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Comparative Antioxidant Potential and Phytochemical Chemical Study on Leaves and Flowers Extracts of *Erythrina suberosa*

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A B S T R A C T

Present study is based on comparison of antioxidant potential and related phytochemicals present in leaves and flowers extracts of *Erythrina suberosa*. Results indicate that different extracts of flowers and leaves (Crude/Methanol, Hexane and Water) show good antioxidant potential in all assays. In DPPH radical de-colourization assay crude extract of flowers found most active with IC_{50} value 49.00 µg/ml while aqueous extract showed lowest potential (IC_{50}, 193.40 µg/ml). On the other hand maximum ability to scavenge ABTS radicals was possessed by aqueous leaves extract (IC_{50}, 09.40 µg/ml) and least by hexane extract of leaves (IC_{50}, 37.20 µg/ml) other have intermediate potential in both assays. Comparison of Total antioxidant capacity and anti-lipid peroxidation, aqueous leaves extract and crude extract of flowers has shown maximum potential respectively in these assays. Overall, high antioxidant potential as well as total phenolic contents were possessed by different flowers extracts.

Keywords: *Erythrina suberosa*, DPPH, Methanol, Hexane, antioxidant potential

A R T I C L E I N F O

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1. Introduction

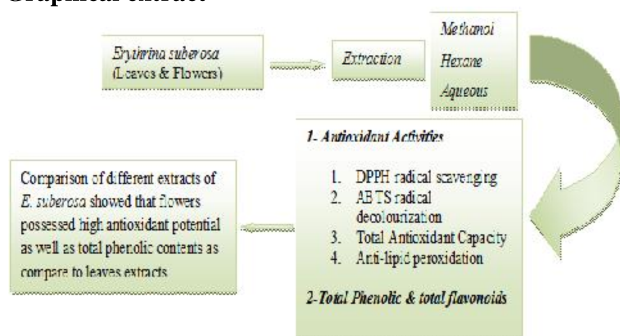
Oxidation process is essential for living organisms in order to attain energy from the metabolism of food. Production of free radicals during food metabolism is usual process. Free radicals especially reactive oxygen species (ROS) play very

important beneficial role in the body like protection of body against infectious agents and in various signaling processes. Excessive and continuous production of free radicals results into cell and tissue damage such as membrane, proteins, lipids and nucleic acid[1]. Oxidative damage that caused by free radicals may also be associated to many diseases like diabetes, atherosclerosis, aging[2,3].

Antioxidants are important nutritional supplements used for good physical health and for the prevention of diseases like cancer and heart diseases. Plants contain polyphenolic compounds which are important free radical scavengers as well as chain braking agents under certain conditions. Flavonoids are important polyphenols act as metal chelators, binds to metals and prevent their involvement in toxic reactions[4].

Erythrina suberosa belongs to the family *Fabaceae*, is an attractive, ornamental big tree with beautiful red coloured flowers. Flowers are in the form of bunches and their aqueous extract in combination with *Hibiscus rosa sinensis* is used in summer season as soothing drink[5]. Because of its medicinal importance and easy availability, *Erythrina suberosa* was selected to carry out the present study. Both flowers and leaves of the plants were used to evaluate and compare their antioxidant potential in different antioxidant assays. In addition to this their total phenolics and flavonoids contents were also determined.

Graphical extract



2. Materials and method

1,1-diphenyl-2-picryl hydrazyl radical (DPPH), , thiobarbituric acid (TBA), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), 2,2´-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), trolox (6-hydroy-2,5,7,8-tetramethylchroman-2-carboxylic acid) all were purchased from Sigma chemicals. All other solvents and chemicals used for this study were of commercially available analytical grade quality.

Plant material, Extraction and Fractionation:

Fresh leaves and flowers of *Erythrina suberosa* were collected from the lawn of Lahore College for Women University (LCWU), Lahore. Dried leaves and flowers were separately powdered and extracted with methanol. For crude extraction 12g of dried leaves powder were soaked in methanol for 6 days. After that soaked leaves were filtered and the filtrate was evaporated. 1g of crude extract was saved for study and remaining part was dissolved water and mixed with hexane in equal volume in a separation funnel. After shaking well hexane layer was separated. This procedure was repeated many times to get maximum contents in hexane. Finally the hexane was evaporated to get crude hexane fraction. Remaining water fraction was lyophilized to get water extract. All fractions (methanol, hexane and water) were saved for further use. Same procedure was repeated with the dry power of flowers.

