Research Article

Evaluation of Antioxidant Activity of Ten Selected Plants of *Zingiberaceae* Family Available in Manipur

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ABSTRACT

The present study was designed to investigate the anti-oxidant activity of the rhizomes of ten selected plants of zingiberaceae family available in Manipur. Anti-oxidant activity was determined by three methods such as 1, 1-diphenyl -2-picryl hydrazyl (DPPH), Ferric – reducing antioxidant power (FRAP) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Scavenging activity. The antioxidant activities of the extracts of the rhizomes in four solvents like petroleum ether, chloroform, methanol and distilled water extracts were reported. Result reveals that for DPPH assay *Alpinia galanga*, *Kaemfria rotunda, Hedychium rubrum* and *Amomum gracil* shows high activity in petroleum ether, chloroform, methanol and distilled water extracts. In ABTS method, *Kaemfria rotunda, Amomum gracil, Curcuma leucorhiza* and *Hedychium rubrum* shows high activity in petroleum ether, chloroform, methanol and distilled water extracts. For FRAP test, *Hedychium rubrum* shows high activity in both petroleum ether and methanol, *Curcuma leucorhiza* and *Alpinia galanga* shows high activity in chloroform and distilled water extract.

Keywords: Antioxidant, DPPH, ABTS, FRAP, *zingiberaceae*, solvent.

INTRODUCTION

Now a day, there has been given more emphasis toward the field of free radical chemistry. In human body, free radicals, reactive nitrogen species and reactive oxygen species are generated by pathological states, exposure to different physiochemical conditions or various endogenous systems. For a proper physiological function it is necessary to keep a balance between free radicals and antioxidants. Oxidative stress is ensured when free radicals overwhelm the body's ability to regulate them. Thus free radicals adversely alter proteins, lipids, DNA and trigger a number of human diseases [1]. Free radicals are chemical species that contains an unpaired electron. They are highly unstable and reactive. They combined easily with other free radicals and can formed free radical chain. They behave as oxidants or reductants as they can either donate an electron to or accept an electron from other molecules [2]. According to Genestra M [3], Hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$) etc are include in free radicals. In living organism, oxidant (not free radical) such as hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH), can easily lead to free radical reactions.

Reactive oxygen species (ROS) include radical and non-radical species. They are responsible for lipid peroxidation, protein oxidation, DNA damage and advanced glycation end-products (AGEs) formation via effects of ROS on cellular signaling pathways and are produced as the by-product of the electron transport chain (ETC) in mitochondria[4]. In human body free radicals and other ROS are derived either from normal essential metabolic processes or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Formation of free radical in the cell occurs continuously as a consequence of both enzymatic and non-enzymatic reaction[5].

We have interested the plants belonging to *Zingiberaceae* family, because *Zingiberaceae* family plants have been found to have potent antioxidant properties in several earlier works [6-11]. The species of *Zingiberaceae* family choosen for our studies are *Alpinia galanga, Alpinia nigra, Amomum gracil, Curcuma amada, Curcuma leucorhiza, Costus speciosus, Hedychium maximum, Hedychium rubrum, Hedychium coronarium,*and Kaemfria *rotunda.* Particularly, these species are growing in all tropical forest. However, earlier researchers have known little about their antioxidant properties. This prompted us to carry out this study of the antioxidant activity of the rhizomes of the plants of Zingiberaceae family. The antioxidant activities of these plants were done by 1, 1-diphenyl -2-picryl hydrazyl (DPPH), Ferric–reducing antioxidant power (FRAP) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Scavenging activity.

MATERIAL AND METHODS

Plant collection and preparation

Healthy rhizome of the ten species (*Alpinia galanga, Alpinia nigra, Amomum gracil, Curcuma amada, Curcuma*

leucorhiza, Costus speciosus, Hedychium maximum, Hedychium rubrum, Hedychium coronarium and *Kaemfria rotunda* were collected during the month January-April 2017 from Kangmong area, Imphal West district, Manipur, India. Those collected plant species were identified by Prof. P. Kumar Singh, Centre for Advance Study, Life Science Department, Manipur University, Cancipur, Imphal, and Manipur. The rhizomes were washed by running water and then by tap water. Those clean rhizomes were cut into small pieces; shade dried about seven days, grounded to fine powder by mechanical shaker and then kept in an air tide container for further use.

