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Research Article

*In vitro* antioxidant activity of *Alangium salvifolium*  
(L.f.) Wang (Alangiaceae) stem

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**ABSTRACT**

Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of stem of *Alangium salvifolium* have been tested using various antioxidant model systems viz., DPPH, hydroxyl, superoxide and ABTS. The ethanol extract of stem of *Alangium salvifolium* showed potent hydroxyl radical scavenging activity, superoxide radical scavenging activity and reducing abilities. The methanol extract exhibited potent DPPH and ABTS radical cation scavenging activity. The inhibitory concentration (IC<sub>50</sub>) in all models viz., DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activity of stem of *Alangium salvifolium* were found to be 19.17, 17.33, 17.29 and 17.37 µg/mL respectively at 1 µg/mL concentration. This study indicates significant free radical scavenging potential of the stem of *Alangium salvifolium* which can be exploited for the treatment of various free radical mediated ailments.

**Keywords:** *In vitro* antioxidant, Scavenging activity, Flavonoid, DPPH, ABTS, *Alangium salvifolium* and Phenol.

**INTRODUCTION**

Reactive oxygen species (ROS) are known to control various normal physiologic functions of organisms by acting as secondary messengers. But excessive stimulation of NAD(P)H by cytokines or mitochondrial electron transport chain, xanthin oxidase and some exogenous sources (such as UV radiation, pathogen invasion, herbicide action, oxygen storage) can overproduce ROS resulting in numerous diseases<sup>1,2</sup>. Organisms possess several defense mechanisms to control the level of ROS<sup>3</sup>. When such defense mechanisms become unbalanced, antioxidant supplement can be used to reduce the oxidative damage. Repairing such damages by naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most acceptable modes of modern therapy<sup>4</sup>.

Herbal medicine represents one of the most important fields of traditional medicine all over the world<sup>5</sup>. In

developing country and particularly in India low income people such as farmer, people of small isolate villages and native communities use folk medicine for the treatment of common infection. Antioxidants are compound that can delay or exhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reaction<sup>6</sup>. Hence, considerable attention has already been focused on the isolation, characterization and utilization of natural antioxidants as potential disease preventing agents.

*Alangium salvifolium* (L.f.) Wang (Family: Alangiaceae) commonly known as Alangi in Tamil was found distributed in South India. The leaves of *Alangium salvifolium* are used as astringent, laxative, refrigerant and used to treat rheumatism, leprosy, syphilis and asthma<sup>7</sup>. The root bark is used as purgative, astringent, anthelmintic, antipyretic, expectorant, anti-inflammatory, emetic, diaphoretic,

anticancer, antimicrobial and antitumor agents<sup>8,9,10</sup>. The root is used as hypotensive agent, anthelmintic and used in the treatment of biliousness, inflammation and snakebite. The stem bark shows antitubercular activity. The fruits are used as laxative, refrigerant, emetic and antiphlegmatic agent.

To the best of our knowledge, no reports are available on the antioxidant potential of various extracts of stem of *Alangium salvifolium*. In this paper, we investigated the antioxidant activity of various extracts that were derived from *Alangium salvifolium*. Furthermore, the total phenolic and total flavonoid content of *Alangium salvifolium* stem were also determined.

## MATERIALS AND METHODS

### Collection of plant sample

Stem of *Alangium salvifolium* (L.f.) Wang was collected from Kottaram, Kanyakumari District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

### Plant sample extraction

The coarse powder (100g) of *Alangium salvifolium* stem was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 mL in a Soxhlet apparatus for 48 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

### Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method<sup>11</sup>. Samples (100 $\mu$ L) were mixed thoroughly with 2 mL of 2% Na<sub>2</sub>CO<sub>3</sub>. After 2 minutes 100  $\mu$ L of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic acid equivalents per 100 gram of dry weight (g 100g<sup>-1</sup>DW) of the plant samples.

