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Original Article

ANTIOXIDANT POTENTIAL OF ZINGIBER NIMMONII (J. GRAHAM) DALZELL

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ABSTRACT

Objectives: Free radicals and other reactive oxygen species(ROS),such as superoxide anions,hydroxyl radicals and hydrogen peroxide are an entire class of highly reactive species derived during the normal metabolism of oxygen or from exogenous factors and agents. Comparative Antioxidant potential was evaluated using Nitric Oxide Scavenging Assay, DPPH, and superoxide scavenging assays since ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA and estimation of total flavonoid content was carried out in three different extracts; n-Hexane, Chloroform and Methanol.

Methods: Comparative Nitric Oxide scavenging,DPPH scavenging and superoxide scavenging were carried out in all the three sequential extracts of *Zingiber nimmonii* so as to calculate the free radical scavenging power of the plant. Estimation of the total flavonoid content was performed in the three extracts using Aluminium chloride method.

Results: n-Hexane extract gave an IC_{50} of 180mgml^{-1},270µgml^{-1}, 326 µgml^{-1} respectively for nitric oxide, DPPH and superoxide scavenging assays. Activities were greater than the positive controls which proved the significance of n-hexane extract compared with chloroform and methanol. Hexane extract also gave the maximum percentage of flavonoid content [20% equivalent to that of Quercetin standard] when compared to Chloroform [18%].

Conclusion: All the antioxidant assays and flavonoid estimation studies revealed that hexane was the most promising and significant extract among hexane,chloroform and methanolic extracts. So the potential extract was chosen to be hexane and was further screened for various characterization studies.

Keywords: *Zingiber nimmonii*, Zingiberaceae, Nitric oxide scavenging, $IC_{50.}$

INTRODUCTION

Herbal products encompass a variety of self-prescribed preparations of plant origin that may be generally categorized as food, dietary supplements, cosmetics and herbal medicinal products [1]. The genus Zingiber has about 85 species of aromatic herbs mostly distributed in East Asia and tropical Australia [2]. Plants belonging to Zingiberaceae(Ginger family) are known for a number of medicinal properties[3-5]. The term 'Zingiber' is derived from the Sanskrit word 'shringavera', owing to their 'hornshaped' rhizomes. Zingiber species are rich in volatile oils and are used in traditional medicine and as spices. *Z. officinale* Roscoe or 'ginger' has been used in Indian traditional medicine for relief from arthritis, rheumatism, sprains, muscular aches and pains, congestion, coughs, sinusitis, sore throats, diarrhoea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills, etc.[6].

Zingiber nimmonii (J. Graham) Dalzell, an endemic species from the Western Ghats in South India, grows both at low and high altitudes, in moist areas under the shades of trees [7, 8].

Zingiber nimmonii rhizome oil is a unique natural product with 69.9% of isomeric caryophyllenes,viz. β-caryophyllene (42.2%) and α-caryophyllene (27.7%), along with traces of isocaryophyllene(0.03%) in it. The major constituents of the rhizome oil of *Z. nimmonii* varied from the rhizome oils of *Z. zerumbet* and *Z. officinale*, the most studied Zingiber oils. The oil showed significant activities against the human pathogenic fungi,Candida glabrata, C.albicans and Aspergillus niger, but no activity against the plant pathogen, Fusarium oxysporum.[9].

Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired,nascent electron. Atoms of oxygen or nitrogen having central unpaired electron, are called reactive oxygen or nitrogen species [10-13]. Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA[14].

Studies have shown that many plants have chemical components and biological activities that produce definite physiological actions in the body and, therefore could be used to treat various ailments[15].The most important of these bioactive constituents of plants are alkaloids, tannins, phenols and flavonoids[16,17]. Flavonoids, a group of polyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action [18, 19]

Nitric oxide scavenging, DPPH, superoxide scavenging assay and estimation studies for total flavonoid content were performed in the hexane, chloroform and methanolic extracts so as to identify the most potential extract.

MATERIALS AND METHODS

Plant Material

Zingiber nimmonii (J. Graham) Dalzell, was collected from Calicut University Campus, Kerala, India and taxonomically identified by Dr.A.K Pradeep, Herbarium curator, University of Calicut, Kerala, India. The voucher specimen (95954) has been retained in Department of Botany, University of Calicut, Kerala, India.