Preparation of crude extract

40gms fine powder of *Hedychium Maximum* was soaked in 400ml of petroleum ether, chloroform, methanol and distilled water solvents respectively, one after another with occasional shaking in the cold condition for 5 days. Each extract was filtered using Whatman No.1 filter paper and evaporated at low temperature. The dried crude extracts thus obtained were directly used for the determination of antioxidant activities.

Chemical material

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-1,3,5-triazine(TPTZ), 2,2'-azinobis-(3-ethylbenzo thiazoline-6-sulfonic acid) (ABTS), ascorbic acid, TBHQ were purchased from Sigma Aldrich. Acetic acid, Methanol, Hydrochloric acid was purchased from MERCK. DMSO and $KHSO_4$ were purchased from HIMEDIA. All the chemicals were of analytical grade.

Antioxidant properties

Antioxidant properties were analyzed by three different methods.

Analysis of antioxidant activity by scavenging of 1, 1-diphenyl -2-picryl hydrazyl (DPPH) [12]

Antioxidant activity of the plant extracts was assessed on the basis of the radical scavenging effect of the stable DPPH free radical, using a modified method of Brand-Williams, W.*et al* 1995. The working solutions of the test extract were prepared in DMSO. We prepared 0.024gm DPPH in 100ml methanol as stock solution; adjust the DPPH absorbance at1.120nm measure at 517 nm for working DPPH. Then 1.9 ml of the working DPPH solution was mixed with 100 µl of different concentrations of the test sample solution. These solution mixtures are then kept in dark for 1 hrs and optical density was measured at 517 nm using spectrophotometer against methanol as reference. A blank solution (100 µl DMSO and 1.9ml of working DPPH solution) was also measured against the reference. The optical density was recorded and percentage of inhibition was calculated using the relation:

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

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

Calculation of IC_{50}

Decolourisation was plotted against the sample extract concentration and a linear regration curve was established in order to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the DPPH free radical by 50 %. All the analysis was carried out in triplicate and the results are expressed as mean.

FRAP (Ferric –reducing antioxidant power) assay[13]

FRAP assay for determining the antioxidant activity in the plant extract was adopted a modified method of Benzie and Strain (1996). The stock solutions included 0.3 M acetate buffer (463ml of 0.3M acetic acid and 37ml of 0.3 M sodium acetate solution), PH 3.6 and 10mM TPTZ (2, 4, 6-tris tripyridyl-s-triazine) solution in 50ml of 40mM HCL, and 20mM $FeCl_3.6H_2O$solution. The fresh working solution was prepared by mixing 50ml of acetate buffer (0.3M. PH 3.6), 5ml of TPTZ solution (156mg TPTZ in 50ml in 40ml 40mM HCL) and 5ml $FeCl_3$ solution (20mM). Working solution of the test samples was prepared in DMSO. Each sample solution 100µl was added in 1.9ml of freshly prepared FRAP, voltex well and kept at dark for 20 minute, absorbance was measured at 593nm using 100µl DMSO and 1.9ml FRAP as control. Ascorbic acid, Trolox and TBHQ were used as standard compound.

Azino bisethyl bezthiozoline sulphonicacid (ABTS) Scavenging

ABTS radical-scavenging activity of the extract was determined according to Arnao *et al*[14]. Working ABTS solution was prepared by mixing 20ml 7.4 mM ABTS with 20ml of 2.6 mM $KHSO_4$. This solution is then incubation at dark for 12hrs. 1ml of working ABTS was mixed with 20ml of methanol and check the absorbance of 1.1(±0.02) at 734 nm. The radical decolourization assay was performed in a volume of 1425µl working ABTS solution and 75 µl of different concentration of the test samples. A blank solution (75 µl DMSO+1425 µl ABTS) was run against methanol .Ascorbic acid was used as standard compound. Percentage of total antioxidant activity (% TAAs) was calculated using the flowing equation.

Total antioxidant activity (TAA %) = (A –B /A) ×100

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

RESULTS AND DISCUSSION

For extraction of the selected plants, four solvents, petroleum ether, chloroform, methanol and distilled water were used. Each of the different extracts of each plant was screened for radical scavenging activity using 1, 1-diphenyl -2-picryl hydrazyl (DPPH), Ferric –reducing antioxidant power (FRAP) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Scavenging activity. Here, Ascorbic acid is used as a standard antioxidant for DPPH and ABTS methods. From the result of calculation, it was observed that, radical scavenging

activity of the Petroleum ether extract of the plants decreases in the order:

Alpinia galangal **>** *Kaempferia rotunda*> *Curcuma amada* > *Costus speciosus* > *Hedychium maximum* > *Amomum gracile* > *Hedychium coronarium* > *Alpinia nigra* > *Hedychium rubrum* > *Curcuma leucorhiza*.

