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## *In vitro* antioxidant and free radical scavenging activity of *Alpinia calcarata*: A novel underutilized crop in Tropical Island

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### Abstract

Since last few decades, there has been an exponential growth in popularising herbal medicine owing to its natural origin and lesser side effects while curing different ailments in the body.

The present study was undertaken to reveal the effect of solvent (methanol, ethanol and aqueous) on phytoconstituents and antioxidant activity of *Alpinia calcarata* rhizome. Methanolic extract had highest phenolic content (21.08 mg/g GAE) followed by ethanolic (17.52) and aqueous extract (8.91 mg/g). DPPH radical scavenging activity was found to be highest in ethanolic extract (83.9%) and lowest in aqueous extract (58.01%). However, ABTS activity was found to be highest in methanolic extract (7.1 mg trolox/g) and lowest in aqueous extract (5.6 mg/g). Similar observations were found for Nitric oxide (NO) and FRAP activity. The metal chelating activity (MCA) was highest in ethanolic extract (84.6%) and lowest in methanolic extract (80.7%). This implied the potentiality ethanolic extract of *Alpinia* rhizome powder to be used in food and herbal industries for the preparation of nutraceutical compounds and herbal supplements.

**Keywords:** Genetic combining ability, specific combining ability, okra, variance, growth, yield and quality

### 1. Introduction

Tropical plant species are good sources of formulating herbal drugs due to presence of secondary metabolites in the form phytochemicals to defend against biotic and abiotic stresses. *Alpinia calcarata* is a rhizomatous perennial herb native to Asia, Australia, and the Pacific Islands belongs to Zingiberaceae family. The Phytochemical screening of mature rhizome revealed the presence of polyphenols, tannins, flavonoids, steroid glycosides and alkaloids in the extracts and essential oil of this plant. The solvent extracts of the rhizomes are known to possess anti-bacterial, anti-fungal, anti-helminthic, antinociceptive, anti-inflammatory, antioxidant, aphrodisiac, gastro protective, and anti-diabetic properties (Arawawala *et al.*, 2012; Rahman and Islam, 2015; Arambewela *et al.*, 2015; Raj *et al.*, 2011; Akhtar M.S *et al.*, 2002; Abu Ahmed *et al.*, 2015) <sup>[3, 23, 2, 1]</sup>, thus its decoction is widely used to treat cough, stomach ache, respiratory ailments, bronchitis, asthma, rheumatism and diabetics. The *in vitro* conservation strategies for the Propagation of *A. calcarata* was demonstrated by Mathew *et al.* (2014) <sup>[17]</sup> who reported for its scientific exploitation for pharmaceutical and commercial purposes. Hema and Nair (2009) <sup>[10]</sup> investigated about the flavonoids and other more polar constituents from *A. calcarata*. This study revealed that *A. calcarata* has considerable chemical similarity with *A. katsumadai* is also a very good source of pinocembrin 6 which induces mitochondrial apoptosis in colon cancer cells. Ramya R. *et al.* (2015) <sup>[22]</sup> reported that majority of the secondary metabolites like flavonoid, steroid, and terpenoids were found in ethanolic extract of *A. calcarata* possessing powerful *in vitro* antioxidant activity in comparison with the various other solvent extracts.

As situated in the subtropical region (10-35° N latitude), plants in Andaman and Nicobar Islands (India) frequently encounter unfavourable climatic factors, such as high rainfall (320cm), temperatures, contamination of soils by high salt concentration and highest UV irradiance. Due to these abiotic stresses, ROS are generated in plant leading to protein denaturation, DNA impairment and cellular damage etc. So, as an adaptive mechanism, rice plant synthesizes high level of phenols as secondary metabolites in combating the damaging effects of oxidative stresses. The suppressive effects of abiotic stress and looming vulnerability of tropical Islands to the Tsunami, 2004; Hudhud, 2015 like climatic aberrations necessitates in searching Island germplasm for stress mitigation. Such germplasm information can be

useful further research in breeding programme. It was observed that basic information regarding phenolic content and effect of different solvents on antioxidant activities of *Alpinia calcarata rhizome* is rarely reported and the information pertaining to tropical climate like Andaman is not reported so far based on our knowledge. So, the present study we determined the phytoconstituents and radical scavenging activities of *Alpinia* rhizome powder for understanding its usefulness for industrial application in formulating herbal drugs.

