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GCMS OF METHANOLIC EXTRACT AND ANTIOXIDANT ACTIVITY OF *CALOTROPIS GIGANTEA* LEAVES AND FLOWERS

"Weeds are the flowers too, once you get to know them."-A. A. Milne

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ABSTRACT

Calotropis gigantea is also known as "Badabadam" or "eruku". The plant is common in Asia and widely available throughout India, Bhutan, Nepal, and Sri Lanka. Gas Chromatography Mass Spectroscopy analysis was performed in methanolic extract of *Calotropis gigantea* leaves and flowers. Antioxidant activity was carried out in Two methods-Nitrous oxide and DPPH (2, 2-Diphenyl-1-picrylhydrazyl).Antioxidant activity was carried out at 10-100µl concentrations. The bioactive compounds are present in *Calotropis gigantea* leaves and flowers Antioxidant activity are determined in *Calotropis gigantea* leaves and flowers. They are used to reduce the impact of oxidative stress which are helpful in preventing diseases such as Cardiovascular diseases, cancer, neurodegenerative diseases, diabetes, irritable bowel disease, immune system ,respiratory, liver disorder and chronic kidney disorder.

Keywords: Gas Chromatography Mass spectroscopy, Antioxidant, 2, 2-Diphenyl-1-picrylhydrazyl, Cardiovascular, neurodegenerative, Badabadam, Irritable Bowel disease, Oxidative stress

INTRODUCTION:

Calotropis gigantea is also known as waste land weed or crown flower or giant milkweed or shallow wort. It is also known as tropical wild herbaceous species **[1]**. Calotropis is a genus which is described in 1810. Giant milk weed is found in Bangladesh, Burma, China, Indonesia, Malaysia, Pakistan, Philippines, Thailand, and Sri Lanka. **[13]**. *Calotropis gigantea* is widely found in India and have been utilized for variety of medical condition in the country's traditional system **[11, 12]**. The plant thrives in variety types of soil and climatic conditions. It is found in tropical and subtropical regions **[11, 12]**.

Classification:

Kingdom-Plantae

Division: Mangliophyta

Class: Magnoliopsida

Order : Gentianales

Family: Apocynaceae

Sub-Family: Asclepiadoideae

Genus: Calotropis

Species: *gigantea*

Gas chromatography-mass spectrometry (GC-MS) is a popular analysis technique for identifying and quantifying concentrations of organic substances. Naima Saeed *et al* ., (2012), Gocer H and Gulcin I(2011),Gulcin(2011)have reported in their paper that Human cells are constantly exposed to reactive oxygen radicals are generally generated by environmental factors, pollutants and stress factors. When this exposure overwhelms endogenous preventive systems, cells are exposed to oxidants, leading to free radicals which induce noxious effects.

Free radical attacks biological molecules such as lipids, proteins, enzymes, DNA, RNA leading to cell injury associated with many diseases including artherosclerosis, heart

diseases, and carcinogenesis. (Sonia Losada-Barreiro *et al., (*2022) reported that Antioxidants are the compounds which act as radical scavengers when added to the food products and prevent radical chain of oxidation reaction, delay or inhibit the oxidation process and increase the shelf life by retarding the process of lipid peroxidation. Antioxidants are used to prevent or delay in onset of major degenerative diseases. They block the oxidation process that can produce free radicals which can be used towards chronic diseases and aging. *Calotropis gigantea* is used as a good source of antioxidant property. These plants have an ability to biosynthesis a wide range of non-enzymatic antioxidants capable of ROS-Induced oxidant damage.

Figure 1: *Calotropis gigantea* leaves

Figure 2: *Calotropis gigantea* flowers

MATERIALS AND METHODS:

Authentication:

The leaves and flowers of *Calotropis gigantea* was authenticated at Herbal Plant Anatomy Research Centre, Tambaram, Chennai.

Extraction:

Fresh plants were collected on roadside of College road, Chennai. 100 grams of leaves and flowers of *Calotropis gigantea* was dissolved in 1000 ml of methanol. The extracts were stored in glass bottles and were given for Gas Chromatography Mass Spectroscopy analysis. Gas Chromatography mass spectroscopy was analysed at Vellore Institute of Technology, Vellore. The extracts were kept for drying in glass petri plates and powdered extracts which was scraped out after drying were used for further applications.

