

Authentication and Photochemical Screening of *Hibiscus Rosa Sinensis*.

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ABSTRACT

Natural products from plants are rich sources used for treating a number of diseases. In this modern era, the knowledge and experience of the usage of herbs are being blend with advanced technology to develop a safe and elegant herbal product. Hence herbal medicines are the staple of medical treatment in many developing countries. The traditional knowledge with its holistic and systematic approach supported through experimental base can serve as an innovative and powerful discovery of natural 5 α -reductase inhibitor. Hibiscus Rosa sinensis is one of the miraculous medicinal herbs. Hibiscus rosasinensis is a perennial ornamental woody plant; belong to Malvaceae family available throughout India. Various parts of this plant like roots, leaves and flowers have been known to possess medicinal properties like anti inflammatory, antifungal, anti-microbial, oral contraceptive, laxative, aphrodisiac, menorrhagic etc. The present paper is an attempt to provide a detailed botanical description, classification, traditional uses, extraction procedure, phytochemical study, pharmacological studies, bioactivity and phytochemical screening of the plant.

Keywords: Antifungal, Anti-microbial, Bioactivity, *Hibiscus rosa sinensis* Linn, Traditional medicine.

Introduction

Plants are one of the most important sources of medicines. The medicinal plants are extensively utilized throughout the world in traditional system of medicines "Ayurveda" [1].

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kinds. India is rich source of medicinal plants and is called "Botanical Garden of the World" with enormous wealth of biodiversity. There are almost 45,000 plant species recorded in India so far [2] of which 7,500 species have been used for medicinal purposes [3]. *Hibiscus rosa-sinens* is a medicinal plant member belonging to the family Malvaceae. The plant *Hibiscus rosa-sinensis* is a perennial shrub with tap root. The leaves are 3.5-12 cm in length and 2-5.5 cm wide. Leaves are simple ovate or ovate-lanceolate. Leaves are entire at the base and coarsely toothed at the apex. Taste is mucilaginous. Flowers are pedicillate, Actinomorphic, pentamerous and complete. Corolla consists of 5 petals, red in colour and about 3 inches in diameter, generally available in many areas within its hardiness range. *Hibiscus rosa-sinensis* are native to tropical Asia. A native of south eastern Asia (China), the plant is commonly found throughout the tropics and as a house plant throughout the world. Most ornamental varieties are hybrids. The present wide range of cultivars is considered to be a complex of inter specific hybrids, between 8 or more different species originating from the African east coast and islands in the Indian and pacific ocean [4]. *Hibiscus rosa-sinensis* is used for the treatment of various diseases including alopecia. Traditionally, apart from this it is also used for anti-dandruff agent. Quercetin is a flavonoid found in *H. rosa-sinensis* flower. In flowers (per gm fresh tissues) included quercetin 7 mg. It can be used as an ingredient in supplements, beverages, or foods. Quercetin supplements have been used for prevention and treatment of cancer. It is a potent 5 α -reductase inhibitor. 5 α -reductase, also known as 3-oxo-5 α -steroid 4-dehydrogenases is involved in steroid metabolism. The prime role of 5 α -reductase being conversion of testosterone to dihydrotestosterone (DHT). Inhibition of DHT formation serves as a strategy for the treatment of alopecia and prostate hyperplasia. Moreover it is useful for hair growth promoting activity, by stimulating hair follicles and improves the blood flow of the hair. Positive results were obtained from study done on mouse for evaluation of hair growth promoting activity. The botanical taxonomy of this plant is given below-

Scientific classification

Kingdom: Plantae
 Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Malvales
 Family: Malvaceae
 Genus: Hibiscus
 Species: *Rosa sinensis*

Vernacular names

English: Chinese hibiscus
 Sanskrit: Japa
 Hindi: Jasum, Gulhar
 Bengali: Jaba
 Tamil: Sembaruthi
 Telugu: Mandara
 Malayalam: Bunga Raya

Fig1: *Hibiscus rosa-sinensis* flowerFig2: *Hibiscus rosa-sinensis* plant.

