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ASSESSMENT OF *IN VIVO* AND *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *NARINGI CRENULATA* (ROXB.) NICOLSON

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ABSTRACT

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. They are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions.The aim of present research was to investigate the antioxidant potential of different plant parts of *N. crenulata* both *in vivo* and *in vitro*.The antioxidant activity of the methanolic extracts of *in vivo* and *in vitro* plant parts of *Naringi crenulata* was determined on the basis of their scavenging activity of the stable 2, 2- diphenyl-2-picryl hydrazyl (DPPH) free radical. The methanolic extracts of *Naringi crenulata* and Ascorbic acid (standard) showed DPPH free radical scavenging activity in a concentration range of 10-1000 µg/ml and its inhibition ranged from 21.3 -83.4 % and 49.68-94.60 % respectively. Methanolic extracts of root were found to have maximum IC_{50} value (898.49 µg/ml) followed by leaf (572.51 µg/ml), callus (279.49 µg/ml) and stem (147.80 µg/ml).Standard antioxidant used was Ascorbic acid (1mg/ml) having 24.79 µg/ml IC_{50} value. Maximum antioxidant potential was found in methanolic extract of stem while minimum was found in methanolic extract of root. This suggests that the methanolic extract of *Naringi crenulata* contains compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.The high antioxidant capacity observed for methanolic extract of *Naringi crenulata* suggested that this plant could be used as an additive in the pharmaceutical industry providing good protection against oxidative damage.

KEYWORDS: ***Naringi crenulata,*** **Antioxidant, ascorbic acid, methanolic extract and DPPH free-radical scavenging assay**

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INTRODUCTION

Antioxidant means "against oxidation." An antioxidant is a substance that retards or prevents deterioration, damage or destruction by oxidation. Human consumption of antioxidants has many health benefits, including the prevention of oxidative damage associated with free radical damage and their contribution to disease such as cancer, the etiology of aging, coronary heart disease, ischemia – reperfusion injury [1], multiple sclerosis, Parkinson's disease, senile dementia, autoimmune disorders and asbestosis.[2]Antioxidant's potential in preventing damage associated with free radicals and their implied role in disease has encouraged the search for compounds with potent antioxidant activity. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench and/or scavenge them and to protect the body against their deleterious effects.[3] Recently there has been increasing interest in free radicals in biological system and their implied role as causative agent in the etiology of a variety of pathological physiologies. Plant based antioxidants are now preferred as an alternative to synthetic ones because of safety concerns. Therefore, many researches regarding antioxidant potential from plant source were carried out. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity viz *Coleus spicatus*[4], *Ficus glomerata*[5],*Justicia californica.*[6] Therefore the aim of the present research was to investigate the antioxidant potential of different plant parts of *N. crenulata* both *in vivo* and *in vitro*. Free radical scavenging activity was detected with stable DPPH and colour formation.

MATERIAL AND METHODS

Collection of Plant material

Plant parts of *N. crenulata* were collected from the campus of University of Rajasthan, Jaipur and specimen was compared with the voucher specimen available at Herbarium of Department of Botany, University of Rajasthan, Jaipur. The fresh plant samples (*N. crenulata*: leaf, stem and root) were collected and washed individually under running tap water to remove soil particles and other dirt. Furthermore, *in vitro* callus obtained on MS medium fortified with NAA (2.0 mg/l) and BAP (0.5 mg/l) was also taken for the present study. The *in vivo* leaf, stem and root were dried in the laboratory at room temperature for 7 days while the callus was dried at 60ºC for 2 days in an oven. All dried samples were ground well into a fine powder in a mixer grinder. The powder was stored in air tight bottles at room temperature before extraction.

Preparation of the Plant extracts

A fixed weight (10 gm) of each powdered material i.e. leaf, stem, root and callus was soxhlet extracted in 250 ml of 80 % methanol for 72 hours. At the end of extraction each extract was passed through Whatman No.1 filter paper and evaporated under vacuum. All extracts were stored at 4ºC in a refrigerator until used for further analysis.

