Research Article

# *INVITRO* ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS OF THE PLANT *GLYCOSMIS PENTAPHYLLA* CORREA

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**ABSTRACT**

This study was conducted to investigate the antioxidant effect of the different crude extracts (Petroleum ether, ethanolic and aqueous) of the plant *Glycosmis pentaphylla* correa. 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. *Glycosmis pentaphylla* correa ethanolic crude extract showed a significant antioxidant activity in DPPH, ABTS, nitric oxide and $H_2O_2$ scavenging methods. The findings of the present study suggested that *Glycosmis pentaphylla* 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.

**Keywords:** *Glycosmis pentaphylla* correa, antioxidant activity, DPPH, ABTS.

## 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 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.[6]

## MATERIALS AND METHODS

### Plant Material

The plants of *Glycosmis pentaphylla* correa were collected from the local areas around the Mangalore, Karnataka, India, and authenticated by the botanist and a voucher specimen (NIMS/2010/NGP) is being maintained in the laboratory of Phytochemistry and Pharmacognosy, NIMS Institute of Pharmacy, Shobha Nagar, Jaipur, India. The whole plant then, including root, stem, leaves and flower were shade dried and chopped into small pieces.

### Preparation of extracts

The shade dried plant *Glycosmis pentaphylla* was powdered (300g) and extracted with three different solvents petroleum ether, ethanol (99.99%), and water in three different soxhlet extractors exhaustively for 20-24 hours. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50$^0$C) using flash evaporator. The dried extracts obtained were used in the study.

### 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 petroleum ether extract, ethanolic extract, and aqueous 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, Steroids etc.[8]

**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 extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37ºC for 30 min. and absorbance was measured at 490 nm using ELISA reader.[9]

**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 minutes at 734nm.[10]

**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.[11,12]

**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.[13]

$IC_{50}$ 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.[14]

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

## RESULTS AND DISCUSION

The percentage yield of petroleum ether, ethanolic and aqueous extracts was found to be 9.8, 11.4 and 14.6. Preliminary phytochemical screening of the crude extracts of the plant *Glycosmis pentaphylla* 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 centered 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.[15] From results it may be postulated that extracts of *Glycosmis pentaphylla* 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 extracts of *Glycosmis pentaphylla* possessed antioxidant activity with maximum $IC_{50}$ value being 26.2mcg/ml for ethanolic extract suggest the free radical scavenging 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.[16] The chromophore formation was not complete in the presence of *Glycosmis pentaphylla* extract, which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts increases.

$H_2O_2$ 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, $H_2O_2$ 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 $H_2O_2$ by extracts of *Glycosmis pentaphylla* may result from its antioxidant and free radical scavenging activity.

Results obtained in the present study indicate that extracts of *Glycosmis pentaphylla* showed a free radical scavenging activity but ethanolic extract showed the maximum antioxidant activity which was significantly comparable to free radical scavenging activity of ascorbic acid. (Table & Figure 1-4).

**Table 1:** DPPH scavenging activity of extracts of *Glycosmis pentaphylla*

| Sample | DPPH Percentage scavenging (Mean ± SEM ) of triplicates | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | 10µg/ml | 20µg/ml | 30µg/ml | 40µg/ml | 50µg/ml | |
| Petroleum ether extract | 17.71 ± 1.29** | 36.23 ± 1.72** | 50.52 ± 3.02** | 68.00 ± 3.19** | 79.71 ± 3.22** | 30 |
| Ethanol extract | 20.83 ± 1.93** | 40.46 ± 2.55** | 53.54 ± 3.09** | 71.34 ± 1.91** | 85.02 ± 3.05** | 28.5 |
| Aqueous extract | 12.21 ± 2.14 | 29.53 ± 1.85 | 41.21 ± 2.46 | 51.72 ± 2.68 | 67.17 ± 1.45 | 38.5 |
| 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 extracts of *Glycosmis pentaphylla*

| Sample | ABTS Percentage scavenging (Mean ± SEM ) of triplicates | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | 10µg/ml | 20µg/ml | 30µg/ml | 40µg/ml | 50µg/ml | |
| Petroleum ether extract | 17.24 ± 1.13** | 31.21 ± 2.02** | 54.75 ± 2.03** | 68.43 ± 1.62** | 74.21 ± 1.81** | 29.8 |
| Ethanol extract | 22.43 ± 1.36** | 42.45 ± 2.43** | 59.11 ± 2.34** | 72.96 ± 3.13** | 79.74 ± 3.53** | 26.2 |
| Aqueous extract | 13.52 ± 1.04** | 26.17 ± 1.27** | 46.31 ± 1.83** | 60.91 ± 2.13** | 66.63 ± 3.27** | 33.6 |
| Ascorbic acid | 28.47 ± 1.24 | 48.32 ± 2.05 | 64.19 ± 3.95 | 71.89 ± 2.98 | 78.18 ± 2.85 | 22.8 |

