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In-vitro Comparative Antioxidant Activity of Ethanolic Extracts of *Glycosmis pentaphylla* and *Bauhinia variegata*

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Abstract

This study was conducted to investigate the antioxidant effect of the ethanolic extracts of the leaves of *Glycosmis pentaphylla and Bauhinia variegata*. The antioxidant activity was evaluated by various antioxidant assays, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), nitric oxide and hydrogen peroxide (H_2O_2) scavenging method. The antioxidant activities were compared to standard antioxidant ascorbic acid. Ethanolic crude extract of the plant *Leucas aspera* showed maximum significant antioxidant activity in DPPH, ABTS, nitric oxide and H_2O_2 scavenging methods. The findings of the present study suggested that these two plants could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

Key Words: *Glycosmis pentaphylla*, *Bauhinia variegata*, Antioxidant activity

Introduction

Nature still serves as the man's primary source for the cure of his ailments. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. However, the potential of higher plants as source for new drugs is still largely explored [1]. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases and have potential benefits to the society. Natural antioxidants from plant sources are potent and safe due to their harmless nature, wild herbs have been investigated for their antioxidant properties [2].

Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods [3].

Glycosmis pentaphylla Correa, Rutaceae is commonly known as tooth-brush plant. Infusion of leaves of *Glycosmis pentaphylla* is used in fever, liver disorders, cough and jaundice, as tonic and appetiser to women after delivery [4,5]. The plant *Bauhinia variegata* Linn. (Caesalpiniaceae) commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic [6].

The biomechanical mechanism of liver injury includes metabolite or coenzyme effects (depletion or stimulation), enzyme effects (inhibition or stimulation), activation to a more toxic form, and membrane disturbances. The antioxidants play an important role in liver protection by inhibiting the free radical formations [7].

Experimental

Plant Material

The leaves of *Glycosmis pentaphylla* correa and *Bauhinia variegata* were collected from the local areas around the Mangalore, Karnataka, India, and authenticated by the botanist and voucher specimens of the same has been maintained in the laboratory of Phytochemistry and Pharmacognosy, NIMS Institute of Pharmacy, Shobha Nagar, Jaipur, India (NIMS/2010/NGP, NIMS/2010/RBV) respectively. The leaves of *Glycosmis pentaphylla*, and *Bauhinia variegata* were shade dried and chopped into small pieces.

Preparation of extracts

The shade dried leaves of *Glycosmis pentaphylla*, and *Bauhinia variegata* were powdered (300g) and extracted with Ethanol (99.99%) in two different soxhlet extractors exhaustively for 20-24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-500 C) using flash evaporator. The dried extracts obtained were used in the study [8].

Chemicals

1, 1 - diphenyl-2-picrylhydrazyl and 2,2'-azino-bis (3 ethylbenzthiazoline-6 sulfonic acid) (Aldrich),Ascorbic acid (SD

Fine Chemicals Ltd.). All chemicals used were of analytical grade.

Phytochemical studies

Preliminary phytochemical investigation of ethanolic extract of the plant materials was carried out for qualitative determination of the groups of organic compounds present in them, by using different tests for alkaloids, carbohydrates, proteins, tannins, flavanoids, steroids etc [9].

DPPH radical scavenging activity

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. The assay was carried out in a 96 well microtitre plate. To 100 µl of DPPH solution, 10 µl of various concentrations of the extracts or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ºC for 30 min. and absorbance was measured at 490 nm using ELISA reader [10].

ABTS radical cation decolourisation assay

ABTS (54.8 mg) was dissolved in 50ml of distilled water to 2mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at734 nm [11].

Scavenging of nitric oxide

Sodium nitroprusside (5µM) in std. phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25 °C for 5 hr. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The activity was compared with ascorbic acid, which was used as a standard antioxidant [12,13].

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide [14].

IC50 value is the concentration of the sample required to scavenge 50 % free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated by using the following formula [15].

Scavenging activity (%) = [{OD control - OD sample}/ OD control] x 100

Results and Discussion

The percentage yield of ethanolic extracts was found to be 11.4 and 8.6. Preliminary phytochemical screening of the crude extracts showed the presence of steroids, alkaloids, glycosides, saponins, flavonoids, tannins and carbohydrates.

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. DPPH is relatively stable nitrogen centred free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent as a result of which electron become paired off forming the corresponding hydrazine. The solution therefore looses colour stoichometrically depending on the number of electrons consumed which is measured spectrometrically at 517 nm [16]. From results it may be postulated that extracts of *Glycosmis pentaphylla,* and *Bauhinia variegata* have hydrogen donors, thus scavenge free radical DPPH.

