Research Article

Antioxidant activities of petroleum ether fraction of *Alpina galanga* (L.) Willd

Raaman N.*, Sivaraj, C, Mahalakshmi, R

Tissue Culture and Natural Products Laboratory, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600025, India

Address: Correspondence for author: Raaman N.

E-mail: raaman55@gmail.com
*Corresponding author

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ABSTRACT

***Alpinia galanga* (L.) Willd. (= *Languas galanga*), commonly referred to as galanga, belongs to family Zingiberaceae and widely cultivated in South-east Asian countries such as Philippines, Indonesia, Thailand, India and China. The purpose of this study was to evaluate antioxidant activities of petroleum ether fraction of rhizomes of *Alpinia galanga.* DPPH radical scavenging assay, $ABTS^{\cdot+}$ radical cation scavenging assay, $OH^{\cdot}$ radical scavenging assay, $NO^{\cdot}$ radical scavenging assay, Fe^{3+} reducing power assay and phosphomolybdenum reduction assay were carried out for antioxidant activities of petroleum ether fraction of rhizomes of *Alpinia galanga.* The results showed that the maximum DPPH, $ABTS^{\cdot+}$, $OH^{\cdot}$ and $NO^{\cdot}$ radical scavenging activities were 59.50%, 63.83%, 57.88% and 56.03% , respectively. The Fe^{3+} and Mo^{6+} reductions were 0.883 and 0.175, respectively. The data indicated that petroleum ether fraction of *Alpina galanga* has significant antioxidant property to inhibit the generation of reactive oxygen species**

KEYWORDS: *Alpinia galanga*, Antioxidant activity, thin layer chromatography, DPPH radical

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ABSTRACT

Alpinia galanga (L.) Willd. (= Languas galanga), commonly referred to as galanga, belongs to family Zingiberaceae and widely cultivated in South-east Asian countries such as Philippines, Indonesia, Thailand, India and China. The purpose of this study was to evaluate antioxidant activities of petroleum ether fraction of rhizomes of Alpinia galanga. DPPH radical scavenging assay, ABTS·+ radical cation scavenging assay, OH• radical scavenging assay, NO• radical scavenging assay, Fe3+ reducing power assay and phosphomolybdenum reduction assay were carried out for antioxidant activities of petroleum ether fraction of rhizomes of Alpinia galanga. The results showed that the maximum DPPH, ABTS·+, OH• and NO• radical scavenging activities were 59.50%, 63.83%, 57.88% and 56.03% , respectively. The Fe3+ and Mo6+ reductions were 0.883 and 0.175, respectively. The data indicated that petroleum ether fraction of Alpina galanga has significant antioxidant property to inhibit the generation of reactive oxygen species.

KEYWORDS: Alpinia galanga, Antioxidant activity, thin layer chromatography, DPPH radical

1. INTRODUCTION

Plant and plant products are being used as a source of aromatic, spicy and sweet in taste for medicine. *Alpinia galanga* (= *Languas galanga*; Family- Zingiberaceae) is used in medication, culinary and cosmetics for centuries [1, 2]. It is widely used in dietary intake as well as in the traditional system of medicine viz., Ayurveda, Unani, Chinese and Thai folk medicine [3]. It has a pungent, hot and spicy taste with an aromatic ginger-like odour [4]. Rhizome has characteristic fragrance as well as pungency; therefore, it is widely used as a condiment for foods and local medicine in China and Thailand [5, 6]. It is officially mentioned in various

pharmacopoeias as well as scientifically reported to possess several therapeutic activities [7]. It is commonly known as Rasna and Sugandhmula in Sanskrit, Kullanjan in Hindi, Koshtkulinjan in Marathi, Arattai in Tamil and Galanga in English [8].