Chemicals

DPPH (Sigma Aldrich, Mumbai), methanol, ascorbic acid (Merk, Germany). All other reagents were of analytical grade.

DPPH Assay

The antioxidant activity of the methanolic extracts of *in vivo* and *in vitro* plant parts of *N. crenulata* was determined on the basis of their scavenging activity of the stable 2, 2- diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1ml of each solution of different concentrations (10-1000µg/ml) of the extracts was added to 2 ml of 0.002% methanolic DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (10-1000 µg/ml).Then the % inhibition was calculated by the following equation:

% radical scavenging activity = (absorbance of blank-absorbance of sample/ absorbance of blank) x 100

From calibration curves, obtained from different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

Procedure

At first, 8 test tubes were taken to make aliquots of 8 concentrations (10, 50, 100, 200, 400, 600, 800 and 1000µg/ml) with the samples.Each plant extract and ascorbic acid were weighed accurately and dissolved in methanol to make required concentrations by dilution technique. Here ascorbic acid was taken as standard.DPPH was weighed and dissolved in methanol to make 0.002% (w/v) solution. To dissolve homogeneously magnetic stirrer was used.After making the desired concentrations, 2ml of 0.002% DPPH solution was applied on each test tube by pipette.The room temperature was recorded and the test tubes were kept for 30 mins in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only methanol was taken as blank.After 30 minutes, the absorbances of each test tube were taken by a UV spectrophotometer.IC_{50} 's were measured from % Inhibition *vs.* Conc graph.

STATICAL ANALYSIS

All the values are expresses as mean ± SD and data was analyzed by One-way ANOVA, using Graphpad INSTAT. The post-hock analysis was carried out by Dunnet's multiple comparison tests to estimate the significance of difference between individual groups ($P<0.001$).

RESULT AND DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract.

In the present study, methanolic extracts of the different plant parts and callus of *N. crenulata* showed potential free-radical scavenging activity. Methanolic extracts of different plant parts of *Naringi crenulata* showed different levels of DPPH activity. Results are summarized in Table 1, Plate -1 Fig A. In DPPH radical-scavenging activity assay, the radical scavenging activity of the methanolic extract of the different plant parts of *Naringi crenulata* increases with increasing concentration (Table 1). The methanolic extract of *Naringi crenulata* and Ascorbic acid (standard) showed DPPH free radical scavenging activity in a concentration range of 10-1000 µg/ml and its inhibition ranged from 21.3 -83.4 % and 49.68-94.60 % respectively. Methanolic extracts of root were found to have maximum IC_{50} value (898.49 µg/ml) followed by leaf (572.51 µg/ml), callus (279.49 µg/ml) and stem (147.80 µg/ml). Standard antioxidant used was Ascorbic acid (1mg/ml) having 24.79 µg/ml IC_{50} value. The results are reported in Plate1 – Fig. B.The highest DPPH radical scavenging activity was detected in methanolic extract of stem followed by methanolic extract of callus, leaf and root respectively. These activities are less than that of ascorbic acid. During the present study the IC_{50} of the methanolic extract of root was maximum followed by leaf, callus and stem. IC_{50}value of *Naringi crenulata* stem and ascorbic acid were found to be 147.80 µg/ml and 24.79 µg/ml respectively. This indicates that the methanolic extract of stem was potently active than other plant parts of *Naringi crenulata.* The highest DPPH radical scavenging activity was detected in methanolic extract of stem followed by methanolic extract of callus, leaf and root respectively. These activities are less than that of ascorbic acid. Maximum antioxidant potential was found in methanolic extract of stem while minimum was found in methanolic extract of root. This suggests that the methanolic extract of *Naringi crenulata* contains compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Similar results in which methanolic extract possess strong antioxidant activity was reported in *Ionidium suffruticosum*[8]. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity viz *Persea americana* and *Cnidosculus aconitifolius*[9], *Pleurotus ostreatus*[10], *Alpinia galangal*[11], *Cocculus hirsutus*[12] ,*Brugeiera Gymnorrhiza and Aegialitis rotundifolia*.[13]

