

# Antioxidant and antibacterial activities of *Hibiscus Rosa-sinensis* Linn flower extracts

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**Abstract:** Antioxidant and antibacterial potential of different solvent extracts of locally grown *Hibiscus rosa-sinensis* Linn was evaluated. The antioxidant activity was assessed by estimation of total flavonoids contents, total phenolic contents, DPPH free radical scavenging activity and percentage inhibition of linoleic acid oxidation capacity. Agar disc diffusion method was used to assess antibacterial potential of crude extract of *H. rosa-sinensis*. The yield of the crude extracts (23.21±3.67 and 18.36±2.98% in 80% methanol and ethanol solvents was calculated, respectively. Methanol and ethanol extract of *H. rosa-sinensis* showed total phenolics 61.45±3.23 and 59.31±4.31 mg/100g as gallic acid equivalent, total flavonoids 53.28±1.93 and 32.25±1.21 mg/100g as catechine equivalent, DPPH free radical scavenging activity 75.46±4.67 and 64.98±2.11% and inhibition of linoleic acid oxidation potential 75.8±3.22 and 61.6±2.01% respectively, was measured. Antibacterial study against three human pathogens such as *staphylococcus sp.*, *Bacillus sp.* and *Escherichia coli* showed growth inhibitory effect in the range of 12.75±1.17 to 16.75±2.10 mm. These results showed *H. rosa-sinensis* indigenous to Kallar Kahar and its allied areas bear promising medicinal values and could be used for developing herbal medicines to target oxidative stress and infectious diseases.

**Keywords:** *H. rosa*, antibacterial activity, Kallar Kahar, antioxidant, diabetes.

## INTRODUCTION

To civilize the human society lifesaving natural sources has played a critical role. Plants in its any form always remained a major source of traditional way of managing health problems and different economic issues (Adriana and Alfieri 2013). Importance of the plants basically originates due to the presence of specific biological active classes of organic compounds (Joshi and Dhawan 2005). The concentration of these classes, however, varies from specie to specie and region to region of plants. On the planet earth, according to one report, more than 258,650 species of higher plants and round about similar number of lower plants exist but not more than 10% of higher plants species are in medicinal use (Shinwari 2010). These 10% flora of the world also has not been completely explored also. Over 50% of all modern clinical drugs are obtained from natural sources (Stiffness and Douros 1982). So in this way natural products have played a vital role in drug development programs of pharmaceutical industries (Baker *et al.*, 1995). Further, due to the long list of side effects of purely synthesized drugs or medicines more than 80% world's population

still emphasize to use traditional and old medicinal system such as Homeopathy, Unani, Ayurveda, Sidha, Naturopathy etc. In very rare cases it was reported that pure natural compounds were used as therapeutic agents, however, natural extracts were in frequent use both in developing functional foods and treating illnesses.

*H. rosa-sinensis* is a member of the family Malvaceae which is considered as an important medicinal family. The specification of plant are very typical i.e. roots are cylindrical of 5-15 cm length, leaves are simple ovate or ovate-lanceolate with coarsely toothed at the apex, flowers are pedicellate with 5 petals of red colour (Lalit 2012). It is widely cultivated as ornamental plants in public and home places; anyhow, it is also extensively planted in northern areas at high altitudes. The plants also grow in northern areas of Pakistan, in the strip of valley Soon Skacer and Kalar Kahaar at high altitude. The medicinal plants of these areas carry more medicinal values as compared to those grow at sea level and plane areas.

The extract of *H. rosa-sinensis* has already been tested for the treatment of oxidative stress diseases including diabetes and tumor. There is emerging evidence that diabetes leads to depletion of the cellular antioxidant

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defence system and increased levels of reactive oxygen species (ROS) (Rösen 2001). In biological systems superoxide anion activates the formation of ROS that involve in initiation of different chronic diseases such as hypertension, diabetes, cancer, renal disorder, inflammation and neurodegeneration (Rhian 2004; Kathy 2003; Koechlin 2004). Strong antioxidant natural extract may reduce the risk of these diseases. Extracts of *H. rosa-sinensis* plant were found in better position to manage the cellular antioxidant defense depletion threat by diabetes (Rösen 2001). These were the triggering characteristics that motivate us to assess medicinal values of flowers of *H. rosa-sinensis* grown at the hills of valley Kallar Kahar, Pakistan. In this work we aimed to investigate the antioxidant and antibacterial potential of *H. rosa-sinensis* flowers grown at the hills of Kallar Kahar using methanol and ethanol solvents.

