Determination of Phenolic Content and Antioxidant Potential of Ethanol Extract of Three Species of Alpinia

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Abstract- *The present study aims to investigate hither to unknown antioxidant activity of ethanol extracts of three species of Alpinia. Alpinia galanga (L.) Willd. commonly called as Rasna, Greater galangal or Kulinjan is a medicinally important rhizome used in Indian traditional system of medicine to cure a number of ailments. Due to the high demand for the rhizome of A. galanga traders are now substituting it with rhizomes of A. calcarata and A. officinarum. Antioxidant activity of ethanol extracts was studied by means of various assays including 1,1-diphenyl 2-picryl hydrazyl, β-Carotene/linoleic acid bleaching and trolox equivalent antioxidant capacity (TEAC). Total phenolic content in each extract was determined with folin-ciocalteu reagent. The trend of phenol content was as: A. galanga (202.56± 0.36 mg/g) > A. officinarum (185.77 ± 0.71 mg/g) > A. calcarata (167.31 ± 0.44 mg/g). Ethanol extracts of A. galanga, having high percentage of phenol content showed potent in vitro antioxidant activity followed by moderate activity of ethanol extracts of A. officinarum and A. calcarata.*

Keywords- Alpinia species, Antioxidant activity, Ethanol extracts, Phenolic content

I. INTRODUCTION

Genus *Alpinia* consist of 230 species from family *Zingiberaceae* . One of these, rhizome of *Alpinia galanga* (L.) Willd. is used in Indian traditional system of medicine for rheumatism, fever, bronchial catarrh, stomach pain, stimulant, carminative, tonic, aphrodisiac, aromatic and to decrease the urine output in diabetic patients etc. It is commonly known as Greater galangal in English, Rasna in Sanskrit and traded as Kulinjan in Indian market. It is native to Eastern Himalaya and distributed in China, Malaya, Indonesia, Thailand, India (Verma et. al., 2011). Philippines and Indonesia. Rhizomes of *A. galanga* are reported for anti-microbial, anti-diabetic, anti-inflammatory, anti-cancer, antiflatulence, anti-fungal in AIDS patients, cytoprotective and anti-allergic activity (Upadhye et. al., 2018). The chemical study reported presence of a wide array of bioactive phytoconstituents in the rhizome of *A. galanga* as galangin, α-pinene, β-pinene, limonene, cineol, terpinen-4-ol, α- terpineol, resin containing galangol, kaempferide, methyl cinnamate, camphor, myricene, methyl eugenol, flavones, alpinin, 3-deoxy-4-methoxy (Chudiwal et. al., 2010) and 1′-acetoxychavicol acetate (Siringam et. al., 2012). Of which, it is a foremost source of flavonol galangin having the diverse source of biological and pharmacological properties, such as anti-mutagenic, anticlastogenic, antioxidative, anti-inflammatory (Tag et. al., 2007), metabolic enzyme modulating, anti-proliferative and anticancer activity (Madhuri and Pandey, 2009). Crude drug samples of *A. galanga* commonly known as 'Kulinjan' were studied for their biological resource. Owing to its high demand in the Indian market, *A. galanga* is seen to be adulterated/substituted with rhizomes of two other species *Alpinia calcarata* (Haw.) Roscoe and *Alpinia officinarum* Hance under the common trade name (Girija and Rema, 2014; Namdeo and Kale, 1984). A perusal of literature showed that reports on comparative antioxidant studies on herb *A. galanga* (rhizome) and its adulterants /substituent were not available. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are closely involved in human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis, liver diseases and atherosclerosis. (Freeman et. al., 1984; Squadrito and Pryor, 1998). There has been an increased interest in identifying antioxidant phytochemical, because these molecules can inhibit the propagation of free radical reactions to protect the human body from diseases (Kinsella et. al., 1993) and retard lipid oxidative rancidity in food (Duthie et.al., 1993).

Hence, it was proposed to evaluate comparative antioxidant efficacy and potency of above species by various assays including 1,1-diphenyl 2-picryl hydrazyl, β-Carotene/linoleic acid bleaching and trolox equivalent antioxidant capacity (TEAC). Total phenolic content in each ethanol extract was also determined. Hence, in present study, a 'battery" of parameters was used to demonstrate antioxidant activity.