### Estimation of Flavonoid

The flavonoid content was determined<sup>12</sup>. An aliquot of 0.5mL of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This

mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

### DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H<sup>13</sup>.

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method<sup>13</sup>. Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (50,100,200,400 & 800 $\mu$ g/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) =  $\{(A_0 - A_1)/A_0\} * 100\}$

Where, A<sub>0</sub> is the absorbance of the control reaction, and A<sub>1</sub> is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

### Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell<sup>14</sup>. Stock solutions of EDTA (1mM), FeCl<sub>3</sub> (10mM), Ascorbic Acid (1mM), H<sub>2</sub>O<sub>2</sub> (10mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA, 0.01mL of FeCl<sub>3</sub>, 0.1mL H<sub>2</sub>O<sub>2</sub>, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50,100,200,400 & 800 $\mu$ g/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by

comparing the results of the test with those of the control using the above formula.

#### **Superoxide radical scavenging activity**

The superoxide anion scavenging activity was measured as described by Srinivasan *et al*<sup>15</sup>. The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800µg/mL), and 0.5 mL Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

#### **Antioxidant activity by radical cation (ABTS +)**

ABTS assay was based on the slightly modified method of Huang *et al*<sup>16</sup>. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

#### **Reducing power**

The reducing power of the extract was determined by the method of Kumar and Hemalatha<sup>17</sup>. 1.0 mL of solution containing 50,100,200,400 &800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

#### **Statistical analysis**

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity,

superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

## **RESULTS**

#### **Total phenolic content and total flavonoid content**

The total phenolic and total flavonoid content of the methanol extract of *A. salvifolium* stem were found to be 0.62g 100g<sup>-1</sup> and 0.99g 100g<sup>-1</sup>.

#### **DPPH radical scavenging activity**

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *A. salvifolium* stem was shown in Figure1. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, methanol extract (800 µg/mL) exhibited highest DPPH radical scavenging activity (112.45%) with lowest IC<sub>50</sub> value (17.18 µg/mL). A higher DPPH radical scavenging activity is associated with a lower IC<sub>50</sub> value.

#### **Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of *A. salvifolium* stem was presented in figure 2. Hydroxyl radical scavenging activity of different extracts of *A. salvifolium* stem increases with the concentration of extracts. At 800 µg/mL ethanol extract which has lowest IC<sub>50</sub> value (17.33 µg/mL) showed highest scavenging activity (102.19%) against hydroxyl radical.

#### **Superoxide radical scavenging activity**

The *A. salvifolium* stem extracts were subjected to the superoxide scavenging activity and the results were shown in figure 3. It indicates that ethanol extract of *A. salvifolium* stem (800µg/mL) exhibited the maximum superoxide scavenging activity (113.80%) which is higher than the standard ascorbic acid whose scavenging effect is 101.23%. IC<sub>50</sub> values (Table 1) of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of *A. salvifolium* stem and standard in this assay were 23.06, 23.24, 23.93, 18.38, 17.29 and 20.94 µg/mL respectively.

#### **ABTS radical cation scavenging activity**

The *A. salvifolium* stem extracts were subjected to ABTS radical cation scavenging activity and the results were presented in figure 4. The methanol extract exhibited potent ABTS radical cation

scavenging activity (113.84%) in concentration dependent manner and petroleum ether extract has least scavenging activity (73.90%). A higher ABTS radical cation scavenging activity is associated with a lower IC<sub>50</sub> value (17.37µg/mL).

#### **Reducing power**

Figure 5 showed the reducing ability of different solvent extracts of *A. salvifolium* stem compared to ascorbic acid. The results clearly indicate that the reducing power of the *A. salvifolium* stem extracts increased in dose dependent manner. Among the solvent tested, ethanol extract exhibited higher reducing activity (0.417OD).

#### **DISCUSSION**

There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants<sup>18,19</sup>.

Phenols and flavonoids are the precursors considered to be beneficial antioxidants as they exhibit the scavenging activity of harmful active oxygen species<sup>20</sup>. The antioxidant activity of this plant may be due to the presence of phenolic substances.

#### **DPPH radical scavenging activity**

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience<sup>21</sup>. From the present results it may be postulated that the extracts of *Alangium salvifolium* stem reduces the DPPH radical to corresponding hydrazine when its reacts with hydrogen donors in antioxidant principles.

