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ANTIOXIDANT PROPERTIES OF SOME EXCLUSIVE SPECIES OF ZINGIBERACEAE FAMILY OF MANIPUR

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KEYWORDS

Zingiberaceae, Antioxidants, DPPH and β-carotene-linoleic acid methods.

ABSTRACT

Antioxidant activity of 6 rhizomes of Zingiberaceae family (*Zingiber cassumunar, Alpinia galanga, Alpinia allughas, Hydychium corancium, Hydychium coccinum* and *Kaempferia galaugalim)* have been measured by DPPH method and β-carotene-linoleic acid method after extraction with two different solvents- methanol and dichloromethane to evaluate the choice of solvent for best results.

INTRODUCTION

Antioxidants, scavenge for free radicals1, and consequently are a very special group of nutritional supplements. The free radicals have a strong tendency to impair the proper functioning of the immune system which leads to infection and a hoard of degenerative diseases. Due to biochemical processes occurring in the body, it is normal for free radicals to be present in the body at all times, however when the free radicals increase to an abnormal level the danger begins. Antioxidants are substances with free radical chain reaction breaking properties. Among the numerous antioxidants available, flavonoids are naturally occurring phenolic compounds in plants. The antioxidative effect of flavonoids had long been recognized. They are known to inhibit lipid peroxidation, to scavenge free radicals and active oxygen, to chelate iron ions and to inactivate lipoxygenase.

We have been interested in Zingiberaceae family species of Manipur as these could be new source of natural antioxidants because most of these wild rhizomes are used as traditional medicines and spices by the local Manipuri people. It is known that several species from Zingiberaceae2-3 displayed antioxidant properties, we were particularly interested in screening some of the abundantly available wild species that grow in Manipur in two different solvents such as dichloromethane(less polar) and methanol (polar) solvents for crude extraction.

The species of Zingerberacea family chosen for our study are *Zingiber cassumunar, Alpinia galanga, Alpinia allughas, Hydychium corancium, Hydychium coccinum and Kaempferia galaugalim* which grow particularly in Manipur, although this family is widely distributed 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 assessment of antioxidant properties of these rhizomes. Most members of the family are easily recognized by the characteristic aromatic fleshy rhizomes.

MATERIALS AND METHODS

Plant material

All the rhizomes (*Zingiber cassumunar*, *Alpinia galanga*, *Alpinia allughas*, *Hydychium corancium*, *Hydychium coccinum* and *Kaempferia galaugalim)* were collected from the forests in Manipur region.

Chemical material

DPPH and β-carotene were purchased from Aldrich Chemical company. Linoleic acid and Tween 40 were purchased from Lancaster. Pyragallol was purchased from S.D.Fine Chemical company. All other chemicals were of the highest analytical grade and purchased from common sources.

Extraction

About 250 g of the rhizome of each plant were separately extracted with dichloromethane and methanol in soxhlet for 8hours. Extracts of each solvent were evaporated under reduced pressure and final residue were used for assessment of antioxidant activity by DPPH-(2,2-diphenyl-1-picrylhydrazyl) method and β-carotene-linoleic acid method.

	% crude mass of rhizome
A = Zingiber cassumunar	3.1%
B = Alpinia allughas	1.06%
C = Hydychium corancium	3.2%
D = Kaempferia galaugalim	2.97%
E = Hydychium Coccinum	1.08%
F = Alpinia galangal	2.93%

Antioxidant properties

Antioxidant properties were analyzed by two different methods:

1. **DPPH method**. The antioxidant properties were assessed by DPPH radical scavenging method (Sanchez-Moreno,1998)4-5. The different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH. 2.8 ml of DPPH solution (45 μg/ ml) were rapidly mixed with 200 μl and 400 μl of methanolic solution of plant extract one at a time in cuvette placed in the spectrophotometer. The absorbance at 515 nm was measured after 5 min. The initial absorbance of the DPPH was 1.2 -1.3. The decline in radical concentration indicated the radical scavenging activity of the sample.