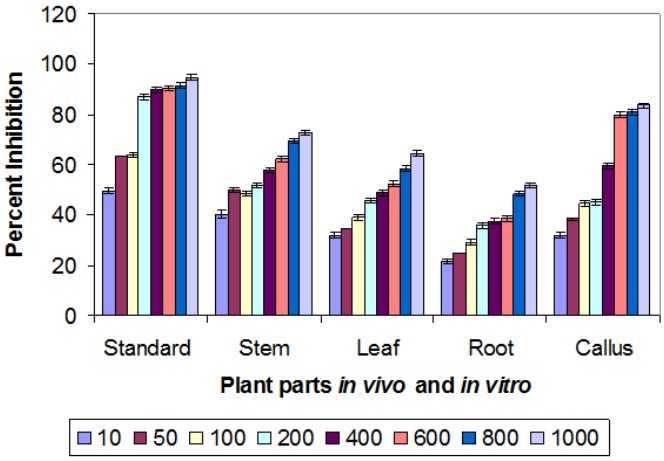

Figure A
Estimation of DPPH free radical scavenging activity

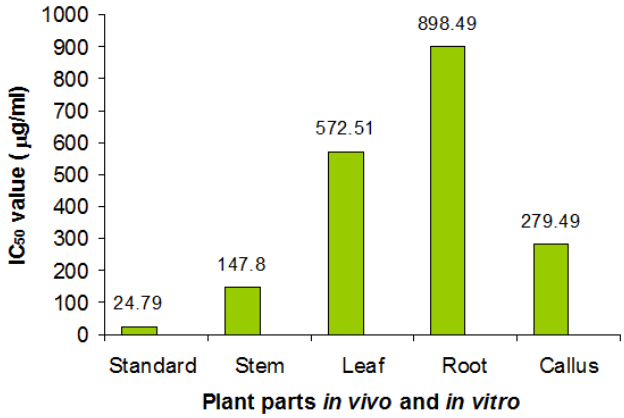

Figure B
Estimation of IC_{50} value PLATE - 1

Table 1
Percentage DPPH radical scavenging activity of methanolic extracts of Naringi crenulata plant parts in vivo and in vitro

S. No	Conc. (µg/ml)	Percentage inhibition by methanolic extract of *Naringi crenulata* ±S.E.M				
		Standard	Stem	Leaf	Root	Callus
1	10	49.68±1.092	40.20±1.850	31.8±1.076	21.3±1.114	32.1±1.104
2	50	63.25±1.122	49.91±1.980	34.6±1.054	24.7±1.108	38.3±1.124
3	100	63.75±1.104	48.49±1.120	39.2±1.049	29.3±1.110	44.8±1.086
4	200	86.87±1.124	51.95±1.124	45.8±1.09	35.7±1.120	45.1±1.112
5	400	89.93±1.086	57.57±1.075	48.8±1.240	37.3±1.091	59.3±1.076
6	600	90.29±1.120	62.19±1.120	52.4±1.180	38.4±1.041	79.7±1.053
7	800	91.55±1.103	69.39±1.114	58.3±1.078	48.2±1.096	81.1±1.096
8	1000	94.60±1.107	72.49±1.108	64.3±1.085	51.7±1.042	83.4±1.042
		IC_{50}=24.79µg/ml	IC_{50}=147.80 µg/ml	IC_{50}=572.51 µg/ml	IC_{50}=898.49 µg/ml	IC_{50}=279.49 µg/ml

Data presented as the mean ± SEM of three measurements.

CONCLUSION

The results of DPPH scavenging activity assay in this study indicates that the methanolic extract of stem was potently active than other plant parts of *Naringi crenulata.* Maximum antioxidant potential was found in methanolic extract of stem while minimum was found in methanolic extract of root. This suggests that the methanolic extract of *Naringi crenulata* contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Thus, the*, Naringi crenulata* methanolic extract as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free radical-mediated diseases. However, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization.Further studies are needed to identify the antioxidative compounds of this plant species in the management of human diseases resulting from oxidative stress.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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