Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *LASIANTHUS LUCIDUS* BLUME

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Received: 29 Mar 2012, Revised and Accepted: 05 Jun 2012

ABSTRACT

Although *Lasianthus lucidus* grow abundant over a long range of tract and sporadic ethno-botanical usage of the plant are reported, systematic study with the plant for evaluation of bioactivity for revalidation of its traditional use is lacking. In present work we made an attempt to assess antioxidant properties of the plant using acetone and methanol as solvent. We also wanted to correlate antioxidant potential with total phenol and total flavonoid contents of the plant. DPPH (2,2-diphenyl-1- picrylhydrazyl) radical scavenging method and FRAP (Ferric Reducing Antioxidant power) method were followed for assessing antioxidant potential. It is observed that DPPH IC_{50} values of the acetone and methanolic extracts were 15.16±1.66 and 24.39±0.97 µg/ml respectively. The total phenolic content of the acetone and methanolic extracts were 29.71±1.55 and 56.24±2.34 mg TAE/100g of leaf extracts respectively, while the total flavonoids were 31.34±5.54 and 65.74±1.09 mg CAE/100g of leaf extracts respectively. The antioxidant activities were correlated with the total phenolic content. The results suggested a direct correlation between phenolic and flavonoid contents of different extracts and their respective antioxidant potency.

Keywords: Antioxidants, Phenolic, Flavonoid, *Lasianthus lucidus.*

INTRODUCTION

Antioxidants are micronutrients that have gained interest in recent years due to their ability to neutralize the actions of free radicals[1]. Free radicals are potentially harmful products generated during a number of natural processes in the body and associated with ageing of cells and tissues. Failure to remove active oxygen compounds, over a long term, can lead to various diseases like cardiovascular diseases, cancer, diabetes, arthritis and various neurodegenerative disorders[2]. Reactive Oxygen Species (ROS), such as hydroxyl radical, hydrogen peroxide, and superoxide anions, are produced as bi-products in aerobic organisms and have been implicated in the pathology of a vast variety of human diseases including cancer, atherosclerosis, diabetic mellitus, hypertension, AIDS and aging.[3,4,5] It is generally assumed that the active constituents present in plants, which are contributing to the protective effects, are antioxidants. In view of several drawbacks of synthetic compounds, preparations of plant origin have received increasing attention. *Lasianthus lucidus* Blume (Rubiaceae) is a shrub native to tropical and subtropical regions of Asia, especially in tropical regions of China[6] and North East India[7]. There is very sporadic information available in literature about the medicinal properties of this plant despite its wide availability, however decoction of leaves have been reported to stop bleeding and applied to fever[8]. The genus *Lasianthus* grow almost exclusively in the understory of primary forests, with the occasional records from secondary or seriously disturbed forests or forest edges. The genus has a great ecological significance and important component of vegetations in tropical forests of Asia[9].The leaves have been reported for its antimicrobial property[10]. In this study we report the antioxidant activity of methanolic and acetone extracts of *L. lucidus* leaf.

MATERIALS AND METHODS

Collection of plants

Lasianthus lucidus Blume was collected from Assam University campus, Assam, India. The plant is identified and authenticated by Botanical survey of India, Shillong. The aerial parts (leaves) were collected during the months of April- May and shade dried.

Extraction

Coarse powder of the plant material was extracted by hot continuous percolation method in soxhlet extractor with various solvents (petrol ether, acetone and methanol) for 48 hours respectively. The extracts were concentrated and dried under reduced pressure.

Screening of antioxidant activity

Qualitative Assay

Methanolic (ME) and acetone (AC) leaf extracts of *L. lucidus* were spotted on a silica gel coated Thin Layer Chromatographic plate and were run on 1:1 petrol- methanol ratio. The TLC plate was air dried and sprayed with .02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the colour changes were noted[11].

Quantitative Assay

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the leaf extracts was determined by DPPH (2,2-diphenyl-1 picrylhydrazyl) method[12]. The crude acetone and methanolic extracts of the leaf is mixed with methanol to prepare the stock solution (40 µg/ml). From this stock solution five sample solutions were prepared to attain the concentrations 2.5 µg/ml, 5µg/ml, 10 µg/ml, 20 µg/ml and 40 µg/ml. Freshly prepared DPPH solution was added in each of this test samples and after 20 minutes absorbance was taken at 550 nm. Ascorbic acid was taken as positive control and the DPPH solution without sample solution was used as control. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. Methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH Radical Scavenging Activity } (\%) = [1-(As/Ac)] \times 100.$$

Ac = absorbance of control, As = absorbance of sample solution.

The % DPPH Radical Scavenging Activity of different extracts was plotted against different concentrations and from the graph IC_{50} was calculated.