   Pyragallol solution (125 μg/ ml) was used as a reference corresponding to 100% radical scavenging activity. Radical scavenging activity or antioxidant properties was evaluated as percentage was calculated as

$$\frac{\left(A_0 - A_{test}\right)}{\left(A_0 - A_{ref}\right)} \times 100$$

where as A_0 is the initial absorbance (DPPH + sample absorbance) and A_{ref} and A_{test} are absorbance after 5 min with pyragallol solution and sample solution.

2. **β-carotene-linoleic acid method**. β- carotene-linoleate method (by Miller 1971)6 A solution of β- carotene is prepared by dissolving 2 mg of β- carotene in 10 ml chloroform. 2 ml of this solution is pippetted into 100 ml RB flask after chloroform was removed under vacuum 40 mg 0f purified linoleic acid , 400 mg of tween 40 emulsifier and 100 ml of aerated distilled water are added to shake vigorously. Aliquots (4.8 ml) of this emulsion are added to test tubes containing different concentrations of the extracts. BHT was used for comparison purpose. As soon as the emulsion was added to each tube, zero time absorbance was measured on UV-VIS spectrophotometer at 470nm. The tubes were then placed in water bath at 50°C and the measurement of absorbance was continued until the color of β- carotene disappeared. a blank devoid of β- carotene was prepared for background correction.

$$AA = \frac{\beta - carotene\, content\, after\, 2\, hours\, of\ assay}{initial\, \beta - carotene\, content}$$

RESULTS AND DISCUSSION

As a preliminary investigation, collection of medicinal plants especially endangered species of Zingiberacea family from various parts of Manipur and their morphological details were worked out. 6 species under 4 genera were collected so far, *viz*., *Alpina* (2 species), *Hedychium* (2 species), *Kaemferia* (1 species) and *Zingiber* (1 species). However, there are many species which have yet to be investigated. The medicinal properties of these plants of Zingiberacea family are supposed to be due to the presence of certain bioactive compounds having antioxidant properties. Antioxidant properties were shown by both the dichloromethane and methanol crude extracts of all the six species of Zingiberaceae family. The absorbance value of the control was also recorded in each case. The antioxidant properties of all the crude extracts were found to be close to or higher than pyragallol and BHT (2,6-di-tert-butylhydroxy toluene). Graphical comparison of different rhizomes by DPPH method is given below in Fig.-1. As shown below the methanolic extract of the six rhizomes showed antioxidant properties of 0.2 and 0.4 ml extract are in the increasing order as *A. galanga*> *A. allughas*> *H. coccinum* > *Z. cassumunar* > *H. corancium*> *K. galaugalim*. The extracts of . Extracts of these plants are dose-dependently and showed an increase in DPPH free radical scavenging activity, in vitro. The extracts, which showed strong DPPH radical scavenging activity are *Alpinia galanga* and *Alpinia allughas*, while the others show moderate antioxidant properties. As shown in Fig.-2, the DCM extract of the six rhizomes showed antioxidant properties of 0.2 and 0.4 ml extract are in the increasing order as *Z. cassumunar* >*A. galanga*> *H. coccinum* > *H. corancium*> *A. allughas* > *K. galaugalim*. The extracts of *Zingiber cassumunar* and *Alpinia galanga* show best result, while the others show moderate antioxidant properties.

It is also noted that some of these rhizomes have substantial amounts of essential oils which may interfere with the antioxidant assay. We are now concentrating on these two species of Alpinia. Column chromatography of the crude extract of both the species of Alpinia to separate various active components and their evaluation of antioxidant properties of each fraction is under progress.