The aqueous-ethanolic extract of aerial parts of *Hibiscus rosa-sinensis* were reported for its use in constipation and diarrhea. The alcoholic extract of flowers of *Hibiscus rosasinensis* has been proved to possess anticonvulsant property. In traditional medicine, the leaves of the plant are used in fatigue and skin disease. Fresh root juice of the plant is given for gonorrhoea and powder root for menorrhagia. Flowers of the plant are used in epilepsy, leprosy, bronchial catarrh and diabetes. An infusion of the petal is widely used in ayurvedic medicine in India as a demulcent refrigerant drink in fever and decoction is given in bronchial catarrh. It has been reported that the plant flower possesses anti-spermatogenic and androgenic, anti-tumour and anticonvulsant activities. The use of flower to treat heart disorders has also been described has demonstrated the anti-diabetic activity of *Hibiscus rosa-sinensis* in diabetic rural population. Infusion of the petals is given as refrigerant and demulcent. Leaves are used as laxative while root is used in cough [5].

Several articles and ancient literature have shown that the flowers of this plant possess antifertility activity. [6] The aqueous ethanolic extract of aerial parts of this plant was reported for its use in constipation and diarrhea [7]. In traditional medicine, the leaves of the plant are used in fatigue and skin disease. Powdered root of the plant is given for menorrhagia and the fresh root juice for gonorrhoea [8]. Flowers of the plant are used in diabetes, epilepsy, bronchial catarrh and leprosy [9, 10]. An infusion of the petal is widely used in Ayurvedic medicine in India as a demulcent refrigerant drink in fever [10] and decoction is given in bronchial catarrh [11]. Previous studies showed that the plant possess anti-phlogistic, anti-diarrhetic and anti-complementary activity [12]. A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn. was found to exhibit significant hypoglycemic activity in glucose induced hyperglycemic rats [13].

Traditional uses:

Hibiscus rosa sinensis has been used in Siddha medicine, a traditional Tamil system from South India, for many centuries. Hibiscus extracts have been used for ages in Ayurveda to cure many ailments. The plants have the natural health benefit that can be used to cure diseases naturally. They are used to cure ailments such as cough cold, hair loss and hair greying also. The flowers and leaves of this plant play a major role in hair treatment. These are ground into a fine paste with water and this is generally used as a shampoo plus conditioner. The plant also helps to improve the overall texture and health of hair. Hibiscus is a sweet sour herb and is used in the preparation of herbal teas. It acts as an antioxidant and also helps in the reduction of cholesterol levels [14]. It has also been used in the traditional medicine for treating colds, loss of appetite, disorders of the respiratory tract. The plant is beneficial as a mild laxative, expectorant and diuretic. The hibiscus *Rosa sinensis* has been found to have emmenagogue effects which can stimulate menstruation and in some women, cause an abortion [15].

Extraction procedure of *Hibiscus rosa-sinensis*

Hibiscus rosa-sinensis flower was purchased from local vender. It was crushed into coarse powder by machine. 500 g of coarse dry power of *Hibiscus rosa-sinensis* (flower) was taken in a 2000 ml conical flask and added 1000 ml of methanol. It was kept for 72 hrs in air tight condition at 25 to 30 °C temperature.

After that, it was filtrated by normal filter paper. Filtrate was kept in a 1000 ml beaker. After filtration, the filtrate was concentrated by rotary evaporator at 40 to 45 °C temperature and other ambient condition. The percentage yield of extraction was 1.85 % w/w. The extract was stored in glass vials in air tight condition at room temperature with proper label.