II. MATERIAL AND METHODS

Plant Material

Rhizomes of *A. galanga* and *A. calcarata* were collected from Dapoli and Naoroji Godrej Centre for Plant Research, Maharashtra, India in October 2016, respectively. The rhizomes of *A. officinarum* were procured from local Pune market in the same year.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, *β*-carotene, linoleic acid, butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (Steinheim, Germany). All the solvents employed for bioassays were of AR grade and purchased from LOBA chemicals, Mumbai, India. All experiments were performed on a spectrophotometer Elcetronics India Model no. 1372.

Preparation of sample solutions

Accurately weighted (10 g) powdered samples were extracted exhaustively with methanol (50 mL) using Soxhlet apparatus with an extraction time of 480 min (8 h). The extracts were concentrated under reduced temperature and pressure using rotary evaporator. Respective yields of methanol extract of samples were *A. galanga* - 0.990 g, *A. calcarata* - 0.867 g and *A. officinarum* -0.9501 g.

Radical-scavenging effect of extracts in DPPH radicals

DPPH radical-scavenging ability was assessed according to the reported method (Jung et al., 2005). Briefly, to a methanolic solution of DPPH (60 mM, 2 ml), 50 µl of each test extracts at different concentrations (5 - 50 µg/ml) dissolved in methanol was added. Absorbance measurements commenced immediately at 515 nm. The decrease in absorbance was determined after 70 min when the absorbance stabilized. The absorbance of the DPPH radical without extracts and control was measured. Ascorbic acid was used as a reference antioxidant. Percent inhibition of DPPH radical in samples was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \text{ X } 100$$

Where $A_{C(0)}$ is the absorbance of the control at $t=0$ min and $A_{A(t)}$ is the absorbance in presence of antioxidant at t =70 min.

β-Carotene/linoleic acid bleaching assay

Antioxidant activity was measured by the standard *β*-carotene linoleic acid assay (Mokbel and Hashinaga, 2005) with slight modifications. *β*-Carotene (3.34 mg), linoleic acid (40 mg) and Tween-20 (400 mg) were added to chloroform (1 ml). Chloroform was then removed at 40°C under vacuum using a rotatory evaporator. The resulting mixture was diluted with distilled water up to 10 ml. The volume of resulting emulsion was further made up to 100 ml with hydrogen peroxide (0.01 M). Methanolic stock solutions (1 mg/ml) of each extract and synthetic standard antioxidant BHT were prepared. Parts of the stock solutions were suitably diluted to obtain formulation of concentration ranging from 1 to 5×10^{-5} ml. *β*-Carotene-linoleic acid emulsion (2 ml) was transferred into test tubes containing test samples (0.1 ml). A solution containing methanol (0.2 ml) and *β*-carotene- linoleic acid emulsion (2 ml) was used as a control. The test tubes were placed in water bath maintained at 40 °C. Absorbance of all the samples was measured at 470 nm at zero time and after every 15 min till the colour of *β*-carotene disappeared (105 min) in the control. All the samples were analyzed in triplicate. The per cent inhibition was determined by the following formula:

$$\% \text{ Inhibition} = [(A_{A(105)} - A_{c(105)})/A_{C(0)} - A_{c(105)}] \text{ X } 100$$

Where, $A_{A(105)}$ is the absorbance of antioxidants at 105 min, $A_{C(105)}$ is the absorbance of control at 105 min, $A_{c(0)}$ is the absorbance of control at 0 min. The sample concentration providing 50 % inhibition (IC_{50}) was calculated and reported as mean±SD. BHT was used as standard.

Evaluation of Trolox equivalent antioxidant capacity (TEAC)

Total antioxidant activity of the samples extract was measured using the TEAC assay **(**Miller et. al., 1995**)** with minor modifications. To measure antioxidants capacity, 0.25 ml of each extract (25 µg/ml) was mixed with an equal volume of $ABTS^{\cdot}+$, H2O2, peroxidase and deionized water. The reaction mixture was stand for 10 min at room temperature. The decrease in absorption at 734 nm after the addition of the reactant was used to calculate the TEAC value. The TEAC value is expressed as the milimolar concentration of Trolox solution having an antioxidant equivalent to a 1000 ppm solution of the sample under investigation. The higher the TEAC value of the sample, the stronger the antioxidant ability.

Total phenolic content

Total phenolic content in each extract was determined with folin-ciocalteu reagent (Slinkard and

Singleton, 1977) using pyrocatechol as a reference standard. 2 ml of 2 % Na_2CO_3 folin-ciocalteu reagent was added and allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. Concentration of total phenol content was determined using the formula:

Absorbance = 0.001 × pyrocatechol (μg) + 0.0033

III. STATISTICAL ANALYSIS

Experimental results were expressed as means ± SD of six replications for each data point.

