Research Article

Antioxidant activity of different leaf extracts of *Ocimium sanctum, Mangifera indica* and *Hibiscus rosa sinensis*

D.Peer basha*[1], K.Ravishankar[2], G.V.N.Kiranmayi[2], Subbarao.M*[3]
[1]Therdose pharma Pvt.Ltd., Hyderabad, Andhra Pradesh, India.
[2]Sri Sai Aditya pharmaceutical sciences & Research, Suram palem, Andhara Pradesh, India.
[3]Acharya nagarjuna University, Guntur, Andhra Pradesh, India.
***Corresponding author's E-mail:** mannamsrao@yahoo.com

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ABSTRACT

The present investigation study was undertaken to investigate the antioxidant properties of three medicinal plants *Mangifera indica*, *Hibiscus rosa-sinensis* and *Ocimum sanctum*. The aqueous, ethanol and hexane extracts of the three plants were prepared and investigated for their antioxidant activity. Free radical scavenger activity estimated by using DPPH, Reducing power method and Phosphomolybdenum Reduction Assay. The antioxidant activity of ethanolic extract of *Hibiscus rosa-sinensis* exhibits strong activity when compared to other two plants. The results of DPPH radical scavenging activity, reducing power method and phosphomolybdenum assay of *Hibiscus rosa sinensis* Ethanolic leaf extract were similar to that of standard Gallic acid.

Keywords: Antioxidant, *Hibiscus rosa-sinensis* and *Ocimum sanctum*, *Mangifera indica*.

INTRODUCTION

Antioxidants are a group of substances which, when present at .low concentration, in relation to oxidizable substances, significantly inhibit or delay oxidative process, while often being oxidized themselves. Antioxidants can retard lipid oxidation through competitive binding of oxygen, retardation of the initiation step, blocking the propagation step by destroying or binding free radicals, inhibition of catalysts or stabilization of hydro peroxides.[1] Antioxidants can scavenge the active forms of oxygen involved in the initiation step of oxidation or can break the oxidative chain reaction by reacting with the fatty acid peroxy radicals to form stable antioxidant radicals, which are either too unreactive for further reactions or form non-radical products .

Tulsi is an important symbol of the Hindu religious tradition. Found in most of the Indian homes and worshipped .Tulsi belongs to plant family Lamiaceae. Tulsi extracts are used in Ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria.

The herb *Hibiscus rosa-sinensis* Linn.belonging to the family Malvacecae and is commonly known as Jasvand.[2] Flowers are used in all kinds of inflammation; internally they are prescribed in the form of decoction of bronchial catarrh, as a becenic and sudorific roots are mucilaginous and demulcent, valuable in.

Mangoes belong to genus *Mangifera indica* which consists of about 30 species of tropical fruiting trees in the flowering plant family Anacardiaceae. The major nutritional antioxidants, vitamin E, vitamin C and β-carotene, may be beneficial to prevent several chronic disorders.[3] considerable interest has arisen in the possible reinforcement of antioxidant defenses, both for chemoprevention and treatment purposes.[4]

The present investigation study was undertaken to investigate the antioxidant properties of three medicinal plants *Mangifera indica*, *Hibiscus rosa-sinensis* and *Ocimum sanctum* by DPPH, Reducing power method and Phosphomolybdenum Reduction Assay.

MATERIALS AND METHODS

Plant Collection

The leaves of *Ocimum sanctum* (OS), *Mangifera Indica* (MI) and *Hibiscus rosa-sinensis* (HS) were collected from the Tirumala hills Sri Venkateswara University, Tirupati.

Drying of plant material

The leaves of these plants were washed with distilled water to remove any impurities and finally dried under shade. Then the .dried leaves were ground into a powder with warring Commercial laboratory blender and further milled (mesh size 850 um).

Preparation of extracts

The extraction was performed in Analytical department, Therdose Pharma. Three solvents were used for the extraction and they are hexane 99% (Rankem, RFCL Limited, New Delhi), ethanol 99.8% (Fisher Scientific, Thermo fisher scientific India Pvt, Mumbai) and Milli Q water.

Chemicals and Equipments

Gallic acid, DPPH, Ferricyanide, Phosphate buffer, Trichloroaceticacid, 0.1% ferric chloride, % potassium ferricyanide, 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate.