## 2. Materials and Methods

### 2.1 Sample preparation

After harvest, the *Alpinia calcarata* rhizome was washed in tap water to remove mud, clay and sand particles adhered to it followed by drying in cabinet dryer at 50<sup>o</sup> C for 2-3 days to reduce the moisture content to 6-7% (wet basis). After drying turmeric slices were grinded into powder form by using high speed blander. One gram sample was mixed with 50 ml of each of solvent (80% methanol, 80% ethanol and distilled water) and kept for 1-2 day followed by centrifugation at 8000rpm for 10 min followed by filtering through Whatman No. 1 filter paper. The extract was concentrated by rotary evaporator and kept in a refrigerator (-4<sup>o</sup>C) for analysis.

### 2.2 Estimation of phytochemicals

#### 2.2.1 Total phenolic content

The Folin-Ciocalteu colorimetric method was used to measure the total phenolic content (Singleton *et al.*, 1999)<sup>[27]</sup> with little modification. Briefly, 200 µl of the extractions were oxidized with 1 ml of 0.5 N Folin-Ciocalteu reagent and then the reaction was neutralized with 1 ml of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a UV-2600 spectrophotometer (Simadzu, Japan) after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent (mg GAE) per 100g of flour weight.

#### 2.2.2 Total flavonoid content

Total flavonoid content was estimated by colorimetric method (Bao *et al.*, 2005). Briefly, 0.5 ml extracts were added to 15 ml polypropylene conical tubes containing 2 ml ddH<sub>2</sub>O and mixed with 0.15 ml 5% NaNO<sub>2</sub>. After reacting for 5 min, 0.15 ml 10% AlCl<sub>3</sub>.6H<sub>2</sub>O solution was added. After another 5 min, 1 ml 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at 415 nm. Qualification was done using the Rutin as standard and the results was expressed as milligrams of rutin equivalent (mg RE) per 100 g of dry weight.

using the Rutin as standard and the result was expressed as milligrams of quercetin equivalent (mg QE) per 100 g of dry weight.

### 2.3 Estimation of free radical scavenging activity

#### 2.3.1 DPPH radical scavenging activity

It was measured with the procedure described by Rattanachitthawat *et al.*, 2010<sup>[24]</sup> with some modification. The working solution of DPPH was freshly prepared by diluting 3.9 mg of DPPH with 95% ethanol to get with an absorbance of 0.856±0.05 at 517nm. The different concentration of extract was mixed with 1.5 ml of working DPPH and the absorbance of the mixture immediately

measured spectrophotometrically after 10 min. BHA was used as standard.

$$\% \text{ inhibition of DPPH radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

Where A<sub>control</sub> is the absorbance of the control (without extract) and A<sub>sample</sub> is the absorbance in the presence of the extract/standard.

The IC<sub>50</sub> (concentration for 50% inhibition) values of all the methods were calculated by using linear regression analysis. All the experiments were repeated thrice. Higher the IC<sub>50</sub> value, lower is the scavenging activity.

#### 2.3.2 ABTS radical scavenging activity

The total antioxidant capacity was determined by a colorimetric method (Re *et al.*, 1999)<sup>[25]</sup> with a little modification. First, ABTS<sup>+</sup> solution prepared and was adjusted with pH to about 0.784±0.01 with 80% ethanol at 734 nm. Then, 3.9 ml ABTS<sup>+</sup> cation solution was added to 0.1 ml (2.5 mg/ml) of extracts and mixed thoroughly. The mixture incubated for 6 min at room temperature and tested the absorbance at 734 nm. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, µM Trolox equivalents per 100 g dry weight).

$$\% \text{ inhibition of ABTS radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

#### 2.3.3 Ferric reducing-antioxidant power (FRAP) assay

It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form at low pH (Benzie and Strain (1999)<sup>[5]</sup>.