GC-MS analysis:

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at

260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Antioxidant activity –DPPH method:

Sánchez-Moreno et al;1999 was performed with 1, 1-Diphenyl -2-picryl hydroxyl radical scavenging of leaves and flower extracts of *Calotropis gigantea*. The extracts and standard reference compounds were prepared with 9.9% ethanol at various concentrations.(50-500µg/ml).1ml of various concentration (50-1000µg/ml) of the extracts and standard reference compounds were dissolved in 1ml of 0.2ml of DPPH and were made upto 3ml in 99% methanol in 10 ml test tube to achieve a final volume of 3 ml. The mixture was vortexed and incubated for 90 minutes at room temperature. The optical density was measured at 517nm and the standards were compared using BHT, Ascorbic acid.

Antioxidant activity-NO Method:

3ml of phosphate buffered saline was taken in the test tube and pH of 7.4 was maintained.10mM sodium nitroprusside in PBS was added and plant extracts (leaves and flowers of *Calotropis gigantea* of 50-1000µg/ml at different concentration were added to the test tubes. The test tubes were incubated at 25˚C for 15 minutes. About 0.5 ml of samples was taken and 0.5ml of Griess reagent added to the test tubes .The optical density was measured at 546 nm. The standards were compared using BHT, Ascorbic acid.

RESULTS

Gas Chromatography Mass Spectroscopic analysis:

Bioactive compound analysis was analysed by using Gas chromatography mass spectroscopy in methanolic extract of *Calotropis gigantea* leaves and flowers. [There are references in which twenty chemical constituents have been identified from ethanolic extract of stem and fruit of *Tribulus terrestris* and *Calotropis procera* by Gas Chromatogram Mass spectrometry (GC-MS) analysis.].

