

**PHYTOCHEMICAL PROFILING OF ETHANOLIC FLOWER
EXTRACT OF *HIBISCUS ROSA-SINENSIS* AND EVALUATION OF ITS
ANTIOXIDANT POTENTIAL**

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ABSTRACT

The medicinal plant is the greatest high-class foundation of life redeemable drugs for the mainstream of the flora and fauna population. They bear to be a significant beneficial support for improving the illnesses of the humanoid. *Hibiscus rosa-sinensis* has belonged family Malvaceae. It grows in warm temperate, topical, and semi-tropical climates. *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. The 5-petaled flowers are 10 cm (4 in) in diameter, with prominent orange-tipped red anthers. *Hibiscus rosa-sinensis* extract from different part of the plant has significant protective effects.^[7,5,6,3,2]

Flowers of the plant are used in epilepsy, leprosy, bronchial catarrh and diabetes.^[2] They have been used to benefit the prevent hair loss, reduce gray hair, inflammation. It improves blood circulation, liver disorders, constipation. The aim of this study is to determine the phytochemical properties of *Hibiscus rosa-sinensis* the flower (Petals) using ethanol extracts and antioxidant activity collected from Islamabad, area. Phytochemical screening of ethanol extracts of the flower (Petals) was tested for the presence of phytochemical constitutes by standard procedures followed by Debela, 20021. Antioxidant screening of ethanol extracts of the flower was tested by Uddin N, 2011 method.^[8] Results showed that Tannins, Saponins, Alkaloids, Terpenoids, Sterol, Glycosides, Flavonoids presented in ethanol extracts of the flower (Petals). Ethanol extracts of the flower (Petals)

show antioxidant activity. In conclusion, the phytochemical examination of the therapeutic plants is critical and has business enthusiasm for both research Center and pharmaceuticals organizations for the assembling of the new medications for treatment of different infections. Additionally ponder are expected to research singular phytochemical compound of the blossom of *Hibiscus rosa-sinensis* and recognizable proof and auxiliary assurance of novel antioxidants fusions will prompt the advancement of therapeutics from these plants.

KEYWORDS: *Hibiscus rosa-sinensis*, antioxidant activity, phytochemical compound.

INTRODUCTION

The medicinal plants are the greatest high-class foundation of life redeemable drugs for the mainstream of the flora and fauna population. They bear to be a significant beneficial support for improving the illnesses of the humanoid. *Hibiscus rosa-sinensis* belongs to family Malvaceae.^[1] It is commonly known as Shoeblack plant or China rose. The term “*rosa-sinensis*” is a Latin word which means “rose of China”.^[2] It grows in warm temperate, topical, and semi-tropical climates. *Hibiscus rosa-sinensis* is a bushy, evergreen shrub or small tree growing 2.5-5 m (8–16 ft) tall and 1.5-3 m (5-10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. The flowers have five petals which are 10 cm (4 inch) in diameter, with prominent orange-tipped red anthers.^[3] *Hibiscus rosa-sinensis* extract from different part of the plant has significant protective effects.^[4-8] Flowers of the plant are used in epilepsy, leprosy, bronchial catarrh and diabetes.^[8] They have been used to benefit the prevent hair loss, reduce gray hair, inflammation. It improves blood circulation, liver disorders, and constipation.^[9]

Moreover, its leaves and flowers are renowned for their therapeutic usage. They possesses hair growth promoting and anti-greying properties. In India, the extract of various parts of *Hibiscus rosa-sinensis* are available in the market as the herbal products intended for hair growth.^[10] All these activities are related to the chemical component present in the plant *i.e.* phenolic and flavanoidal compounds are well-known for their antioxidant potential, fatty acid and fatty acid esters are renowned for antibacterial potential, aromatic compounds are famous for aroma and etc.^[11-13] Phytochemical screening is a procedure of evaluating a plant’s phytochemical constituents by means of standard established tests and delivers direct knowledge of a plant’s phytochemical constituents where instrumentation such as NMR spectroscopy is lacking. It’s a prerequisite for chromatographic separation of plant extracts

for known and unknown natural products constituents. The present study focused on the investigation of phytochemical screening and antioxidant potential present in the ethanolic extracts of *Hibiscus rosa-sinensis*.

