*Research Article*

# Evaluation of antioxidant and antibacterial efficacy of flowers of *Calotropis gigantea*

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**Abstract**

**Objective:** The main objective of the present work is to screen the antioxidant and antibacterial activity of various extracts obtained from flowers of *Calotropis gigantea* belonging to the family *Apocynaceae*. **Materials and Methods:** Both organic (Pet ether, Benzene and Acetone) and aqueous (Hydroalcohol) extracts of *Calotropis gigantea* flowers were prepared by using Soxhlet and cold maceration technique respectively. The extracts were concentrated using rotary evaporator. By using invitro paradigms, DPPH, nitric oxide and hydroxyl free radical scavenging activity were carried out. Meanwhile all the extarcts were subjected for antibacterial activity using various gram+ive and gram-ive bacterias in the substrate medium of Muller agar media with Diffusion assay method. **Results:** In the antioxidant activity, Pet ether extract showed a significant activity than other extracts. Also increase in concentration of the extract showed an increase in inhibition of the generfated free radicals. At most the pet ether extract at a higher concentration of 320 mcg/ml showed an increased inhibition of DPPH (73.5±0.2%), Nitric oxide (56.1±0.3%) and Hydroxyl (78.1±0.8%). Meanwhile antibacterial activity of hydroalcohol extract showed an increased zone of inhibition for various organisms used in this method than other extracts and significant to the standard, Pefloxacin. **Conclusion:** It was investigated that antioxidant activity showed a potential inhibition on generated free radicals and both gram+ive and gram-ive bacterial strains. Further studies are needed to evaluate the structural elucidation of active ingredients present in those extracts.

***Keywords:*** *Calotropis gigantea,* extraction, antioxidant, antibacterial

## Introduction

The development of resistance for existing antibiotixcs or synthetic agents is increasing public concern over environmental pollution and toxicity generated new antibiotics. During the last two decades plant based products are most of the plants developed a lot of pharmacological evidence to prove as antimicrobial and antioxidant strategy.

*Calotropis gigantea* (family: Apocynaceae), being a shrub is commonly called as *Aslcepias gigantea*, milk weed or swort. It is commonly called as Palerukku (Lindley, 1985). But it is a native species of various South East Asian countries mainly Thailand (Bingtao et al., 1811). It has inflorescence stalk (5cm) and petals (2.5-4cm). It is used by native tribes for various ailments for healing both epidemic and endemic diseases as well as disorders (Upadhyay, 2014). The various parts of the plant have noteworthy medicinal value in the middle of human beings. The roots are used as emetic. Latex is used as purgative and expectorant. The barks are used as tonics in minimal doses for intermittent fevers. The flowers are used as digestive, stomachic and anti- asthmatic. The present study is carried out to screen the antioxidant and antibacterial activity of the flowers extracts obtained from the plant.

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## Materials and Methods

### Collection of plant materials

The whole plant of *C. gigantea* L was collected from farms of Acharya Ranga Agricultural University, Nellore which was

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identified by Dr.Ramesh Reddy, Senior taxonomist of the same university and authenticated by Dr. S. M. Khasim, Professor, Department of Botany, Acharya Nagarjuna University, Guntur.

**Chemicals**

All the chemicals used in this work are analytical grade and were procured from S.D.fine Chemicals Pvt Ltd Mumbai and were purest form.

**Experimental Procedure**

The flowers of *C. gigantea* were gathered from college campus and washed with fresh water to remove the soily adhered matters. They were dried under shade at room temperature, fumigated and packed in polythene bags. Then they were powdered by using a pulveriser and sieved with 40 mesh to obtain fine powder. About 1kg of powder was weighed and subjected to successive soxhlete extraction with petroleum ether (60-70°C), benzene and acetone for a period of 48 hours. Subsequently the dried marc was subjected to cold maceration by using hydro alcohol (50:50) for 3 consecutive days. Finally all the extracts such as Pet ether extract (PE), Benzene extract (BE), Acetone Extract (AE) and Hydroalcohol extract (HAE) were filtered through muslin cloth. Then they were processed under reduced pressure and dried in vacuum condition to get a semisolid consistency whose yields are assessed (Kokatae et al., 2010).

**Antioxidant activity**

The *in vitro* antioxidant activity was carried out on various extracts by using three various paradigms and they are as follows:

***DPPH radical scavenging activity***

In this model free radicals were generated by using 2, 2-diphenyl-picryl-hydrazil (DPPH) (Blois et al., 1958). About 0.1 ml of DPPH in methanol was prepared and 1ml of solution was added to 3 ml of solutions of different extracts of the plant using methanol at concentration range of 10-320 µg/ml. Ascorbic acid acted as a standard drug and its concentration was also prepared like the test extracts. The mixtures were shaken vigorously and kept under incubation for 30 minutes. Then absorbance of all the mixtures were measured at 517 nm using UV-Vis Spectrophotometer.

