ANTIOXIDANT ACTIVITY OF THE SUCCESSIVE EXTRACTS OF *CAESALPINIA PULCHERRIMA* FLOWERS

G.S.CHAKRABORTHY

School of Pharmacy and Technology Management, Faculty of Pharmacy, SVKM's, NMiMS University,Shirpur, Maharastra 425 405

Summary

Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like Analgesic, Antiinflammatory, Antioxidant, hypoglycemic agents and many more. The successive extracts of *Caesalpinia pulcherrima* flowers were screened for *in vitro* antioxidant properties using the standard procedures. The successive extracts such as petroleum ether, ethyl acetate, methanol and water and 50% crude methanol extracts exhibited IC_{50} values of respectively in DPPH and respectively in nitric oxide radical inhibition assays. The values are comparable with the standards such as ascorbic acid and quercetin. The *Tagetes erectus* flowers are showing antioxidant activity**.**

Key words: *Caesalpina pulcherrima*, Antioxidant, DPPH, Nitric Oxided, Peroxidation, Free radical scavenging.

Address for correspondence
School of Pharmacy and Technology Management
SVKM's, NMIMS University,
Shirpur Campus,
Dist: Dhulia,
Maharashtra 425 405,
(India)
Email: phdgs77@indiatimes.com

Introduction

Caesalpinia pulcherrima flowers belong to the family Caesalpiniaceae commonly called as Red Bird of Paradise which is widely used in our Traditional System of Medicine. It is a shrub growing to 3 m tall, native to tropical America. The leaves are bipinnate, 20-40 cm long, bearing 3-10 pairs of pinnae, each with 6-10 pairs of leaflets 15-25 mm long and 10-15 mm broad. The flowers are borne in racemes up to 20 cm long, each flower with five yellow, orange or red petals. The fruit is a pod 6-12 cm long (1). The leaves are used in the treatment of purgation, as antipyretic and sometimes used as a substitute for senna.

The dried leaves are used in the treatment of erysipelas. Flowers as anthelmintic, remedy for cough and catarrh. Root part is used for curing intermittent fevers. Bark is used as emmennagogue and abortifacient. The plant contains flavonoid as myricitroside. Tannins, benzoic acid and gallic acid are present in leaves, flowers and in fruits. Presence of cyaniding-3,5- diglucoside is reported from leaves (2). From the literature cited very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for its therapeutic activity.

Lipid peroxidation is the outmost important biochemical assay which is involved in pathogenesis of many diseases like diabetes mellitus, atherosclerosis, tumor, myocardial infraction and also in the process of ageing. Free radicals generally called as reactive oxygen species (ROS) are synthesized *in vivo* from a various biochemical reactions and tends to form a chain in the system (3, 4). These free radicals are the major points in lipid peroxidation (5, 6). Plants containing Flavonoids have been reported to possess strong oxidant properties. Thus in the present investigation the successive extraction of *Caesalpinia pulcherrima* flowers was screened for *in vitro* antioxidant properties using standard operating procedures.

Material and Methods

Chemicals

Chemicals used in this study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, sodium nitrite, trichloroacetic acid, Folin-Ciocalteu reagent, butylated hydroxyl anisole (BHA), ascorbic acid (Merck [India]), Gallic acid, linoleic acid (Sigma). All reagents used for the experiments were of analytical grade (AR).

Collection of plant material: The plant was collected from the wild sources of Shirpur forest, Maharashtra, India in the month of May 2008. The plant was identified and authenticated from standard resources.

Preparation of extracts and Standards: The successive extracts of the shade dried powdered flowers (50- gram) of *Caesalpina pulcherrima* was prepared with different solvents as per the order of their polarity in Soxhlet apparatus. The solvents were evaporated with the help of rotary evaporated to get a solid residue (12-gram). The solid residue was placed in a vacuum desicator and was further used for the experiments (7, 8, 9). The *in vitro* experiments, a weighed quantity (5-gram) of the extract was dissolved in Dimethyl Sulphoxide (DMSO) or methanol and used. Solution of ascorbic acid and quercetin were used as standards for *in vitro* studies were prepared in distilled DMSO.

Estimation of total phenolics: The total phenolic contents of ethanol extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton (10) and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25-ml volumetric flask, to this 10 ml of distilled water, and 2ml of phenol reagent (20%v/v) was added, and then the volume was made up with 29% sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Antioxidant Assay

Free radical scavenging activity using DPPH radical: The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical (11, 12). A total of 100 µL of the methanolic extract (from 20 to 40 µg ml in DMSO solution). After the incubation period at 37°C for 50 mim.
The absorbance of each solution was determined atb490 nm the corresponding blank readings were also noted and the remaining DPPH was calculated and tabulated in Table 1. IC_{50} values is the concentration of sample required to scavenging 50 % DPPH free radical.