Plant Extraction

The collected rhizomes were dried under shade and then powdered with mechanical grinder. The dried powder material was extracted

with Hexane, Chloroform and Methanol in a soxhlet apparatus sequentially. These extracts were further used for different assays and analysis.

Chemicals

n-Hexane, Chloroform and Methanol used for the extraction process, Potassium acetate and folins reagent for analysis were obtained from Merck. Sodium nitroprusside, sulphanilamide, napthylethylenediamine dichloride, phosphoric acid, Ascorbic acid, Aluminium chloride were purchased from Merck, India. Butylated hydroxytoluene, 2,2-Diphenyl-1-picrylhydrazyl, Ethylene diaminetetraacetic acid, Nitroblue tetrazolium and Riboflavin were purchased from Sigma-Aldrich and the rest of the chemicals were of analytical grade.

Nitric Oxide Scavenging Assay [20]

Nitric oxide scavenging activity was measured spectrophotometrically. Extracts prepared in Hexane, Chloroform and Methanol, were added to different test-tubes in varying concentrations (0-600mg/ml).Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter,1.5ml of Griess reagent(1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. A Comparative graph was plotted so as to compare the potential of the n-Hexane, Chloroform and Methanolic extracts.

$$\text{Nitric Oxide scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples of extracts.

DPPH Radical scavenging Assay [21]

Various concentrations of plant extracts (4.0ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.1 mM. The mixture were shaken vigorously and left to stand for 30 min and the absorbance was measured at 517 nm. BHT was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\text{\% decolorization} = [1-(ABS_{sample}/ABS_{control}] \times 100$$

Superoxide Radical Activity [22]

This method is based on the inhibition of production of nitroblue tetrazolium of the superoxide ion by the plant extract and is measured spectrophotometrically. The assay tubes containing 20 µl of methanolic extract, 0.2 ml of EDTA,0.1 ml of nitroblue tetrazolium,0.05 ml of riboflavin and 2.25 ml of phosphate buffer and control tubes were set up without the extract. Similarly the activity of the standard antioxidants was also carried out. The initial optical uniformly with a fluorescent lamp for 30 min. A 560 nm was measured again and difference in optical density was taken as the quantum of superoxide production. The percentage inhibition was calculated by comparing with the optical density of the control tubes.

$$\text{\% scavenging of Superoxide} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination [23,24,25]. Briefly,0.5 ml solution of each plant extracts were separately mixed with 1.5 ml of methanol,0.1 ml of 10% aluminum chloride,0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water,and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm with a double beam Thermo Scientific UV2100 UV/Visible spectrophotometer. Total flavonoid contents were calculated as Quercetin from a calibration curve. The calibration curve was prepared by preparing Quercetin solutions at concentrations 20 to 100µg/ml in methanol.

RESULTS AND DISCUSSION

Nitric Oxide Scavenging Assay

It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis[26]. IC50 values were calculated for each of the extracts and compared with Ascorbic acid standard. Hexane extract gave the most promising results.IC50 values were 180mgml-1,200mgml-1,301mgml-1 for hexane, chloroform and methanol respectively as compared to the 190mgml-1 of the ascorbic acid standard [Fig 1].The IC50 values of the hexane and chloroform extracts showed significant activity than A.purpurata of the same family.

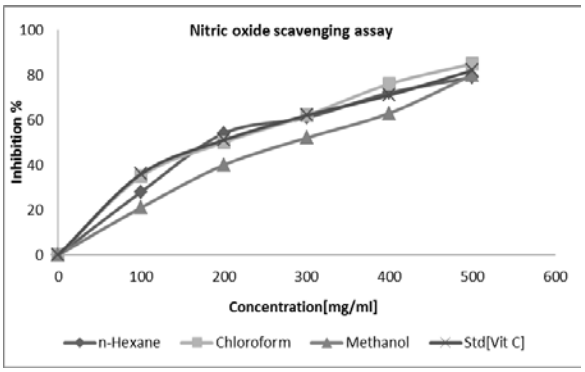

Fig. 1: Nitric Oxide scavenging activity of different extracts and Ascorbic acid[Std]