MATERIAL AND METHODS

Plant Material

Fresh and disease resistant flowers of *Hibiscus rosa sinensis* were collected in the month of June from Islamabad, Pakistan. A voucher specimen of the flower was identified and authenticated by the taxonomist at the Department of Botany, University of Karachi. The collected flowers were dried in the shade and stored in an airtight container at room temperature (35 °C).

Preparation of extract

Dried flowers of *Hibiscus rosa sinensis* were soaked in ethanol for 48 hours at laboratory temperature (~25 °C), extract was filtered, concentrated under reduce pressure and obtained a thick brown residue (HRSE) which was further used for the phytochemical screening and evaluation of antioxidant potential.

Preparation of Reagent for Phytochemical Analysis

Lead acetate solution: 10% lead acetate solution was used for the detection of tannins.

Ferric Chloride solution: A 5% w/v solution of ferric chloride in 90% alcohol is used for the detection of tannins.

Wagner's reagent: For the detection of alkaloids wagner's reagent was used. To prepare this reagent, iodine (1.27 g) and potassium iodide (KI) (2 g) was dissolved in 5 mL of distilled water and the volume was made 100 mL.

Phytochemical Qualitative Analysis

The ethanolic flower extract were assessed for the existence of the phytochemical analysis by using the following standard methods.^[14-16]

Tests for Alkaloids

Wagner's Test

About 0.5 mL of extract was taken to the test tube in which 2-3 drops of wagner's reagent was added. A reddish-brown precipitate was appeared which indicated the presence of alkaloids in the extract.

Test for tannins***Lead sub-acetate test***

0.1 g of the ethanolic extract (HRSE) was weighed and placed in a test tube and 2-3 drops of 10% lead acetate solution were added. The presence of tannin was confirmed as creamy gelatinous precipitate formed.

Ferric Chloride Test

About 0.5 mL of extract was dissolved in 10 mL of distilled water, then filtered. Few drops of ferric chloride solution was added to the filtrate. Blue-black precipitate formed, indicated hydrolysable tannins.

Tests for Saponins***Foam test***

About 0.5 mL of extract was added to 2-3 mL of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponin.

Tests for Terpenoids and sterol***Salkowski test***

About 0.5 mL of extract was mixed with 2 mL of chloroform and concentrated H₂SO₄ (3 mL) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive result of the presence of terpenoids and sterols

Libermann-buchard test

About 1 mL of extract was taken to the test tube and 1 mL chloroform, 0.5 mL acetic anhydride and few drops of H₂SO₄ was added to the tube. The formation of dark green colour indicated the presence of terpenoids and sterols.

Tests for Flavanoids***Shinoda test***

About 0.5 g of extract was taken to the test tube dissolved in 2 mL of methanol by heat. Metallic magnesium and four to five drops of conc. HCl were added. Orange colour indicated the presence of flavanoic aglycones.

Sodium hydroxide (NaOH) test

About 1 mL of extract was taken to the test tube and few drops of aqueous NaOH was added a yellow colouration shows the presence of flavanoid.

Tests for Glycosides

Keller-killani test

About 2 mL of extract was taken to the test tube and glacial acetic acid, one drop of 5% FeCl₃ and conc. H₂SO₄ were added to the tube. Reddish brown color appeared at junction of the two liquid layers and upper layer appears bluish green, confirming the presence of glycosides.

Tests for Fats and oils

Stain test

A little amount of the extract was constrained in the middle of the two filter papers. No oil stained on the filter papers which showed that fat and oil was not presence in the extract.

Tests for Lignins

Labet test

About 2 mL of extract was taken to the test tube and gallic acid was added; no change in the solution was observed which specified that no lignins present in the extract.

