 20 minutes. The supernatant was collected in 100 ml volumetric flask and made up to a known volume. The 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of distilled water. To this, 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml. It was shaken well and left for 30 minutes and the absorbance was read at 700 nm against a reagent blank[15].

(d) Determination of total carbohydrate

100 mg of dried sample powder was homogenized with 5 ml of 80 % ethanol and centrifuged at 2000 rpm for 10 minutes. Then it was re-extracted with the same solvent and centrifuged again. The supernatant were pooled. To the supernatant, equal volume of petroleum ether was added to remove the chlorophyll pigments using separation funnel. The lower layer was taken as sample. 1 ml of protein free carbohydrate solution was mixed with 4 ml of the anthrone reagent. The reaction mixture was heated for 5 minutes in a boiling water bath at 100ºC with the marble on the top of the test tube to prevent loss of water by evaporation. Suitable reagent blank was prepared. The colour intensity was measured at 620 nm[16].

(e) Determination of total protein content

100 mg of sample was homogenized with 5 ml of ice-cold phosphate buffer and centrifuged at 2000 rpm for 5 minutes. To the supernatant solution, equal volume of 10% ice-cold trichloro acetate was added and incubated for 10 minutes at 4°C for an hour. The precipitated protein was centrifuged and the pellet was dissolved in 1 ml of 0.1N sodium hydroxide. 0.5 ml of the protein solution was mixed with 5 ml of alkaline copper reagent. It was shaken well and allowed to stand at room temperature for 10 minutes. Then, 0.5 ml of folin–ciocalteau reagent was added and the volume was made up to a known quantity using distilled water. Blank was prepared without the sample extract. After 30 minutes

the absorbance of the solution was read at 660 nm[17].

Estimation of vitamins

(a) Thiamine

5 g of sample was homogenized in 50 ml ethanolic sodium hydroxide. Its 10 ml filtrate was added to 10 ml potassium dichromate and absorbance was recorded at 360 nm after development of colour.

(b) Niacin

5 g of sample was treated with 50 ml of 1 N sulphuric acid for 30 minutes and 0.5 ml of ammonia solution was added to it. It was filtered, to 10 ml of this filtrate 5 ml of potassium cyanide was added and then acidified with 5 ml 0.02N sulphuric acid. The absorbance of the resulting solution was recorded at 420 nm[18].

(c) Riboflavin

5 g sample was extracted with 100 ml ethanol for 1 hour. 10 ml of this filtered extract, 10 ml 5% potassium permanganate and 10 ml 30% hydrogen peroxide was added and allowed to stand on hot water bath for 30 minutes. To this 2 ml of 40% sodium sulphate was added. The volume was made up to 50 ml and absorbance was recorded at 510 nm[19].

(d) Ascorbic acid

Accurately, 1 g of each sample was weighed and taken in a 25 ml conical flask. Then 10 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and placed in the sample for 24 hours, to provide the required reaction time. After 24 hours, the samples were filtered through 0.45 µm filter paper. Then 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added. Subsequently, meta phosphoric acid was added separately with acetic acid (0.5 ml), sulphuric acid (5% v/v) solution (1 ml) and ammonium molybdate solution (2 ml) in each volumetric brown flask and the volume was made up to 25 ml with distilled water. The absorbance was measured at 760 nm on a UV/visible spectrophotometer[20].

(e) Elemental analysis:

The elemental analysis of calcium, phosphorus and iron were performed. The ground sample of *Hibiscus rosa sinensis* Linn and *Hibiscus syriacus* Linn flowers was sieved with a 2 mm sieve and 5 g of sample was subjected to dry ashing in a well cleaned porcelain crucible at 550^{0}C in muffle furnace. The resulting ash was dissolved in 10 ml HNO_3/HCl /H2O (1:2:3) and heated gently until brown fumes disappeared. To the remaining material of crucible 5 ml distilled water was added and heated until colourless solution was obtained. The mineral solution of crucible was transferred to 100 ml volumetric flask by filtering through Whatmann filter No. 42 and volume was made up with distilled water. This solution was used for elemental analysis by Atomic Absorption Spectrophotometer. The concentration each element was calculated as percentage of dry matter[21].