FRAP (Ferric Reducing Antioxidant Potential) Assay

The total antioxidant capacity of the leaf extract was determined using the iron III reduction method[13]. 1 ml of sample solutions (2.5 µg/ml, 5µg/ml, 10 µg/ml, 20 µg /ml and 40 µg/ml) were mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and 1% aqueous potassium hexacyanoferrate solution (2.5 ml). After 30 minutes of incubation at 50°c, trichloroacetic acid was added and the mixture was centrifuged at 3000rpm for 10 minutes. Then, the upper layer solution was mixed with water and an aqueous ferric chloride (0.1%) solution. The absorbance was read at 700nm and ascorbic

acid was used as positive control. The antioxidant content was determined by using a standard curve of ascorbic acid (0-10 µg/ml). The total antioxidant contents of leaf extracts were expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of leaf weight[13].

Total phenolic assay

Plant extracts (200µl, 1mg/ml) was oxidised with 1ml of 10% Folin Ciocalteu's reagent (v/v) and neutralised by adding 0.8 ml of 7.5% sodium carbonate. The absorbance of the resulting mixture was measured at 765 nm after incubating at 30°c for 1 hour. Results were expressed as mg of tannic acid equivalent TAE/100g of leaf extract[14].

Total flavonoid content

4.2 g of extract was dissolved in 5ml of 50% methanol followed by addition of 1ml of 5% (w/v) sodium nitrate solution. After 6 minutes, 1 ml of a 10% (w/v) aluminium chloride solution was added. The mixture was made up to 50ml with distilled water and mixed well. The absorbance was recorded at 500nm after 15minutes. Rutin was used for calibration curve and a concentration range of 5-50 µg/ml was prepared. The total flavonoid content was expressed as mg rutin equivalents RE/100 g dry plant material[15].

Statistical analysis

All data presented are means of three determinations along with standard deviations (SD). Statistical analysis used the MS Excel software (CORREL Statistical function) to calculate rutin, ascorbic acid and tannic acid equivalents, to determine inhibition percentage and to establish linear regression equations. The correlation between the dose and response were checked by regression analysis. Correlation coefficient ($R^2 \geq 0.90$) was taken as highly correlated.

RESULT AND DISCUSSION

Total results of the work have been presented in table 1 and figure 1 and figure 2.

Total phenolic and flavonoid content

Flavonoid and Phenolic compounds are rich in plants and also are the most abundant secondary metabolites in plants. Over the past few years, investigations for phenolic and flavonoids in medicinal herbs have gained importance due to their high anti-oxidative properties[16]. The highest ($P<0.05$) amount of phenolic content was found in the methanolic extract (56.24±2.34 TAE/100g) followed by the acetone extract (29.71±1.55 TAE/100g). The flavonoid contents were the most ($P<0.05$) in the methanolic extract (65.74±1.09 RE/100g) followed by the acetone extract (31.34±5.54 RE/100g).

Antioxidant assays

The utility of antioxidant therapies in many diseases are well recognised. Therefore, our finding of the effective inhibition of free radicals by the leaf extract of *L. lucidus* is an important finding, especially from a therapeutic point of view. The antioxidant activity of the extracts was determined using a DPPH scavenging assay and ferric oxide reducing activity method.

The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds[17].The degree of colour change (yellow on purple background) on TLC plate indicates the presence of antioxidant in the plant extract. The results of free radical scavenging potential from leaf extracts of the plant at different concentrations tested by DPPH free radical scavenging method are depicted in Figure 1. The acetone and methanolic extracts of the leaf exhibited a logarithmic dose dependent inhibition of DPPH activity with a 50% inhibition (IC_{50}) at a concentration of 24.39±0.97 µg/ml. The corresponding IC_{50} for acetone extract was 15.16±1.66 µg/ml (Table 1). In both the cases IC_{50} value was low compared to that of the reference compound ascorbic acid which had an IC_{50} value of 38.79±1.98 µg/ml. There is evidence of a good correlation between phenolic contents of the different extracts ($R^2 = 1$) and their IC_{50} DPPH values. These results were consistent with the findings of many research groups who reported such correlation between total phenolic content and IC50 antioxidant activity.[18,20]

The FRAP assay measured the ability of phenolic contents to reduce Fe^{3+} to Fe^{2+}. Here a linear increase in reducing power was observed over the concentration range 2–10 µg/ml sample and ascorbic acid (Fig:-2). The methanolic extract has potent reducing power followed by the acetone extract. Significant correlations are found between the data from the DPPH and FRAP antioxidant assays ($R^2 = 0.94593$). These correlations confirmed the antioxidant activity of the crude extract obtained from leaf of *L. lucidus*. Variations in free radical capacity of different concentrations may be attributed to the amount of various metabolites in crude extract.