DPPH Scavenging effect (%inhibition) =1- [$A_{517}$ (sample) - $A_{517}$(control) / $A_{517}$(control)] × 100

***Determination of NO radical scavenging activity***

In this model nitric oxide free radicals were generated by using Griess reagent (Rao, 1997). About 5 ml of Griess reagent (1% w/v of sulphanilamide, 2%v/v of phosphoric acid and 0.1%w/v of naphthylenediamine dihydrochloride) was treated with nitric oxide generating system [10%w/v of sodium nitroprusside and 2 ml of phosphate buffered saline (0.9%w/v)]. To the reaction mixture each extract in spectrum alcohol with various concentrations (10-320 µg/ml) of 3ml was added and diluted with 3 ml of distilled water. The standard, ascorbic acid was also carried out as above. The mixtures were shaken vigorously and kept under incubation for 30 minutes. Then absorbance of all the mixtures were measured at 546 nm using UV/Vis Spectrophotometer.

NO Scavenging activity (%inhibition) =1-[$A_{546}$ (sample) - $A_{546}$(control) / $A_{546}$(control)] × 100

***Determination of hydroxyl radical scavenging activity***

In this method hydroxyl free radical was generated by Ferrous-ascorbate-EDTA-$H_2O_2$ system (Fenton reaction) (Elizabeth and Rao, 1990).The composition of fenton reagent is 2-deoxy-2-ribose (2.8 mM), potassium dihydrogen phosphate (20 mM, pH-7.4), Ferric chloride (100 µM), EDTA(100 µM) and hydrogen peroxide (1.0 mM) with various concentrations (10-320 µg/ml) of *C. gigantea* extracts and L-ascorbic acid was used as standard. Then they were incubated for an hour and the absorbance was measured at 532 nm.

Superoxide Scavenging effect (%inhibition) = [$A_{cont}$-$A_{test}$ / $A_{cont}$] × 100

**Antibacterial activity**

Anti-bacterial activity of various extracts of flowers obtained from *C.gigantea* was performed by paper disc diffusion assay method (Sathyanarayana et al., 2009). Bacterial strains such as *Bacillus subtilis* (NCIM 2063), *Streptococcus aureus* (NCIM 2019), *Escherchia coli* (NCIM 2065) and *Klebsiella pneumoniae* (NCIM 2036) were engaged for screening this activity. All the selected organisms were maintained using Mueller Hinton agar (MHA) medium at 37°C. The discs of uniform size (6 mm) were prepared using Whatmann filter paper No.1 and sterilized in hot oven at 160°C for 1hr. Then the discs were impregnated with minimum inhibitory concentration of different concentrations (50, 100 and 250 µg/ml) of various extracts and standard, Pefloxacin. The solvent DMF is used as a control. The plates were prepared by MHA media and extracts of various dilutions were allowed to solidify and dried. Different impregnated discs in various concentrations of extracts were prepared in the solidified agar plates and were labeled. Then a loop of bacterial culture was inoculated with the MHA media at labeled spots. The plates were inoculated at 37°C for 24 hrs and the zone of inhibition was noted.

**Results and Discussions**

In this study the pet ether extract showed a significant

activity in scavenging the generated free radicals by diphenyl picrohydrazyl (Figure 1), nitric oxide (Figure 2) and hydroxyl (Figure 3) paradigms than ascorbic acid. The extract also showed an increase in percentage of inhibition on concentration dependent. All the three paradigms are widely used for preliminary antioxidant activity. The radical formed in these models are stable radicals. Antioxidants upon interaction with auto generated DPPH radicals, transfer a proton to these radicals bydirect abstraction of phenolic $H^+$ ions and electron transfer process. They neutralize the formed free radicals. The neutralization of free radical is visually observed by colour change from purple to yellow colour. This discoloration indicates the scavenging potential of the antioxidants (Villano et al., 2007;

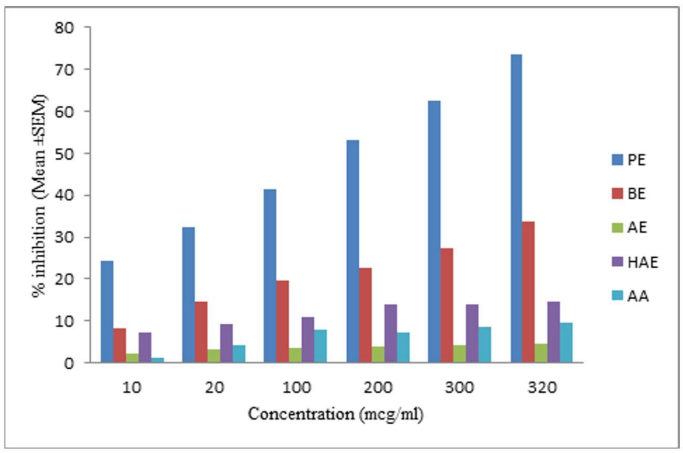


**Figure 1.** DPPH free radical Scavenging activity of Various extracts at different concentration and standard Ascorbic acid.

