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Research Article

# EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF CALOTROPIS PROCERA FRUIT EXTRACT

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**ABSTRACT**

The plant *calotropis procera* (family-Asclepiadaceae) commonly known as " Aak " is used in many ayurvedic formulations.The present study was designed to evaluate the In vitro antioxidant potential of ethanolic fruit extract of *calotropis procera*. In vitro antioxidant activity was carried out by DPPH (1,1-Diphenyl-2-picryl hydrazyl) method, reducing power assay and phosphomolybdenum assay. The ethanolic fruit extract (100,250 and 500 µg/ml) was compared with Gallic acid (1, 2.5 and 5 µg/ml) as a standard. The results revealed that the fruit extract of *calotropis procera* possess significant antioxidant activity.

**Keywords**: *calotropis procera*, reducing power assay, phospohomolybdenum assay.

## INTRODUCTION

Reactive oxygen species are involved in a number of degenerative diseases such as cancer, cirrhosis, atherosclerosis, diabetes, heart failure[1-4] and also in wound healing[5]. Plant-derived antioxidants such as tannins, stilbenes, phenolic acids, flavones could delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid per oxidation, protein damage, DNA strand breaking[6] because of their redox properties, which help them to act as hydrogen donors, reducing agents and free radical scavengers[6, 7]. They are also strong chelators of metal ions[8]. Antioxidants play a potential role in the treatment and prevention of several diseases[9]. *Calotropis procera* (Ait.) R.Br. (giant milkweed) belongs to the family Asclepiadaceae locally known as "Aak" is distributed in tropical and sub-tropical Africa and Asia. *Calotropis procera* have large bushy shrub, leaves decussate, inflorescence extra axillary umbellate panicale, corolla purple, lobes erect[10]. The fruits are inflated, grey-green in colour and release flat, brown seeds with a tuft of white hair at one end[11]. The entire plant has been reported to contain alkaloids, sterols, flavonoids, cardiac glycosides, saponins, triterpinoids and uscharin[12].

*Calotropis procera* possess a wide range of biological activities such as analgesic, anti-inflammatory, antidiabetic, antiarthritic, antioxidant, antihelminthic, anticandidal, wound healing, anticonvulsant, antitumour, antiasthmatic and hepatoprotective.

## MATERIALS AND METHODS

### Collection of plant material

Fresh fruits were collected from the *Calotropis procera* plant growing in the surrounding areas of West Godavari district, A.P. The plant was identified and Authenticated by Dr. T. Raghuram, Taxonomist, Maharani's college, Peddapuram.

### Preparation of extract

The freshly collected fruits of the plant were cleared from dirt and dried under shade and then coarsely powdered manually. The dried

fruit powder was macerated in ethanol for a period of 7 days and later subjected to hot percolation for 8 hrs. Then the solution was filtered, concentrated and subjected to drying.

**Chemicals and Instruments**

All the chemicals used are of Analytical grade. DPPH has been procured from Research lab fine chemical Industries, Mumbai, Gallic acid was a gifted sample. Trichloro acetic acid, sodium phosphate, ammonium molybdate and sulphuric acid were used for this study are of analytical grade. The Instruments used for this study are PH Meter, UV- Visible double beam spectrophotometer (ELICO-SL-210), centrifuge machine and electronic balance were used for the analysis.

**IN-VITRO ANTIOXIDANT STUDY**

**DPPH Free Radical Scavenging Activity[13]**

The free radical scavenging activity was evaluated by the DPPH method, 0.1mM solution of DPPH in ethanol was prepared, Gallic acid was taken as reference standard, different concentrations of the extract (100, 250 and 500 µg/ml) and standard drug (1, 2.5 and 5 µg/ml) were prepared using ethanol. 1.0 ml of 0.1 mM of DPPH solution was mixed with 3.0 ml of all the concentrations of extract and standard separately. A blank was prepared using 0.1 mM DPPH and ethanol mixture without adding extract. These mixtures are kept in dark about 30 min and the optical density was measured at 517 nm. The experiment was repeated triplicate. The percentage inhibition of the DPPH activity was calculated by using the following formula.

