IN VITRO ANTIOXIDANT ACTIVITY OF FLOWERS OF *HIBISCUS ROSA-SINENIS* LINN.

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Summary

Antioxidant activity of alcoholic flower extract of *Hibiscus rosa-sinenis* Linn. (Malvaceae) was studied in four in vitro models viz. radical scavenging activity by DPPH reduction Assay, Scavenging of $O_2^{\cdot -}$, Scavenging of H_2O_2 and NO Scavenging. Alcoholic flower extract of *Hibiscus rosa-sinenis* Linn possessed significant antioxidant activity in all the models. In all the modes at 250µg/ml and 500µg/ml shows significant activity while in DPPH reduction Assay shows significant activity at 50 and 100µg/ml.in conclusion Alcoholic flower extract of *Hibiscus rosa-sinenis* Linn showed promising free radical scavenging activity.

Key words: *Hibiscus rosa-sinenis* Linn, Alcoholic extract, free radical scavenging.

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Introduction

Hibiscus rosa-sinenis Linn is a conspicuous, ornamental, evergreen, glabrous, showy 1.5 to 1.4 m high shrub cultivated throughout India up to 1200m in the hills[1]. *Hibiscus rosa-sinesis* are native to Tropical Asia. A native of Southeastern Asia (China), the plant is commonly found through out the tropics and as a houseplant throughout the world. The plant has a great medicinal value; its stem bark is used as a mild, pleasant and safe purgative. A decoction of root is used for venereal diseases and fevers. Leaves are emollient, aperients, anodyne and laxative. Flowers are astringent, demulcent, emollient, refrigerant, constipating, hypoglycemic, and aphrodisiac. Oil made by mixing the juice of fresh petals and olive oil for stimulating hair growth [2, 3].

Hibiscus rosa-sinesis flowers mainly contains Anthocyanins and Flavonoids, these are cyaniding-3, 5-diglucoside, cyaniding-3-sophoroside-5-glucoside, quercetin-3, 7-diglucoside, quercetin-3-diglucoside. Other minor constituents are A cyclopeptide alkaloid, cyaniding chloride, quercetin, hentriacontane and vitamins i.e. riboflavin, ascorbic acid and thiamine[1]. Epidemiological studies have found that the intake of antioxidants such as Vitamin C reduces the risk of coronary heart disease and cancer[4].In the present study we have studied the antioxidant activity of alcoholic extract of Hibiscus *rosa-sinesis* flowers of using *in vitro* model.

Materials and methods

In the present study, the matured flowers of *Hibiscus rosa-sinenis* Linn were collected from Local areas of Belgaum, Karnataka. The flowers were authenticated from Botanical Survey of India, Pune India. After authentification, all the flowers were dried at room temperature until they were free from the moisture and subjected to physical evaluation with different parameters. The parameters which were used for evaluation are nature, odour, color, taste, size, shape, width, length.

Finally flowers were subjected to size reduction to get coarse powder and then passed through sieve no.40 to get uniform powder. 100 gm of powder was subjected to successive hot continuous extraction (soxhlet) with alcohol. Each time before extracting

with the next solvent the powdered material was air dried in hot air oven below 50^0C. After the effective extraction, the solvent was distilled off, the extract was then concentrated on water bath. The obtained extracts was subjected to chemical investigation and tested for antioxidant activity.

Free radical scavenging assays

DPPH assay:

One milliliter solution of the extract (in methanol) was added to 0.5 ml of 0.15 ml DPPH solution (in methanol). The contents were mixed vigorously and allowed to stand at 20^0C for 30 minutes. The absorbance was read at 517 nm. IC_{50} value (the concentration required to scavenge 50% DPPH free radicals) was calculated.

Scavenging of $O_2^{\cdot-}$:

The method was adopted from Yen and Chen[5]. The reaction mixture, comprising 1ml of extract solution in distilled water, 1ml of phenazine methosulphate (60µM) in phosphate buffer (0.1M,pH 7.4), 1ml NADH (450µM) in phosphate buffer, was incubated at 25^0C for 5min.the absorbance was then read at 560 nm against blank samples.

Scavenging of H_2O_2:

Scavenging of H_2O_2 by the extract was determined by the method of Ruch etal[6]. One milliliter of extract solution [prepared in phosphate buffer saline (PBS)] was incubated with 0.6 ml of 4 ml H_2O_2 solution (prepared in PBS) for ten minutes. The absorbance of the solution was measured at 230nm against a blank solution containing the extract without H_2O_2. The concentration of H_2O_2 was spectrophotometrically determined from absorption at 230nm using the molar absorptivity of $81M^{-1}cm^{-1}$.

Table 1

Scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) by Alcoholic extract.

Group (µg/ml)	**DPPH (%Control)**	**$O^{\cdot-}_2$ (%Control)**	**H_2O_2 (%Control)**	**NO (%Control)**
Control	100.0 ± 4.000	100.7±3.913	100.4±3.557	100.3±3.524
Extract-50	46.72± 3.987***	94.48±5.706	94.60±4.241	81.67±2.193**
Extract-100	24.20 ± 3.382***	85.88±5.107*	71.97±4.063***	73.40±2.264***
Extract-250	14.26 ± 0.6337***	51.15±2.194***	61.98±3.874***	31.28±0.3781***
Extract-500	1.220 ± 0.2131***	47.56±3.124***	51.03±4.476***	29.89±0.6373***

Note: Statistical analysis was carried out by Student's' test.

***P<0.0001 is considered significant. **P= 0.001, *P= 0.02

NO Scavenging:

Scavenging of NO was determined by incubating SNP (5mM, in PBS) with different concentration of extract at 25^0C. After 120 minutes, 0.5ml of incubation solution was withdrawn and mixed with 0.5ml of griess reagent[7]. The absorbance was measured at 550nm. The amount of nitrate was calculated from standard curved constructed by sodium nitrite.

Results and discussions

The potentially reactive derivatives of oxygen, ascribed as ROS such as hydrogen peroxide Super oxide radical anion and nitric oxide, are continuously generated inside the human body as consequences of exposure to a plethora of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA[8]. This oxidative damage is a crucial etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in the ageing process[9].

In the present study alcoholic extract showed good radical scavenging activity against DPPH radical in all the concentrations. Extract showed moderate activity against oxygen free radical (O^{-}_{2}) scavenging between the concentration ranges 250-500 µg/ml, Hydrogen peroxide (H_2O_2) scavenging between the concentrations ranges 100-500 µg/ml and nitric oxide (NO) scavenging between the concentrations ranges 100-500 µg/ml. Statistical analysis was carried out by Student's' test and is as shown in the table. Free radical scavenging effect may be due to the presence of flavonoid in the plant.

Conclusion:

Herbal formulations are attracting the attention of physician, patients significantly for their versatile diversed biological properties. Hibiscus which is a good antioxidant is been experimentally tested for the various methods of free radical scavenging activity. Thus there is a vast scope for development of polyherbal formulation in future.

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