## MATERIALS AND METHODS

### *Collection of sample*

Samples of *H. rosa-sinensis* were collected from the hills of Kallar Kahar situated at 635 meters above sea level with coordinate 324660 N and 72420 E in DMS (Degrees Minutes Second). The collected flowers samples were authenticated in the research laboratory of Botany Department, Government College University Faisalabad, Pakistan. Mature flowers with uniform structure were chosen and washed with doubly distilled water. Then samples of flowers were air dried till constant weight was calculated. Dried sample of flowers were ground to powder by using commercial blender. The samples of flowers were stored in air tight polythene bags for further periodical use.

### *Chemicals*

Methanol and ethanol were purchased from Merck Chemicals Co. Water was distilled twice and filtered using a decompressing pump. Folin-ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazil (DPPH), ascorbic acid (vitamin C), butylated hydroxytoluene (BHT), gallic acid and butyl hydroxyanisol (BHA) were purchased from Sigma-Aldrich Chemical Co. A Millipore type filter (0.22  $\mu$ m and 0.45  $\mu$ m) was obtained from Sartorius, USA. For absorption measurement double beam U-2800 UV-visible spectrometer, HITACHI, Japan was used. Bacterial strains were obtained from University of Health Sciences, Lahore.

### *Extract preparation*

The dried flowers powder was extracted with 80% methanol or ethanol using 1:10 (sample to solvent) ratio through orbital shaker (Gallenkamp, UK.). The extracts were separated from the residues by filtering through Whatman No.1 filter paper. Filtrates were then concentrated under reduced pressure at 40°C, using a rotary evaporator (EYELA, N-N series, Rikakikai Co. Ltd.

Tokyo, Japan). Dried crude extracts were then weighed and stored in a refrigerator at -4°C. The percentage yield of extracts were calculated using the expression  
Yield (%) = Amount of dried extract/wt. of sample  $\times$  100

### *Determination of total phenolic contents (TPC)*

TPC were determined by using Folin-ciocalteu reagent according to the method described by Kulisic *et al.*, (2004). Briefly 50mg of dry mass of extracts was mixed with 0.5mL of Folin-ciocalteu reagent and 7.5mL deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% sodium carbonate (w/v) was added. The mixture was heated in a water bath at 40°C for 20 min and then cooled in an ice bath. Finally absorbance at 755 nm was measured with UV-visible spectrophotometer. Concentration of phenolics was calculated using a calibration curve of gallic acid (10-100 ppm). The results were expressed as gallic acid equivalents (GAE) gram per 100 grams of dry mass of dry matter. Each sample was analyzed in triplicate.

### *Determination of total flavonoid contents (TFC)*

The concentration of TFC was measured following the procedure reported by Dewanto *et al.*, (2002). To the aqueous solution of 1mL of plant extract (0.01 g/1 mL of dry matter) 5mL of distilled water, 0.3mL NaNO<sub>2</sub> (5%, w/v) was added. The resulting solution mixture was incubated for 5 min. Then 10% AlCl<sub>3</sub> (0.6mL) was mixed and incubated for other 5min. To this mixture, 1 M NaOH (2mL) was added and total volume of the mixture solution was made 10mL deionized water. Absorbance was noted at 510 nm with the help of spectrophotometer. Total flavonoid concentration was calculated as catechin equivalents g/100 g of dry matter. The data was expressed as average of triplicate analysis.