Roots are adventitious, in groups, fibrous, persistent in dried rhizomes, about 0.5 to 2 cm long and 0.1 to 0.2 cm in diameter and yellowish brown in colour. Rhizomes are cylindrical, branched, 2 to 8 cm in diameter, longitudinally ridged with prominent rounded warts marked with fine annulations; scaly leaves arranged circularly, externally reddish brown, internally orange yellow; odour pleasant and aromatic, spicy and sweet taste. The herb is mostly propagated by rhizomes, grows best in shaded areas away from direct sunlight, it also requires well drained soils to grow in and is usually propagated by dividing and replanting the rhizomes during the spring. The 4 to 6 year plants are harvested for their rhizomes at the end of growing season. The rhizomes of A. *galanga* are unearthed and collected in the early autumn and late summer in China, where the plant is extensively cultivated [9, 10]. In the present study, the preliminary phytochemical screening and the antioxidative activities of rhizome of *Alpinia galanga* were carried out.

2. MATERIAL AND METHODS

2.1 Collection and authentication of plant material

Rhizomes of *A. galanga* were collected from the market, Chennai, India. The plant was authenticated by Prof. Dr. N. Raaman, Director, CAS in Botany, University of Madras, Chennai, India. Rhizomes were thoroughly washed and dried in shade for 30 min. Dried rhizomes were made into small pieces using blender and stored in air tight container till further use.

2.2 Preparation of extract

The pieces of rhizome material (250 g) were soaked in methanol and extracted by maceration method for 72 h. Then, the supernatant was filtered through filter paper and concentrated using rotary evaporator which yielded a greenish-black coloured sticky residue. To this residue, petroleum ether was poured and soluble fraction was collected. The process was repeated till petroleum ether fraction changed to be colourless. The fraction should be condensed at room temperature.

2.3 Thin layer chromatography

Thin layer chromatography (TLC) was carried out for methanol extract of rhizomes of *A. galanga* on Merck TLC aluminium sheets, silica gel 60 F_{254} (20 x 20 cm), precoated plates [11]. The Petroleum ether fraction of rhizomes of *A. galanga* was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 356 nm (Harborne, 1998). The R_f values of the coloured spots were recorded [12].

2.4 Phytochemical screening

The petroleum ether fraction of rhizomes of *A. galanga* was subjected to preliminary phytochemical screening using standard methods [13]. The petroleum ether fraction was screened for different classes of phytoconstituents such as alkaloids, steroids, terpenoids, flavonoids and phenolic compounds using specific standard reagents [14, 15].

2.5 Antioxidant activities

2.5.1 DPPH radical scavenging assay

The antioxidant activity of petroleum ether fraction of rhizomes of *A. galanga* was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al [16] with slight modifications. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of petroleum ether fraction of varying concentrations (100-600 µg/mL). Ascorbic acid was used as reference standard. Mixer of 1 mL methanol and 1 mL DPPH solution was used as control. The decrease in absorbance was measured at 517 nm after 30 min in dark using UV-Vis spectrophotometer. The percentage of DPPH radical inhibition was calculated as

2.5.2 $ABTS^{\bullet+}$ radical cation scavenging assay

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity, following the procedure described by Delgado-Andrade et al [17]. Briefly, $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of 1 mL of petroleum ether fraction of

varying concentrations (10-60 µg/mL) to 1 mL of diluted $ABTS^{\bullet+}$ solution, the absorbance was measured after 10 min. The ABTS $^{\bullet+}$ radical-scavenging activity of the samples was expressed as

2.5.3 Hydroxyl (OH·) radical scavenging assay

The hydroxyl radical scavenging capacity of petroleum ether fraction was evaluated by the method described by Olabinri et al [18]. Sixty µL of FeSO4.7H2O (1 mM) was added to 90 µL of aqueous 1,10-phenanthroline (1 mM), 2.4 mL of 0.2 M phosphate buffer pH 7.8 was added to the above mixture, followed by addition of 150 µL of hydrogen peroxide (0.17 mM) and 1.0 mL of different concentrations of petroleum ether fraction. The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm against blank. Ascorbic acid was used as the standard reference. The percentage inhibition was calculated by following equation

