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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF FRUIT CONSTITUENTS OF *AMOMUM SUBULATUM* ROXB.

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Abstract

Reactive oxygen Species (ROS) are highly toxic to cells. Cellular antioxidant enzymes and the free radical scavengers normally protect the cell from toxic effects of the ROS. The dietary ingredients of Indian food play an important role in preventing the generation of ROS. *Amomum subulatum* Roxb**.** One of the important spices in Indian food was evaluated for its antioxidant activity. Amomum fruits were extracted with acetone and methanol. Both the extracts were subjected to Ferric reducing power (FRAP) test and DPPH radical scavenging activity using Ascorbic acid as reference standard. Result in FRAP test shows significant efficacy as compared to ascorbic acid. The activity was found to be concentration dependant. The DPPH radical scavenging activity has shown that it was concentration dependent as with increasing concentration it was increasing. And IC_{50} value of both the extract was at 20 µg /ml. Thus it has been concluded that spices like *Amomum subulatum* Roxb. Used in Indian food may be helpful in preventing oxidative stress.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals[1, 2] like super oxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All this radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second[3].when generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates[4-6] and this leads to a number of physiological disorders. Free radicals are involved in the development of degenerative diseases[6]. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation cancer, cardiovascular disorders, neurological disorders, and in the process of aging[7]. Most living species have an efficient defense system to protect themselves against the oxidative stress induced by ROS[8]. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases[9-11]. Many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins etc.[12], and can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Spices and condiments are widely used in Indian recipes as adjuncts to enhance the flavour and taste of food. The traditional medical literature describes their potential role as a source of many vitamins and a domestic remedy for many of the human disorders[13]. Antioxidant effect of spices like Cinnamon (*Cinnamomum verum* J S Presl syn. *Cinnamomum zeylanicum* Blume; Lauraceae; bark) and Cardamom (*Amomum subulatum* Roxb; Zingiberaceae; seeds) have been reported[14-15]. Effect of *Amomum subulatum* Roxb. On oxidative stress and atherosclerosis in cholesterol fed rabbits were found.[16]

With this background information, the present study was undertaken to screen the In vitro Free radical scavenging activity of fruit constituents of *Amomum subulatum*

Roxb. *Amomum subulatum* Roxb. (Synonym: Sthulaila) belongs to family Zingiberaceae is an herb with leafy stem and perennial root stock; cultivated in swampy places along the sides of mountain streams in Bengal and Assam[17]. The seeds are aromatic and pungent, with a sharp, pleasant taste; they are similar in properties to the true cardamom. The seeds on steam distillation yield a dark brown, mobile essential oil (2.5%) having a characteristic odour of cineol. The principal constituent of the oil is cineol (64.94%). The oil also contains: bisabolene, sabinene, terpinene, terpineol, terpinyl acetate, and polymerized oil18. In Indian traditional system of medicine the plant seeds are widely used as stomachic, carminative, cardiac stimulant and expectorant. Oil extracted from them is applied to the eyelids to ally inflammation. It is an agreeable aromatic and stimulant and is used for flavoring. Decoction of cardamom is used as a gargle in affections of the teeth and gums. In combination with the seeds of melon it is used as a diuretic in cases of kidney stones. It is used in liver affection such as congestion of the liver. It is used as an aphrodisiac, and also used as an antidote to snake bite and scorpion sting[1]

MATERIALS AND METHODS

PLANT MATERIAL

The fruits of *Amomum subulatum* Roxb. Were collected from local market of Modasa and authentified by Dr. M.S. Jangid of Botany Department, Sir. P. T. Science College, Modasa.

PREPARATION OF EXTRACT[16, 20]

The coarsely powdered drug was extracted in Soxhlet apparatus with acetone. This extract was concentrated & then air dried. The residue was further extracted with Methanol & the extract was concentrated & air dried.

In-vitro Free Radical scavenging activity

CHEMICALS AND REAGENTS

1. Ferric reducing antioxidant power (FRAP): Potassium ferricyanide (K3Fe (CN) 6, of purity 98.0%), Ferric Chloride (FeCl3.6H2O, of purity 97.0%), Ascorbic acid (purity 98.3%), Potassium hydrogen phosphate, Sodium hydroxide, Trichloro acetic acid

2. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity: α- α diphenyl β

picryl hydrazyl DPPH. A solution of 1.3 µg/ml in methanol was made & protected from light by covering the test tubes with aluminum foil.