### *Determination of %age inhibition of lipid peroxidation in linoleic acid system*

This study was performed following the method described by (Iqbal and Bhanger 2005) making minor modification. Shortly, 20 mgflowers extract was mixed to a solution mixture of linoleic acid (0.10mL), 99.5% ethanol (8 mL) and 0.2 M sodium phosphate buffer of pH 7 (8 mL) and total dilution of mixture was done up to 20 mL with distilled water and incubate it for 75 h at 40°C. At the end of the incubation period the extent of oxidation was measured following thiocyanate method reported by Yen *et al.* (2000); with 10 mL of ethanol (3:1, ethanol:water), 30% of 0.2mL of an aqueous solution of ammonium thiocyanate and 0.2mL each of sample solution and 20 mM in 3.5% HCl ferrous chloride (FeCl<sub>2</sub>) solution was added sequentially. After continuous 3 min stirring, the absorption was measured with UV-visible spectrophotometer at 500 nm were taken as peroxide contents. A blank sample with linoleic acid but without extracts was taken as control. Synthetic antioxidants, butylated hydroxytoluene (BHT) was used as a positive control. The minimum peroxidation level was measured

after 75 h (3 days) in the blank sample Percent inhibition of linoleic acid per oxidation (I) was calculated using following relation:

$$I\% = 100 - [(Abs. \text{ increase of sample at 75h} / Abs. \text{ increase of control at 75h}) \times 100]$$

#### DPPH free radical scavenging assay

The antioxidant potential of the *H. rosa-sinensis* extracts was assessed by measuring the scavenging 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was performed as described by Bozin *et al.* (2006). The samples were mixed with 1 mL of 90  $\mu$ M DPPH solution and filled up with 95% methanol, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. Scavenging of DPPH free radical was recorded spectrophotometrically at 515 nm using UV-visible spectrophotometer. Percent scavenging of DPPH free radical was calculated using following relation.

$$\% \text{ scavenging} = 100 - [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}]$$

#### Determination of Antibacterial activity

The methanolic and ethanolic extract of *H. rosa-sinensis* flower extracts was investigated against three pathogenic microorganisms, including; *Bacillus subtilis*, *Staphylococcus sp.* and *Escherichia coli (E. coli)* by disc diffusion method reported by Kelen and Tepe (Kelen and Tepe, 2008). Shortly, 100  $\mu$ L of bacterial suspension ( $5 \times 10^5$  CFU/mL) in nutrient agar were taken in glass plates and inoculated. About 10  $\mu$ L of each extracted sample dissolved in methanol was dropped on the sterile filter discs (5 mm diameter) and placed very carefully on the inoculated nutrient agar containing bacterial strains. Livofloxacin, a potent antibiotic (25  $\mu$ g/disc) was used as positive control, while sterile filter disc without extract taken as negative control. All the glass plates were incubated for 24 h at 37°C.

## RESULTS

#### Extraction yield, total phenolic and flavonoid contents determination

Extraction of the bioactive compounds was carried out in methanol and ethanol. Better extraction yield (23.21  $\pm$  3.67%) was obtained in methanol solvent as compared to the ethanol solvent (18.36  $\pm$  2.98%). Table shows the extraction yield, TPC and TPC in flowers of the *H. rosa-sinensis* using methanol and ethanol solvents. Antioxidants was found in promising amount, TPC 61.45  $\pm$  3.23 and 59.31  $\pm$  4.31 mg/100g as gallic acid equivalent and TFC 53.28  $\pm$  1.93 and 32.25  $\pm$  1.21 mg/100g as catechine equivalent in methanol and ethanol crude extract of *H. rosa-sinensis* flowers, respectively.

#### Antioxidant activity measurement in linoleic acid system

Crude extracts of flower of *H. rosasinensis* showed effective inhibition of peroxidation in linoleic acid as

shown in fig. 1. Methanol extract showed greater inhibition activity (75.80  $\pm$  3.22%) as compared to the ethanol extract (61.6  $\pm$  2.01%). The control used in this study was BHT which shows 82.34  $\pm$  4.66% inhibition power.

#### DPPH free radical scavenging activity measurement

Fig. 2 indicates the DPPH free radical scavenging potential of methanol extract, ethanol extract and BHT (standard). The results showed the scavenging ability in the following order BHT > methanol extract > ethanol extract which were 77.54  $\pm$  4.77, 75.46  $\pm$  4.67 and 64.98  $\pm$  2.11%, respectively.