The antioxidant activity of rhizome extracts and BHT at 100 ppm concentration as measured by the bleaching of β-carotene, is presented in Fig. 3. It can be seen that rhizome extracts prepared by different solvents exhibited varying degrees of antioxidant activity. Methanol was found to give the maximum antioxidant activity. The mechanism of bleaching of -carotene is a free-radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. β-carotene, in this model system, undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their

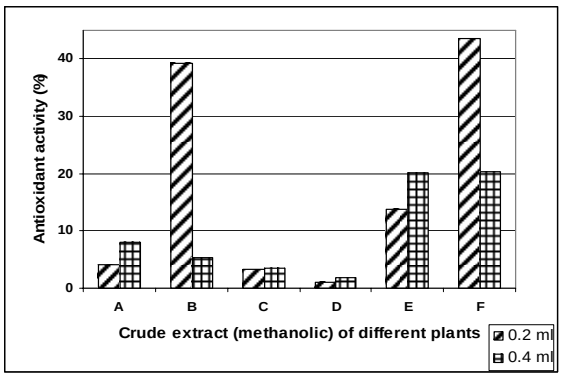

Figure-1 Antioxidant activity of crude methanolic extract of different plants

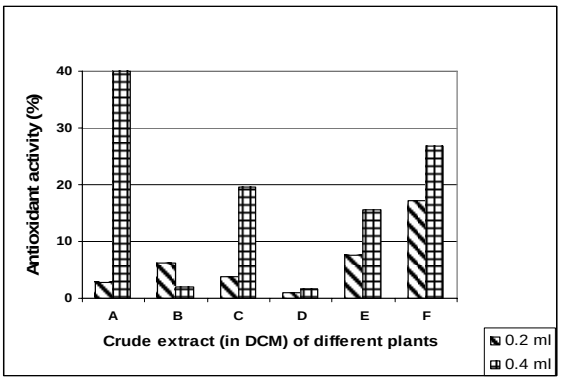

Figure-2 Antioxidant activity of crude DCM extract of different plants

A = Zingiber cassumunar, B = Alpinia allughas,C = Hydychium corancium, D = Kaempferia galaugalim, E = Hydychium coccinum, F = Alpinia galanga

double bonds by oxidation, the compound loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. The presence of different extracts can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

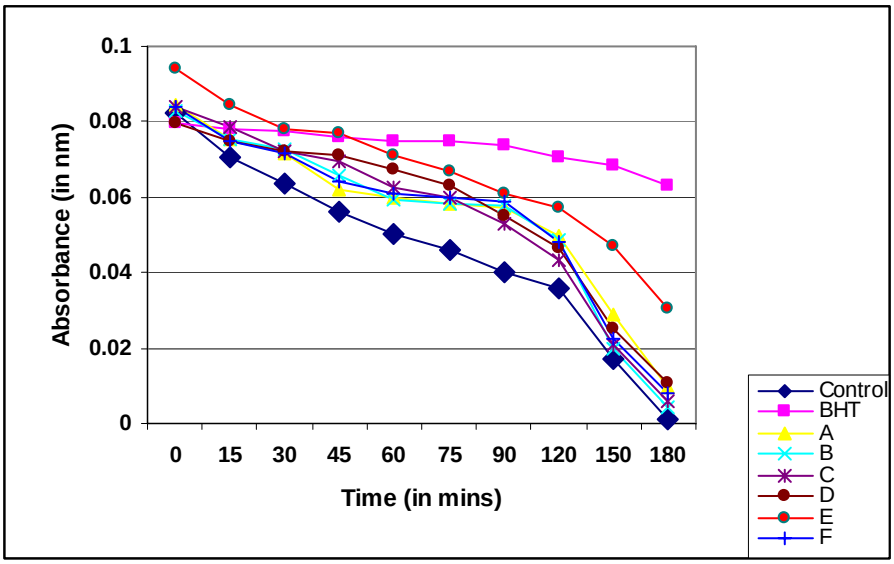

Figure-3. Reduction in Absorbance by β- carotene-linoleate method

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 six species of Zingiberaceae family showed moderate to good antioxidant properties. During the screening of six plants in this work, *Alpinia galanga*, *Alpinia allughas* and *Zingiber cassummar*, in this order, were found to be the most promising ones. The methanolic extract of the plants show better results for DPPH analysis than the DCM extract showing stronger activity as compared to BHT in some cases.

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