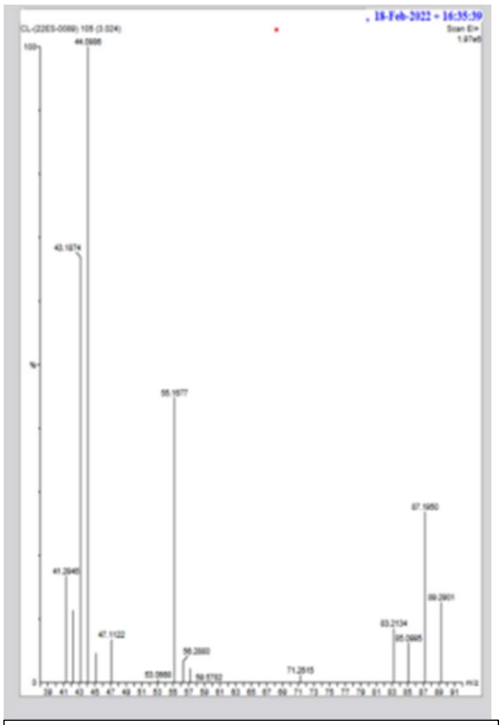


Figure 3: GC-MS peaks of GCMS of *Calotropis gigantea* leaves

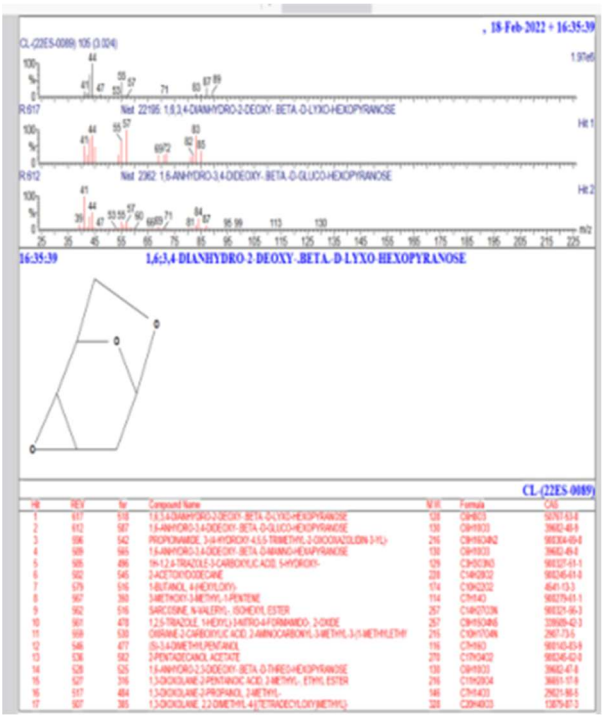


Figure 4: Bioactive Compounds found in *Calotropis gigantea* leaves

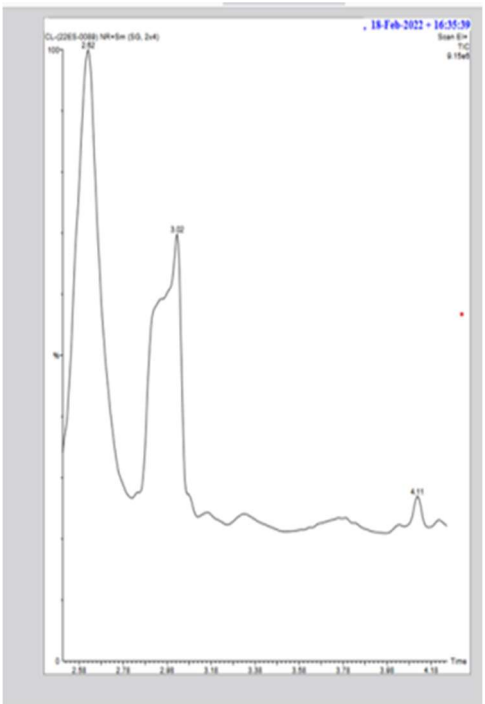


Figure 5: GC MS peak in *Calotropis gigantea* leaves

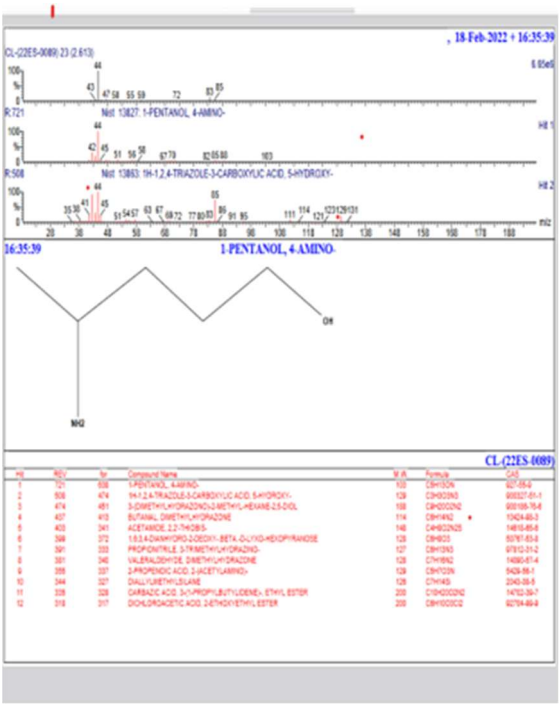


Figure 6: Bioactive Compound present in *Calotropis gigantea* leaves

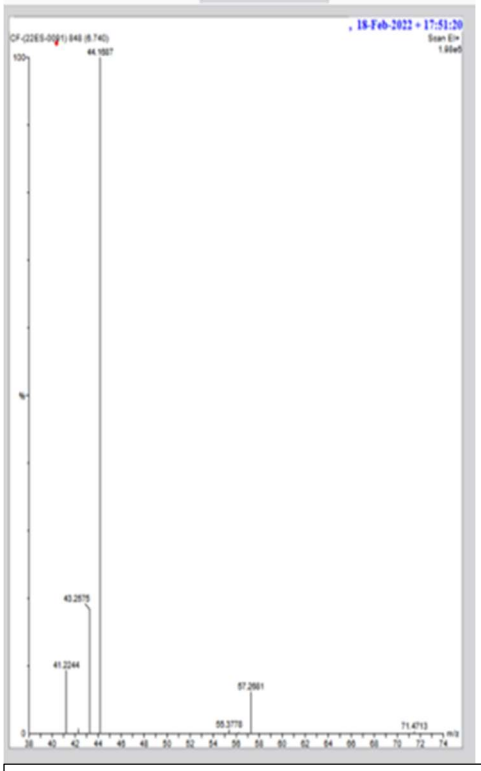


Figure 7: GCMS peaks of *Calotropis gigantea* flower

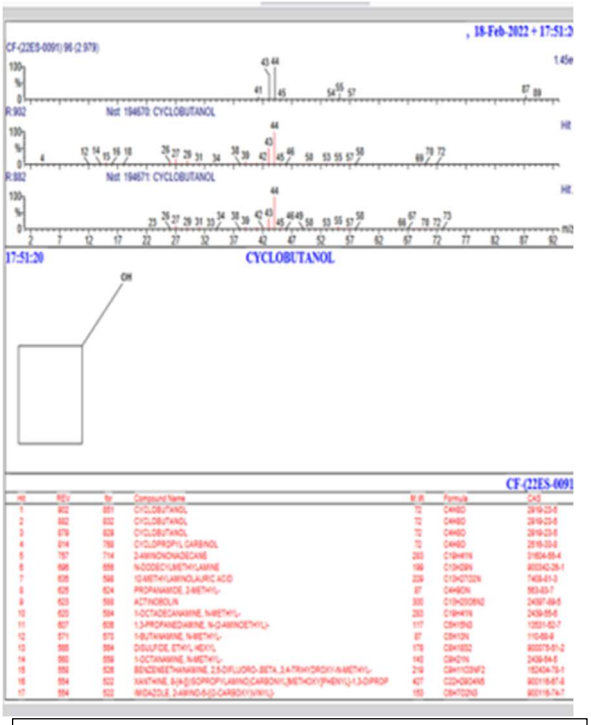


Figure 8: Bioactive Compound of *Calotropis gigantea* flower

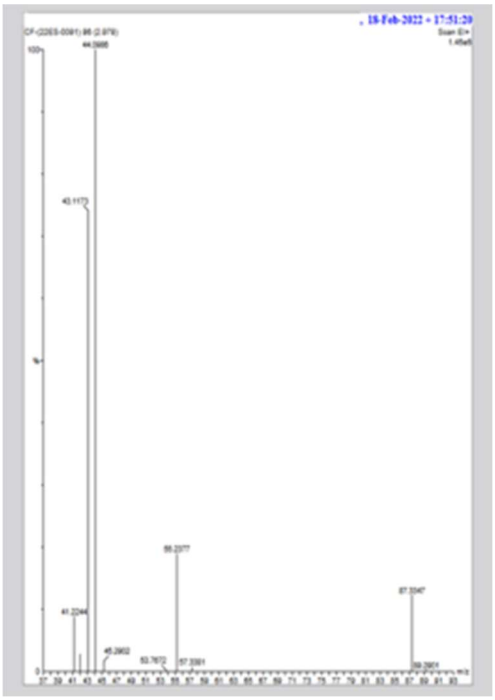