DPPH radical scavenging assay:

DPPH Free radical scavenging ability of various fractions of *Erythrina suberosa* leaves and flowers was assessed by reported method of Blois et al., with certain changes[6]. Reaction mixture contained 0.5 ml of varying concentrations of sample solution in methanol and 2.5 ml DPPH radical solution (Final concentration, 0.1mM). The mixture was thoroughly mixed and incubated for thirty minutes at 37ºC. Absorbance was measured at 517 nm by UV/Vis spectrophotometer. % radical scavenging was calculated as:

% Inhibition = [1-Absorbance $_{(sample)}$/ Absorbance $_{(control)}$] x 100

$ABTS^{\bullet+}$ Decolourization assay:

$ABTS^{\bullet+}$ radical cation decolourization potential of samples and standard compounds was evaluated by reported method[7]. 7.0 mM ABTS stock solution was oxidized by 2.45 mM $K_2S_2O_4$ to generate $ABTS^{\bullet+}$ radical cation. Absorption of the resultant solution was adjusted up to 0.70±0.10 at 734 nm. Reaction mixture contained 2.5 mL $ABTS^{\bullet+}$ and 0.5 mL sample solution was incubated at room temperature for 5 minutes and percentage quenching was estimated. Trolox was used as standard reference compound.

Total Antioxidant Capacity:

In order to determine total antioxidant capacity of different extracts, 0.5 ml of each sample was added in 4ml of reagent solution containing 0.6M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate. This mixture was incubated for 90 minutes at 95ºC. Optical density of the solution was observed at wavelength 695nm with a spectrophotometer. In blank experiment, methanol was added in place of extract. Same procedure was adopted to check antioxidant capacity of ascorbic acid[8].

Anti-lipid peroxidation Assay:

A slightly modified Halliwell et al. method was used to estimate anti-lipid peroxidation activity of samples and reference standards[9]. In brief, 1.15 % KCl (1.5 mL) and 10.0 % egg yolk (1.0 mL) were added to different series of sample's concentration. To this solution 0.5 mL of 0.2mM ferric chloride was added and reaction mixture was incubated for 1h at 37°C. Reaction was then stopped by adding 2.0 mL of ice-cold HCl (0.25 N) containing 0.38 % thiobarbituric acid, 15.0 % trichloroacetic acid and 0.5 % butylated hydroxytoluene. It was heated for 1 h at 80°C, followed by centrifugation at 3000 rpm. Pink colour was measured spectrophotometrically at 532 nm.

Total Flavonoids:

Reaction mixture containing 250µl of the sample extract, 1.50 ml distilled water and 90µl sodium nitrate solution (5%) was mixed thoroughly and allowed to stand for 5 to 6 min. After that 0.18ml of alluminium chloride solution (10%) was added to it and further stand for 5 min. Whole

mixture was mixed with 0.6ml NaOH (1M) and deionized water was added to make it up to 3ml before measuring absorbance at 510 nm. Same method was performed to prepare a calibration curve of quercetin as standard and the results were stated as quercetin equivalent (mg g^{-1} dry mass)[10].

Total phenolic content: Total phenolic contents were estimated using Folin-Ciocalteu reagent. 20 µL of each sample solution was mixed with 100µL Folin-Ciocalteu reagent and 1.58 mL deionized water and allowed to stand for 10 min. After that 300 µL of 25% Sodium carbonate solution was added to this reaction mixture and incubated for 30 min at 40°C. Absorbance was recorded at 765 nm and results were documented as gallic acid equivalent (GAE mg g-1 dry wt)[11].

Statistical analysis:

All determinations were carried out in triplicate and presented as their mean values in the results. Statistical comparisons of the mean values were performed by analysis of variance (ANOVA).

3. Results and Discussion

Antioxidant Assays

DPPH radical scavenging assay

In this assay different concentrations of each extract (both leaves and flowers) were allowed to scavenge DPPH a stable radical and the reaction was monitored at 517 nm after 30 min incubation (37ºC). Results presented in table-1 indicate that among different fractions of leaves extract aqueous extract possessed least radical scavenging potential (IC_{50}, 133.00µg/ml), hexane extract showed highest potential (IC_{50}, 59.00µg/ml) while intermediate activity was shown by ethanol extract (IC_{50}, 128.00 µg/ml). Similarly among different flower extracts aqueous extract showed least activity with IC_{50} value 193.40 µg/ml however, in this case highest potential of DPPH radical scavenging was shown by ethanol extract (IC_{50}, 48.90 µg/ml) and hexane fraction showed intermediate ability (IC_{50}, 122.00 µg/ml) as indicated in Table-1.

$ABTS^{•+}$ Decolourization assay:

% AI for various extracts in ABTS radical decolourization assay was determined at 200 µg/ml. Results indicated that among all the extracts aqueous extract of both leaves and flowers extracts showed maximum decolourization about 99.00%. IC_{50} (50% inhibitory concentration) values for all extracts were determined. According to the results mentioned in Table-1, the order of efficiency of leaves extracts is aqueous > crude > hexane with IC_{50} values 9.40 µg/ml, 25.70 µg/ml and 37.20 µg/ml respectively. More or less same order was observed in case of flower extracts with exception where hexane extract is batter (IC_{50}, 30.40µg/ml) than the crude (IC_{50}, 35.50µg/ml). Comparison of two assays shows that ABTS radical decolourization assay is more sensitive than DPPH radical scavenging assay.

