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Antioxidant Activities of the rhizomes of different Zingiberaceae plants of North-East India

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Abstract

In the present investigation, different fractions of rhizomes different plants of Zingiberaceae family were tested for their *in vitro* anti-oxidant activities. Protocols such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methods and β-carotene-linoleic acid methods have been used for evaluation. The antioxidant activities of the extracts of the rhizomes in different solvents like methanol, chloroform and petroleum ether were reported. All the different solvent extracts of each plant shows different absorbance values indicating their bioactive contents. The study reveals that the highest activity of anti-oxidant was observed in methanol extract in some plants while high activity (anti-oxidant) was observed in Chloroform extract in some plants.

INTRODUCTION

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plants. In recent past, there is a resurgence of interest in the study and use of medicinal plants. The juice of the boiled rhizome has also been used as a medicine for worm infestation in children [1]. The North-Eastern (NE) region of India is located between 87°3"E to 97°52'E latitude and 21° 34'N to 29°50'N latitude and is known over the whole world for its genetic resources. North-East India politically consists of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura.

Plants belonging to the Zingiberaceae family have been found to have antioxidant properties in several earlier studies [2-5]. The anti-oxidative potential of plant extracts and pure compounds can be measured using numerous *in vitro* assays. Each of these assays is based on one feature of antioxidant activity, such as the ability to scavenge free radicals or to inhibit lipid peroxidation. However, the total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals [6]. Two or more methods should always be employed in order to evaluate the total anti-oxidative effects of vegetables. Some studies on the anti-oxidant activities of *Zingiber zerumbet* rhizomes had been reported [7, 8].

Antioxidants are substances with free-radical chain reaction breaking properties. They are inhibitors of lipid peroxidation and are important not only for food protection, but also for the defense of living cells against oxidative damage. Antioxidant activity is essential for life to counteract the strongly oxidizing environment in which we live [9]. Many biological functions, such as protection from mutagenesis, carcinogenesis and aging, among others, are due to anti-oxidative effects [10]. The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of new drugs. In the search for sources of natural antioxidants, some medicinal plants have recently been extensively studied for their antioxidant activity and radical scavenging activity by many investigators [11-15].

The species of Zingiberaceae family chosen for our study are *Alpinia galanga* Linn., *Alpinia allughas (*Retx) ROS., *Curcuma longa* Linn., *Curcuma caesia* Roxb., *Curcuma augustifolia* Roxb., *Hedychium aurantiacum* Wall ex Roscoe, *Hedychium coccineum* Buch.-Ham.ex Sm, *Kaempferia galanga* Linn. , *Zingiber cassumunar* Roxb., *Zingiber officinale* Rosc which grow particularly in all tropical forests. However, little is known about their antioxidant properties and compounds responsible for antioxidant activity in these species. This prompted us to carry out this study of the antioxidant activity of the rhizomes of the plants of Zingiberaceae family thereby to isolate and identify the active components present in these plants. The antioxidant activities of these plants were carried out by scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and the β-carotene-linoleate methods.

MATERIALS AND METHODS

Plant materials:

Rhizomes of ten ginger species (*Alpinia* galanga Linn. (IBSD/Z-1), *Alpinia allughas* (Retx) ROS. (IBSD/Z-5), *Curcuma longa* Linn. (IBSD/Z-20a), *Curcuma caesia* Roxb. (IBSD/Z-19), *Curcuma augustifolia* Roxb. (IBSD/Z-17), *Hedychium aurantiacum* Wall ex Roscoe. (IBSD/Z-26), *Hedychium coccineum* Buch.-Ham.ex Sm. (IBSD/Z-27), *Kaempferia galanga* Linn. (IBSD/Z-37), *Zingiber cassumunar* Roxb. (IBSD/Z-39), *Zingiber officinale* Rosc. (IBSD/Z-41a) were collected from different parts of Manipur. All the ginger species were collected during the month of January-February 2008-2009 and identified by the taxonomist of the instititute.

