

## Comparative Phytochemical Profiles and Antioxidant Properties of *Antigonon leptopus*, *Artabotrys hexapetalus* and *Allamanda blanchetii* Leaf Extracts

Manikumar Bollapragada, Manjula Shantaram\*

Department of Studies and Research in Biochemistry, PG Centre, Mangalore University, Jnana Kaveri, Chikka Aluvara, Karnataka, India 571232.

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### ABSTRACT

Oxidative stress, which is frequently induced by an overproduction of free radicals, poses a high risk to human health. A molecule that has an unpaired electron is called a free radical, which is highly reactive, and could cause damages to cellular components such as DNA, or the cell membrane inside the human body. Antioxidants to neutralize free radicals are produced by human body but ageing and oxidative stress conditions would increase the formation of free radicals, therefore exogenous antioxidants are needed. *Antigonon leptopus*, *Artabotrys hexapetalus* and *Allamanda blanchetii* leaves are popular choices in traditional herbal medicine practice. Those plants are traditionally used to treat diabetes mellitus, inflammation, cytotoxic, thrombolytic and antimicrobial activities. In the present study, leaves of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* were used to analyze the presence of phytochemicals and evaluation of *in vitro* antioxidant property. Quantitative determination of total flavonoids and total phenolics was evaluated using spectrophotometric equivalents of the standards, such as quercetin and gallic acid respectively. The antioxidant activities of the plant extracts were determined using DPPH, ferric reducing antioxidant power and total antioxidant capacity. From this study, it is inferred that methanolic extract of *A. leptopus* is a rich source of secondary metabolites and antioxidant potency when compared to *A. hexapetalus* and *A. blanchetii*. Therefore, this plant may serve as a source of natural product and be utilized to treat oxidative stress mediated diseases.

**Keywords:** *Antigonon leptopus*, *Artabotrys hexapetalus*, *Allamanda blanchetii*, antioxidant, Phytochemicals.

### INTRODUCTION

Medicinal plants are considered to be the source of natural compounds that have been used by humans to treat many ailments since ancient times. They are the most successful basis for drug clues with lesser toxicity, as documented in literature<sup>1,2</sup>. Natural products in the form of plants served as the most important source of therapeutic agents and were in use even from *vedic* period<sup>2,3</sup>. Natural products and their derivatives are thus looked upon not only as a source of affordable healthcare but also as an important commodity of international trade and commerce<sup>4</sup>. Traditional use of plants and other natural product-derived drugs in the treatment of ailments like cardiovascular diseases, cancer and neurological diseases, envisages well for future utilization<sup>3</sup>. Research reported that polyphenols can act both as antioxidants and pro-oxidants based on their concentration and cellular environment<sup>5</sup>.

*Antigonon leptopus* belongs to family polygonaceae and it is commonly found in tropical Asia especially in China and India, Africa, the Caribbean and the Americas<sup>6</sup>. *A. leptopus* plant flowers are also used in omelets<sup>7</sup>. A hot tea prepared from the aerial portion of this plant, is used as a treatment for cough and throat constriction in Jamaica and considered as one of the important medicinal plants in their

folk-medicine<sup>8,9</sup>. Previous studies have shown that *A. leptopus* plant extracts, exhibited potential anti-thrombin and anti-diabetic activities<sup>10-12</sup> and reported its anthelmintic activities properties<sup>13</sup>.

*Artabotrys hexapetalus* belongs to family annonaceae and is widely distributed in the southern part of China and is used in traditional Chinese medicine for the treatment of malaria<sup>14</sup>. This species is distributed worldwide mainly in India, Sri Lanka, and South China. It is indigenous to south India and very commonly cultivated in gardens throughout the country. The bark and fruit parts are used to treat diarrhea, dysentery, bruises, cuts, pains, sprain, inflammation, gout, helminthiasis, leprosy, skin diseases, wound, ulcers, tumors, amenorrhea, dysmenorrhea, cough, asthma, bronchitis, colic and constipations<sup>15</sup>.

*Allamanda blanchetii* commonly known as purple Allamanda, is an ornamental plant of Allamanda genus and it belongs to apocynaceae family. All parts of the plant are poisonous if ingested. *A. blanchetii* is commonly used as an ornamental plant. Only a few reported that *A. blanchetii* shown to have antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities<sup>16</sup>.

Considering the medicinal activities of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* and in order to clarify the

biological activity of leaf parts of these plants, the aim of this study was to investigate the *in vitro* antioxidant properties and the phytochemical activities of leaves in methanolic extract. To the best of our knowledge, this is the first study that has evaluated and compared activities of leaf extracts.