Total antioxidant capacity assay:

Total antioxidant capacity (TAC) of different flower and leaves extracts was determined as mg ascorbic acid equivalent. In this assay Mo(VI) is reduced to Mo(V) complex which is green in colour and the colour intensity is measured at 695 nm[12]. Both leaves and flower extracts showed various level of total antioxidant capacity. Highest level was achieved by crude extract of leaves while aqueous extract of leaves showed lowest antioxidant activity (Table-2).

Lipid per-oxidation assay:

Oxidative degradation of lipids is well known as lipid peroxidation. This process proceeds by a free radical chain reaction mechanism mostly effecting polyunsaturated lipids resulting into lipid peroxides. Lipid peroxides are very unstable undergo additional decomposition to form complex series of compounds like reactive carbonyl compounds. Polyunsaturated fatty acid peroxides further react to form malonaldehyde (MDA) most widely reported analytes for the purpose of estimating the effect of oxidative stress on lipids[13]. Anti lipid peroxidation property of different leaves and flowers extracts was carried out at fixed concentration of 5 mg/ml. Comparison of the results as indicated in figure-1 showed that maximum inhibition of lipid peroxidation is possessed by crude flowers extract (79.6 % at 5 mg/ml) of E. subberosa. Among different fractions of leaves, hexane extract showed highest potential (75 %) while aqueous extract of leaves possessed least inhibitory property i. e., 7.6 % at 5 mg/ml concentration.

Total flevonoid and phenolic contents:

Plant phenolics possess strong radical neutralization potential that is why they are gaining more importance now days. Flavoniods are polyphenols are reported as strong antioxidants due to their radical scavenging ability[14]. According to the results as mentioned in table-2, hexane fraction of flowers extract has shown highest total phenolic contents i.e., 680.0 mg/g gallic acid equivalent while hexane part of leaves has shown maximum total flavonoids (37.2 mg/g quercetin equivalent).

Table-1: Comparison of IC_{50} values of different extracts of flowers and leaves of *Erythrina suberosa* in DPPH and $ABTS^{•+}$ decolourization assay.

Sr.No	Extracts	DPPH IC_{50}(µg/ml)		$ABTS^{•+}$ IC_{50}(µg/ml)	
		Leaves	Flowers	Leaves	Flowers
1	Crude	128.00±0.43	49.00±0.33	25.70±0.12	35.50±0.54
2	Hexane	059.10±0.24	122.10±0.02	37.20±0.09	30.40±0.31
3	Aqueous	133.00±0.04	193.40±0.53	09.40±0.06	29.10±0.26

All experiments were performed in triplicate. IC_{50} values are mean of three readings

Standard: Gallic acid [IC_{50}, DPPH (6.17±0.02 µg/ml), $ABTS^{•+}$ (0.60±0.08 µg/ml)]

Table-2: Comparison of Total Phenol, Total flavonoid contents and Total Antioxidant Capacity of Leaves as well as Flowers extracts of *Erythrina suberosa*.

Sr. No	Extracts	Total phenolic (mg/g gallic acid equivalent)		Total flavonoid (mg/g quercetin equivalent)		Total Antioxidant Capacity assay (mg ascorbic acid equivalent)	
		Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
1	Crude	159.00±0.20	213.00±0.33	25.70±0.12	35.50±0.54	61.0±0.03	51.0±0.06
2	Hexane	240.00±0.09	680.00±0.02	37.20±0.09	30.40±0.31	51.0±0.10	37.5±0.08
3	Aqueous	263.00±0.40	245.00±0.53	09.40±0.06	29.10±0.26	08.0±0.01	52.0±0.04

All experiments were performed in triplicate.

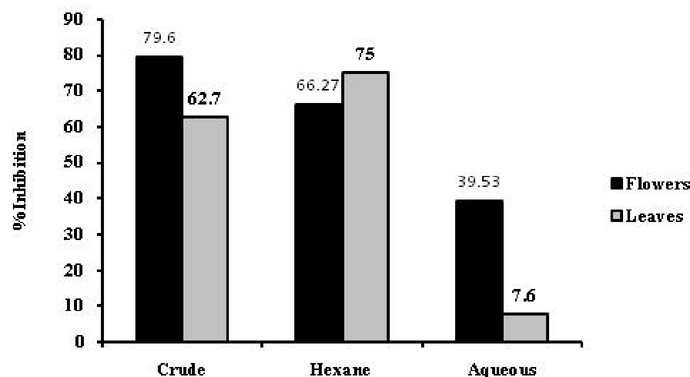


Figure 1: Comparison of % Inhibition of different Leaves and Flowers extracts of *Erythrina suberosa* in Lipid Per-oxidation Assay (concentration: 5 mg/ml), **Standard:** Gallic acid (90% at 5 mg/ml)

4. Conclusion

Comparison of different extracts of *E. suberosa* leaves and flowers showed that flowers possessed high antioxidant potential as well as total phenolic contents as compare to leaves extracts.

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