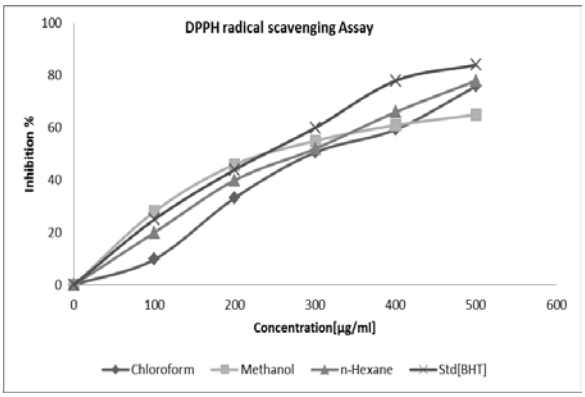

Fig. 2: DPPH scavenging activity of different extracts and BHT [Std]

DPPH radical scavenging Assay

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified. IC_{50} values were calculated for each of the extracts and compared with BHT standard. Hexane extract gave the most promising results.IC_{50} values were 270µgml^{-1},300µgml^{-1},330µgml^{-1} for hexane, chloroform and methanol respectively as compared to the 250µgml^{-1} of the BHT standard [Fig 2].The IC_{50} values of the hexane and chloroform extracts showed significant activity than *W.fruticosa*.

Superoxide scavenging Assay

Superoxide anion radical is known as an initial radical and plays an important role in the formation of other reactive oxygen-species such as hydrogen peroxide or singlet oxygen in living systems[27]. IC_{50} values

were calculated for each of the extracts and compared with Ascorbic acid standard. Hexane extract gave the most promising results.IC_{50} values were 326µgml^{-1},360 µgml^{-1},425µgml^{-1}for hexane, chloroform and methanol respectively as compared to the 401µgml^{-1} of the Ascorbic acid standard[Fig 3].The IC_{50} values of the hexane and chloroform extracts showed significant activity similar to *A.lanata*.

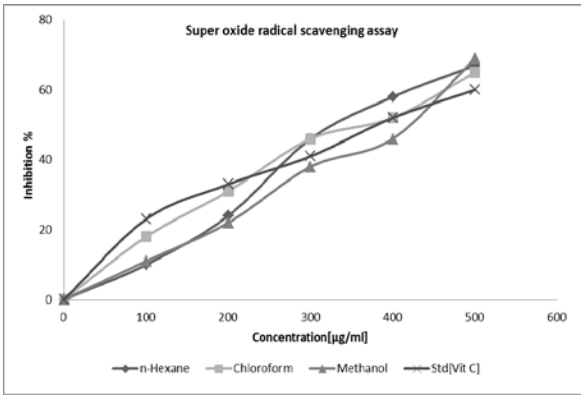

Fig. 3: Superoxide scavenging activity of different extracts and Ascorbic acid [Std]

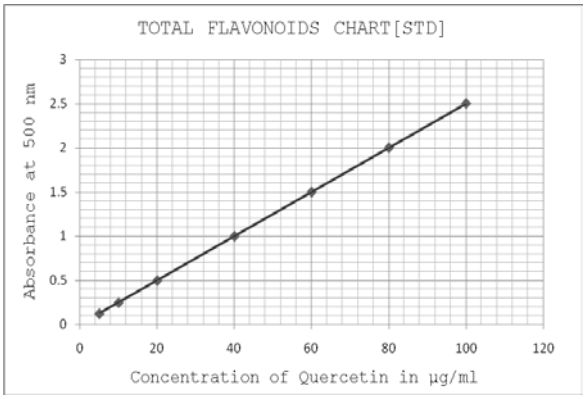

Fig. 4: It shows estimation of total flavonoids-standard graph of Quercetin

Estimation of total flavonoid

The Hexane sample contained 0.18% flavonoid equivalent to that of Quercetin. The Chloroform sample contained 0.22% equivalent to that of Quercetin, whereas methanolic extract gave negative results indicating the absence of Flavonoids in the sample. The readings were compared with the standard graph of Quercetin [Fig2].The *Z.nimmonii* showed activity similar to *Cyamopsis tetragonoloba.*

CONCLUSION

On the basis of the results obtained from the evaluation of nitric oxide, DPPH and superoxide scavenging; best IC_{50} values were shown by hexane extract in all the three assays when compared to the respective standards. So isolation of the bioactive molecules will be extremely significant since the findings are supported by flavonoid content in the hexane and chloroform extracts.