INSTRUMENTS

UV Spectrophotometer (Shimadzu UV 1601)
Centrifuge Machine (Eltek research Centrifuge-TC-4100D)

METHODS

I. Preparation of sample extracts:-

Both the extract above mentioned was taken in range of 10-200 µg/ml in methanol.

II. Preparation of standard solution:-

Ascorbic acid was used as standard. Aliquots of 10-200 µg/ml in methanol were prepared.

III. Ferric reducing antioxidant power (FRAP)[21]

The solutions of extracts & standard were spiked with 2.5 ml of phosphate buffer (pH 6.6) & 2.5ml, 1% Potassium Ferricyanide. The mixture was kept in water bath at 50˚ C for 20 min. the resulting solution was cooled rapidly. To this 2.5ml of 10% Trichloro acetic acid was added. It was centrifuged at 3000 rpm for 10 minutes. Supernant 1ml was mixed with 1ml of distilled water & 0.5ml of 0.1% Ferric Chloride Solution. The absorbance was measured at 700nm.

IV. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity[22-25]

2.5ml of DPPH solution was added to 5ml water & absorbance was taken after 30 minute at 517nm for control reading. Different concentrations of extracts and sample were diluted to 5ml with water. To it 2.5ml of DPPH was added. The mixture was kept in dark for 30 minutes and absorbance was measured at 517nm after 30 minutes. The absorbance of control reduce dose dependently.

The % reduction was calculated as follow

% Reduction = (AB – AA / AB) X 100

AA is the absorbance of the tested sample after 30 minutes.

AB is the absorbance of blank sample.

IC50 is the concentration required to reduce % reduction by 50 %.

RESULT AND DISCUSSION

IC_{50} values of both the extract was found 20 µg /ml which were comparable to IC_{50} value of Ascorbic acid (15 µg /ml). Here percentage inhibition was observed concentration dependant. Acetone extract

showed highest 90.73% inhibition at 200 µg /ml and methanol extract showed highest 76.76% inhibition at 200 µg /ml while ascorbic acid showed 91.11% inhibition at same concentration, which shows significant antioxidant activity of *Amomum subulatum.*

CONCLUSION

On the basis of the results obtained in the present study, we conclude that both the acetone and methanol extracts of *Amomum subulatum* Roxb. have significant amounts of antioxidant activity and this activity may be due to (1) Protocatechuic acid (2) 1,7-bis(3,4-dihydroxyphenyl)hepta-4E,6E-dien-3-one (3)2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H benzocycloheptene and (4) Protocatechualdehyde.

Thus spices like *Amomum subulatum* Roxb. Used in Indian food may be helpful in preventing oxidative stress.

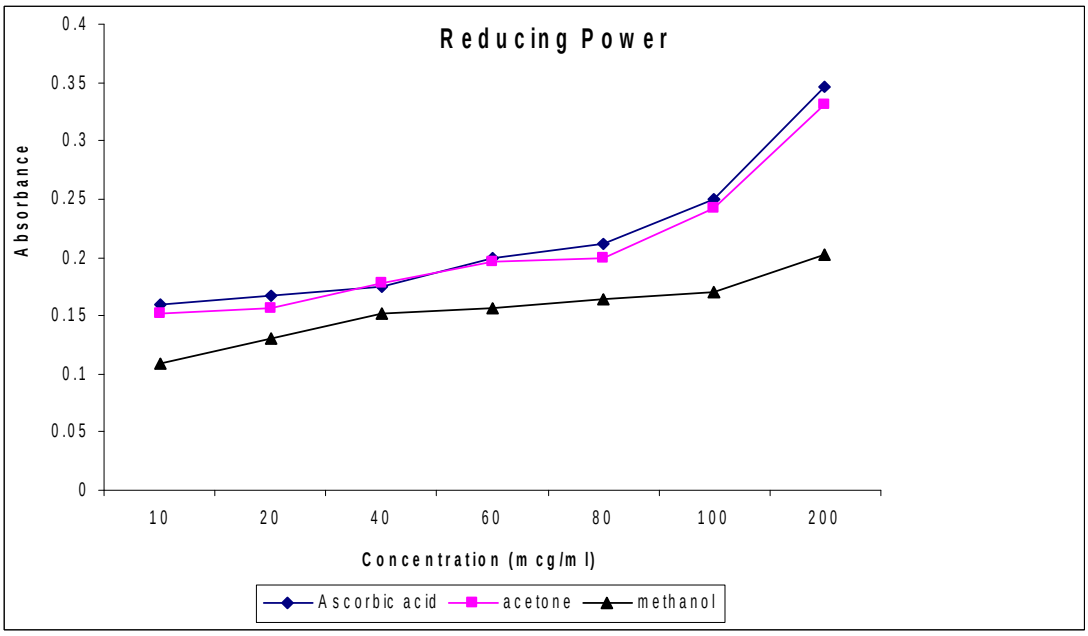
Further studies are needed to evaluate the in-vivo antioxidant potential of this extract in various animal models.

