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Evaluation of antioxidant activity of large cardamom (leaves of *Amomum subulatum*)

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Abstract

Amomum subulatum, is commonly used spice. In the present study the ethanolic and aqueous extracts of leaves of *Amomum subulatum* is evaluated for antioxidant activity by the 1,1Diphenyl -2-picrylhydrazyle (DPPH) free radical scavenging activity, β-carotene bleaching assay and total phenolic contents methods. The ethanolic extract showed significant antioxidant activity. The IC_{50} of ethanolic extract, total phenolic content, and mean antioxidant activity are 8.25±2.0 µg/ml, 11.04±0.2, 41.2±1.5% respectively and that of ascorbic acid was 2.0±0.14 µg/ml whereas BHA was found to be 50.3±0.6. The study showed that the ethanolic extract consumption could exert beneficial effects due to its antioxidant activity.

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INTRODUCTION

Free radicals (Reactive oxygen species and Reactive nitrogen species) are responsible for DNA damage which leads to inflammatory diseases and progression to cancer [1]. In the past several years, unprecedented progress has been made in the recognition and understanding of roles of reactive oxygen species in many diseases. These include atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age

pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis, which are age-related. The body protects itself from the potential damages of reactive oxygen species. Its first line of defense is superoxide dismutases, glutathione peroxidases, and catalase. Scientists have indicated that antioxidant nutraceuticals supplied from daily diets quench the reactive oxygen species or are required as cofactors for antioxidant enzymes [2].

An hypothesis for the role of free radicals in cancer was elaborated by D. Harman in 1962 who suggested that it might be possible to reduce the extent of damage caused by free radicals through three dietary changes: (*i*) caloric reduction, i.e., lowering the level of free radical reactions arising in the course of normal metabolism; (*ii*) minimize dietary components that tend to increase the level of free radical reactions (e.g., polyunsaturated fats); and (*iii*) supplement the diet with one or more free radical reaction inhibitors (anti-oxidants). With respect to (*ii*) and (*iii*), lipid peroxidation exemplifies the type of chain reaction initiated by free radicals, with unsaturated fatty acids being the primary center of free radical attack. Anti-oxidants act as free radical scavengers and are able to terminate these reactions [3].

Apart from many dietary components, there are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. Current research reveals the different potential applications of antioxidant /free radical manipulations in prevention or control of disease. Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity [4].

The antioxidant role of some other spices such as garlic, gingoviola, green tea, cumumin, *Terminalia chebula*, *Salvia officanalis*, *Calamintha incana*, *Thymus vulgaris* [5] were already studied. On the other hand clove and large cardamom (seeds) flavouring spices are extensively studied for their antioxidant activity [6].

Amomum subulatum (greater or large cardamom, bara elachi), a herb with leafy stem and perennial roots stalk is cultivated in swampy places along the side of mountains streams in Nepal, India (Bengal, Sikkim, Assam), they are used in preparation of sweet meats [7]. *Amomum subulatum* showed antiulcerogenic effects [8], hepatoprotective activity [6], and as fungitoxitant[9].

Volatile oil of *Amomum subulatum* contains 1, 8 cineole, pinene terpineole, pinene, terpinyl acetate as main constituent [10].Still no work is performed on *Amomum subulatum* leaves as antioxidant. Thus main objective of this study to explore the antioxidant activity of ethanolic and aqueous extracts of plant leaves.

2. Materials and methods

1,1Diphenyl -2-picrylhydrazyle (DPPH), β- carotene, Linoleic acid (>=99%), Butylated hydroxyanisole (BHA) were obtained from Sigma Aldrich Co. All other chemicals used were of analytical grade.

2.1. Preparation of Plant Extract

Plant was collected from Kukrail forest research centre, Lucknow and authenticated from NBRI Lucknow (Ref. No: NBRI/CIF/Re./08/2008/32). Plant material consisted of fresh leaves of *Amomum subulatum*, Extraction was performed using soxhlet assembly About 20 g of plant material was extracted in 250 mL of 98% ethanol at 40 °C, and 250 mL distill water at 60 °C, separately for 9 h. The final extracts were passed through No.1 Whatman filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4 °C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% methanol and distill water to obtain a stock solution of 1000 µg/mL. The stock solutions were

serially diluted with the respective solvents to obtain lower dilutions (1, 2, 4, 6, 8, 10, 15, 25, 40, 50, 75, 100, 250, and 500 µg/mL).

2.2. Preliminary Phytochemical Screening of the Extracts

Extracts were screened by applying general chemical tests for alkaloids, glycosides, Sugars, Phenolic compound, Sterols, Tannins, proteins, Saponin and free amino acids.

2.3. Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Ethanolic Extract

Antioxidant activity of the plant extracts and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [5], the diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 100µg/mL. We prepared 0.002% DPPH in methanol. Then 2 mL of this solution was mixed with 2 mL of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using a Shimadzu spectrophotometer against methanol. The blank was used as 2 mL of methanol with 2 ml of DPPH solution (0.002%).

The optical density was recorded and percent of inhibition was calculated using the formula given below [11].

% of inhibition of DPPH activity = A–B/A × 100

Where A is optical density of the blank and B is optical density of the sample.

2.4. Evaluation of antioxidant activity

The antioxidant activity of the extract was evaluated using a β-carotene/linoleate model system. A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 mL of chloroform. One milliliter of this solution was then pipette out into a round-bottom flask. After chloroform was rotary evaporated at 40 °C under vacuum, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier and 50 mL of distilled water were added to the flask under vigorous shaking. Aliquots (5 mL) of this emulsion were transferred into a series of tubes containing 2 mg of each extract or 2 mg of BHA (butylated hydroxyl anisole) for comparison. An aliquot (5 mL) of emulsion without any further addition was used as control. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 10-min intervals by keeping the sample in a water bath at 50 °C until the visual color of -carotene in the control sample disappeared (about 120 min) [12].

Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using a formula from Ismail and Hong [13].

$$AA = \left(1 - \frac{(A_0 - A_t)}{(A^\circ_0 - A^\circ_t)}\right) \times 100$$

where A_o and A^o_o are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and A^o_t are the absorbance values measured in the samples or standards and control at t = 120 min.

2.5. Total phenol content (TPC) TPC was determined using the Folin-Ciocalteu's reagent [14]. Samples of 0.3 mL were introduced into test tubes followed by 1.5 mL of Folin- Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give a reading of less than 1. Total phenol contents were expressed in Gallic acid equivalents (mg per 100 g fresh fruit). The Gallic acid standard line has the equation y = 0.0111x - 0.0148 (R^2 = 0.9998), where y is absorbance at 765 nm and x is concentration of Gallic acid in mg/L. All Absorptions were measured in three replicates.

2.6. Statistics

All the analyses were carried out in triplicate and the results are expressed as mean ± SD. Statistical analysis was performed using GraphPad Prism 5.0 Softwares (Inc, San Digeo USA).

3. Result and Discussion

Ethanolic, aqueous extracts of *Amomum subulatum* and standard ascorbic acid tested for in-vitro antioxidant activity using the DPPH method, the ethanolic and aqueous extract showed antioxidant activity with IC_{50} value of 8.25±2.0 µg/mL, and 21.6±2.0 µg/mL. The IC_{50} value for Ascorbic acid was 2.0±0.14 µg/mL. The result indicates that the antioxidant activity of ethanolic extract was found higher than aqueous extract of *Amomum subulatum* whereas lesser than that of ascorbic acid.

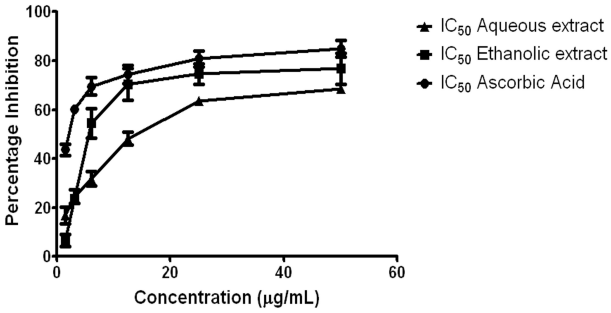

Figure 1: DPPH free radical scavenging activity of aqueous, ethanolic extracts of *A. subulatum* leaves and ascorbic acid

The antioxidant activity of extracts of the *Amomum subulatum* examined by using a β-carotene/linoleate model system, BHA is presented in Table 1. According to the preventive activity against bleaching of β-carotene, ethanolic and aqueous extracts showed the antioxidant activity (41.2±1.5) and 35.96±1.2 respectivly, comparable to that of BHA (50.3±0.6). Ethanolic extract showed lower antioxidant activity than BHA in the β-carotene/ linoleic acid model system (Figure 2).

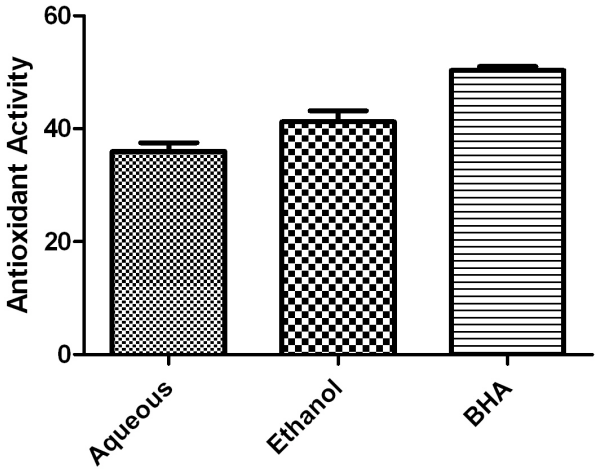

Figure 2: Antioxidant activity of aqueous, ethanolic extracts of *A. subulatum* leaves and BHA

Table1: Antioxidant Activity of ethanolic extract of *Amomum subulatum*

Test compound	DPPH assay IC_{50} (µg/mL)*	β-carotene/linoleic acid (%AA)	TPC (in GAE)*
A. subulatum (Ethanolic)	8.25±2.0	41.2±1.5	11.04±0.2
A. subulatum (Aqueous)	21.6±2.0	35.96±1.2	6.87±0.7
Ascorbic acid	2.0±0.14	-	-
BHA	-	50.3±0.6	-

*Values are means ± SD of three determinations

The content of phenolic compound (mg/g) in ethanolic and aqueous extracts expressed in GAE is 11.04±0.2 and 6.87±0.7 respectively. The extracts showing free radical scavenging activity and total Antioxidant activity contain phenolic compound, suggesting that the antioxidant activity may be due to a great extent to the polyphenol content.

4. Conclusion

Above *in vitro* studies shows that ethanolic extract of *A. subulatum* leaves possess promising antioxidant, it may be due to polyphenol and other chemical constituent present in extract. Identification of all chemical constituent in leaf extract that are responsible for antioxidant activity requires further investigation, The crude ethanolic extract merits further experiments in vivo. However, present study

showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics.

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CONFLICT OF INTEREST DECLARATION

I hereby declare that I have no competing interests.

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