RESULTS AND DISCUSSION

Quantitative estimation of phytochemicals, vitamins and trace elements from flowers of *Hibiscus rosa sinensis* and *Hibiscus syriacus* were analyzed and summarized in TABLE 1, TABLE 2 and TABLE 3 respectively. The quantity of total phenols, tannins, flavonoids was found maximum in *Hibiscus syriacus* than *Hibiscus rosa sinensis* whereas carbohydrate and protein content was higher in *Hibiscus rosa sinensis* than in *Hibiscus syriacus*. The result of vitamins analysis obtained showed the higher concentration of thiamine, niacin, ascorbic acid and riboflavin in *Hibiscus rosa sinensis* than *Hibiscus syriacus*.

TABLE 1 : Quantitative phytochemical evaluation in flower powder of Hibiscus species

S.No.	Phytochemicals	*Hibiscus rosasinensis*	*Hibiscus syriacus*
1.	Flavonoids	0.171 mg/g	0.278 mg/g
2.	Total phenolic	0.092 mg/g	0.1 mg/g
3.	Tannins	0.073 mg/g	0.089 mg/g
4.	Carbohydrate	0.356 mg/g	0.227mg/g
5.	Protein	0.247 mg/g	0.224 mg/g

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. They have anti cancer, anti inflammatory activities and a large effect in lower intestinal tract and heart disease. Flavonoids as antioxidants from *Hibiscus rosa sinensis* and *Hibiscus syriacus* provide anti-inflammatory action. Phenols and phenolic compounds modify the prostaglandin pathways

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and due to this action, they prevent platelets from clumping and have the ability to block specific enzymes that cause inflammation; antioxidant, immune enhancers, anti clotting and hormone modulators.

TABLE 2 : Quantitative vitamin evaluation in flower powder of Hibiscus species

S.No.	Vitamins	*Hibiscus rosasinensis*	*Hibiscus syriacus*
1.	Thiamine	0.072 mg/g	0.069 mg/g
2.	Niacin	0.075 mg/g	0.060 mg/g
3.	Ascorbic acid	0.0339 mg/g	0.0193 mg/g
4.	Riboflavin	0.087 mg/g	0.082 mg/g

TABLE 3 : Quantitative screening of elements in flower powder of Hibiscus species

S.No.	Trace element	*Hibiscus rosa sinensis*	*Hibiscus syriacus*
1.	Calcium	0.0127%	0.0136%
2.	Phosphorus	0.4113%	0.4342%
3.	Iron	0.771%	0.7216%

Vitamins are important and their deficiencies cause adverse effects on the metabolism of the human body and even in a trace amount, they are very essential for the body metabolism. Sufficient amount of ascorbic acid in the diet is important for the body, in that its deficiency causes scurvy disease. Niacin is a constituent in two pyrimidine nucleotide coenzymes, NAD and NADP, and it is active in preventing pellagra disease in humans. Hence the flowers of *Hibiscus rosa sinensis* and *Hibiscus syriacus* will be source for water soluble vitamins supplement.

Minerals are essentials not only for health and performance, but more importantly for growth and development, as they play a central role in many biochemical and physiological processes. Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases. The trace elements such as calcium and phosphorus were greater in *Hibiscus syriacus* whereas the content of iron was higher in *Hibiscus rosa sinensis*.

Iron is an essential element for human beings and animals and is an essential component of haemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes, its deficiency causes anemia, weakness, depression, poor resistance to infection. Calcium and phosphorus play important role in building and maintaining strong bones and teeth also large part of human blood and extra cellular fluids. It is also necessary for normal functioning of cardiac muscles, blood coagulation, milk clotting and regulation of cell permeability. Its deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus.

CONCLUSION

The results from this study indicate that analyzed flowers of *Hibiscus rosa sinensis* and *Hibiscus syriacus* are a rich source of primary metabolites like protein and carbohydrate the secondary metabolites like flavonoids, tannins and phenolics also the trace elements such as iron, calcium and phosphorus could be of equal value to those which have been characterized as medicinal properties.

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