## MATERIALS AND METHODS

### Collection of plant leaves

Leaves of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* were collected from Sindhanur taluk, Raichur district, Karnataka state, India, during the period between January and March 2016. The leaves were then washed in running tap water, dried and macerated.

### Preparation of extracts

One hundred grams of the dried macerated leaves of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* were soaked in 1000 mL of absolute methanol for 24 hours with occasional stirring. The extracts were then filtered using a double layered muslin cloth and the filtrate was concentrated to dryness using a rotary evaporator at reduced pressure. The dried extracts were stored at 4°C till further use.

### Qualitative phytochemical screening

Phytochemical screening of the plant samples was carried out using described protocols of Harbone, Sofowora, Trease and Evans with minor modifications. A stock solution of each extract, with a concentration of 10 mg extract/ml distilled water, was prepared and used for the phytochemical screening<sup>17,19</sup>.

The different chemical tests were performed for establishing profile of the leaf extract for its chemical composition. The chemical tests for various phyto-constituents in the ethanol extract was carried out as described below:

### Test for the presence of alkaloids

Approximately 3 ml of extracts were added to 3 ml of 1% HCl and heated for 20 min. The mixtures were then cooled and used to perform the following tests:

#### Mayer's test

To the 1 ml of plant extract in a test tube, 1 ml of Mayer's reagent was added drop by drop. The formation of a greenish color or cream precipitate indicated the presence of alkaloids.

#### Dragendoff's test

To the 1 ml of plant extract in a test tube, 1 ml of Dragendoff's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

#### Wagner's test

To the 1 ml of plant extract in a test tube, 1 ml of Wagner's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

### Test for the presence of saponins

About 3 ml of plant extract was added to 3 ml of distilled water and shaken vigorously. The formation of a stable persistent froth was taken as a positive test for saponins.

### Test for the presence of flavonoids

About 2 ml of the plant extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was

added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicative of the flavonoids.

### Test for the presence of tannins

About 2 ml of plant extract was diluted in 5 ml distilled water in a test tube, and then added a few drops of 1% lead acetate solution. A red precipitate shows the presence of tannins.

### Test for the presence phenols

Two ml of 5% solution of FeCl<sub>3</sub> was added to 1 ml crude extract. A black or blue-green color indicated the presence of tannins and phenols.

### Test for the presence of terpenoids

Approximately 2 ml of chloroform and 3 ml of H<sub>2</sub>O<sub>2</sub> were added to 5 ml of plant extracts. A reddish-brown coloration was taken as positive test for terpenoids.

### Determination of total phenolic content

The total polyphenols of the methanolic extract was determined by Folin-Ciocalteu method earlier with minor changes<sup>20</sup>. Extracts of different volumes were mixed with aqueous Na<sub>2</sub>CO<sub>3</sub> (1.5 ml) followed by adding 500 µl of Folin-Ciocalteu reagent and incubated for 5 min. at room temperature. Then the solution was incubated in dark for 2 h. The absorbance was measured at 734 nm using UV/visible spectrophotometer. The result was analyzed as gallic acid equivalent (GAE) using a (0-100 µg/ml) standard curve. Data of total phenolic contents are expressed as milligrams of GAE per gram dry weight. All samples were analyzed in duplicates.

### Determination of total flavonoids

The total flavonoid content of methanolic extracts was determined by the method, described earlier with minor modifications<sup>21</sup>. Quercetin (0–100 µg/ml in methanol) was used as a standard reference. The standard and the extract solutions (mg/ml) were mixed with 0.1 ml of 10% (W/V) aluminum chloride, 0.1 ml of potassium acetate, 1.5 ml of methanol, and 2.8 ml of water. For the blank, both potassium acetate and aluminum chloride were added and their volume was replaced by water. The reaction mixture was incubated for 30 min. at room temperature, and the absorbance was taken at 415 nm. The result was analyzed in quercetin equivalent using a 0–100 µg/ml standard curve.

### Determination of total antioxidant capacity

The total antioxidant capacity of extracts was evaluated by the phospho-molybdenum method described earlier with minor modifications<sup>22</sup>. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate Mo (V) complex at acidic pH. In this assay, 0.1 ml of extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (10–100 µg/ml) with methanol.