Briefly, 0.9ml of prepared FRAP reagent is mixed with 0.1ml of diluted sample and the absorbance at 595 nm is recorded after a 15 min incubation at 37 °C and the results were expressed in mM of Fe<sup>2+</sup> equivalents per g dry weight.

#### 2.3.4 Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured using the procedure described by Marcocci *et al.*, 1994<sup>[16]</sup>. SNP (10 mM) in phosphate buffer saline (PBS) was mixed with different concentrations of the extract and incubated at 25°C for 150 min. The samples were added to Griess reagent (1% sulphanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthylethylenediamine dihydrochloride).

The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was measured by the following formula:

$$\% \text{ inhibition of NO radical} = (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}) \times 100$$

#### 2.3.5 Metal chelating activity (MCA)

The chelating of ferrous ion was measured using the method of Dinis *et al.*, 1994<sup>[6]</sup>. The crude extracts were reacted with 0.05 mL of 2.0 mM FeCl<sub>2</sub>.

The mixture was then added with 0.2 mL of 5.0 mM ferrozine. After which, the reaction was shaken and incubated at room temperature for 10 min and the absorbance of the red color was measured at 562 nm. EDTA was used as a positive control. The percentage of metal chelating activity was calculated by the following equation:

$$\% \text{ Metal chelating activity} = (\text{Ac} - \text{As}) / \text{Ac} \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the extract/standard.

### 2.3.6 Reducing power (RP) assay

This method involves in the mechanism of single electron transfer system using ferric to ferrous reducing activity as determined spectrophotometrically from the formation of Perl's Prussian blue colour complex. This method is based on the principle of increase in the absorbance of the reaction mixtures described by Oyaizu (1986) [19]. 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of  $\text{K}_3\text{Fe}(\text{CN})_6$  (1% w/v) are added to 1.0 mL of sample dissolved in distilled water.

The resulting mixture is incubated at 50° C for 20 min, followed by the addition of 2.5 mL of Trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of  $\text{FeCl}_3$  (0.1%, w/v). The absorbance is then measured at 700 nm against blank sample. The higher the absorbance of the reaction mixture the greater is the reducing power.

## 2.4 Statistical analysis

### Statistical analysis

Data were analysed by MS-Excell and SPSS 17.0 software for Duncan's multiple range test (DMRT) to assess the statistical differences among the means at  $p < 0.05$ .

## 3. Results and discussions

### 3.1 Phenolic and flavonoid content

Total phenolic and flavonoid content of three extract is shown in table 1. Methanolic extract had highest phenolic (21.08 mg/g GAE) followed by ethanolic (17.52) and aqueous extract

(8.91 mg/g). Similar results were reported by Wong *et al.*, (2008) [29] in other species of *Alpinia*. They reported that the phenolic content of *Alpinia galanga* had 2.14 mg GAE/g which was 10 times less that our result. The reason may be due to different growth period, geographic location, storage type, genetic diversity etc. Similarly, the flavonoid content was varied between 60.7 mg rutin/g (methanolic extract) and 14.3 mg rutin/g (aqueous extract). Methanol, besides having higher extraction efficiency, is more efficient in cell wall degradation as compared with other two solvents (Lapornik *et al.* 2005) [14]. The higher values for methanolic extract may be due to the extraction power of solvent (80% methanol) to the flavonoid compounds to the dilution media. The levels of total phenolics determined in this way are not absolute measurements of the amounts of phenolic compounds, but are in fact based on their chemical reducing capacity relative to gallic acid. It has been observed that the phenol antioxidant index is a combined measure of the quality and quantity of antioxidants in vegetables (Elliot, 1999). The phenolic compounds are the dominant antioxidants that exhibit scavenging efficiency on free radicals, and reactive oxygen species are numerous and widely distributed in the plant kingdom (Zia-Ul-Haq *et al.*, 2011a) [30].