For $CHCl_3$ extracts radical scavenging activity follows the order: *Kaempferia rotunda* > *Hedychium rubrum* > *Costus speciosus* > *Curcuma leucorhiza* > *Alpinia nigra* > *Hedychium maximum* > *Hedychium coronarium* > *Curcuma amada* > *Alpinia galangal* > *Amomum gracile*.

For CH_3OH extracts radical scavenging activity follows the order: *Hedychium rubrum* > *Alpinia nigra* > *Curcuma leucorhiza* > *Kaempferia rotunda* > *Alpinia galangal* > *Amomum gracile* > *Hedychium maximum* > *Curcuma amada* > *Hedychium coronarium* > *Costus speciosus*.

For H_2O extracts radical scavenging activity follows the order: *Amomum gracile* > *Hedychium rubrum* >*Alpinia galangal* > *Alpinia nigra* > *Hedychium maximum* > *Curcuma leucorhiza* > *Kaempferia rotunda* > *Curcuma amada* > *Costus speciosus* > *Hedychium coronarium*.

Therefore, different solvent extracts of each plant shows varying radical scavenging activity. Here, lower the value of IC_{50} higher the radical scavenging activity and vice versa. Ascorbic acid is also used as standard antioxidant. For a few plants, the petroleum ether and $CHCl_3$ extract show high activity, while in some either CH_3OH or H_2O extract shows high activity compared to the other solvent extracts. Of all the ten selected plants, *Hedychium rubrum* show highest radical scavenging activity in $CHCl_3$, CH_3OH and H_2O extracts. In *Kaempferia rotunda* highest activity is observed in $CHCl_3$, CH_3OH and petroleum ether extracts. *Amomum gracile* shows highest scavenging activity in H2O and CH_3OH extracts. In *Alpinia galangal* highest scavenging activity is observed in Petroleum ether, CH_3OH and $CHCl_3$ extracts. In *Alpinianigra,* and *Curcuma leucorhiza* highest activity is observed in CH_3OH extract. While in *Costus speciosus* highest activity is observed in $CHCl_3$ extracts.

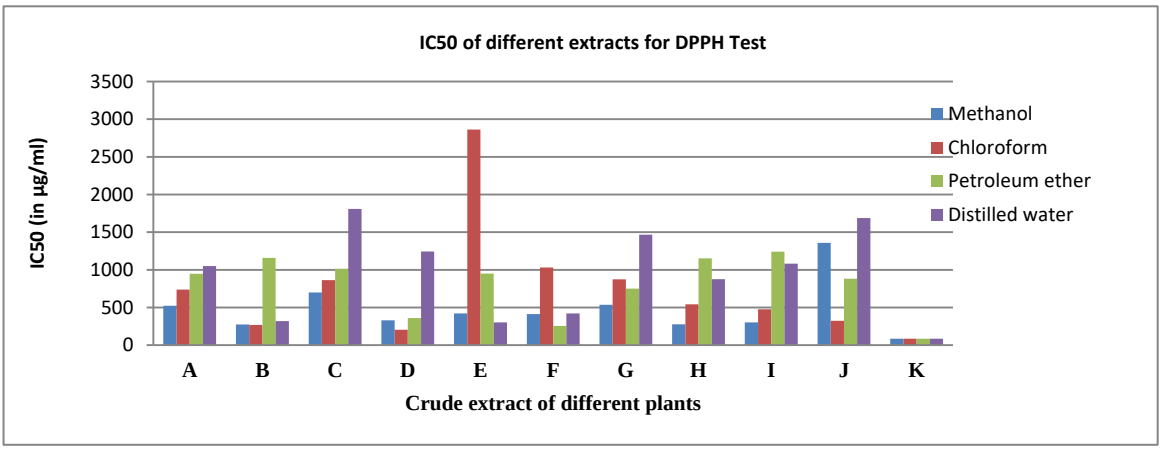

Figure 1: IC_{50} of crude extract of different plants (DPPH) method

A**.** *Hedychium maximum,* B. *Hedychium rubrum*, C. *Hedychium coronarium*, D. *Kaempferia rotunda.* E. *Amomum gracile*, F.*Alpinia galangal,* G. *Curcuma amada,* H. *Alpinia nigra,* I. *Curcuma leucorhiza*, J. *Costus speciosus*, K. Ascorbic acid