Tests for Quinones

About 1 mL of extract was taken to the test tube and 1 mL of concentrated sulphuric acid was added. No formation of red color which indicated the absence of quinones.

Determination of (antioxidant activity)

The antioxidant activity of plant extracts were measured by scavenging of free radicals by following the standard procedure with little modifications.^[17] Radical which is used for screening of these extracts is 1, 1-diphenyl-2-picrylhydrazyl (DPPH) which serves as a stable radical and shows maximum absorbance at 520 nm. The DPPH radical was dissolved in methanol and its initial colour is purple but as the radical scavenges its colour and absorbance minimize. The scavenging of radical is due to active antioxidant constituent in samples. The change in absorbance was monitored by double beam spectrophotometer (BMS, UV-2800). 2 mL of methanolic DPPH solution was mixed with 1 mL of test samples. Reaction mixture was incubated in dark at room temperature for 30 mins and then change in absorbance was measured at 520 nm. The reference contains 1 mL of DMSO as the samples dissolved in DMSO. IC₅₀ value (at which 50% of radical scavenges) was determined by EZ-Fit Enzyme Kinetic Program.

RESULTS AND DISCUSSION

Many naturally-occurring compounds found in plants have been shown to possess antioxidant potential and could thus serve as a source of traditional drugs (Kim et al., 1995). Table 2 showed the antioxidant activity of ethanolic extract of flowers of *Hibiscus rosa sinensis*. The radical scavenging activity were compared with the standard Gallic acid and *N*-acetyl cysteine. The antioxidant activity observed could be due to the presence of secondary metabolites. Some other reports are also reported that, various parts of this plant showed that antioxidant activity. Based on the preliminary phytochemical screening of ethanolic extract of flowers of this plant (Table 1), showed that tannins, saponins, alkaloids, terpenoids, sterol, glycosides, and flavonoids are present. If properly screened by using additional solvents, could yield new antioxidant drugs. Further research is therefore recommended to isolate, purify and characterize these chemical constituents.

Table 1: Physico-chemical tests of ethanolic flower extract of *Hibiscus rosa sinensis*.

S. No	Phytochemical test details	Observation	HRSE (Ethanolic extracts)
1	Tannins (Lead sub-acetate test) (Ferric chloride test)	Creamy gelatinous precipitate Blue-black precipitate	+ +
2	Saponins (Foam test)	Formation of foam	+
3	Alkaloids Wagner's test	Reddish-brown precipitate	+
4	Terpenoids and sterol (Salkowsky's test) Liebermann-buchard test	Reddish brown colouration Dark green colour	+ +
5	Glycosides (Keller-killani test)	Reddish brown colour at junction of the two liquid layers and upper layer appears bluish green	+ +
6	Flavanoids (Shinoda test) (NaOH test)	Orange colouration Yellow colouration	+ +
7	Fats and oils (stain test)	No stain on paper	-
8	Lignins (labet test)	No change observed	-
9	Quinones (Conc. H ₂ SO ₄ test)	No change observed	-

Table 2: *In-vitro* antioxidant activity of ethanolic flower extract of *Hibiscus rosa sinensis*.

S.NO	Samples	IC ₅₀ ± SEM mg/mL	Radical Scavenging Activity %RSA
1	HRSE	231.110 + 1.59	94.910
2	Gallic acid (Standard)	23.436 + 0.43	93.93
3	Acetyl cysteine (Standard)	111.44 + 0.7	95.95

CONCLUSION

In conclusion, the phytochemical examination of the therapeutic plants is critical and has business enthusiasm for both research Center and pharmaceuticals organizations for the assembling of the new medications for treatment of different infections. Additionally ponder are expected to research singular phytochemical compound of the blossom of *Hibiscus rosa-sinensis* and recognizable proof and auxiliary assurance of novel antioxidants fusions will prompt the advancement of therapeutics from these plants.

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