**Table 1:** Phytochemicals in *Alpinia calcarata* rhizome powder

Extract	Phenol (mg GAE/g)	Flavonoids (mg rutin/100g)	DPPH (mg BHT/g)	ABTS (mg trolox/g)	FRAP (mg $\text{Fe}^{+2}$ /g)	NO (mg Vit-C/g)	MCA (mg EDTA/g)	RP (mg BHT/g)
Ethanolic	17.52	60.7	5.82	6.96	2.17	11.65	11.90	11.02
Methanolic	21.08	47.3	5.25	7.1	1.90	11.40	12.35	15.01
Aqueous	8.91	14.3	5.52	5.6	1.59	11.05	11.70	6.1

(DPPH- 2,2-diphenyl-1-picrylhydrazyl; ABTS- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid MCA-Metal chelating activity)

**Table 2:** IC 50 values ( $\mu\text{g/ml}$ ) *Alpinia calcarata* rhizome with different antioxidant methods

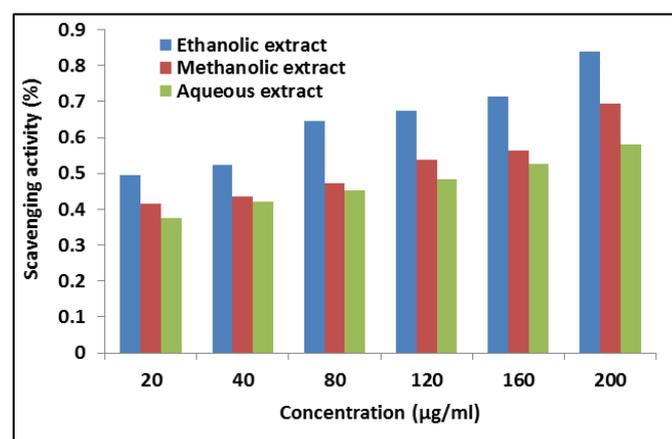
	DPPH	ABTS	NO	MCA
Ethanolic extract	36	141.75	83	2083
Methanolic extract	142.5	146.25	167	1389
Aqueous extract	176	237.1	191	2005

## 3.2 In vitro free radical-scavenging activity

### 3.2.1 DPPH radical scavenging activity

This is based on the reduction of DPPH- solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extracts were able to reduce the stable radical DPPH- to the yellow-coloured diphenylpicrylhydrazine. Results from present study indicated highest DPPH activity in ethanolic extract (5.82 mg BHT/g) followed by methanolic extract (547.5 mg/g) and aqueous extract (532.5 mg/g). There was steady increase in the inhibition of the radicals with concomitant increase in concentration of the extract establishing dose dependence of the extract in scavenging DPPH radicals. DPPH radical scavenging activity of three extracts were increased with increasing concentration (20-200  $\mu\text{g/ml}$ ), being highest in ethanolic extract (83.9%) and lowest in aqueous extract (58.01%) at 200  $\mu\text{g/ml}$  as shown in fig 1. The higher scavenging activity was due to high level of phenolic content in methanolic extract. Phenolic compounds are high level antioxidants (Hall and Cuppett, 1997) [9]

because they possess the ability to adsorb and neutralize free radicals, quench active oxygen species and decompose superoxide and hydroxyl radicals (Duh *et al.*, 1999) [7]. IC50 (concentration required for 50% reduction of scavenging activity) is shown in table 2. Smaller IC50 value correlated with higher DPPH scavenging activity. Highest IC50 value was found in ethanolic extract (36  $\mu\text{g/ml}$ ) suggesting high efficiency in quenching DPPH radical followed by methanolic (142.5  $\mu\text{g/ml}$ ) and aqueous extract (176  $\mu\text{g/ml}$ ).



**Fig 1:** DPPH radical scavenging activities of *Alpinia calcarata* rhizome powder

### 3.2.2 ABTS radical scavenging activity

The ABTS assay is based on the inhibition by antioxidants of the absorbance of the ABTS radical cation (ABTS<sup>•+</sup>) (Sanchez-Moreno, 2002). ABTS activity was found to be highest in methanolic extract (7.1 mg trolox/g) and lowest in aqueous extract (5.6 mg/g) as shown in table 1. A concentration-dependent activity was observed in this assay (Fig. 2). The highest activity in methanolic extract corresponds to high phenolic content compared to other two extract.