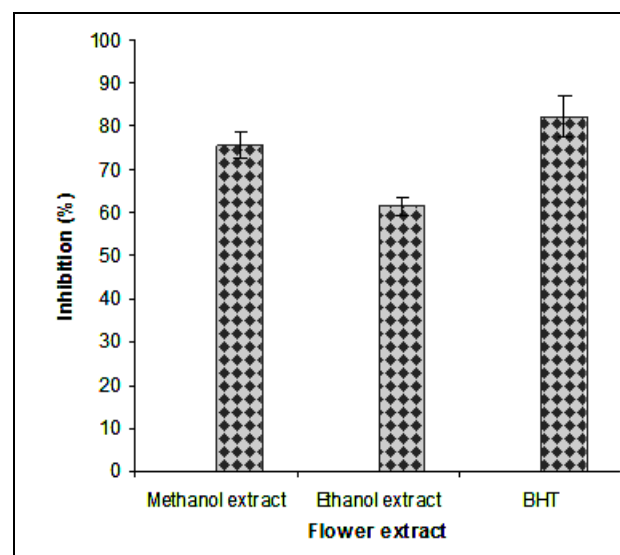


Fig. 1: Inhibition of lipid peroxidation (%) in Linoleic acid system of *H. rosa-sinensis* flower extracts.

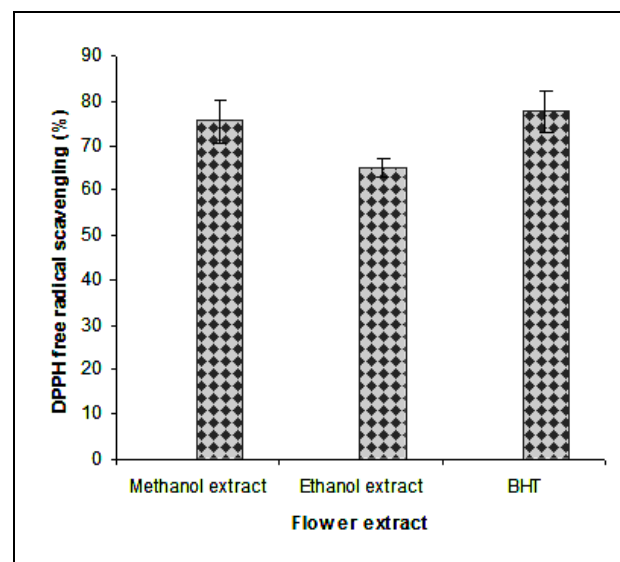
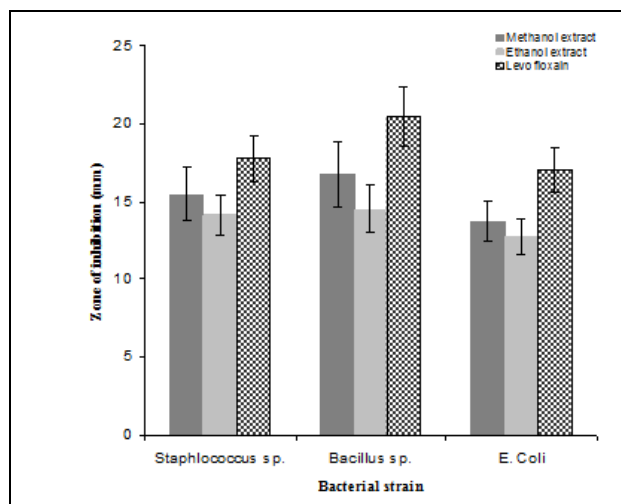


Fig. 2: DPPH free radical scavenging activity (%) of methanol and ethanol extracts of *H. rosa-sinensis* flowers.

**Table:** Extraction yield, total phenolic and total flavonoid content of methanol and ethanol extract of flower of *H. rosa-sinensis*.

S. No	Flower Extraction	Yield g/100g dry extract	Total phenolic contents (mg/100g dry extract)	Total flavonoid contents (mg/100g dry extract)
1	Methanolic	23.21±3.67	61.45±3.23	53.28±1.93
2	Ethanollic	18.36±2.98	59.31±4.31	32.25±1.21

The values are presented as mean±S.D (n=3)



**Fig. 3:** Antibacterial activity of methanol and ethanol extract of *H. rosa-sinensis* flower extracts against human pathogens.