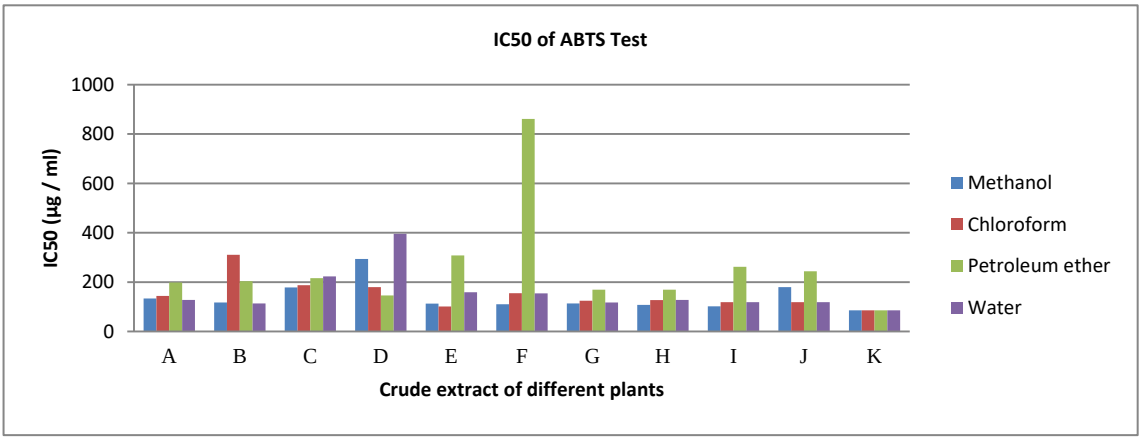

Figure 2: IC_{50} of crude extracts of different plants (ABTS) method

A. *Hedychium maximum,* B. *Hedychium rubrum*, C. *Hedychium coronarium*, D. *Kaempferia rotunda.* E. *Amomum gracile*, F. *Alpinia galangal,* G. *Curcuma amada,* H. *Alpinianigra,* I. *Curcuma leucorhiza*, J.*Costus speciosus*, K. Ascorbic acid.