2.5.4 Nitric oxide (NO·) radical scavenging assay

Nitric oxide was generated from sodium nitroprusside previously bubbled with nitrogen and measured by the Greiss reaction. The nitric oxide radical was measured by following the method of Gulcin, 2006 [19]. Two mL of sodium nitroprusside (10 mm) was mixed with 1 mL of the test extract in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min. To 0.5 mL of the incubated solution, 0.5 mL of sulphanilic acid reagent was added and allowed to stand for 5 min for completing diazotization. To this, 0.5 mL of 0.1% napthyl ethylene diamine dihydrochloride was added and incubated at room temperature for 30 min. Absorbance was measured at 540 nm. Ascorbic acid was used as positive control. The percentage of $NO^{\bullet}$ radical scavenging activity was calculated using the following formula

2.5.5 Ferric (Fe^{3+}) reducing power assay

The reducing power assay of petroleum ether fraction of rhizomes of *A. galanga* was determined according to the method of Yen and Chen, 1995 [20]. One mL each of varying concentrations of plant extract solution (20 - 120 µg/mL) were mixed with 1 mL of 0.2 M phosphate buffer, pH 6.6, and 1% solution of potassium ferricyanide. The mixture was incubated at 50°C for 20 min, during which period ferricyanide was reduced to ferrocyanide. Then, 1 mL of 1% trichloroacetic acid was added to the mixture, which was then centrifuged at 5,000 *g* for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ and the absorbance at 700 nm was measured to determine the amount of ferric

ferrocyanide (prussian blue) formed. Increased absorbance of the reaction mixture indicates the increase in reduction of ferricyanide.

2.5.6 Phosphomolybdenum reduction assay

The antioxidant capacity of the petroleum ether fraction of rhizomes of *A. galanga* was assessed as described by Prieto et al [21]. The petroleum ether fraction of rhizomes of *A. galanga* in dilution from 20 - 120 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 90°C for 90 min. The absorbance of the colored complex was measured at 695 nm. Ascorbic acid was used as standard reference. Increased absorbance of the reaction mixture indicates the increase in phosphomolybdenum reduction.

3. RESULTS AND DISCUSSION

Oxidative stress, the consequence of an imbalance of pro-oxidants and antioxidants in the organism, is rapidly gaining recognition as a key phenomenon in chronic diseases. It is directly involved in the pathogenic mechanism of risk factors and in the protection exerted by various environmental factors [22]. And the quantification of oxidative stress in populations appears to be a possible indicator for the magnitude of environmental risk factors. Oxidative stress is now recognized to be associated with more than 100 diseases, as well as with the normal aging process [23]. Antioxidants are intimately involved in the prevention of cellular damage the common pathway for cancer, aging, and a variety of diseases. 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.

Thin layer chromatography

TLC analysis was carried out for petroleum ether fraction of rhizomes of *A. galanga* by using toluene (2 mL) as the solvent. The separated bands were visualized by UV light at 254 nm. The R_f values of the separated compounds were measured (Table 1, Figure 1).

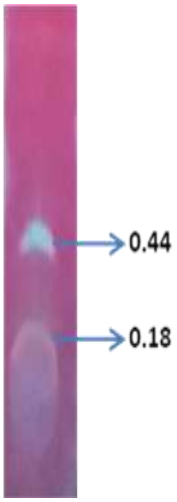

Fig.1: TLC of petroleum ether fraction of rhizomes of *A. galangal*

Spots observed	R_f value (UV 254 nm)
1	0.18
2	0.44

Table 1: R_f values of compounds from petroleum ether fraction of rhizomes of *A. galanga* separated by thin layer chromatography.

Phytochemical screening

The phytochemical analysis was carried out according to the standard procedures. The results showed the presence of alkaloids, terpenoids, steroids in the petroleum ether fraction of rhizomes *A. galanga* (Table 2).