Phytochemical constituents of *Hibiscus rosa-sinensis*

The edible portion of the flower (61.6 %) was reported to have the following nutrient composition (per 100 g): moisture 89.8 %, nitrogen 0.064 %, fat 0.36 %, crude fiber 1.56 %, calcium 4.04 mg, phosphorus 26.68 mg, iron 1.69 mg, thiamine 0.031 mg , riboflavin 0.048 mg, ascorbic acid 4.16 mg and niacin 0.61 mg . Petals of *Hibiscus rosa-sinensis* were reported to contain quercetin-3-di-*O*- β -D-glucoside; quercetin-3-7-di-*O*- β -D-glucoside; quercetin-3-*O*- β -D-sophorotrioside; and kaempferol and kaempferol-3-*O*- β -xylosylglucoside. The major anthocyanin contained in the red flowers of *H. rosa-sinensis* was cyanidin-3-sophoroside. Red-petalled varieties of *H. rosa-sinensis* were found to have more number of anthocyanin bands compared with that observed in yellow-yellow orange varieties. The varieties in the different coloured groups differed in the quantitative distribution of anthocyanins, leucoanthocyanins, flavonol and carotenoids. Flavonoid aglycones found in the flowers (per gm fresh tissues) included quercetin 7 mg and cyanidin 36 mg. The flowers were also reported to contain the following flavones: quercetin-3-diglucoside, quercetin-3, 7-diglucoside, cyanidin-3, 5-diglucoside and cyanidin-3-sophoroside-3-5 glucoside from deep yellow and white flowers and from ivory white flowers is kaempferol-3-xylosylglucoside

. Leaves and stems contain β -sitosterol, stigma sterol, taraxeryl acetate and three cyclopropane compounds and their derivatives. Fatty acids, fatty alcohols and hydrocarbons were identified from *Hibiscus rosa sinensis* leaves. Quercetin, β -sitosterol and linoleic acid can be selected as bioactive markers for quantification of *Hibiscus rosa sinensis* flower.

Table1: Phytochemical constituents reported on various part of plant

Sl. No.	Plant part	Constituent reported
1	Flowers	Thiamine, Riboflavin, Niacin and Ascorbic acid, Apigenidin, citric acid, fructose, glucose, oxalic acid, pelargonidin, quercetin.
2	Leaves	Alkaloids, glycosides, reducing sugars, fatty materials, resin and sterols, Fatty acids, fatty alcohol, and hydrocarbon, sterculic and malvalic acid.
3	Stem	Teraxeryl acetate, β -sitosterol and the cyclic acids sterculic and malvalic acids.
4	Root	Glycosides, tannins, phytosterols, fixed oils, fats, proteins, amino acids, flavonoids, Saponins, gums and mucilage.

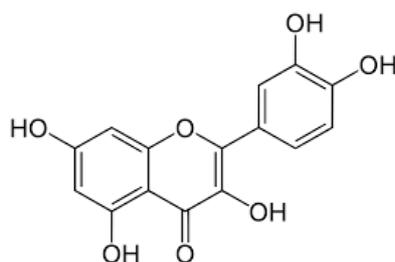


Fig3: Quercetin

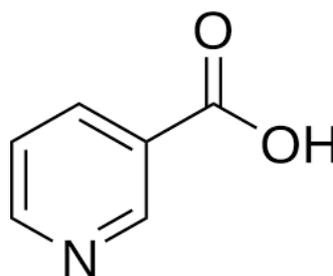


Fig4: Niacin

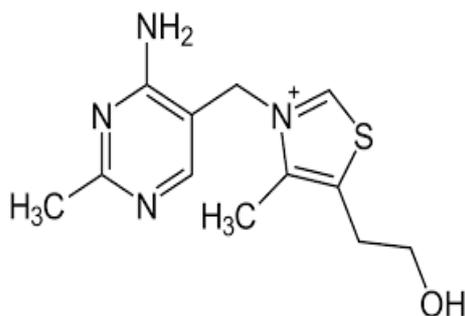


Fig5: Thiamine

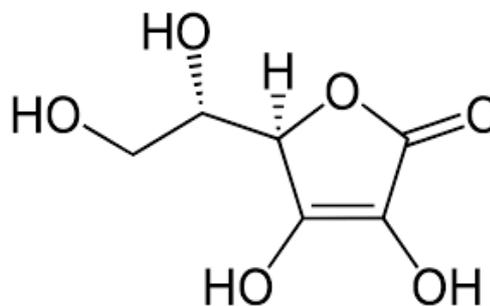


Fig6: Ascorbic acid

Pharmacological activities:

It is found that the leaves and flowers of *Hibiscus Rosa sinensis* have a wide range of pharmacological benefits. The aqueous-ethanolic extract of its aerial parts has been reported for its use in constipation and diarrhoea [16]. It is a blood purifier and also helps in treatment of cystitis, that is, inflammation of the bladder. It is a very good natural source of vitamin C and is used for curing syphilis and gonorrhoea also. Other varieties of hibiscus have also been found to be advantageous in the reduction of blood pressure, similar to captopril [17].