With the increase in concentration (20-200 µg/ml), the scavenging activity of powder to ABTS radical scavenging activity increased from 35.5 to 60.5% (ethanolic extract) to 33.2 to 57.8% (methanolic extract) and 32.8 to 46.9% (aqueous extract) (Fig. 4). So, at higher concentration, the extracts had high scavenging activity. As compared to DPPH activity, the scavenging effect is very low. The relative low scavenging potential for aqueous extract may be due to its low extract potential to remove the phytochemicals from its complex tissue to the solvent medium.

Different radical scavenging capacity of the methanol extract against the tested radicals (DPPH and ABTS) may be due to the different mechanisms involved in the radical-antioxidant reactions. Some compounds, though possessed ABTS<sup>•+</sup> scavenging activity, did not exhibit DPPH scavenging activity (Wang *et al.*, 1998)<sup>[28]</sup>. Thus, comparison of assays is difficult and ranking of antioxidant activity is strongly dependent on the test system and on the substrate to be protected by the antioxidants (Frankel and Meyer, 2000). Due to low power of aqueous extract, its IC<sub>50</sub> value is two times higher than corresponding ethanolic extract (table 2).

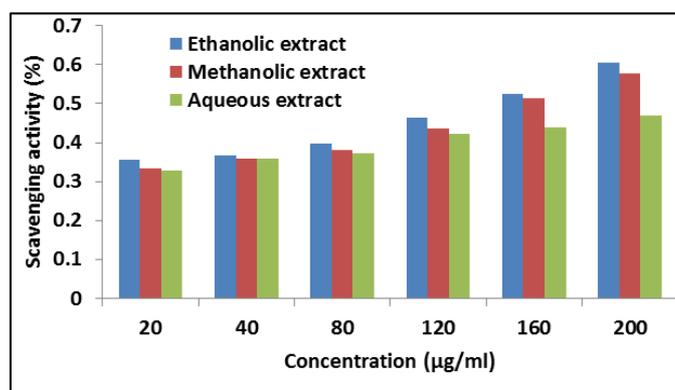


Fig 2: ABTS radical scavenging activities of *Alpinia calcarata* rhizome powder

### 3.2.3 Ferric reducing antioxidant power (FRAP) assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe<sup>3+</sup>-TPTZ] complex and producing a coloured ferrous tripyridyltriazine [Fe<sup>2+</sup>-TPTZ]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction (Guo *et al.*, 2003)<sup>[8]</sup>. FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples (Halvorsen *et al.*, 2006; Pellegrini *et al.*, 2003). Halvorsen *et al.* (2006)<sup>[12, 20, 12]</sup> suggested most of the

secondary metabolites are redox-active compounds that will be picked up by the FRAP assay.

In current study, FRAP activity was measured to be 217.22, 190 and 150.94 mg Fe<sup>2+</sup>/g in ethanolic, methanolic and aqueous extract. With the increase in concentration of sample (0.2-2 mg/ml), the absorbance value increased, being maximum in ethanolic extract (0.52) and minimum in aqueous extract (0.46) as shown in fig. 3. According to Oktay *et al.*, (2003)<sup>[18]</sup> positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. Recently, Li *et al.* (2003)<sup>[15]</sup> reported that the presence of phytochemicals in several plants significantly correlated with high FRAP values. Since, the extract have the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential nutraceuticals when ingested along with nutrients. In this study the ethanolic extract possesses the better hydrogen donation capacity which suppresses the free radicals.