#### **Hydroxyl radical scavenging activity**

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage<sup>22</sup>. They were produced in this study by incubating ferric-EDTA with ascorbic acid and H<sub>2</sub>O<sub>2</sub> at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH<sup>14</sup>. When the extracts of *A. salvifolium* stem were added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The IC<sub>50</sub> value indicates that the plant extract is a better

hydroxyl radical scavenger than the standard ascorbic acid.

#### **Superoxide radical scavenging activity**

Numerous biological reactions generate superoxide radical which is a toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potential precursors of highly reactive species such as hydroxyl radical and thus the study of the scavenging of this radical is important. Superoxide radicals were generated in a PMS-NADH system and are assayed by the reduction of NBT<sup>23</sup>. Superoxide anion plays an important role in the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and singlet oxygen which induce oxidative damage in lipids, proteins and DNA<sup>24,25</sup>. The extracts demonstrate the dose-response inhibition of the superoxide anion radicals. Among the five extracts of stem for superoxide anion scavenging activity, higher activity was shown by ethyl acetate extracts when compared with the other extracts.

#### **ABTS radical cation scavenging activity**

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants<sup>26</sup>. The extract efficiently scavenged ABTS radicals generated by the reaction between 2, 2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium persulfate (Figure 4). The activity was found to be increased in a dose-dependent manner. The ABTS radical scavenging activity of methanol extract of *A. salvifolium* stem (113.84%) indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

#### **Reducing Power Assay**

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals (ie antioxidant) causes the conversion of the Fe 3+ / ferricyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue at 700nm, the Fe 2+ concentration can be monitored; a higher absorbance at 700nm indicates a higher reducing power<sup>27</sup>. The relative reducing power of the different solvent extract of *A. salvifolium* stem from the results are benzene>ethanol>petroleum ether>methanol>ethyl acetate.

The higher absorbance of benzene extracts may be due to its strong reducing power potential. The reducing power of the extracts may be due to the

biologically active compounds in the extract which possess potent donating abilities. This assay further confirmed the antioxidant properties of the extracts.

### CONCLUSION

The findings of the present study clearly indicate the potential antioxidant properties of the different solvent extracts of *A. salvifolium* stem. The promising results of this plant extract with antioxidant assay models proved the plant to be a potential antioxidant agent. This efficacy of the plant could be attributed to the synergistic effect of various

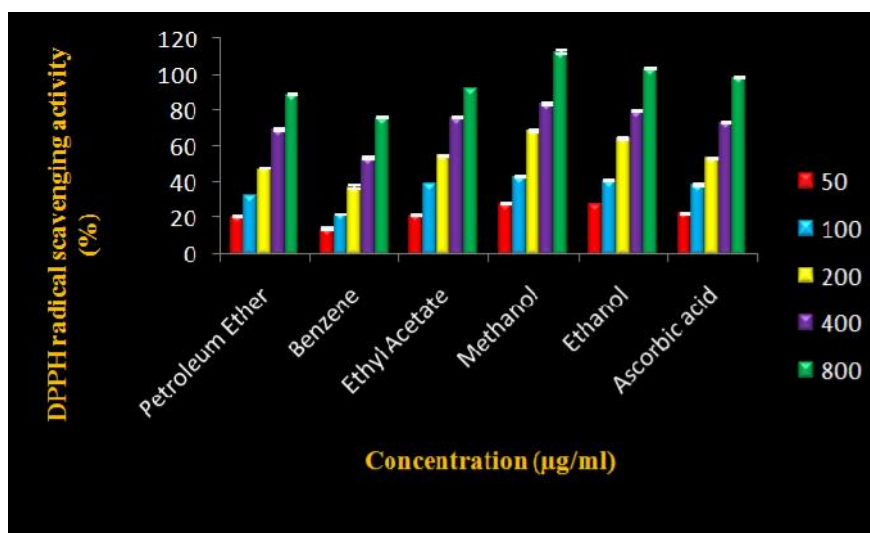
phytochemicals like flavanoids, steroids and polyphenolic compounds. Further detailed investigation needs to be undertaken in order to evaluate the mode of action of this plant extracts at the molecular level.