The present study suggested that *L. lucidus* leaf extracts have moderate antioxidant activity. More detail studies on chemical composition of the leaf extracts, as well as *in vivo* assays are essential to characterize the antioxidants. Although traditionally the plant is used for curing a number of ailments, scientific record regarding medicinal activity of the plant is available only on antibacterial property[10]. We are therefore reporting the antioxidant potency of the plant for the first time based on our in vitro study.

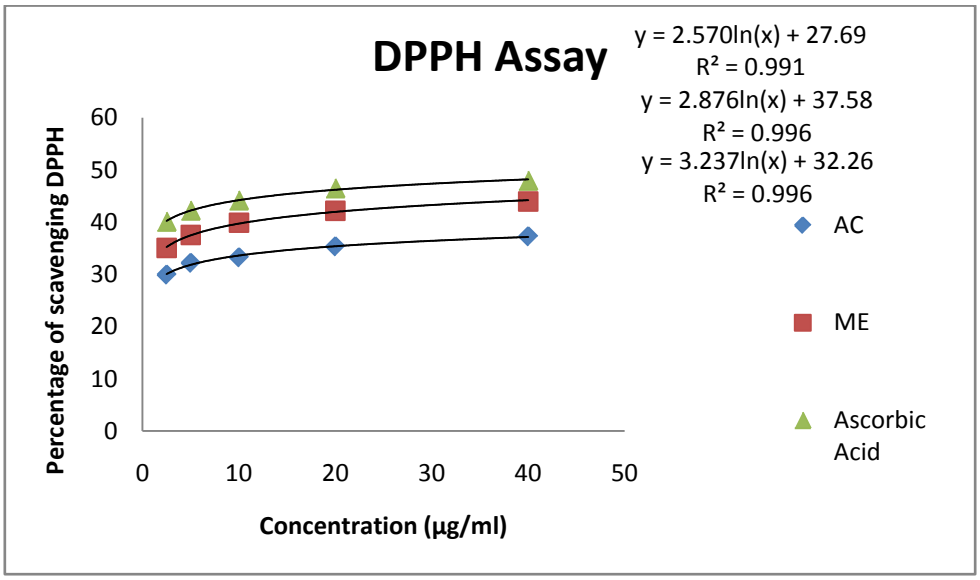

Fig. 1: Antioxidant activity of the plant extract estimated by DPPH radical–scavenging assay

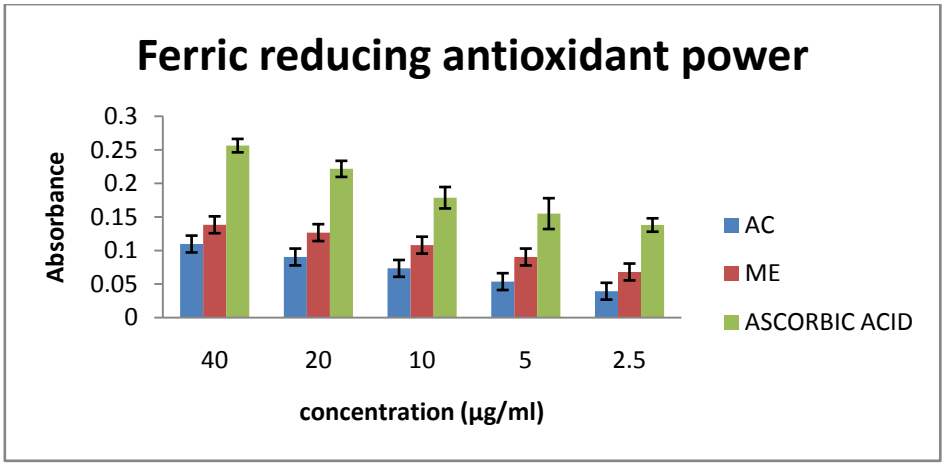

Fig. 2: Antioxidant activity of the plant extract estimated by Ferric Reducing Antioxidant Potential (FRAP) assay

Table 1: Total contents of phenolics, flavanoids and antioxidants and antioxidant activity of various *L. lucidus* leaf extracts.

Leaf extracts	Total phenolic (TAE/100g)	Total flavanoids (RE/100g)	Total antioxidants (AEAC/100g)	Antioxidant activity DPPH (µg/ml)
Acetone (AC)	29.71±1.55	31.34±5.54	29.62406	15.16±1.66
Methanol (ME)	56.24±2.34	65.74±1.09	31.6438	24.39±0.97

CONCLUSION

Our study proposes *L. lucidus* leaves as potent source of antioxidants. The anti oxidant activity of the extracts is directly related to phenolic/flavonoid contents. Isolation and characterization of phenolic compounds and flavonoids from the plant may lead to discovery of new antioxidant compounds.

ACKNOWLEDGEMENT

Authors sincerely acknowledge the literature support received from Bioinformatics Centre, Assam University, Silchar, India.

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