Chemical materials:

2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), β-Carotene were purchased from Sigma Chemical Co. (St. Louis, MO, USA), linoleic acid, tween 80 emulsifier, chloroform, methanol and petroleum ether were purchased from Merck Chemicals, Mumbai, India.

Extract preparations:

The rhizomes of the ginger species were washed thoroughly

with tap water. They were sliced into 5-6 mm slices. The sliced rhizomes were dried in shade for about one week. The dried rhizomes were grounded into a powder with the help of a Waring blender and were extracted with different solvents starting from lower polarity to higher polarity i.e. petroleum ether, chloroform and methanol using cold extraction in shaker for 24hrs at room temperature. Each extract was filtered using Whatman No.1 filter paper and concentrated under reduced pressure to dryness below 40°C using Buchi Vacuum evaporator. The dried extracts thus obtained were directly used for the determination of antioxidant activities.

Antioxidant properties:

Antioxidant properties were analyzed by two different methods.

1. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay: The antioxidant properties for each of the different crude extracts of each plant were screened for radical scavenging activity using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) method [16]. The different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl). 2.8 mL of DPPH solution (45µg/mL) was rapidly mixed with 200µL and 400µL of methanol solution of plant extract one at a time in cuvette placed in the spectrophotometer (SHIMADZU-1700). The absorbance at 515nm was measured after 5 mins. BHT (Butylated hydroxy toluene) solution (125g/mL) was used as a reference corresponding to 100% radical scavenging activity. The decline in radical concentration indicated the radical scavenging activity of the sample.

Calculation

$$\text{Radical Scavenging \%} = \frac{A_o - A_{test}}{A_o - A_{ref}} \times 100$$

Where

A_0 = *Initial absorbance (DPPH + Sample)*

A_{test} = *Absorbance of the sample and DPPH after 5 mins.*

A_{ref} = *Absorbance of DPPH and BHT after 5 mins.*

2. -Carotene Linoleic acid assay: -carotene linoleic acid assay was carried out as described by Miller [17]. A solution of -Carotene was prepared by dissolving 2mg of -Carotene in 10ml of chloroform. 2ml of this solution was pipetted into a 100ml round bottom flask. After chloroform was removed under vacuum, 40mg of purified linoleic acid, 40mg of tween 80 emulsifier and 100ml of aerated distilled water were added to the flask with vigorous shaking. Aliquots (2.8ml) of this emulsion were transferred into different test tubes containing different extracts. BHT (Butylated hydroxytoluene) is used as reference. As soon as the emulsion was added to each tube, zero time absorbance was measured on UV-VIS spectrophotometer at 470 nm (SHIMADZU-1700). The tubes were then placed in water bath at 50°C and the measurement of absorbance was continued until the color of -Carotene disappeared, a blank devoid of -Carotene was prepared for background correction.

AA = *-Carotene content after two hours / Initial -Carotene content*

RESULTS AND DISCUSSION

The three solvents petroleum ether, chloroform and methanol were used for the extraction of the selected plants. Each of the different extracts of each plant was screened for radical scavenging activity using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and β-carotene-linoleate method. From the results of the calculation, it was observed that, radical scavenging activity of the P.E. extract of the plants decrease in the order:

Alpinia galanga Linn.> *Zingiber officinale* Rosc.> *Curcuma longa* Linn.> *Zingiber cassumunar* Roxb.> *Curcuma augustifolia*