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REFERENCES

1. Kosalec I, Cvek J, Tomić S. Contaminants of medicinal herbs and herbal products. Arhiv za higijenu rada i toksikologiju 2009;60(4):485-501.
2. J. D, A. Mabberley Plant-Book: Dictionary of the Higher Plants1990.
3. Basu SK. Herbal medicines concepts and perspectives Environmental perspectives and human responses. S Graphics Kolkata India National service scheme NSS Govt of India p 2002;3:27-44.
4. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of ethnopharmacology 2006;107(2):182-8.
5. Prajapathi ND, Prajapathi T, Jaipura S. Advances in medicinal plants. Jodhpur Asian Med Plant Heal Care Trustp 2005;1:59-71.
6. Medicinal V, S. P, A. of 500 Species, vol. 5. Orient Longman, Madras;.p.431. Arch Surg 1996.
7. Bhat, G, Studies on Zingiberaceae of Karnataka. In: Higher Plants of Indian Subcontinent, Vol. 4. Bishen Singh Mahendra Pal Singh, Dehradun, 1993.p.39-102.
8. Sabu M. Revision of the genus Zingiber in South India. Folia Malaysiana 2003;4:p25-52.
9. Baby S, Mathew D, Anil J, Rajani K, Pradeep N S, Renju KV, Varughese G, Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity, Phytochemistry 2006;67:2469–2473.
10. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408(6809):239-47.
11. Halliwell B. The antioxidant paradox. Lancet 2000;355(9210):1179-80.
12. Pietta PG. Flavonoids as antioxidants. Journal of natural products 2000;63(7):1035-42.
13. Visioli F, Keaney JF, Halliwell B. Antioxidants and cardiovascular disease; panaceas or tonics for tired sheep? Cardiovascular research 2000;47(3):409.
14. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International journal of biomedical science : IJBS 2008;4(2):89-96.
15. Omale J, Okafor J, N. P. Biotech ; 7. Arch Surg 2008;17:3129-33.
16. Hill A. F, Economic Botany. A textbook of useful plants and plant products Company Inc NewYork 1952.
17. Edeoga H, Okwu D, Mbaebic B. O, E, O, Phytochemical constituents of some Nigerian medicinal plants. AfricJBiotech 4 2005;7:685-8.
18. Frankel E, Nutritional benefits of flavonoids. International Conference of food factors: Chemistry and cancer prevention, Hamamatsu, Japan, Abstract C6-2,1995.
19. Pourmorad F, Hosseinimehr S, Shahabimajd N. J, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology 2006;5(11):1142-5.
20. Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AKS, Mehrotra S, et al. Studies on the antioxidant activities of Desmodium gangeticum. Biological & pharmaceutical bulletin 2003;26(10):1424-7.
21. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.
22. Liu F, Ooi VE, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life sciences 1997;60(10):763-71.
23. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey. Pharmacology online 2008;2:560-7.
24. Ebrahimzadeh MA, Bahramian F. Antioxidant activity of Crataegus pentaegyna subsp. elburensis fruits extracts used in traditional medicine in Iran. Pakistan journal of biological sciences: PJBS 2009;12(5):413-9.
25. Ebrahimzadeh MA, Ehsanifar S, Eslami B. Sambucus ebulus elburensis fruits: A good source for antioxidants. Pharmacognosy Magazine 2009;4(19):213-8.
26. Tylor BS, Kion YM, Wang QI. Nitricoxide down regulates hepatocyte-inducible nitric oxide synthase gene expression. Arch Surg 1997;132(11):1177-83.
27. Stief TW. The physiology and pharmacology of singlet oxygen. Medical hypotheses 2003;60(4):567-72.