**DPPH Scavenged (%) = $[(A_0 - A_1) / A_0] \times 100$**

Where $A_0$ is the absorbance of control reaction and $A_1$ is the absorbance of the sample extract. The antioxidant activity of the ethanolic fruit extract was expressed as $IC_{50}$ and compared with standard. The $IC_{50}$ Value was defined as the concentration (in µg / ml) of extract that Scavenges the DPPH radicals by 50%

**Reducing power method[14]**

Different concentrations of the extract (100,250 and 500 µg / ml) and standard drug (1, 2.5 and 5 µg/ml) were prepared using distilled water. 1% potassium ferricyanide, 10% Trichloroaceticacid, 0.1% ferric chloride and 0.2 M Phosphate buffer (pH 6.6) were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1 ml of each concentration of extract and standard were taken separately and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide. Incubate all these samples at 50$^o$c for 20 min. Then add 2.5 ml of 10% Trichloroaceticacid and centrifuge at 3000rpm for 10 min. now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5 ml of freshly prepared ferric chloride. Then the absorbances were measured at 700nm.

**Phosphomolybdenum Reduction Assay[15]**

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method. The assay is based on the reduction of MO (VI)-MO (V) by the extract and subsequent formation of green phosphate / MO (V) complex at acid pH. 0.3ml of each concentration of the extract and standard were taken separately and mixed with 3 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95$^o$c for 90 min. Then the absorbance of the solution was measured at 695nm using spectro photometer against blank after cooling to room temperature. Ethanol (0.3ml) in the place of extract was used as blank.

**RESULTS AND DISCUSSION**

**DPPH Free Radical Scavenging Activity**

The DPPH radical scavenging activity is a sensitive method for the antioxidant screening of plant extract[16]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517nm, which is induced by antioxidants. **Table 1** shows the percentage of DPPH radical scavenged by Gallic acid and ethanolic fruit extract at various concentrations (µg **/** ml). **Figure 1a, 1b** illustrates a decrease in the concentration of DPPH radicals due to the scavenging ability of the soluble constituents in the ethanolic fruit extract of *Calotropis procera* and Gallic acid as a reference standard. The $IC_{50}$ values were found to be 4.10 and 3.3 µg/ml for ethanolic fruit extract *of calotropis procera* and Gallic acid respectively.

**Reducing Power Assay**

Reducing power assay is based on the principle that substances, which have reduction potential, react with potassium ferricyanide ($Fe^{+3}$) to form potassium ferrocyanide ($Fe^{+2}$), which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as

a significant indicator of its potential antioxidant activity. **Table 2** shows the reducing power of ethanolic fruit extract of *calotropis procera* and standard Gallic acid. From **Figure 2a, 2b** it was found that the absorbance of the extract increased with an increase in concentrations.

**Phosphomolybdenum Reduction Assay**

Phosphomolybdenum assay is based on the reduction of MO (VI) – MO (V) and forms green colour Phosphomolybdenum (V) complex, which shows maximum absorbance at 695nm.**Table 3** shows the antioxidant capacity of ethanolic fruit extract of *calotropis procera* and standard Gallic acid. From **Figure 3a, 3b** it was found that the absorbance of the extract increased with an increase in concentrations.

**Table 1: DPPH Free Radical Scavenging Activity**

| Tested Material | Concentration (µg/ml) | % Inhibition ± SEM | $IC_{50µg/ml}$ |
|---|---|---|---|
| Standard Gallic acid | 1<br>2.5<br>5 | 24.32 ± 1.09<br>40.68 ± 0.05<br>66.16 ± 0.42 | 3.3 µg/ml |
| Ethanolic fruit extract of *calotropis procera* | 100<br>250<br>500 | 67.59 ± 0.14<br>75.92 ± 0.49<br>78.82 ± 0.53 | 4.1 µg/ml |

**Table 2: Reducing power method**

| Tested Material | Concentration (µg/ml) | Absorbance ± SEM |
|---|---|---|
| Standard Gallic acid | 1<br>2.5<br>5 | 0.3096 ± 0.0002<br>0.3723 ± 0.0003<br>0.5292 ± 0.0001 |
| Ethanolic fruit extract of *calotropis procera* | 100<br>250<br>500 | 0.086 ± 0.0001<br>0.122 ± 0.0005<br>0.275 ± 0.0005 |