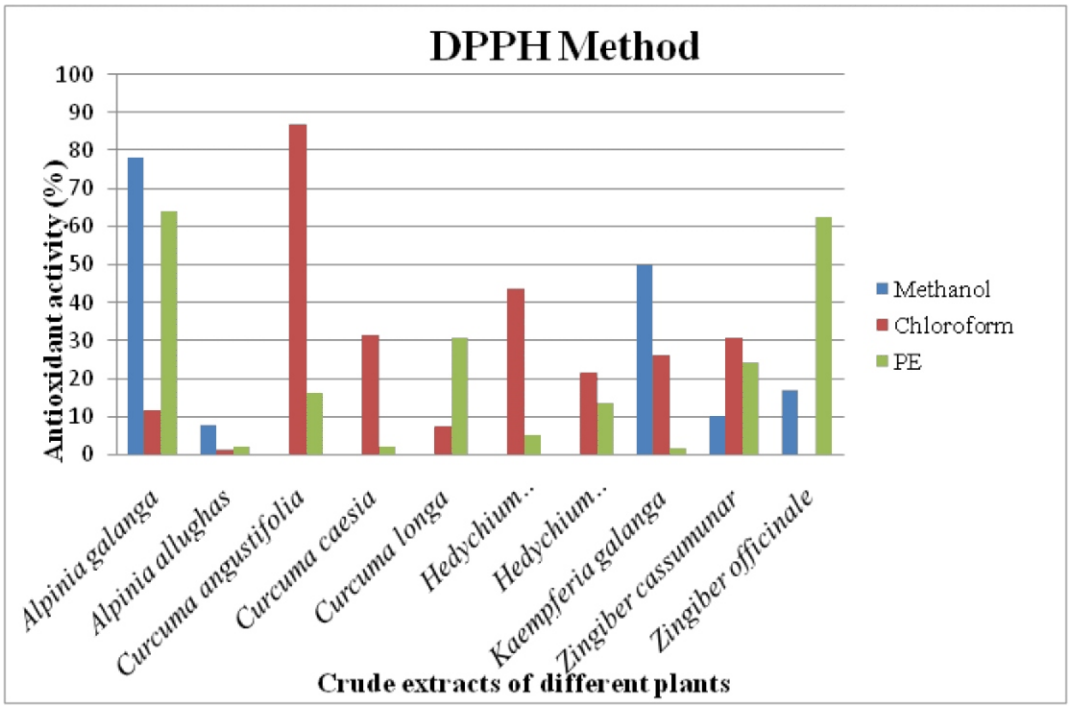

Fig.1: Antioxidant activity of crude extracts of different plants (DPPH) method

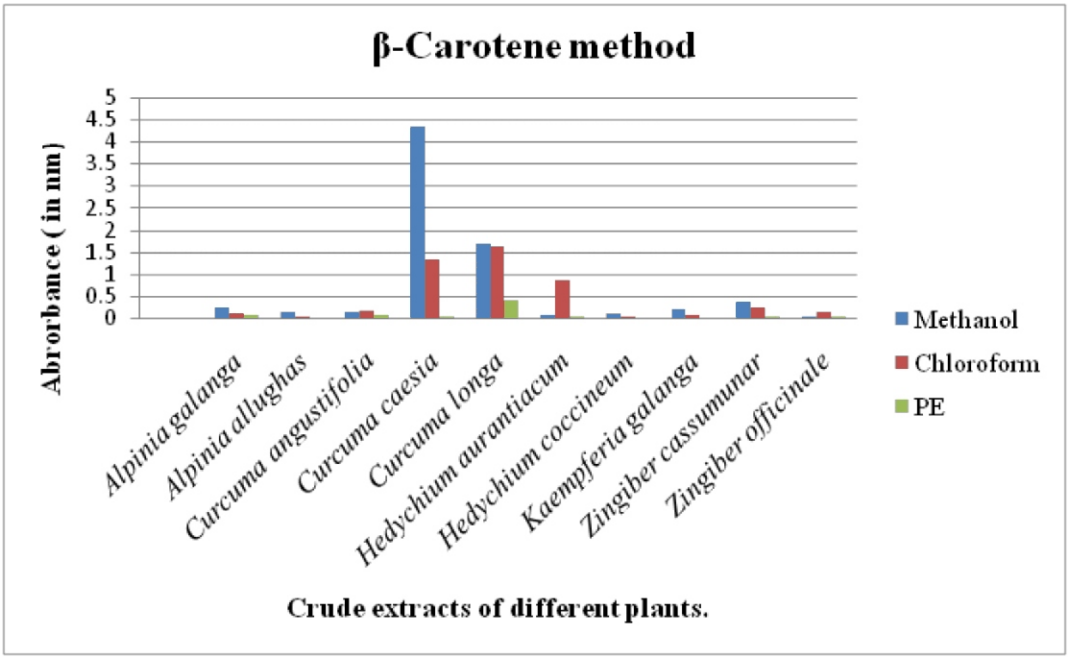

Fig.2: Antioxidant activity of crude extracts of different plants (β-carotene-linoleate method)