### ACKNOWLEDGEMENT

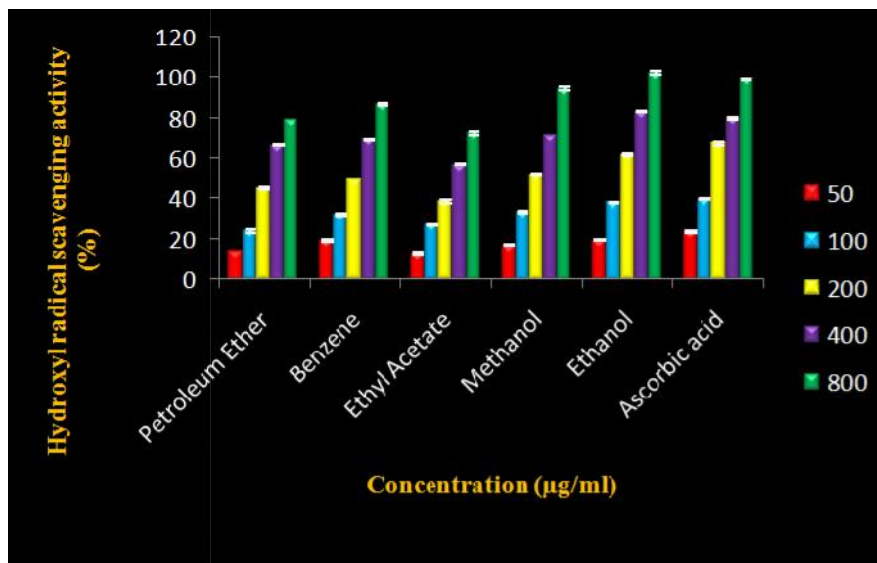
The authors are thankful to Dr. R. Sampathraj, Honorary Director, Samsun Clinical Research Laboratory, Thirupur for providing necessary facilities to carry out this work.

**Table 1**  
**IC<sub>50</sub> values of different solvent extracts of stem of *Alangium salvifolium***

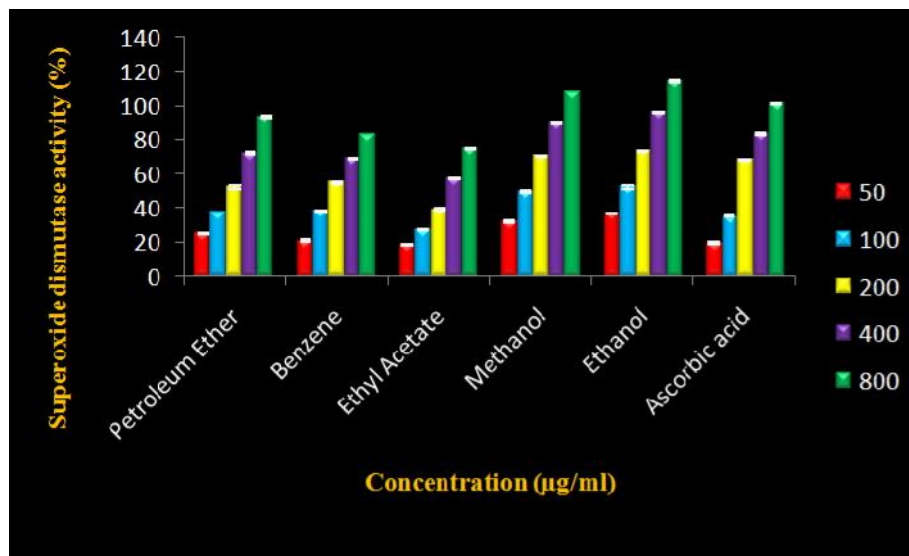
Different solvent extracts	DPPH assay	Hydroxyl assay	Superoxide dismutase activity	ABTS assay
Petroleum ether	21.64	21.96	23.06	23.18
Benzene	23.23	21.03	23.24	22.96
Ethyl acetate	20.78	22.54	23.93	22.02
Methanol	17.18	19.83	18.38	17.37
Ethanol	19.17	17.33	17.29	18.46
Standard (Ascorbic acid)	18.35	17.84	20.94	-
Standard (Trolox)	-	-	-	22.15