**Table 3: Phosphomolybdenum Assay**

| Tested material | Concentration (µg/ml) | Absorbance ± SEM |
|---|---|---|
| Standard Gallic acid | 1<br>2.5<br>5 | 0.1626 ± 0.00003<br>0.3149 ± 0.0015<br>0.4291 ± 0.0003 |
| Ethanolic fruit extract of *Calotropis procera* | 100<br>250<br>500 | 0.1416 ± 0.0002<br>0.2445 ± 0.0001<br>0.3084 ± 0.0003 |

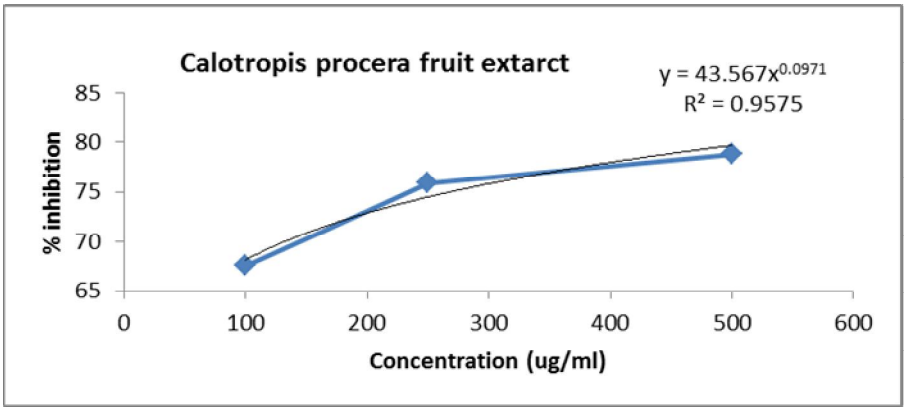


**Fig. 1a: DPPH Free Radical Scavenging Activity of *Calotropis procera***

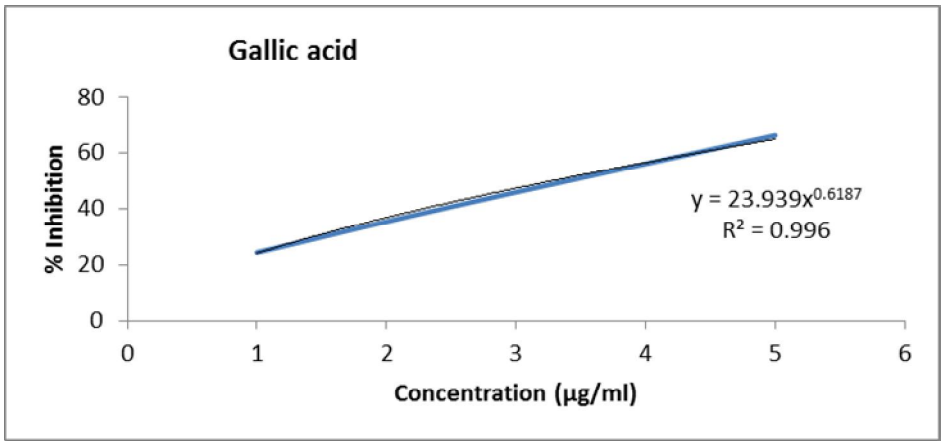


**Fig. 1b: DPPH Free Radical Scavenging Activity of Gallic acid**

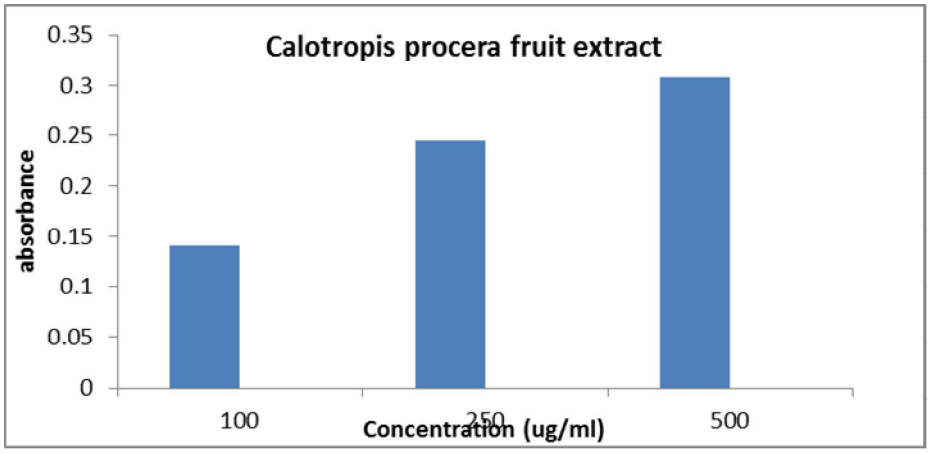


**Fig. 2a: Reducing Power of *Calotropis procera***

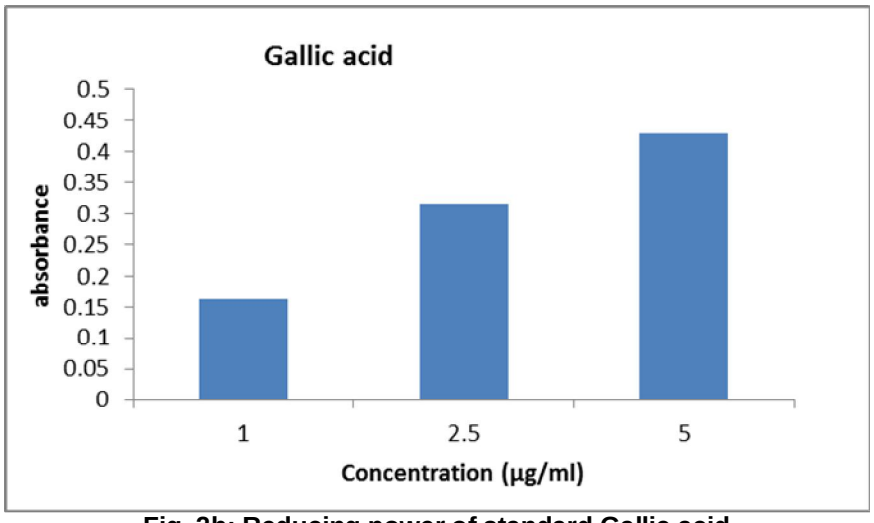


**Fig. 2b: Reducing power of standard Gallic acid**

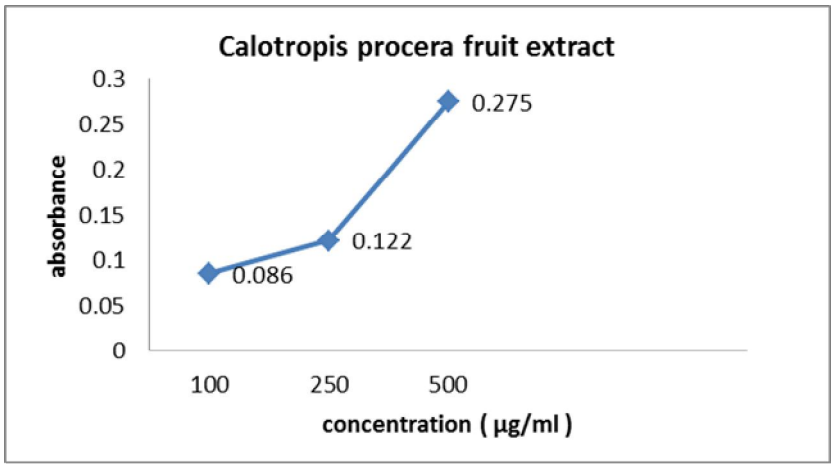


**Fig. 3a: Phosphomolybdenum Antioxidant Assay of *Calotropis procera***

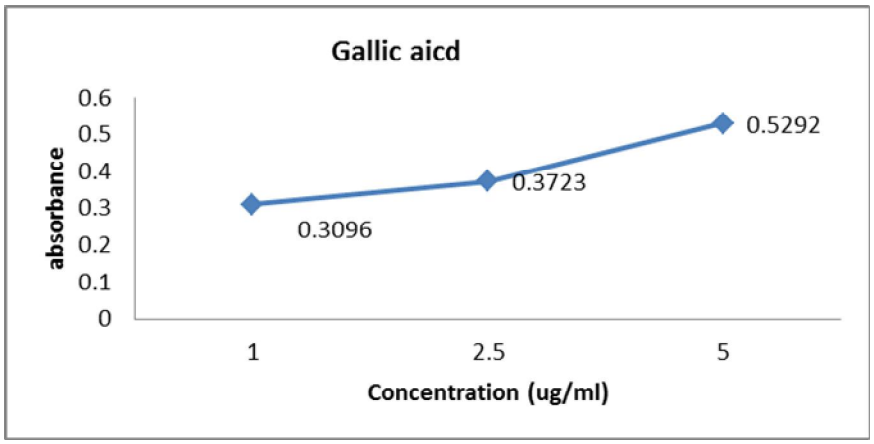


**Fig. 3b: Phosphomolybdenum Antioxidant Assay of Gallic acid**

## CONCLUSION

The present study was designed to evaluate the in vitro antioxidant activity of ethanolic fruit extract of *calotropis procera*. The results obtained indicates the significant antioxidant activities in all the three methods and the results were compared with standard Gallic acid. Further work can be carried out to isolate the compounds and screen for their biological activities.