The principle behind the ABTS assay technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation (ABTS+) a blue green chromogen. In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS, the absorbance of which is measured at 734nm. The free radical scavenging activity by this method of ethanolic extracts of *Glycosmis pentaphylla,* and *Bauhinia variegata* suggests for the antioxidant activity.

The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for NO scavenging activity [17]. The chromophore formation was not complete in the presence of different test extracts, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts increases.

H2O2 is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H2O2 can probably react with Fe+2 and possibly Cu+2 to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H2O2 by extracts of *Glycosmis pentaphylla* and *Bauhinia variegata* may result from its antioxidant and free radical scavenging activity.

Results obtained in the present study indicate that extracts of *Glycosmis pentaphylla,* and *Bauhinia variegata* showed a free radical scavenging activity which was significantly comparable to free radical scavenging activity of ascorbic acid. (Table 1-4).

Table 1: DPPH scavenging activity of ethanolic extracts of leaves of *Glycosmis pentaphylla* and *Bauhinia variegate*

Sl. no.	Sample	DPPH Percentage scavenging (Mean ± SEM) of triplicates					IC_{50}
		10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
1	GPEE	20.83 ± 1.93**	40.46 ± 2.55**	54.54 ± 3.09**	71.34 ± 1.91**	85.02 ± 3.05**	28.5
2	BVEE	20.11 ± 1.41**	39.36 ± 1.52**	52.81 ± 1.69**	70.27 ± 2.12**	83.72 ± 2.21**	29.3
3	Ascorbic acid	24.73 ± 1.84	48.64 ± 3.39	64.64 ± 3.80	77.23 ± 5.12	92.09 ± 4.36	23

Table 2: ABTS scavenging activity of ethanolic extracts of leaves of *Glycosmis pentaphylla and Bauhinia variegate*

Sl. no.	Sample	ABTS Percentage scavenging (Mean ± SEM) of triplicates					IC_{50}
		10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
1	GPEE	22.43 ± 1.36**	42.45 ± 2.43**	59.11 ± 2.34**	72.96 ± 3.13**	79.74 ± 3.53**	26.2
2	BVEE	21.25 ± 1.42**	41.71 ± 1.71**	58.14 ± 0.38**	71.18 ± 2.34**	77.82 ± 2.98**	27.7
3	Ascorbic acid	28.47 ± 1.24	48.32 ± 2.05	64.19 ± 3.95	76.89 ± 2.98	87.18 ± 2.85	22.8

Table 3: NO radical scavenging activity of ethanolic extracts of leaves of *Glycosmis pentaphylla* and *Bauhinia variegate*

Sl. no.	Sample	Nitric oxide radical scavenging (Mean ± SEM) of triplicates					IC_{50}
		10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
1	GPEE	32.97 ± 0.93**	43.65 ± 2.01**	49.51 ± 1.45**	54.19 ± 2.07**	61.06 ± 2.35**	31.0
2	BVEE	31.81 ± 0.89**	42.61 ± 1.79**	48.52 ± 1.93**	53.46 ± 2.14**	60.37 ± 1.46**	33.4
3	Ascorbic acid	38.55 ± 0.94	48.06 ± 1.82	54.32 ± 1.93	59.42 ± 1.75	65.21 ± 3.04	24.0

Table 4: Hydroxyl radical scavenging activity of ethanolic extracts of leaves of *Glycosmis pentaphylla* and *Bauhinia variegate*

Sl. no.	Sample	Hydroxyl radical scavenging (Mean ± SEM) of triplicates					IC_{50}
		10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
1	GPEE	26.12 ± 0.53**	42.24 ± 1.19**	55.21 ± 3.45**	64.53 ± 2.53**	70.37 ± 2.47**	26.2
2	BVEE	24.18 ± 1.92**	41.34 ± 2.03**	53.23 ± 1.84**	61.92 ± 2.91**	68.81 ± 1.89**	7.4
3	Ascorbic acid	38.73 ± 0.97	52.85 ± 1.85	64.19 ± 3.95	71.89 ± 2.98	78.18 ± 2.85	8.3

n=3, values are mean ± S.D; **Significant P < 0.01 as compared with ascorbic acid (used as Standard); GPEE:- Ethanolic extract of leaves of *Glycosmis pentaphylla*; BVEE:- Ethanolic extract of leaves of *Bauhinia variegata*

Conclusion

The results obtained in present study indicate that extracts of leaves of *Glycosmis pentaphylla,* and *Bauhinia variegata* inhibits free radical scavenging activity. The overall antioxidant activity of these extracts might be attributed to its flavonoids, phenolic and other phytochemical constituents. These could be a source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

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