**Table 3:** NO radical scavenging activity of different extracts of *Glycosmis pentaphylla*

| Sample | Nitric oxide radical scavenging (Mean ± SEM ) of triplicates | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | 10µg/ml | 20µg/ml | 30µg/ml | 40µg/ml | 50µg/ml | |
| Petroleum ether extract | 28.76 ± 1.19 | 38.98 ± 1.95 | 45.42 ± 2.03 | 50.98 ± 2.95 | 55.86 ± 1.93 | 36.5 |
| Ethanol extract | 32.97 ± 0.93 | 43.65 ± 2.01 | 49.51 ± 1.45 | 54.19 ± 2.07 | 61.06 ± 2.35 | 31.0 |
| Aqueous extract | 26.98 ± 0.89 | 36.36 ± 1.79 | 42.05 ± 0.93 | 48.94 ± 2.97 | 52.07 ± 1.86 | 41.5 |
| Ascorbic acid | 38.55 ± 0.94 | 43.06 ± 1.82 | 54.32 ± 1.93 | 57.42 ± 1.75 | 65.21 ± 3.04 | 24.0 |

**Table 4:** Hydroxyl radical scavenging activity of different extracts of *Glycosmis pentaphylla*

| Sample | Hydroxyl radical scavenging (Mean ± SEM ) of triplicates | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | 10µg/ml | 20µg/ml | 30µg/ml | 40µg/ml | 50µg/ml | |
| Petroleum ether extract | 16.62 ± 2.31** | 29.24 ± 2.92** | 39.49 ± 1.74** | 51.34 ± 2.32** | 60.15 ± 2.48** | 38.1 |
| Ethanol extract | 26.12 ± 0.53** | 42.24 ± 1.19** | 55.21 ± 3.45** | 64.53 ± 2.53** | 70.37 ± 2.47** | 26.2 |
| Aqueous extract | 12.38 ± 0.59** | 20.63 ± 1.93** | 32.75 ± 2.38** | 42.49 ± 1.29** | 51.08 ± 2.98** | 48.0 |
| Ascorbic acid | 38.73 ± 0.97 | 52.85 ± 1.85 | 64.19 ± 3.95 | 71.89 ± 2.98 | 78.18 ± 2.85 | 18.3 |

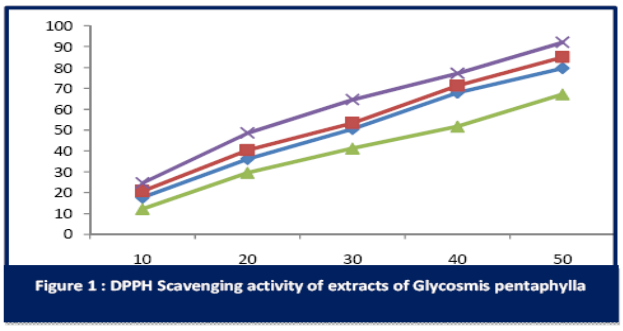

Figure 1 : DPPH Scavenging activity of extracts of Glycosmis pentaphylla

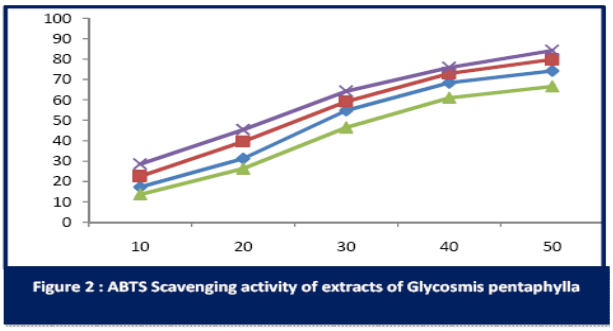

Figure 2 : ABTS Scavenging activity of extracts of Glycosmis pentaphylla

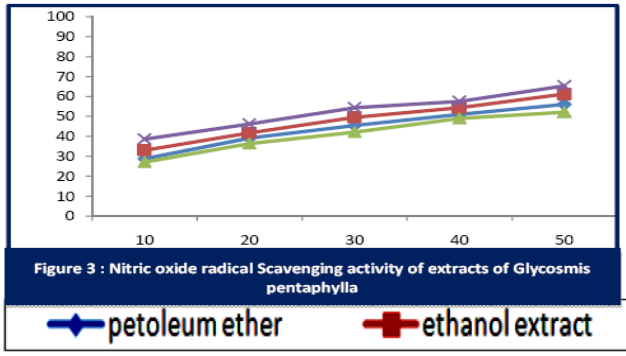

Figure 3 : Nitric oxide radical Scavenging activity of extracts of Glycosmis pentaphylla

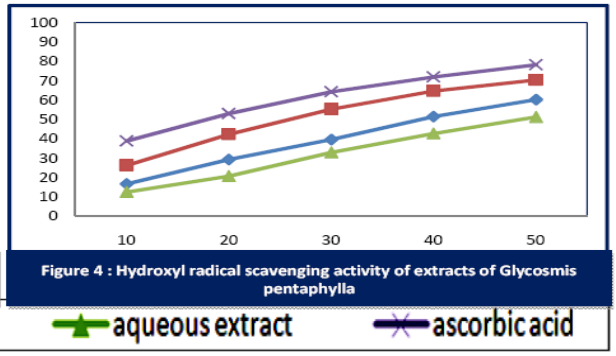

Figure 4 : Hydroxyl radical scavenging activity of extracts of Glycosmis pentaphylla

petoleum ether ethanol extract aqueous extract ascorbic acid

## CONCLUSION

The results obtained in present study indicate that extracts of *Glycosmis pentaphylla* inhibits free radical scavenging activity and have hepatoprotective action also. The overall antioxidant and hepatoprotective activity of *Glycosmis pentaphylla* extract might be attributed to its triterpenoid and polyphenolic content and other phytochemical constituents. It 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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