Table 1

Ferric reducing antioxidant power (FRAP)

Table-1:

	Absorbance		
Conc. (µg/ml)	Ascorbic acid	Acetone extract	Methanol extract
10	0.16	0.151	0.109
20	0.167	0.157	0.13
40	0.174	0.178	0.151
60	0.199	0.196	0.156
80	0.211	0.2	0.164
100	0.25	0.242	0.17
200	0.347	0.331	0.202



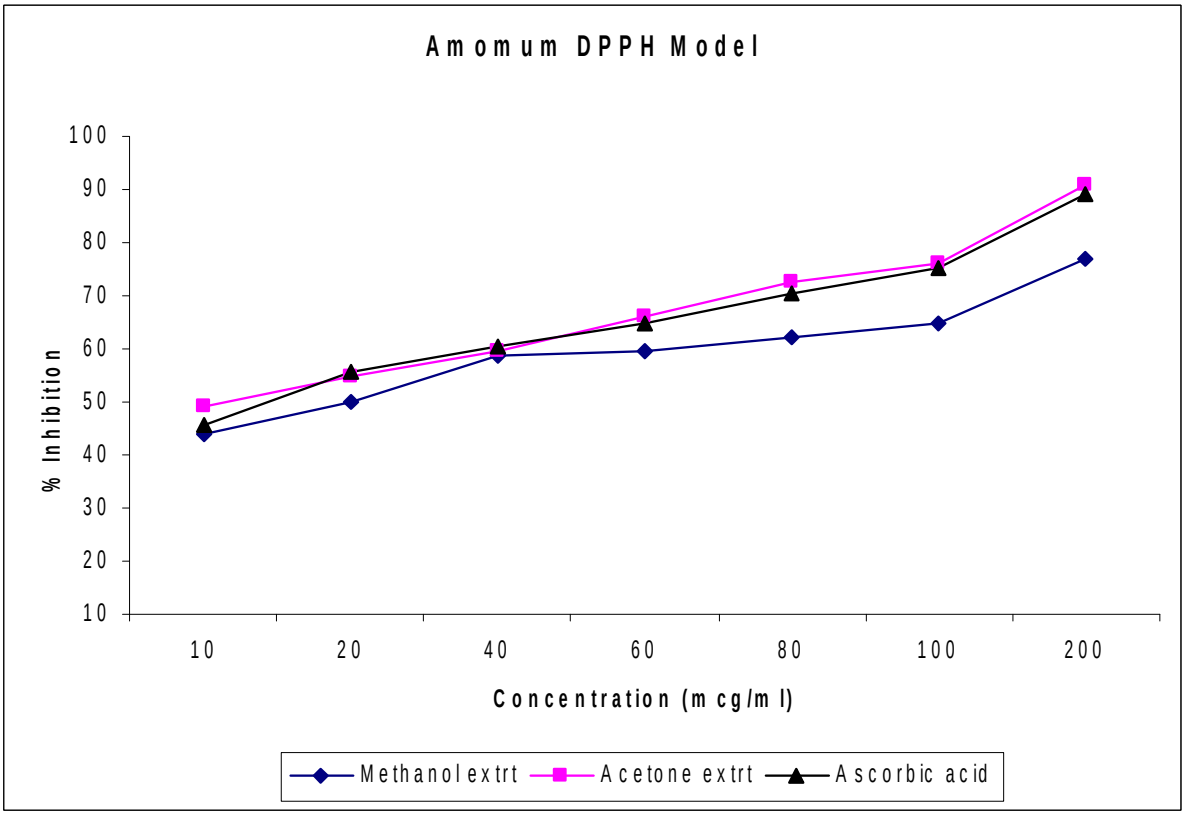
Graph 1.