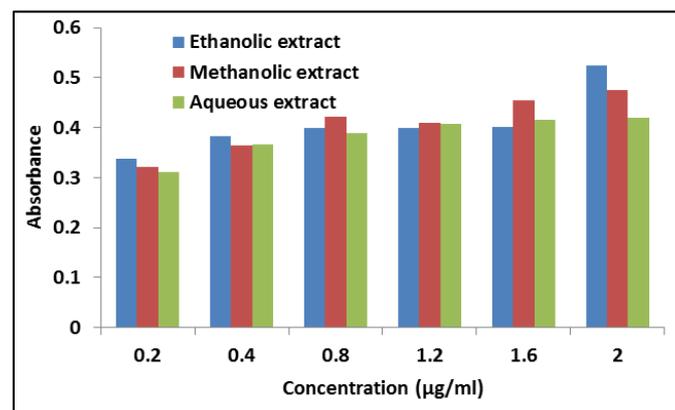


Fig 3: FRAP activities of *Alpinia calcarata* rhizome powder

### 3.2.4 Nitric oxide scavenging activity

The toxicity and damage caused by NO and O<sub>2</sub> is multiplies as they react to produce reactive peroxynitrite, which leads to serious toxic reactions with biomolecules, such as protein, lipids, and nucleic acids. Here, all extracts effectively reduced the generation of NO<sup>•</sup> Radical where the activity was highest in ethanolic extract (11.65 mg Vit-C/g) and lowest in aqueous extract (11.05 mg Vit-C/g). Non-significant difference between three extracts were observed. With the increase in concentration (0.2-2 mg/ml), the scavenging activity increased from 46.5 to 73%, 49.2 to 72% and 46.8 to 71% for ethanolic, methanolic and aqueous extract (Fig. 4). Increasing the sample concentration range from 0.2 to 2 mg/ml, the scavenging effect also increased in the dose dependent manner.

Hence the ethanolic extract of *A. calcarata* has better nitric oxide radical scavenging activity in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions were accordance with the earlier reports of Balakrishnan and Kokilavani (2011)<sup>[4]</sup>. Thus, it suggested for the possibility of utilizing tropical rhizome as a viable source of antioxidant for nutraceuticals and functional foods to curb against NO radical mediated disorder in the body. IC<sub>50</sub> was found to be 83, 167 and 191 µg/ml for ethanolic, methanolic and aqueous extract suggesting higher efficiency of ethanolic extract for 50% inhibition to NO radicals.

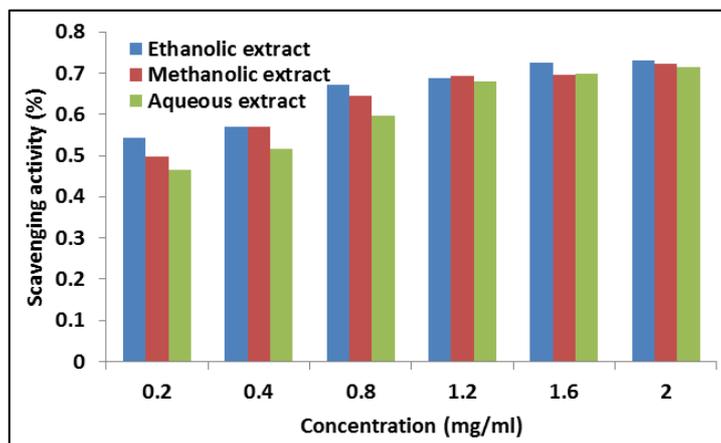


Fig 4: Nitric oxide radical scavenging activities of *Alpinia rhizome* powder

### 3.2.5 Metal chelating activity (MCA)

Metal ion chelating capacity plays a significant role in antioxidant mechanisms, since it reduces the concentration of the catalysing transition metal in LPO (Duh *et al.*, 1999) [7]. It was reported that chelating agents, which form  $\sigma$ -bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Kumaran and Karunakaran, 2006) [13]. Here, it is interesting to note that the MCA of all extracts were at par with each other (11.7-12.35 mg EDTA/g) which showed the effectiveness of rhizome to defend for oxidative damage to cell even with aqueous extract. From fig. 5, it is seen that with the increase in the concentration of (20-200  $\mu$ g/ml) in all three extracts, the chelating activity increased in dose dependent manner where it was highest in ethanolic extract (84.6%) and lowest in methanolic extract (80.7%) at 200  $\mu$ g/ml. Similar results were also reported by Wong *et al.* (2008) [29]. This suggests that ligands in *A. Calcarata* rhizome compete well with ferrozine. Ligands in both parts of the plant effectively sequester ferrous ions by intercepting all coordination sites of metal ions, thus suppressing the formation of hydroxyl radical via Fenton reaction. The results of the present study suggest that *A. Calcarata rhizome* exhibits good chelating activity on ferrous ions, probably due to their content of flavonoids (Hendrich *et al.*, 1999) [11].