Effect on hair growth:

Petroleum ether extracts of the leaves and flowers of *Hibiscus Rosa-sinensis* have been used to check hair growth potential by both in vivo and in vitro methods. An in vivo method, a 1% extract, made with liquid paraffin, was applied topically over the shaved skin of albino rats and was kept under systematic review for a period of time. Length of hair and the different cyclic phases of hair follicles, like anagen and telogen phases were monitored at different intervals of time. In vitro method, the hair follicles from neonate albino rats were isolated and cultured in DMEM supplemented with 0.01 mg/ml petroleum ether extract of leaves and flowers. It was observed that the leaf extract exhibited higher potency than the flower extract on hair growth [18].

Effect on estrous cycle:

The roots of *Hibiscus Rosa sinensis* have been tested for their anti-implantation and also estrogenic properties. Ethanol extract was found to cause the loss of implantation that might be due to anti-zygotic, blastocytotoxic or anti implantation activity [19]. Oral administration of the ethanol extract of roots of *Hibiscus Rosa sinensis* in ovariectomized immature female rats increases the uterine weight and also stimulates uterine growth suggesting estrogenic activity. Hence administration of estrogen may have uterotrophic effects in immature female rats and mice [20, 21].

Effect on blood glucose level:

Streptozotocin induced diabetic rats have been monitored to determine their blood glucose and total lipid levels after orally administering the ethanol flower extract of *Hibiscus Rosa sinensis*. After a period of 7 and 21 days of oral administration of extract, along with glibenclamide, a hypoglycaemic effect has been observed. After 21 days, maximum diminution in blood glucose, that is 41-46% and insulin level of 14% has been noticed. The extract was also found to lower the total cholesterol and serum triglycerides by 22 and 30% respectively. The hypoglycaemic activity of this extract can be compared to that of glibenclamide, but is not mediated through insulin release [22].

Green synthesis of gold and silver nanoparticles:

The leaf extract of *Hibiscus Rosa sinensis* has been used in the biological synthesis of gold and silver nanoparticles by a simple, cost-effective method. The particles are stable for long time. Self-assembly of gold and silver nanoparticles can be attained by carrying out this synthesis at room temperature in an aqueous environment. The shape and size of gold nanoparticles can be modulated by altering the ratio of metal salt and extract in the reaction medium. For the synthesis of gold nanoparticles, 5ml of hibiscus extract and 30 ml aqueous solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (5×10^{-4} M) have been used. The colloids were found to be stable for two months. For the synthesis of silver nanoparticles, 20 mL hibiscus extract has been used with 25 ml aqueous solution of AgNO_3 (0.8×10^{-3} M) with stirring for 1 minute. To initialize the reduction of Ag ions, the pH of the solution was been adjusted to be 6.8 using NaOH. These colloids were found to be stable for four months. To ascertain the formation and stability of metal nano-particles in aqueous solution, UV- visible spectroscopy technique has been used [23].

Bioactivity of hibiscus components:

Hibiscus is one of the medicinal plants that generally find applications in treating diseases such as oxidative stress like hypertension and cancer. This is because they are made up of strong antioxidants like alkaloids and flavonoids or anthocyanins. Anthocyanins are useful in therapy against cardiovascular diseases and cancer [24] and age related conditions such as dementia or Alzheimer's disease [25]. Flavonoids have the ability to induce human protective enzyme systems. They have also been found to have protective effects against many infectious bacterial and viral diseases including degenerative diseases such as cardiovascular diseases, cancers, and other age-related disorders [26]. Flavonoids also have antibacterial, antiviral [27], anticancer and anti-inflammatory properties. Flowers of the plant contain ascorbic acid whose biological role is to act as a reducing agent by donating electrons in several enzymatic reactions and a few non-enzymatic ones. The one-and two-electron oxidized forms of vitamin C that is semi hydro ascorbic acid and

dehydro ascorbic acid, respectively, can be reduced in the body by glutathione and NADH- dependent enzymatic mechanisms [28]. The leaves and stem contain cyclopropane compounds that are inactive at the GABA and glycine receptors. They instead act as an NMDA- receptor antagonist [29]. It inhibits the AMPA receptor and nicotinic acetylcholine receptors, and activates certain K channels [30].