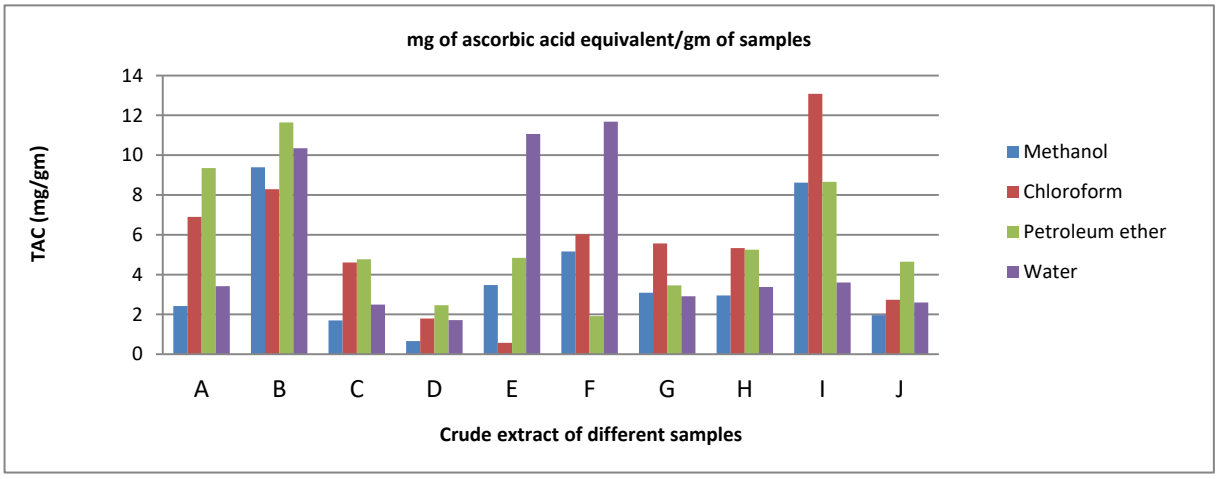

Figure 3: TAC (mg of ascorbic acid equivalent/gm of samples) of FRAP Test

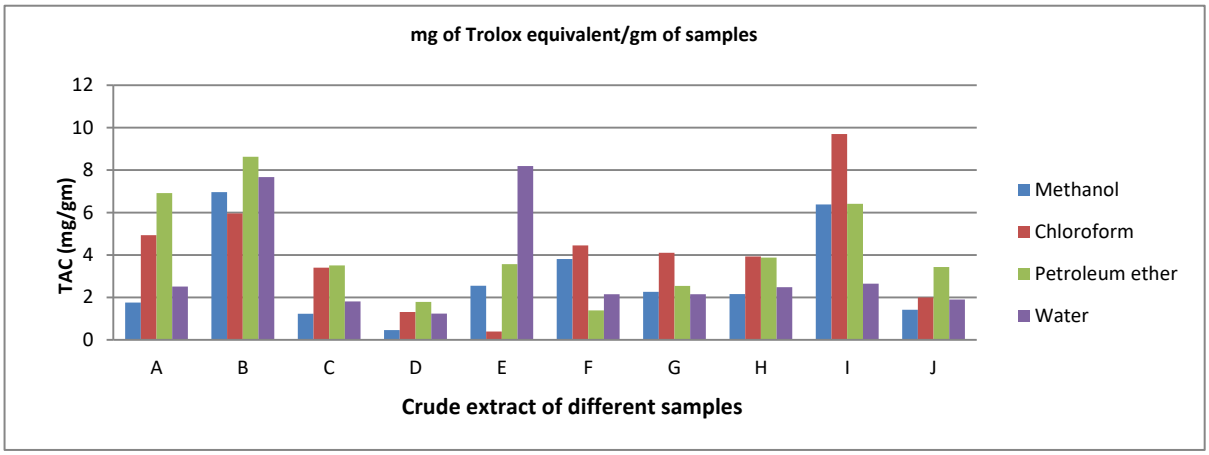

Figure 4: TAC (mg of Trolox equivalent/gm of samples) of FRAP Test

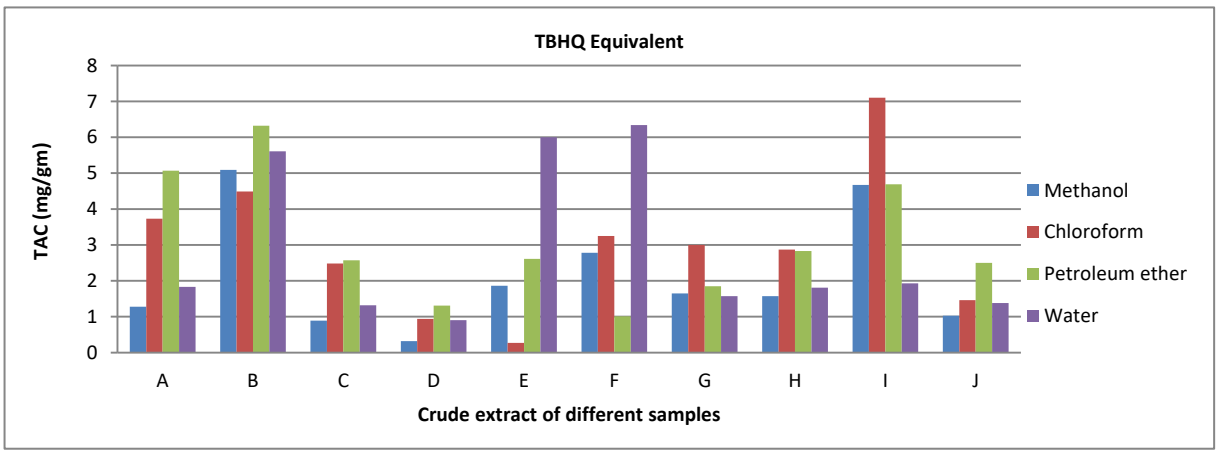

Figure 5: TAC (mg of TBHQ equivalent/gm of samples) of FRAP Test

A. *Hedychium maximum,* B. *Hedychium rubrum*, C. *Hedychium coronarium*, D. *Kaempferia rotunda.* E. *Amomum gracile*, F. *Alpinia galangal,* G. *Curcuma amada,* H. *Alpinianigra,* I. *Curcuma leucorhiza*, J. *Costus speciosus*

CONCLUSION

The extracts from ten selected plant species of Zingiberaceae family showed moderate to good antioxidant properties. In DPPH test *Hedychium rubrum* showed high activity in three solvent, chloroform, methanol and distilled water. *Kaempferia rotunda* showed high activity in chloroform, distilled water and petroleum ether extract. *Amomum gracile, Alpinia galangal*, *Alpinianigra* and *Costus speciosus* showed high activity in distilled water, petroleum ether, methanol and chloroform. For ABTS, both *Curcuma amada* and *Alpinia nigra* showed high activity in all the solvents.

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