#### Antibacterial potential of *H. rosa-sinensis* extracts

The antibacterial activities of the crude extract of *H.rosa-sinensis* flowers were carried out against three human pathogens such as *Staphylococcus sp.*, *Bacillus sp.* and *E. coli* as shown in fig. 3. Levofloxacin antibiotic was taken as a reference antibacterial agent. Both, methanol and ethanol extracts showed promising zone of inhibition of growth in the range of 12.75±1.17 to 16.75±2.10 mm. In comparison to these levofloxacin showed 17.75±1.5, 20.5±1.88 and 17±1.38 mm zone of inhibition against *Staphylococcus sp.*, *Bacillus sp.* and *E. coli* pathogens, respectively.

## DISCUSSION

#### Total phenolic and flavonoid contents

Phenolic contents and flavonoid contents present in the plant body mainly give the idea of its medicinal importance. These are considered the index of the antioxidant and free radical scavenging strength of the plants, as these components are involved in deleting, neutralizing or scavenging free radicals (Duh *et al.*, 1999; Pietta 2000) due to the presence of conjugated ring systems and carboxylic groups. These functional group in phenolic and flavonoid compounds also involve strongly to inhibit lipid peroxidation (Rice-Evans *et al.*, 1995). In

this study both extracts of *H.rosa-sinensis* showed promising quantity of the TPC and TFC. High amount of these two types of antioxidants emphasized on the medicinal potential of plant. However, the extract from flowers showed less quantity of TPC and TFC as compared to present in crude extract of whole plant (Rajesh 2011).

#### Antioxidant activity

DPPH free radical has been considered most reliable to assess free radical scavenging potential of different plants or parts of plants extracts due to its excellent stability. The reduction of DPPH free radical by crude extract either by donating an electron or hydrogen to DPPH make the basis of the assay (Soares *et al.*, 1997) and also the validity of the sample as an antioxidant and free radical scavenger. Considerable scavenging potential of methanol extract showed promising antioxidant behavior of *H. rosa-sinensis* flower extract. Further, most of the biological damage may be attributed to lipid peroxidation process which triggers certain chain reactions via free radical mechanism. Inhibition of lipid peroxidation involves in protecting the body from lethal diseases. Previously, Rajesh *et al.* (2011) studied the total antioxidant activity of *H. rosa-sinensis* plant extracts and reported inhibition of lipid peroxidation in the range of 94-96% which are, however, slightly greater than our investigations. Scavenging of DPPH free radical and inhibition of lipid peroxidation commonly related to the presence of TPC and TFC in a tested extract. Flower extract contain relatively lesser amount of compounds of these two classes as shown in Table as compared to value reported by Rajesh *et al.* (2011) in total plant extract. The presence of lesser quantity in flower extract certainly decrease the antioxidant activities but results still showed promising antioxidant behavior.

#### Antibacterial potential

Antibacterial effect of crude extracts and levofloxacin showed small gap among their values. This may be due to two reasons; flower extract of *H. rosa-sinensis* contain set of more potent component or due to the increased bacterial resistance to the routine antibiotics as compared to the set of natural components present in plant extracts. The later reason has been reported by different research groups (Scalbert 1991; Saravanan 2011; Morand 2000; Ionela and Ion 2007; Begum 2004). Our results are in close agreement with previously reported

antibacterial activities by Ruban and Gajalakshmi (2012) and Sangeetha *et al.* (2009) while disagree with the results reported by Borhan Uddin *et al.* using ethanol flower extract against *S. aureus*. These variations may arise due to the geographical composition and climate difference.

## CONCLUSION

Our findings indicated that geographical and climate difference may change the profile of biological active components to some extent. The flowers of *H.rosa-sinensis* collected from Kallar Kahar showed promising medicinal activities (antioxidant and antibacterial activities) and it can play a therapeutic role against number of epidemic and pathogen born diseases. On the basis of the results and promising activities of methanol extract of flowers it could be subjected to isolate most active components for drug development program and therapy of infection diseases.

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