Table 1: Qualitative phytochemical screening

Sl.no	Tests	<i>Antigononleptopus</i>	<i>Artabotryshexapetalus</i>	<i>Allamanda blanchetii</i>
1.	Alkaloids	+++	+++	+++
2.	Saponins	+	++	+
3.	Flavonoids	++	+++	++
4.	Tannins	+++	++	++
5.	Phenols	+++	++	++
6.	Terpenoids	++	++	+

Key: (+) Low concentration, (++) Moderate concentration, (+++) High concentration

#### Determination of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity(DPPH) assay

The effect of plant extracts on DPPH radical was determined according to the method described with minor modification<sup>23</sup>. One hundred  $\mu$ M solution of DPPH in methanol was prepared. The plant extracts containing different concentrations (0-100  $\mu$ g) were mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm. The control contained all the reagents except extracts. The capacity to scavenge DPPH radical was calculated using the following formulae.

The activity is represented as % radical scavenging that is calculated by:

$$\% = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100$$

A blank: absorbance of the control reaction (containing all reagents except the test compound).

A sample: absorbance of the test compound. Extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph using inhibition percent v/s extract concentration.  $IC_{50}$  values of samples were compared to  $IC_{50}$  of a standard antioxidant in this case ascorbic acid, obtained by the same procedure.

#### Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was analyzed by deoxyribose method described earlier with minor modification<sup>24</sup>. Hydroxyl radical was generated using Fenton reagent (ascorbate-EDTA- $H_2O_2$ - $Fe^{3+}$  method). The total reaction mixture contained 2-deoxy-2-ribose (2.6 mM), ferric chloride (20  $\mu$ M),  $H_2O_2$  (500  $\mu$ M), ascorbic acid (100  $\mu$ M), and plant extracts with various concentrations. The total reaction volume was made up to 1 ml with phosphate buffer (100  $\mu$ M, pH 7.4). The reaction mixture was incubated at 37°C for 1 h to initiate the reaction. After incubation period, 0.8 ml of the reaction mixture was added to the 2.8% TCA (1.5 ml), followed by 1% tertiary butyl alcohol (1 ml) and 0.1% safety data sheet (0.2 ml). The reaction mixture was then heated to 90°C for 20 min. to obtain color, later cooled and 1 ml of double-distilled water was added and absorbance was read at 532 nm with respective blank. The percentage of inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = (A_0 - [A_1 - A_2]) / A_0 \times 100$$

where  $A_0$  is the absorbance of the control without a sample,  $A_1$  is the absorbance in the presence of the sample and deoxyribose, and  $A_2$  is the absorbance of the sample without deoxyribose.

#### Determination of ferric reducing antioxidant power(FRAP) assay

The reductive potential of the *A. leptopus* leaf extract was determined using standard method described earlier with minor modifications<sup>25</sup>. Different concentrations of *A. leptopus* leaf extract in 0.5 ml of water were mixed with equal volumes of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide [ $K_3Fe(CN)_6$ ]. The mixture was incubated for 20 min. at 50°C. At the end of incubation, an equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3200  $\times$ g for 10 min. The supernatant was mixed with distilled water and 0.1% ferric chloride at 1:1:0.2 (v/v/v) and the absorbance was measured at 700 nm. An increase in the absorbance of the reaction mixture indicates the reducing power potential of the sample. Ascorbic acid was used as a standard for comparison.

## RESULTS

The results of phytochemical screening are depicted in table 1. More phytochemicals were detected in the methanol extract of *A. leptopus* (MEAL) leaves than in methanol extract of *A. hexapetalus* (MEAH) and methanolic extract of *A. blanchetii* (MEAB) leaves. Alkaloids, flavonoids, tannins, reducing sugars, saponins, steroids, terpenoids and cardiac glycosides were detected. More percentage of phytochemicals was present in *A. leptopus* when compared to other two plants.

The levels of polyphenol contents in the different plant extracts obtained from leaves are represented in figure 1. The result showed that MEAL 132.5  $\pm$  4.85, mg/g d.w.GAE, MEAH contains 63.63  $\pm$  3.6 mg/g d.w.GAE and MEAB 43.8  $\pm$  2.7. mg/g d.w.GAE. Moreover, MEAL extracts had a higher total flavonoid contents 44.8  $\pm$  1.54 mg/g d.w.QE, MEAH 21.3  $\pm$  0.8 mg/g d.w.QE and MEAB 16.9  $\pm$  0.56 respectively as showed in figure 2.