Roxb.> *Hedychium coccineum* Buch. Ham.ex Sm> *Hedychium aurantiacum* Wall ex Roscoe> *Curcuma caesia* Roxb.> *Alpinia allughas* (Retx) ROS.> *Kaempferia galanga* Linn.

For chloroform extracts radical scavenging activity follows the order: *Curcuma augustifolia* Roxb.> *Hedychium aurantiacum* Wall ex Roscoe> *Curcuma caesia* Roxb.> *Zingiber cassumunar* Roxb.> *Kaempferia galanga* Linn. .> *Hedychium coccineum* Buch. Ham.ex Sm> *Alpinia galanga* Linn.> *Curcuma longa* Linn.> *Alpinia allughas* (Retx) ROS. *Zingiber officinale* Rosc shows no radical scavenging activity in chloroform extract.

For methanol extracts, radical scavenging activity follows in the order: *Alpinia galanga* Linn.> *Kaempferia galanga* Linn. *Zingiber officinale* Rosc.> *Zingiber cassumunar* Roxb.> *Alpinia allughas* (Retx) ROS. *Hedychium coccineum* Buch. Ham.ex Sm, *Hedychium aurantiacum* Wall ex Roscoe, *Curcuma longa* Linn., *caesia* Roxb. and *Curcuma augustifolia* Roxb. show no radical scavenging activity.

Therefore, different solvent extracts of each plant shows varying radical scavenging activity. For a few plants, the P.E. extract shows high activity while in some either $CHCl_3$ or CH_3OH extract shows higher activity compared to other solvents. Also in some plants, either one of the three solvent extracts show no radical scavenging activity.

All the different solvent extracts of each plant shows different absorbance values indicating their bioactive contents. Methanol extract shows highest absorbance in the following plants namely, *Alpinia galanga* Linn. *Alpinia allughas* (Retx) ROS., *Hedychium coccineum* Buch. Ham.ex Sm, *Zingiber cassumunar* Roxb., *Curcuma longa* Linn., *Kaempferia galanga* Linn. and *Curcuma caesia* Roxb. Highest absorbance values of $CHCl_3$ (Chloroform) extract were observed in *Zingiber officinale* Rosc., *Hedychium aurantiacum* Wall ex Roscoe and *Curcuma augustifolia* Roxb. Only *Alpinia galanga* Linn. showed highest absorbance value in the petroleum ether extract. In this method, the more the absorbance value indicates that the plant extract has more bioactive substances.

CONCLUSION

Often it is difficult to decide in a screening for antioxidants from natural sources which of the plant species studied can be considered the best one, as each of them exhibits different antioxidant and/or scavenging activities. The extracts from ten selected species of Zingiberaceae family showed moderate to good antioxidant properties.

Each of the different extracts of each plant was screened for radical scavenging activity using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and β-carotene-linoleate method. Among the ten selected plants of the Zingiberaceae family, *Alpinia galanga* Linn., *Alpinia allughas* (Retx) ROS., *Zingiber cassumunar* Roxb. and *Kaempferia galanga* Linn. shows high radical scavenging activities in all the three solvents used. In *Alpinia galanga* Linn., *Kaempferia galanga* Linn.and *Alpinia allughas* (Retx) ROS., the highest activity is observed in CH_3OH (Methanol) extract, while in *Zingiber cassumunar* Roxb., highest activity is observed in $CHCl_3$ (Chloroform) extract.

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