## REFERENCES

1. Aboutwerat A, Pemberton PW, Smith A, Burrows PC, McMahon RFT, Jain SK, *et al*: Oxidant stress is a significant feature of primary biliary cirrhosis. *Biochim etBiophys Acta – Molecular Basis of Disease*, 2003; 1637: 142–150.
2. Arouma OI: Free radicals, oxidative stress and antioxidants in human health and disease. *J Am Oil Chem Soc*, 1998; 75: 199–212.
3. Baynes JW: The role of oxidative stress in development of complications in diabetes.*Diabetes*, 1991; 40: 405–412.
4. Dreher D, Junod AF: Role of oxygen free radicals in cancer development. *Eur J Cancer*, 1996; 32A: 30–38.
5. Bodeker G, Hughes MA: Wound healing, traditional treatments and research policy. In H. D. V. Prendergast, N. L. Etkin, D. R. Harris, & P. J. Houghton (Eds.), *Plants for food and*

*medicine,*1998; (pp. 245–359). Kew, London: Royal Botanic Gardens.
6. Govindarajan R, Vijayakumar M, Pushpangadan P: Antioxidant approach to disease management and the role of 'Rasayana' herbs of ayurveda. *J Ethnopharm.* 2005*;* 99: 165–178.
7. Robards K, Prenzler PD, Tucker G, Swaitsitang P, Glover W: Phenolic compounds and their role in oxidative processes. *Food Chem*, 1999; 66: 401–436.
8. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB: The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radical Res, 1995; 22: 375-383
9. Ajitha M, Rajnarayana K: Role of oxygen free radicals in human diseases. *Indian Drugs,* 2001; *38*: 545–554.
10. Murti Y, Yogi B, Pathak D. Pharmacognostical standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). Inter J Ayur Res. 2010; 1(1): 14-17.
11. Rahman M.A. and Wilcock, C, C: A Taxonomic revision of Calotropis procera (Asclepiadaceae). Nordic.J. botany. 11 (3), 240-246 (1991).
12. Edman M.D: Nutrient and Cardenolide Composition of solvent extracted Calotropis procera.J.Agri.Food. Chem.313, 509-513 (1983).
13. Fatema Nasrin; Antioxidant and Cytotoxic Activities of ageratum conyzoides stems: International current pharmaceutical Journal: 2(2) 33-37; (2013).
14. P.Jayanth, P.Lalitha: Reducing power of solvent extracts of Eichorna crassipes (Mart) SOLMS; International Journal of Pharmacy and Pharmaceutical Sciences: 3:126-128; (2011).
15. Mani Ranis; Md. Ashraful; Raushanara A, Rumana J; Invitro Free radical Scavenging Activity of Ixora coccinea L; Bangladesh Journal of Pharmacology: 3; 90-96 (2008).
16. Hemaltha,S., Lalitha, P. and Arul Priya,P;Antioxidant activities of extracts of aerial roots of pothos aurea (Linden Exandre): Der. Pharma chemical: 2(6); 84-86(2010).