TABLE 1. ANTIOXIDANT ACTIVITY OF *Caesalpina pulcherrima* FLOWERS EXTRACTS USING DPPH METHOD

Test Compound	IC_{50} values ± SE *(µg/mL)
Petroleum ether extract	251.16 ± 1.57
Ethyl acetate extract	16.00 ± 0.57
Methanol extract	26.67 ± 1.20
50% Methanol crude extract	27.34 ± 1.86
Aqueous crude extract	174.82 ± 1.35
Ascorbic acid	74.66 ± 1.52
Quercetin	55.00 ± 0.77

* Average of 8 determination

Nitric oxide scavenging activity: Aqueous solution of Sodium nitropruside at physiological pH spontaneously released nitric oxide, which can be estimated with oxygen to produce nitrite ions, which can be estimated by the use of Griess IIIosvoy reaction (13). The scavengers of nitric oxide reduce the production of nitric oxide. The reaction mixture (3 mL) containing sodium nitropruside (10 mM, 2 ml), phosphate buffer saline (0.5) and the extract or the standard solution (0.5 ml) was incubated at 25 C for 2.5 h. After incubation, 0.5 ml of the reaction mixture containing nitric was pipette out and were mixed with 1 ml of sulphanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min. for completion diazotization. 1 ml of 1- naphthylaimne (5 %) was added, mixed and allowed standing for 30 min. a pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions and is tabulated in Table 2. IC_{50} values is defined as the concentration of sample required to inhibit 50 % of the nitric oxide radical.

TABLE 2. ANTIOXIDANT PROPERTY OF *Caesalpina pulcherrima* FLOWERS EXTRACTS USING NITRIC OXIDE RADICLE INHIBITION ASSAY

Test Compound	IC_{50} values ± SE *(µg/mL)
Petroleum ether extract	23.00 ± 0.85
Ethyl acetate extract	46.02 ± 0.57
Methanol extract	55.09 ± 1.23
50% Methanol crude extract	72.68 ± 1.05
Aqueous crude extract	151.33 ± 0.84
Ascorbic acid	22.66 ± 0.98
Quercetin	18.50 ± 0.88

* Average of 8 determination

Results and Discussion

In vitro assay: The successive extracts of *Caesalpina pulcherrima* exhibited antioxidant activity in DPPH and nitric oxide radical inhibition assay as evidence by the lowering of IC_{50} values (Table 1 and 2). The successive extracts such as petroleum ether, ethyl acetate, methanol, water and 50 % crude methanol extract exhibited IC_{50} values 251.16 ± 1.57, 16.00 ± 0.57, 26.67 ± 1.20, 174.82 ± 1.35 and 27.34 ± 1.86 µg/mL espectively in DPPH and 23.00± 0.85, 46.02 ± 0.57, 55.09 ± 1.23, 151.33 ± 0.84 and 72.68 ± 1.05.98 µg/mL respectively in nitric oxide radical inhibition assay. These values were observed to be more than those which were obtained from the ascorbic acid and quercetin used as standards. Thus it can be stated that free radical oxidative stress has a major role in the pathogenesis of a wide range of clinical disorders resulting from different natural antioxidant defences. Among the five extracts of *Caesalpina pulcherrima* flowers and 2 standards tested for antioxidant activity using DPPH method, the ethyl acetate successive extract showed the maximum antioxidant activity with IC_{50} values of 15.00 ± 0.57 µg/mL respectively. The methanol extract showed antioxidant activity with IC_{50} values 26.67 ± 1.20 µg/mL. The 50 % crude methanolic extract showed IC_{50} values 27.34 ± 1.05.98 µg/mL respectively. However petroleum ether extract exhibited the lowest antioxidant activity with an IC_{50} value of 251.16 ± 1.57 µg/mL. The standards exhibited IC 50 values 74.66 ± 1.52 and 55.00 ± 0.77 µg/mL respectively. Thus from the above investigation it can be stated that antioxidant are essential as they play an important role in the defensive and ageing process of mankind.

References

1. The Wealth of India, C.S.I.R. publication, New Delhi, India,
2. Khare CP. Encyclopedia of Indian Medicinal Plants, Springer-Verlag Berlin Heidelberg, New York.
3. Halliwell B. How to characterize an antioxidant: an update. *Biochem. Soc. Symp,* 1995. 61: 73-101.
4. Squadriato Gl.; Pelor WA. Free Radical Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Biology and Medicine* 1998, 25: 392-403.
5. Sasaki S. Ohta T. Decker EA. Antioxidant activity of water soluble fractions of salmon spermary tissue. J. Agric. Food Chem. 1996, 44: 1682-1686.
6. Miller NJ. Diplock AT. Rice-Evans CA. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *J. Agric. Food Chem* 1995, 43:1794-1801,
7. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi.
8. Harbone JB. Phytochemical Methods-A Guide to Modern Technique of Plant Analysis, Chapman and Hall, London.
9. Garrat DC. The Quantitative Analysis of Drugs, Chapmam and Hall Ltd., Japan
10. Slinard K, Singleton VL, Total phenol analysis automation and comparison with manual methods. *Am J Enol Vitic* 1977, 49–55.
11. Gupta MK; Lagarkha R; Sharma DK. Antioxidant activity of the Successive Extracts of Grewia asiatica Leaves. *Asian. J. Chem.* 19 (5): 3417-3420, 2007
12. Yen GH; Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* 43: 27-32, 1995.
13. Sreejayan, Rao MNA, Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol*1997, 49: 105–107.