S. No	Phytoconstituents	Chemical reagents	Result
1.	Alkaloids	Mayer's reagent	+
2.	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+
3.	Steroids	Acetic anhydride + conc. H_2SO_4	+
4.	Oils	Filter paper test	+

{+ : present, - : absent}

Table 2: Qualitative phytochemical screening of petroleum ether fraction of rhizomes of *A. galanga*

DPPH radical scavenging activity

The ability of petroleum ether extract of *A. galanga* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). This was compared with a standard (ascorbic acid). Petroleum ether extract of *A. galanga* demonstrated high capacity for scavenging free radicals as shown by the data by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and this capacity increases with increasing concentration [24]. The maximum DPPH radical scavenging activity was 59.50% at 120 µg/mL concentration (Table 3).

S. No.	Concentration (µg/mL)	% of inhibition
1	20	24.30±1.94
2	40	35.84±2.73
3	60	40.58±3.01
4	80	47.95±3.41
5	100	52.70±3.66
6	120	59.50±4.47

Table 3: DPPH radical scavenging activity of petroleum ether fraction of rhizomes of A. *galanga*

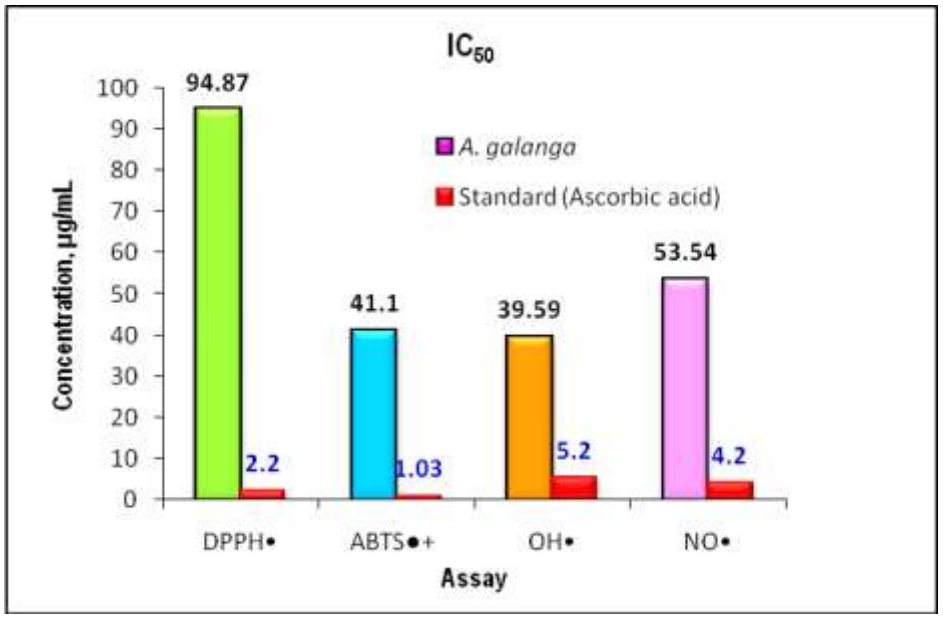

Fig.2: IC_{50} of petroleum ether fraction of rhizomes of *A. galanga* and standard (Ascorbic acid)

It was compared with standard ascorbic acid and the IC_{50} of DPPH radical scavenging activity was 94.87 µg/mL concentration (Figure 2). The scavenging ability of the petroleum ether fraction of rhizomes of *A. galanga* may be due to its bio compositions such as phenolic acids and flavonoid. The radical scavenging activities of the extracts were determined by using DPPH a stable free radical at 517 nm. 1,1-diphenyl-2-picrylhydrazyl is a nitrogen-centred free radical, color of which changes from violet to yellow on reduction by donation of H or e^- by the petroleum ether fraction of *A. galanga.* Saha et al [25] have reported that the DPPH radical scavenging activity was increased by increasing the concentration as reported for in the petroleum ether fraction of rhizomes of *A. galanga*.