The total antioxidant content of the plant extracts was measured spectrophotometrically by phosphor-molybdenum method. The reduction of Mo (VI) to Mo (V) by the extracts is the main principle of this assay. The formation of green colored Mo (V) end product was measured at 695 nm. The total antioxidant activity of the extracts in decreasing order; MEAL > MEAH > MEAB and is presented in figure 3.

*In vitro* assays can be used to monitor the ability of plant extracts to quench radicals and in this study, radical scavenging activity was monitored against synthetic DPPH radical. The results of the DPPH free radical scavenging activities of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* leaf extracts are showed in figure 4a with the  $IC_{50}$  values. The results show that at lower concentrations, (10 – 50  $\mu$ g/mL) the *A. leptopus* extract

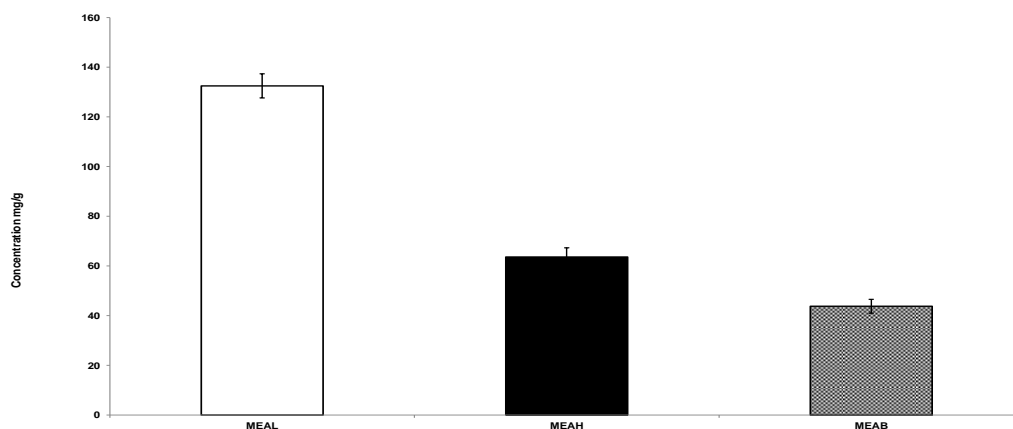


Figure 1: Total phenolics contain of leaf methanolic extract of a leptopus (MEAL), a hexapetalus (MEAH) and a blanchetti (meab). The value were expressed in mg/ g dw. The values are mean of three replicates  $\pm$  standard deviation.

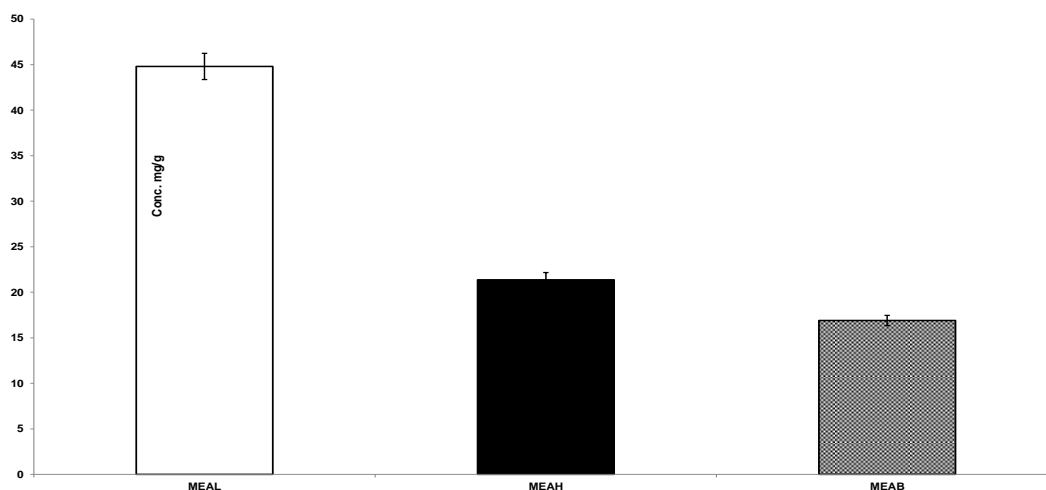


Figure 2: Total flavonoid contain of leaf methanolic extract of a leptopus (MEAL), a hexapetalus (MEAH) and a blanchetti (MEAB). The value were expressed in mg/ g dw. The values are mean of three replicates  $\pm$  standard deviation.

was a better inhibitor of the DPPH radical than the *A. hexapetalus* and *A. blanchetii* extract, whereas, at higher concentrations (25 –150  $\mu\text{g/mL}$ ), the MEAL had a better DPPH radical scavenging activity than the MEAH and MEAB extracts. The  $\text{IC}_{50}$  values were 33.95  $\mu\text{g/mL}$  for the MEAL, 107.29 for MEAH and 102.04  $\mu\text{g/mL}$  for MEAB extract in contrast to that of ascorbic acid (33.2 $\mu\text{g/mL}$ ).