Phytochemical screening of *Hibiscus rosa-sinensis* extract

Phytochemical tests of the methanolic extract of *Hibiscus rosa-sinensis* flower were performed following the methods

Detection of Phytosterols

Liebermann-Burchard Test: - 10 mg of extract was dissolved in 1mL of chloroform. 1 ml of acetic anhydride was added followed by the addition of 2mL of concentrated sulphuric acid but no reddish violet colour was developed, indicating the absence of steroids.

Detection of Triterpenoids

Nollar's test: - In the test tube 2 ml of 0.01% anhydrous stannous chloride in thionyl chloride solution and test solution was added. Purple colour formed changed to deep red colour after few minutes indicates the presence of Triterpenoids.

Detection of Flavonoids

Shinoda test: - To the extract magnesium turnings and then conc. hydrochloric acid (HCl) was added & red colour was produced.

Detection of Alkaloids

Mayer's test: - 1.2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloid.

Dragendroff's test:-0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orange brown coloured precipitate suggested the presence of alkaloid. Wagner's test 2 ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicated the positive response for alkaloid.

Detection of Alkaloids

Biuret test: - 1 ml of 40% NaOH mixed with 2 drops of 1% copper sulphate was added to the extract, a violet colour indicated the presence of proteins.

Detection of Protein and Amino Acids

Ninhydrin test:-Extract solution was treated with ninhydrin (Tri-ketohydrindene hydrate) at the pH range of 4-8. Development of purple colour indicated the positive response for amino acids.

Detection of Glycosides

Legal test: - Extract was dissolved in pyridine; sodium nitroprusside solution was added to it and made alkaline. Pink red colour was produced.

Borntrager's test: - Few ml of dil. sulphuric acid added to the test solution. Boiled, filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink red colour was produced in organic layer.

Keller Killiani test: - Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulphuric acid. At the junction of liquid reddish brown colour was produced which gradually becomes blue.

Detection of Phenolic compounds and tannins

Ferric chloride test: - 5 ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

Potassium dichromate test: - 5 ml of the extract was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

Detection of Saponins

Foam test:-1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. Development of stable foam suggested the presence of saponins.

Potassium dichromate test: - 1 ml extract was treated with 1% lead acetate solution.

Formation of white precipitate indicated the presence of saponins.

Results

Phyto-chemical screening of the methanolic extract of *Hibiscus rosa-sinensis* flower showed presence of different type of phyto-constituents as depicted in table 2.

Table 2. Phytochemical screening of the methanolic extract of *Hibiscus rosa sinensis* flower

Test	Methanolic extract of <i>Hibiscus rosa sinensis</i> flower
Phytosterols	+
Triterpenoids	+
Flavonoids	+
Alkaloids	+
Protein and amino acids	+
Deoxy sugars	-
Reducing sugars	+
Glycosides	-
Phenolic compounds and Tannins	-
Saponins	-

("+" Indicates positive; "-" indicates negative)

Conclusion

Thus *Rosa sinensis* is a powerful medicinal plant that is proven to have many valuable properties. Due to its various applications, hibiscus and its oil have a wide scope for future research especially in the field of pharmacology and as cosmeceuticals. The medicinal plant *Hibiscus rosa sinensis* is having many pharmacological activity, therapeutic activity, various parts of this plant like flowers, leaves and roots have been known to possess medicinal properties like oral contraceptive, laxative, aphrodisiac, menorrhagic etc. Hence most work could be done on the above plant to reveal the unknown mysteries which would help the need of the present pharmaceutical world.

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