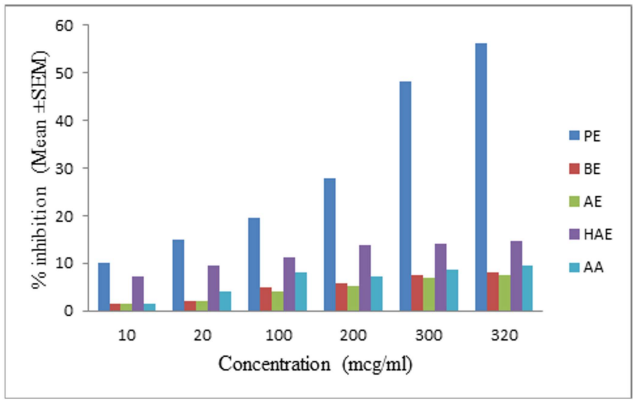


**Figure 2.** Nitric Oxide free radical Scavenging activity of Various extracts at different concentration and standard Ascorbic acid.

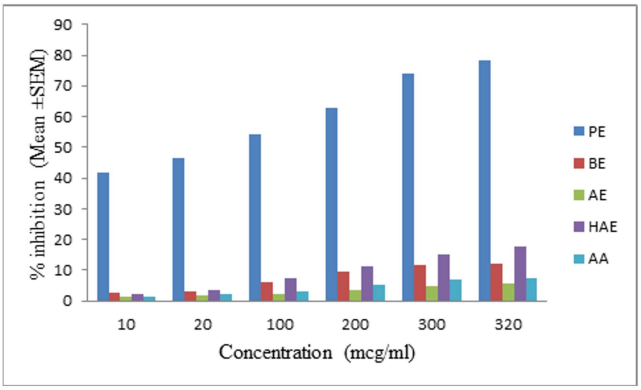


**Figure 3.** Hydroxyl free radical Scavenging activity of Various extracts at different concentration and standard Ascorbic acid.

**Table 1.** Antibacterial activity of various extracts obtained from *C. gigantea* flowers at different concentrations and Standard Pefloxacin

| **Various extracts and different concentrations (mcg/ml)** | **Zone of inhibition (mm)** | | | |
|---|---|---|---|---|
| | ***B. subtilis*** | ***S. aureus*** | ***E. coli*** | ***K. Pneumonae*** |
| Petroleum ether (PE) | | | | |
| 50 | -- | -- | 07 | 09 |
| 100 | -- | 08 | 09 | 11 |
| 200 | -- | 11 | 12 | 14 |
| Benzene (BE) | | | | |
| 50 | 07 | 08 | -- | -- |
| 100 | 12 | 11 | -- | -- |
| 200 | 13 | 13 | -- | 06 |
| Acetone (AE) | | | | |
| 50 | -- | -- | 07 | -- |
| 100 | -- | 07 | 08 | 06 |
| 200 | 07 | 09 | 11 | 09 |
| Hydroalcohol (HAE) | | | | |
| 50 | 09 | 07 | 08 | 09 |
| 100 | 14 | 11 | 12 | 15 |
| 200 | 21 | 16 | 17 | 24 |
| Pefloxacin | | | | |
| 100 | 29 | 34 | 31 | 26 |
| DMF | 05 | 07 | 06 | 05 |

DMF: Dimethyl Formamide; (---): Inhibition

Foti et al., 2004). The antioxidant principleof the extract competes with oxygen to react with nitric oxide released during diazotization reaction of naphthyl ethylenediamine. Thus inhibits the generated nitrite radicals (Ialenti et al., 1993) which indicates the scavenging potential. Meanwhile hydroxyl radicals are the most reactive species, initiating peroxidation of the cell membrane (Halliwell et al., 1987). The lipid radical thus generated, initiates chain reaction in presence of oxygen by giving rise to lipid peroxide which break down to aldehydes such as malondialdehyde which are known to be mutagenic or carcinogenic (Miyake, 1997). These radicals which on interaction with the extract showed a yellow colour formation which is an indication of scavenging and showed an intensity of hunting potential of the extract. So the petroleum ether extract reduced the free radical with increase in concentration by dose dependent manner.

In case of the antibacterial activity, it was observed that the hydro alcohol extract showed excellent antibacterial activity and a significant efficacy was showed by it on comparing with standard, pefloxacin (Table 1). Also increase in concentration of the extract showed an increase in zone of inhibition. The primary target binding site of standard, pefloxacin is DNAgyrase and topoisomerase. The primary inhibition is happened for the gyrase mediated DNA supercoiling while secondary inhibition is caused by inhibition of DNA replication of due to bacterial topoisomerase activity. Drugs possessing a concentration of 10-320µg/ml inhibit gyrase-mediated DNA supercoiling and produce bacteriostatic effect (Hooper, 2000a).So the hydroalcohol extract may also follow the same action in inhibiting the bacterial biosynthesis.

The *invitro* antioxidant and antibacterial activity may be due to the presence of an active new pregnanone compound namely Calotropone (Zhu-Nian Wang et al., 2008) which was isolated from aqueous and organic solvents of the flowers of the plant.

**Conclusion**

From our studies we conclude that the active new preganone and glycosides are responsibe for the antioxidant and antibacterial activity. Further studies are under process for isolationa dn biological studies of the reported phytochemicals.

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