Figure 9: GCMS peaks of peaks in *Calotropis gigantea* flower

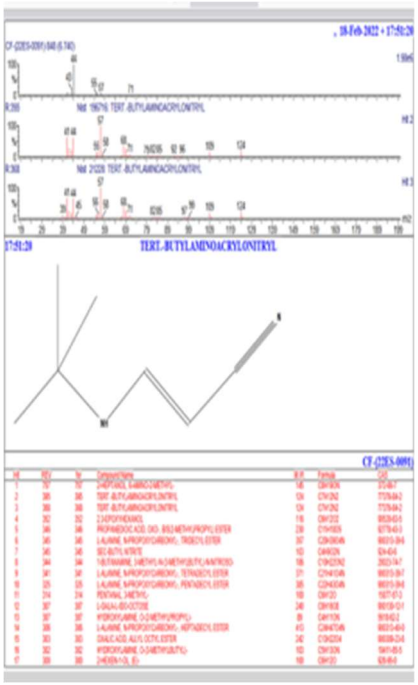
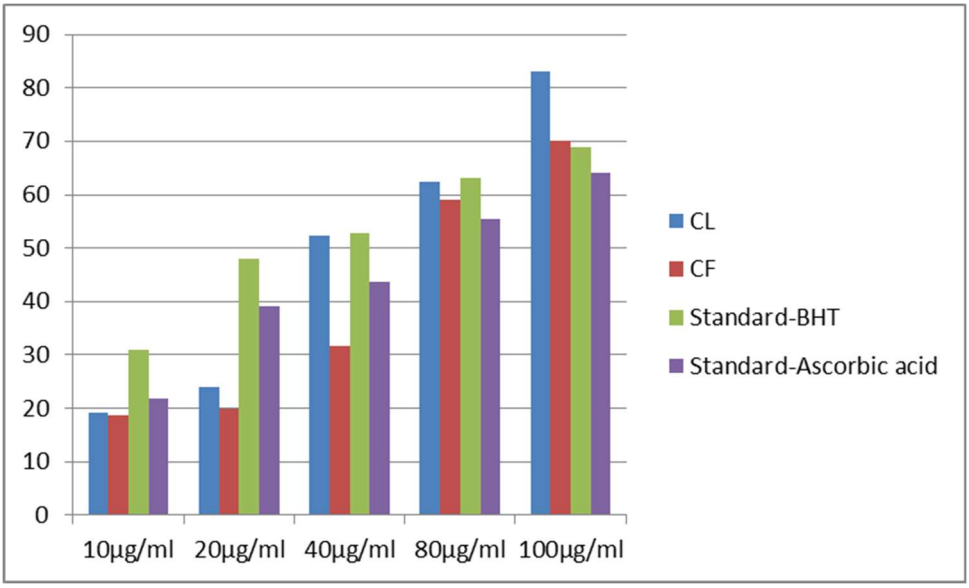


Figure 10: Bioactive Compound present in GCMS of *Calotropis gigantea* flower

(i) Antioxidant activity-Nitrous oxide method:

Antioxidant activity was carried out using Nitrous oxide method and was carried out in different concentrations from 10-100µg/ml. It was performed in leaves and flowers of *Calotropis gigantea* compared to standard such as Butylated hydroxytoluene and Ascorbic acid. There are references in which would healing assay were observed in tested models with leaves of *Calotropis gigantea* dry extract.