IV. RESULTS AND DISCUSSIONS

Antioxidant activity is a complex biochemical process as multiple active species, reaction characteristics and various mechanisms are involved in oxidative stress. Therefore, the mechanism of action is not accurately reflected by single universal method. Thus evaluation of the antioxidant capacity for pure compounds/ extracts/ oil should be carried out by using different *in vitro*/*in vivo* biochemical assays (Karadag et al., 2009; Niki, 2010). Assays are based on mechanism of antioxidant action such as ability to scavenge the free radicals, inhibition of lipid perioxidation. In the present study, essential oil was evaluated for antioxidant potential using DPPH, ABTS and *β*-carotene/linoleic acid methods.

DPPH is commercially available stable free radical commonly used as substrate, to evaluate antioxidant capacity. Antioxidants from the sample scavenge free radical by hydrogen donation which causes a reduction in the intensity of absorption at 517 nm. In the present study, the ability of EOP to scavenge DPPH radical was determined on the base of their concentration providing 50 % inhibition (IC_{50}). IC_{50} value is negatively related to antioxidant capacity, lower the IC_{50} value higher is the antioxidant capacity of test sample. DPPH radical as compared to standard and IC_{50} values of each were as *A. galanga* 11.5±0.67, *A. officinarum* 40.4±0.89 and *A. calcarata* 110 .6±0.74 μg/ml comparable with standard BHT (IC_{50} 47.17±0.90 μg/ml).

The ABTS radical is also widely used in testing antioxidant capacity. In contrast to DPPH, the sample to be tested was added after generation of a certain amount of ABTS•+ radical cation and the remaining ABTS•+ concentration after reaction with the antioxidant sample was then quantified. The trend of TEAC values i.e ABTS scavenging activity with an IC_{50} of *A. galanga* 103.5±0.67, *A. officinarum* 2 40.4±0.89 and *A. calcarata* 263.5±2.67 μg/ml.

The capacity of ethanol extracts of *Alpinia* species to inhibit lipid peroxidation was evaluated using *β*-carotene /linoleic acid bleaching method. The oxidation of linoleic acid generates peroxyl free radicals which then oxidize the highly unsaturated *β*-carotene. The oxidation of *β*-carotene decreases by the antioxidants present in the sample. *A. galanga* showed high capacity towards the inhibition of *β*-carotene bleaching with low IC_{50} value (98.8±2.33 μg/ml) comparable to other two species *A. officinarum* 207.4±0.89 and *A. calcarata* 274.5±2.67 μg/ml. This activity is comparable with standard BHT (IC_{50} 81.44±1.33 μg/ml).

The key role of phenolic compounds is the ability to scavenge free radicals and ROS such as singlet oxygen, superoxide free radical, and hydroxyl radicals. Phenolic compounds in the medicinal plants extracts are frequently responsible for the antioxidant status (Hall, 1997) Taking this into count total phenol content of extracts was assayed. The trend of phenol content was as: *A. galanga (*202.56± 0.36 mg/g) > *A. officinarum* (185.77 ± 0.71 mg/g) > *A. calcarata* (167.31 ± 0.44 mg/g). A significant correlation was shown by total phenolic content and free radical scavenging activities of all extracts. The results confirmed that greater antioxidant activity of three species of *Alpinia* was probably due to their highest amount of phenolic compounds.

V. CONCLUSION

Rhizome of *Alpinia galanga* (L.) Willd. is used in Indian traditional system of medicine for rheumatism, fever, bronchial catarrh, stomach pain, stimulant, carminative, tonic, aphrodisiac, aromatic and to decrease the urine output in diabetic patients etc. However, *A. galanga* is seen to be adulterated/substituted with rhizomes of two other species *Alpinia calcarata* and *Alpinia officinarum* under the common trade name . The ethanol extract of *A. galanga*, showed potent *in vitro* antioxidant activity followed by moderate activity of the ethanol extract of *A. calcarata* and *A. officinarum* as compared to the standard. The preliminary chemical examination of the ethanol extract of above three samples has shown potent of phenolic compounds. The possible mechanism of antioxidant activity includes reducing ability, trolox equivalent antioxidant capacity (TEAC) which may be due to the presence of higher phenolic phytoconstituents in the ethanol extracts. Further, detailed investigations on comparative accounts of efficacy of *A. galangal* and its adulterates/substitutes are warranted for pharmaceutical purpose.

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