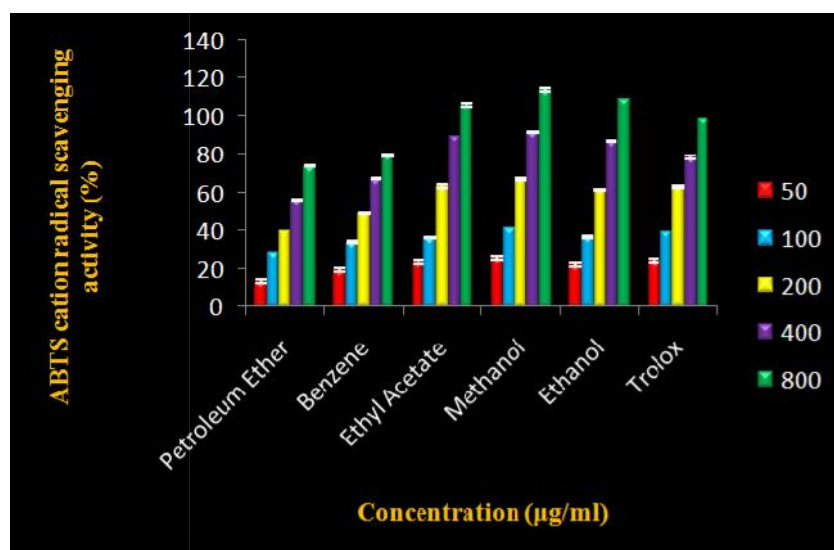
**Fig 1**  
**DPPH radical scavenging activity of different solvent extracts of stem of *Alangium salvifolium***



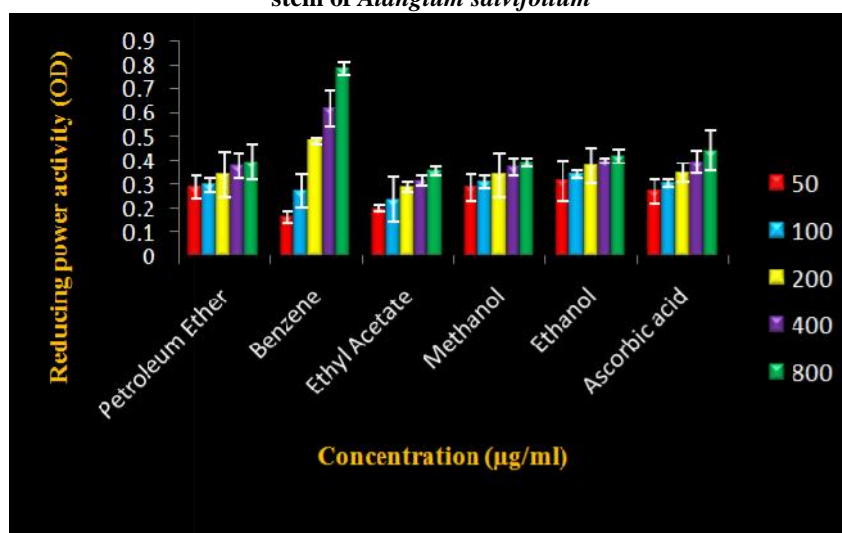
**Fig 2**  
Hydroxyl radical scavenging activity of different solvent extracts of stem of *Alangium salvifolium*



**Fig 3**  
Superoxide radical scavenging activity of different solvent extracts of stem of *Alangium salvifolium*



**Fig 4**  
ABTS radical cation scavenging activity of different solvent extracts of stem of *Alangium salvifolium*



**Fig 5**  
Reducing power ability of different solvent extracts of stem of *Alangium salvifolium*

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