$ABTS^{\bullet+}$ radical scavenging activity

In the total antioxidant activity, ABTS+• is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified [26]. The maximum ABTS+• radical cation scavenging activity was 63.83% at 60 µg/mL concentration (Table 4).

S. No.	Concentration (µg/mL)	% of inhibition
1	10	27.67±1.93
2	20	39.01±2.73
3	30	42.98±3.00
4	40	48.66±3.40
5	50	52.23±3.65
6	60	63.83±4.46

Table 4: $ABTS^{\bullet+}$ radical scavenging activity of petroleum ether fraction of rhizomes of *A. galanga.*

It was compared with standard ascorbic acid. This demonstrates its high anti-oxidative activity with its IC_{50} at 41.10 µg/mL (Figure 2). According to Kavimani et al [27], the potent radical scavenging activity is concentration dependent manner as reported in ABTS+• radical cation scavenging assay.

Hydroxyl ($OH^{\bullet}$) radical scavenging activity

Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical which enables it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [28]. The maximum OH radical scavenging activity was 57.88% at 30 µg/mL concentration (Table 5).

S. No.	Concentration (µg/mL)	% of inhibition
1	5	14.17 ± 0.10
2	10	17.07 ± 1.19
3	15	27.75 ± 1.94
4	20	39.87 ± 2.79
5	25	44.98 ± 3.14
6	30	57.88± 4.05

Table 5: Hydroxyl (OH•) radical scavenging activity of petroleum ether fraction of rhizomes of *A. galanga*.

It was compared with standard ascorbic acid. This demonstrates its high anti-oxidative activity with its IC_{50} at 39.59 µg/mL (Figure 2) concentration. The petroleum ether fraction of *A. galanga* significantly inhibits generation of NO· Radicals in a dose-dependent manner as reported earlier by Sai Krishna et al [29].

Nitric oxide (NO•) radical scavenging activity

In this spectrophotometric method, the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylenediamine dihydrochloride was measured. NO, being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions, reacts with superoxide anion and form a potentially cytotoxic molecule, the peroxynitrite (ONOO) [30]. Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems. The maximum NO• radical scavenging activity was 53.03% at 60 µg/mL concentration (Table 6) and inhibit nitric oxide radical in a dose dependent manner as reported earlier by Rana et al [31]. It was compared with standard ascorbic acid. The IC_{50} of NO• radical scavenging activity was 53.54 µg/mL concentration (Figure 2).

S. No	Concentration (µg/mL)	% of inhibition
1	10	7.75±0.54
2	20	11.20±0.78
3	30	21.55±1.50
4	40	25.86±1.81
5	50	34.48±2.41
6	60	56.03±3.92

Table 6: Nitric oxide ($NO^{\bullet}$) radical scavenging activity of petroleum ether fraction of rhizomes of *A. galanga.*

Ferric (Fe^{3+}) reducing power activity

Studies were made on total reduction ability of Fe^{3+} to Fe^{2+} transformation in the presence of petroleum ether fraction of *A. galanga* and found increasing in showing reduction ability in a dose dependent manner, with increasing concentrations. Since the reducing capacity of the petroleum ether fraction of *A. galanga* serve as a significant indicator of its potential antioxidant activity, the reducing ability was 0.883 at 120 µg/mL concentration of petroleum ether fraction of *A. galanga* (Table 7). It was compared with the standard (0.289) ascorbic acid (Figure 3). The antioxidant activity has been reported to be concomitant with development of reducing power [32].

S. No.	Concentration (µg/mL)	Absorbance at 700 nm
1	20	0.045±0.00
2	40	0.198±0.01
3	60	0.431±0.03
4	80	0.736±0.05

5	100	0.83±0.05
6	120	0.883±0.06

Table 7: Ferric (Fe^{3+}) reducing power assay activity of petroleum ether fraction of rhizomes of *A. galanga*.