In-Vitro Antioxidant Study

DPPH Free Radical Scavenging Activity[5]

The free radical scavenging activity was evaluated by the DPPH method, 0.1mM solution of DPPH in ethanol was prepared, Gallic acid was taken as reference standard, different concentrations of the extracts (100, 300 and 500 µg/ml) and standard drug (1, 2.5 and 5 µg/ml) were prepared using ethanol. 1.0 ml of 0.1 mM of DPPH solution was mixed with 3.0 ml of all the concentrations of extract and standard separately. A blank was prepared using 0.1 mM DPPH and ethanol mixture without adding extract. These mixtures are kept in dark about 30 min and the optical density was measured at 517 nm. The experiment was repeated triplicate. The percentage inhibition of the DPPH activity was calculated by using the following formula.

$$\text{DPPH Scavenged (\%)} = [(A_o - A_1) / A_o] \times 100$$

Where, A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. Gallic acid was used as positive controls.

Reducing power method

Different concentrations of the extracts (100, 300 and 500 µg/ml) and standard drug (1, 2.5 and 5 µg / ml) were prepared using distilled water. 1% potassium ferricyanide, 10% Trichloroaceticacid, 0.1% ferric chloride and 0.2 M Phosphate buffer (pH 6.6) were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1 ml of each concentration of extract and standard were taken separately and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide. Incubate all these samples at 50°c for 20 min. Then add 2.5 ml of 10% Trichloroaceticacid and centrifuge at 3000rpm for 10 min. now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5 ml of freshly prepared ferric chloride. Then the absorbance was measured at 700 nm.

Table 1: DPPH Free Radical Scavenging Activity of different extracts of the three plants

Tested Material	Concentration (µg/ml)	% Inhibition ± SEM	$IC_{50\mu g/ml}$
Standard Gallic acid	1 2.5 5	24.32 ± 1.09 40.68 ± 0.05 66.16 ± 0.42	3.3 µg/ml
Aqueous extract of *HS*	100 300 500	62.32±0.16 72.65±0.68 79.36±0.23	3.85 µg/ml
Ethanolic extract of *HS*	100 300 500	68.23±0.23 76.29±0.32 83.25±0.52	**2.43 µg/ml**
Hexane extract of *HS*	100 300 500	67.59 ± 0.14 75.92 ± 0.49 78.82 ± 0.53	4.1 µg/ml
Aqueous extract of *MI*	100 300 500	60.32±0.26 68.65±0.48 72.36±0.13	3.52 µg/ml
Ethanolic extract of *MI*	100 300 500	64.23±0.22 70.23±0.33 78.25±0.12	2.98 µg/ml
Hexane extract of *MI*	100 300 500	60.12 ± 0.13 69.32 ± 0.19 72.32 ± 0.23	3.63 µg/ml
Ethanolic extract of *OS*	100 300 500	62.29 ± 0.18 68.92 ± 0.42 74.82 ± 0.23	3.18 µg/ml
Hexane extract of *OS*	100 300 500	64.23±0.23 69.29±0.12 75.25±0.42	8.67 µg/ml
Aqueous extract of *OS*	100 300 500	63.32±0.26 67.65±0.18 73.36±0.43	2.28 µg/ml

Table 2: Reducing power method of different extracts of the three plants

Tested Material	Concentration (µg/ml)	Absorbance ± SEM
Standard Gallic acid	1	0.3096 ± 0.0002
	2.5	0.3723 ± 0.0003
	5	0.5292 ± 0.0001
Aqueous extract of *HS*	100	0.086 ± 0.0001
	300	0.122 ± 0.0005
	500	0.275 ± 0.0005
Ethanolic extract of *HS*	100	0.079±0.0004
	300	0.222±0.0003
	500	0.4235±0.0005
Hexane extract of *HS*	100	0.0752±0.0004
	300	0.135±0.0003
	500	0.325±0.0005
Aqueous extract of *MI*	100	0.072±0.0004
	300	0.132±0.0003
	500	0.239±0.0005
Ethanolic extract of *MI*	100	0.081±0.0004
	300	0.142±0.0003
	500	0.321±0.0005
Hexane extract of *MI*	100	0.0653±0.0003
	300	0.159±0.0004
	500	0.432±0.0008
Aqueous extract of *OS*	100	0.087±0.0005
	300	0.132±0.0006
	500	0.459±0.0008
Ethanolic extract of *OS*	100	0.092±0.0007
	300	0.152±0.0006
	500	0.325±0.0005
Hexane extract of *OS*	100	0.065±0.0003
	300	0.232±0.0004
	500	0.321±0.0008