Result shows significant antioxidant activity as compared to standard. The activity was found to be concentration dependant

Table 2

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity

	%Reduction		
Conc. (µg/ml)	Ascorbic acid	Acetone Extract	Methanol Extract
10	45.56	49.23	43.84
20	55.5	54.77	50.2
40	60.33	59.47	58.5
60	65.0	66.11	59.47
80	70.22	72.47	61.96
100	75.15	76.07	65.0
200	89.11	90.73	76.76



Graph 2

REFERENCES

1. Yu BP: Cellular defenses against damage from reactive oxygen species, physiol Rev, 1994; 74: 139.

2. Halliwell B and Gutteridge JMC: in free radicals in biology and medicine, 2nd Ed (Clarendon Pres, Oxford) 1988: 1.

3. Lata H and Ahuja GK: Role of free radicals in health and disease, Ind J Physio & Allied Sci, 2003; 57: 124.

4. Cotran RS, Kumar V and Collins T: in Robbin's pathological basis of diseases, 6th Ed Thomson Press (I) Ltd, Noida, India. 1999: 1.

5. Yu BP, Suescun EA and Yang SY: Effect of age-related lipid peroxidation on membrane fluidity and phospholipasse A2: modulation by dietary restriction, Mech ageing Dev. 1992; 65: 17.

6. Campbell I C and Abdulla EM: strategic approaches to *in vitro* neurotoxicology, in Approaches and methods: Neurotoxicology (Academic Press, London) 1995: 495.

7. Marx JL: Oxygen free radicals linked too many diseases, Science. 1987; 235: 529.

8. Stato M, Ramarathnam N, Suzuki Y, Ohkubo T, Takeuchi M and Ochi H: Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. J. Agri. Food Chem. 1996; 44: 37-41.

9. Stajner D, Milic N, Mimica-Dukic N, Lazic B and Igic R: Antioxidant abilities of cultivated and wild species of garlic. Phytother: Res. 1998; 12: 513-514.

10. Sanchez-Moreno C, Larrauri JA and Saura-Calixto F: Free radical scavenging capacity an inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res. Intern. 1999; 32:407-512.

11. Malencic DJ, Gasic O, Popovie M and Boza P: Screening for antioxidant properties of Salvia reflexa Hornem. Phytother. Res. 2000; 14: 546-548.

12. Pratt DE: Natural antioxidants from plant material, in Phenolic compounds in food and their effects on health II: Antioxidants and cancer prevention (ACS Symposium Series 507) edited by M Hang, C Ho & C Lee (American Chemical Society, Washington DC. 1992: 54.

13. Rastogi RP and Mehrotra BN: Compendium of Indian medicinal plants, (CSIR, New Delhi) 1991.

14. Sambaiah K and Srinivasan K: Indian J Biochem Biophys, 1989; 26: 254.

15. Wu FJ and Su JD: J Chinese Agric Chem Soc. 1996; 34: 438.

16. SC Joshi and Vishesta Joshi: Effect of Amomum subulatum on oxidative stress and atherosclarosi in cholesterol fed rabbits, Pharmacologyonline 2007; 1: 451-463.

17. The Ayurvedic Pharmacopoeia of India, Gov. of India, part-I, Vol-II, 1st edition, 158.

18. The Wealth of India, Raw Materials Vol-I, Revised Edition, Reprinted 2006: 229.

19. LD Kapoor: CRC Handbook of Ayurvedic Medicinal Plants, 35.

20. Kikuzaki H, Kawai Y and Nakatani N: 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging active compounds from greater cardamom, J Nutr Sci Vitaminol (Tokyo). 2001; 47 (2): 167-71.

21. A. Kumaran and R. Joel Karunakaran: In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India, *LWT-Food Science and Technology*, 2007; **40**(2): 344-352.

22. A. Kumaran and R. Joel Karunakaran: Antioxidant activity of polyphenols from Phyllanthus Debilis Klein ex Willd, J Nat. Remedies. 2006; 6(2): 141-146.

23. Adam Matkowski and Magdalena Piotrowska: Antioxidant and free radical scavenging activities of some medicinal plants from Lamiaceae, Fitoterapia 2006; 77: 346-353.

24. Firoj Ahmed and IZ Shahid: Free radical scavenging activity of some Mangroves available in Bangladesh, Oriental Pharmacy and Experimental Medicine 2006; 6 (1): 58-64.

25. JR Baheti, V Kumar, GB Shah and RK Goyal: Free radical scavenging activity of aqueous extract of Rhus Succedanea galls, J Nat Remedies. 2005; 5(1): 15-18.