The FRAP results are shown in figure 4b. The results revealed that the *A. hexapetalus* extract had a significantly higher ( $p < 0.05$ ) value. The results of this assay indicate that there was a gradual increase in absorbance in a dose dependent manner with an increase in the concentration of different extracts. The antioxidant activity trend of different extracts was in the decreasing order; MEAL > MEAH > MEAB as shown in fig 3C.

Hydroxyl radical scavenging potential of plant extracts was analyzed using 2-deoxy-2-ribose method.  $\text{IC}_{50}$  value of MEAL was  $171.08 \pm 2.88 \mu\text{g/ml}$ , MEAH was  $200.3 \pm 3.11 \mu\text{g/ml}$ , MEAB was  $239.7 \pm 6.88 \mu\text{g/ml}$  and for

standard ascorbic acid was  $84.94 \pm 2.4 \mu\text{g/ml}$  as shown in figure 4C respectively.

## DISCUSSION

Nowadays, living organisms are repeatedly exposed to several oxidizing agents and it is well known, that an increased consumption of antioxidant-rich foods and food supplements are obtained often from natural sources. Free radicals are mainly produced in normal physiological process which is very noxious and the cause of many chronic diseases like cancer, diabetes, atherosclerosis etc. Imbalance in levels of free radicals and antioxidants in the body causes oxidative stress<sup>25</sup>. Antioxidants are present in natural molecules having resemblance in chemical structure that can act as inhibitors or quenchers for free radicals, and it delays cellular damage and senescence. *A. leptopus*, *A. hexapetalus* and *A. blanchetii* were used by many populations to treat various ailments, as mentioned above and scientific evidences reporting its common use is increasing.

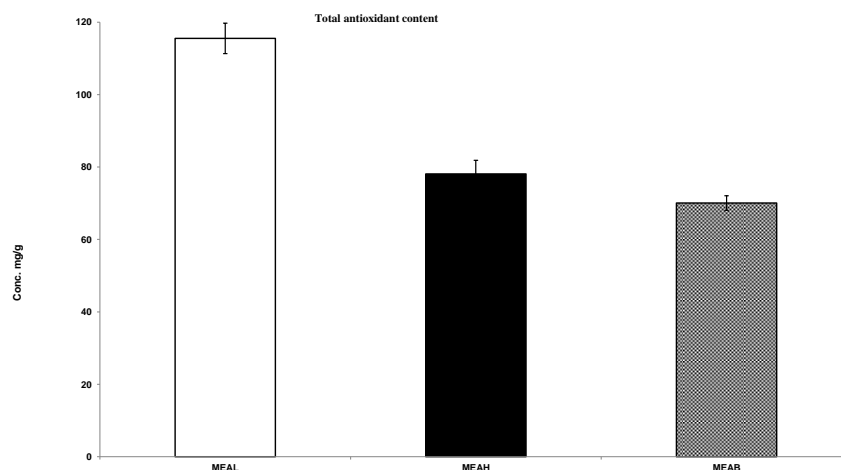


Figure 3: Total antioxidant contain of methanolic extract of a leptopus (MEAL), a hexapetalus (MEAH) and a blanchetti (MEAB) leaves. Data represent means  $\pm$  standard deviation.