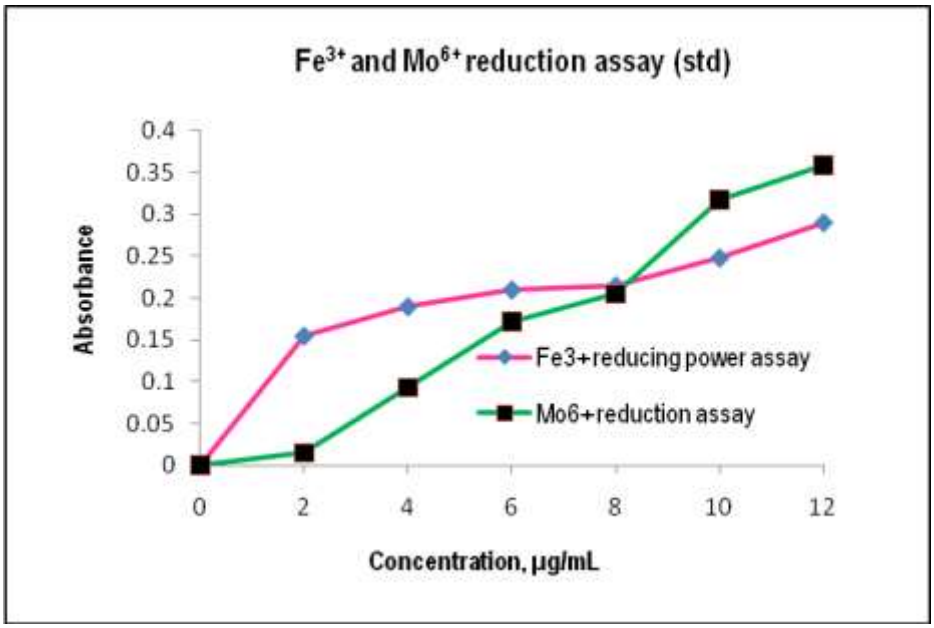

Fig.3: Ferric (Fe^{3+}) reducing power and phosphomolybdenum (MO^{6+}) reduction assay of standard (Ascorbic acid)

Phosphomolybdenum reduction assay activity

The total antioxidant activity of petroleum ether fraction of rhizomes *A. galanga* was measured spectrophotometrically by the phophomolybdenum method, which is based on the reduction of Mo (VI) by the petroleum ether fraction and the subsequent formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption 695 nm. It evaluates both water-soluble and fat-soluble antioxidants with a high absorbance value of the petroleum ether fraction indicated its strong antioxidant acitivity [33]. The maximum absorbance was 0.175 at 120 µg/mL concentration (Table 8). It was compared with the standard (0.359) ascorbic acid (Figure 3). Earlier authors [34] have observed a direct correlation between antioxidant activity

and reducing power of certain plant extracts as reported in petroleum ether fraction of rhizomes *A. galanga*.

S. No.	Concentration (µg/mL)	% of inhibition
1	20	0.04±0.00
2	40	0.048±0.00
3	60	0.059±0.00
4	80	0.086±0.00
5	100	0.119±0.00
6	120	0.175±0.01

Table 8: Phosphomolybdenum reduction assay activity of petroleum ether fraction of rhizomes of *A. galanga*

CONCLUSIONS

The results of the present work indicated that the petroleum ether fraction of rhizomes of *A. galanga* is a potential source of natural antioxidants and significantly inhibit free radicals by dose-dependently. The difference in the antioxidant activity may be ascribed to their different group of phenolic and flavonoids compounds. The petroleum ether fraction of *A. galanga* showed higher phenolic content contributes to the higher antioxidant activity. Based on the results obtained, it can be concluded that the plant contains essential phytochemical constituents and possess active antioxidant property. Further investigations on the isolation of the active component of the extract will throw more information on the mechanism of action.

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