CL-*Calotropis gigantea* leaves

CF-*Calotropis gigantea* flowers

BHT-Butylated Hydroxy toluene

Figure 11: Antioxidant activity of *Calotropis gigantea* leaves and flowers extract by Nitrous oxide method

(ii) Antioxidant activity-DPPH (2,2-diphenyl-1-picryl-hydrazyl) method:

Antioxidant activity was carried out using DPPH method. Bioactive compound was determined using Gas chromatography. Antioxidant activity was carried out in leaves and flowers of *Calotropis gigantea* compared to standard concentration such as Butylated hydroxytoluene and Ascorbic acid. It was carried out at 10-100µg/ml concentration.

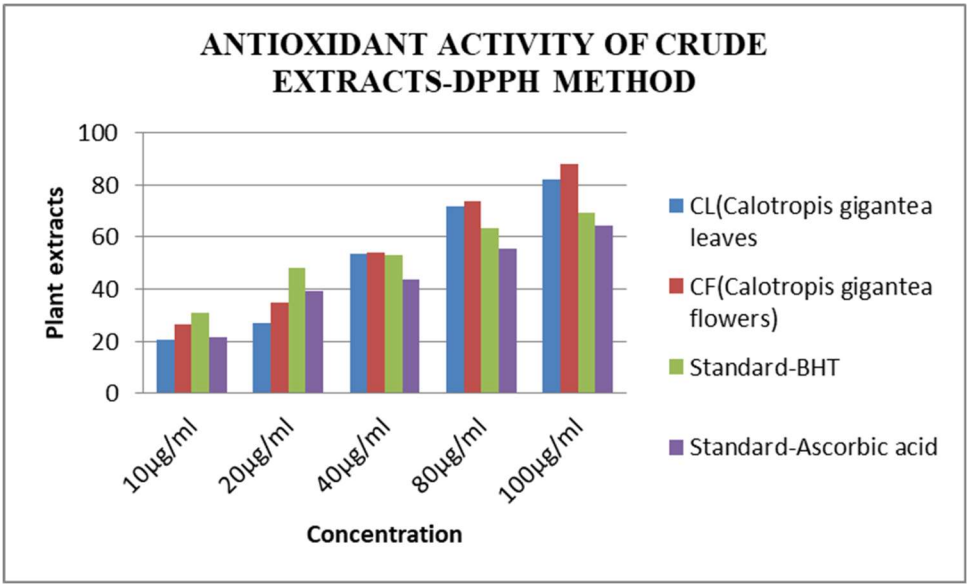


Figure 12: Antioxidant activity of *Calotropis gigantea* leaves and flowers was carried out by DPPH method. As the concentration increases, percentage efficacy also increases

DISCUSSION:

The antioxidant activity studies of *Calotropis gigantea* using DPPH and NO method reveals the significant source of natural antioxidant.

(i) DPPH Method: The observed concentration-dependent activity demonstrated that the extract is rich in compounds capable of donating hydrogen atoms or electrons to neutralize free radicals.

(ii) NO Method: The significant of NO scavenging activity indicates the ability of the plant's bioactive compounds to mitigate oxidative stress caused by reactive nitrogen species.

CONCLUSION:

Gas chromatography mass spectroscopy revealed the presence of Bioactive compounds in *Calotropis gigantea* leaves and flowers. Antioxidant activity was carried out in *Calotropis gigantea* leaves and flowers by using two methods such as nitrous oxide and DPPH method was carried out 10-100µl concentration. The identification of bioactive compound was analysed using GCMS analysis. Antioxidants are known to neutralise free radicals and reduce oxidative stress which is linked to various chronic diseases. The research has contributed by identifying and characterizing bioactive compounds via GCMS. It has also provided us the insights into antioxidant properties of *Calotropis gigantea.* It is crucial for its potential applications in medicines and natural antioxidant source.

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[21] https://pubchem.ncbi.nlm.nih.gov/compound/Propionamide,3-(4-Hydroxy-4,5,5-Trimethy-2-oxoozolidin-3-yl).

[22] https://pubchem.ncbi.nlm.nih.gov/compound/ Cyclopropyl Carbinol.

[23] https://pubchem.ncbi.nlm.nih.gov/compound/ 2-Acetoxydodecane.

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