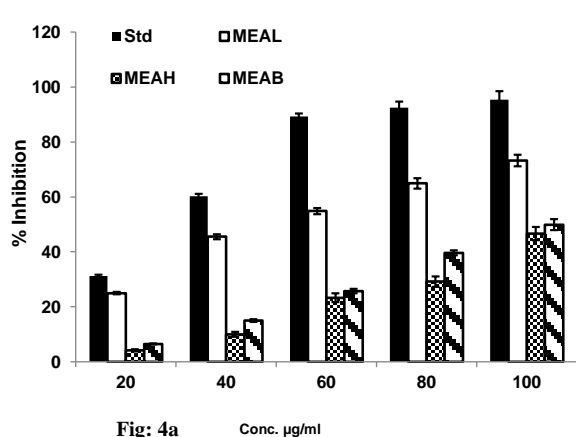


Fig: 4a

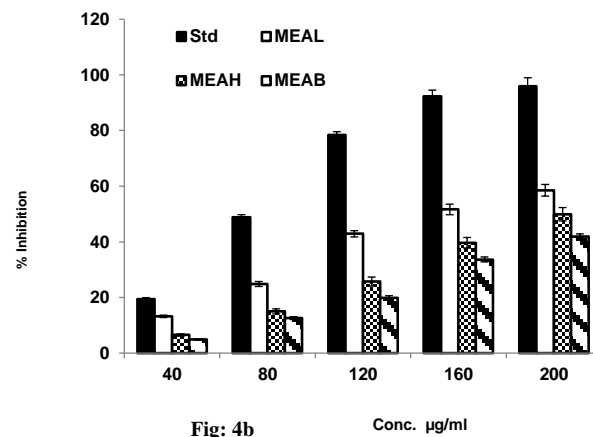


Fig: 4b

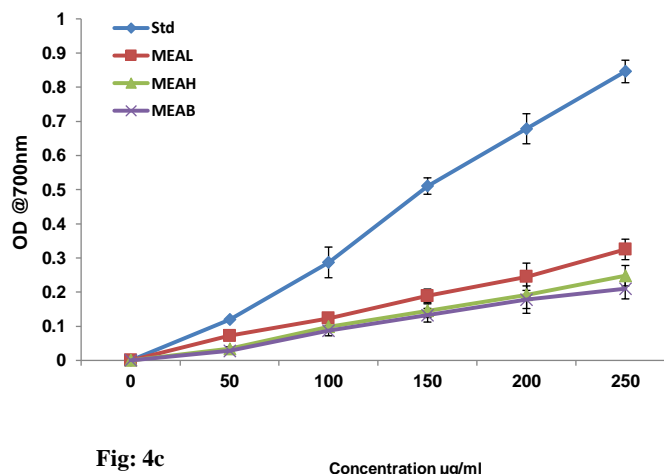


Fig: 4c

Figure 4: Antioxidant activities of methanolic extract of a leptopus (MEAL), a hexapetalus (MEAH) and a blanchetti (MEAB) leaves. (a) 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) (b) hydroxyl radical scavenging activity, (c) ferric reducing antioxidant power. Ascorbic acid was used as positive control. The data points represents means + standard deviation of three independent determinations.

The result of the phytochemical analysis of methanolic extracts of *A. leptopus*, *A. hexapetalus* and *A. blanchetii* leaves revealed the presence of several phytochemicals, viz, saponins, polyphenols, terpenoids, cardiac glycosides, reducing sugars, alkaloids, tannins and steroids. Among these three, MEAL extract is found to be rich in secondary

metabolites when compared to other two plant extracts. These findings are consistent with those of findings of Abdel Rehman<sup>26</sup> who also detected the presence of some of these phytochemicals. Phytochemical screening of *A. hexapetalus* leaf extract revealed the presence of flavonoids, tannins, alkaloids and reducing sugars. Cardiac

glycosides, steroids, terpenoids and saponins were not detected. These results recently corroborate, with the findings of Li *et al.*,<sup>22</sup>. The differences between both research findings may be due to the different extracting solvents and different geographical variations.

Phenolic compounds are present in major amount in *A. leptopus* extracts of leaves as shown in figure 1. Moreover, MEAL was also found to be rich in flavonoids when compared to MEAB and MEAH plant extracts.

In our current study, the free radical scavenging potential was performed by *in vitro* DPPH, OH and ferric reducing activity assays. Our results highlighted that all the investigated extracts have a dose dependent activity, similar to the reference standard. The comparison among the extracts showed that MEAL has the highest radical scavenging potential than MEAB and MEAH. Finally, extracts such as MEAL showed higher free radical scavenging activity and also was rich in phytochemical components such as polyphenol and flavonoid components. Our findings suggest that the plant *A. leptopus* has potential and can be used as an alternative and complementary medicine for many therapeutic ailments. However, further study is in progress to isolate and characterize the active components present in the extracts.

## CONCLUSION

The current study shows that *A. leptopus*, *A. hexapetalus* and *A. blanchetii* are rich in diverse pharmacological activities. The methanol extracts of *A. leptopus* has more significant antioxidant activity when compared to *A. hexapetalus* and *A. blanchetii*. *A. leptopus* can be a potential source of new therapeutically valued bioactive compounds against various diseases. Still, further studies are necessary to isolate and characterize the active components present in the extracts.

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