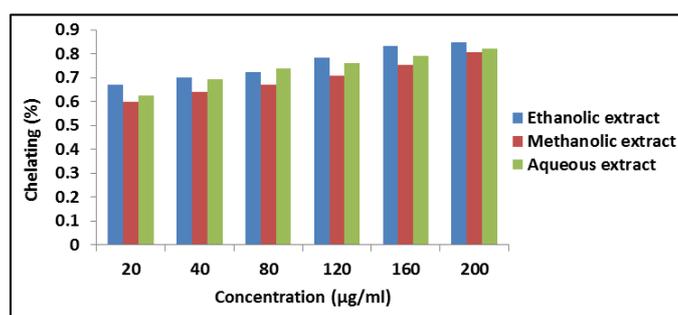


Fig 5: Metal chelating scavenging activities of *Alpinia rhizome* powder

### 3.2.7 Reducing power

The reducing properties are generally associated with the presence of reductones (Pin-Der- Duh, 1998) [21], which have been shown to exert antioxidant action by breaking the free radical chain, by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing capacity of extracts suggested that reductone-associated and hydroxide groups of compounds can act as electron donors and can react with free radicals to convert them to more stable products, and thereby terminate radical chain reactions. The

reducing power of the rhizome increased and correlated well with the increasing concentration. A high absorbance value of the sample indicates its strong antioxidant activity. With the increase in concentration from 0.2 to 2 mg/ml (Fig. 6), the absorbance increased from 20 to 43% (ethanolic extract), 23% to 44.8% (methanolic extract) and 26.3 to 39.3% (aqueous extract). From this study it is revealed that the extracts of *A. Calcarata* was weak in donating electrons to reactive radicals and reducing them into more stable and unreactive species as evident from ABTS scavenging activity.

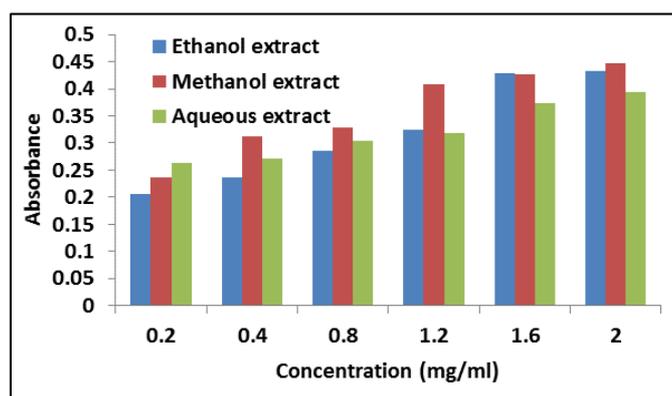


Fig 6: Reducing power of *Alpinia calcarata* rhizome powder

## 4. Conclusions

In living systems free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicine. The determination of total phenolics and flavonoid content along with the total antioxidant capacity of three extract of *A. calcarata* rhizome showed that this plant can be one of the potential sources of safer natural antioxidants. On the basis of the results of this present study, the decreasing order of the antioxidant activity of the extracts assayed through all the three extracts was found to be in the order of ethanol > ethanol > water extract. This showed ethanol was the powerful solvent to extract phytochemical in the study in dose dependent manner. Since reactive oxygen species are thought to be associated with food deterioration and the pathogenesis of chronic infections, and inflammatory diseases, the observed inhibitory potential may partially explain the helpful effects of *A. calcarata* in treating different disease conditions. It is however, worthwhile to further investigate the *in vivo* potentials, different antioxidant mechanisms which may

appear to be a most promising candidate. The extracts can be used for minimizing or preventing lipid oxidation in food or pharmaceutical products, delaying the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf life of food or pharmaceutical materials. Further research will be useful to enable them to fit for industrial needs and they may replace the existing unsafe and undesirable synthetic antioxidants currently used in industry.

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**Conflict of Interest:** The authors declare no conflict of interest for reviewing of the manuscript.

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