Table 3: Phosphomolybdenum Assay of different extracts of the three plants

Tested material	Concentration (µg/ml)	Absorbance ± SEM
Standard Gallic acid	1	0.1626 ± 0.00003
	2.5	0.3149 ± 0.0015
	5	0.4291 ± 0.0003
Aqueous extract of HS	100	0.1416 ± 0.0002
	300	0.2445 ± 0.0001
	500	0.3084 ± 0.0003
Ethanolic extract of *HS*	100	0.1521 ± 0.0003
	300	0.3245 ± 0.0006
	500	0.4261 ± 0.0008
Hexane extract of *HS*	100	0.132 ± 0.0003
	300	0.2321 ± 0.0007
	500	0.310 ± 0.0005
Ethanolic extract of *MI*	100	0.134 ± 0.0004
	300	0.251 ± 0.0006
	500	0.3165 ± 0.0008
Aqueous extract of *MI*	100	0.1321 ± 0.0003
	300	0.2145 ± 0.0006
	500	0.312 ± 0.0008
Hexane extract of *MI*	100	0.111 ± 0.0002
	300	0.2211 ± 0.0006
	500	0.362 ± 0.0004
Ethanolic extract of *OS*	100	0.1330 ± 0.0001
	300	0.1045 ± 0.0001
	500	0.2104 ± 0.0002
Aqueous extract of *OS*	100	0.1220 ± 0.0002
	300	0.1224 ± 0.0005
	500	0.3246 ± 0.0007
Hexane extract of *OS*	100	0.100 ± 0.0002
	300	0.1321 ± 0.0006
	500	0.260 ± 0.0004

Phosphomolybdenum Reduction Assay

The antioxidant activity of the extracts (100, 300 and 500 µg/ml) was evaluated by the phosphomolybdenum method. The assay is based on the reduction of MO (VI)-MO (V) by the extract and subsequent formation of green phosphate / MO (V) complex at acid pH. 0.3ml of each concentration of the extract and standard were taken separately and mixed with 3 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°c for 90 min. Then the absorbance of the solution was measured at 695nm using spectrophotometer against blank after cooling to room temperature. Ethanol (0.3ml) in the place of extract was used as blank.

RESULTS AND DISCUSSION

Antioxidant activity

The results indicated that Ethanolic leaf extract of *Hibiscus rosa sinensis* has a noticeable effect of scavenging free radicals evident from Table 1. It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic condition.[6] Extracts of three plants react with free radicals which are the major initiator of the autoxidation chain of fat, there by terminating the chain reaction.[7,10] It is thus apparent that extracts of three plants are free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. DPPH radical scavenging

activity of *Hibiscus rosa sinensis* Ethanolic leaf extract are similar with that of standard Gallic acid.

The antioxidant activity of Ethanolic leaf extract of *Hibiscus rosa sinensis* and Gallic acid *have* been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[1,8,9] The reducing capacity of various extracts of three plants and Gallic acid indicate their potential antioxidant activity Table 2.

The antioxidant capacity of extracts of three *plants* were determined by phosphomolybdenum assay and the highest absorbance was recorded for Ethanolic extract of *Hibiscus rosa sinensis* Table 3. The antioxidant capacity of the extracts of three plants were measured by phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/ Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of extracts was found to increase with increase in concentration.[11]

CONCLUSION

The total antioxidant capacity of extracts of three *plants* was determined by phosphomolybdenum assay and the highest absorbance was recorded for Ethanolic extract of *Hibiscus rosa sinensis* Table 3. The antioxidant capacity of the extracts of three plants was measured by phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/ Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of extracts was found to increase with increase in concentration.

In this present study an attempt has been made to identify the biological potentiality of the plants to evaluate for their biological activities and for this study the leaf extracts of *Ocimum sanctum, Mangifera indica* and *Hibiscus rosa-sinensis* were collected from the Tirumala hills. After preparation of extracts, three selected plants they were subjected for screening of invitro antioxidant. Based on the significant results obtained the following conclusions were postulated.

From the results obtained it is thus apparent that extracts of three plants are free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. The results of DPPH radical scavenging activity, reducing power method and phosphomolybdenum assay of *Hibiscus rosa sinensis* ethanolic leaf extract were similar to that of standard Gallic acid.

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