EFFICACY OF BIOACTIVE COMPOUNDS AND NANOPARTICLES FROM LEAVES AND STEM OF *CALOTROPIS GIGANTEA (LINN) R. BR* TO THEIR BIOACTIVITY

SYNOPSIS SUBMITTED TO MADURAI KAMARAJ UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF

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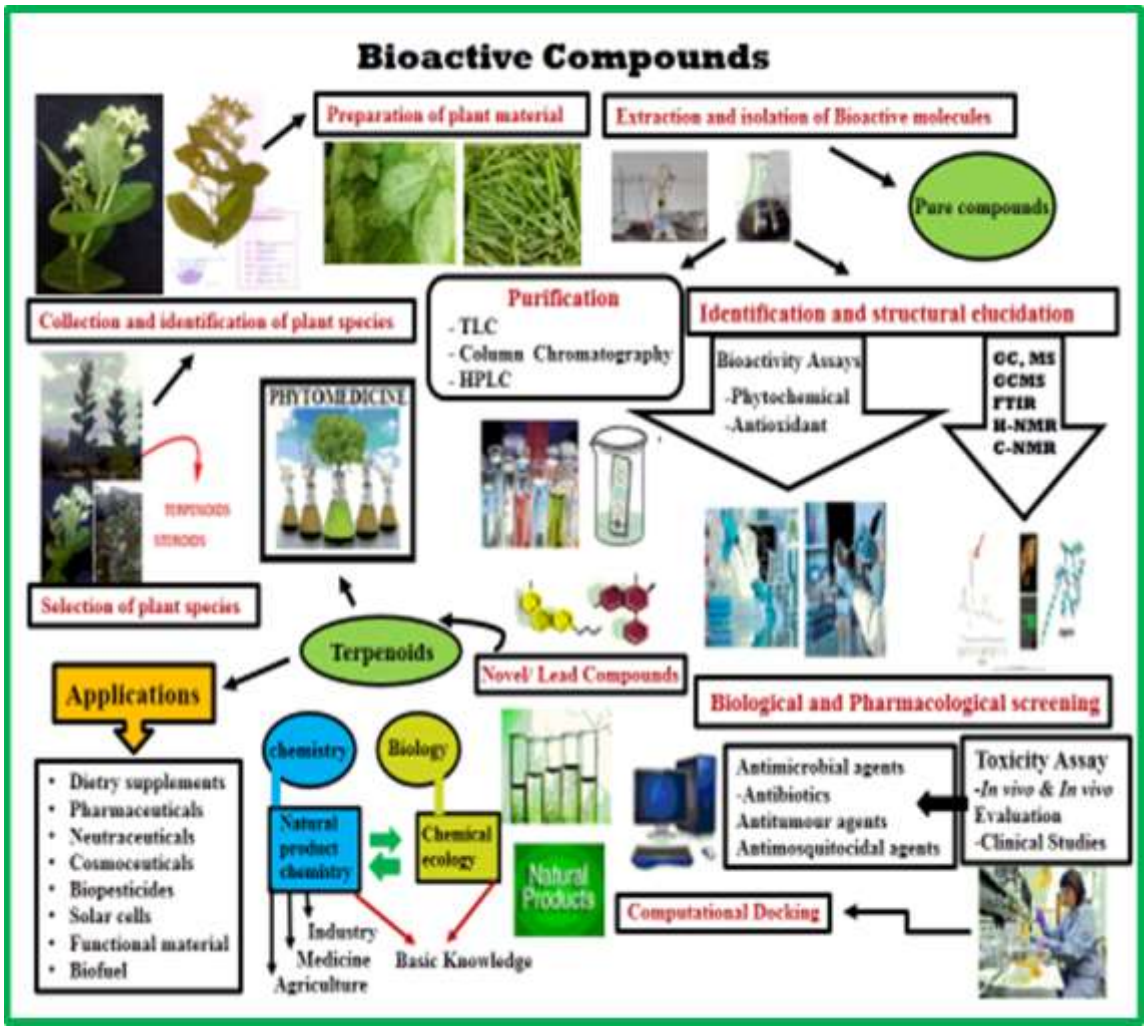
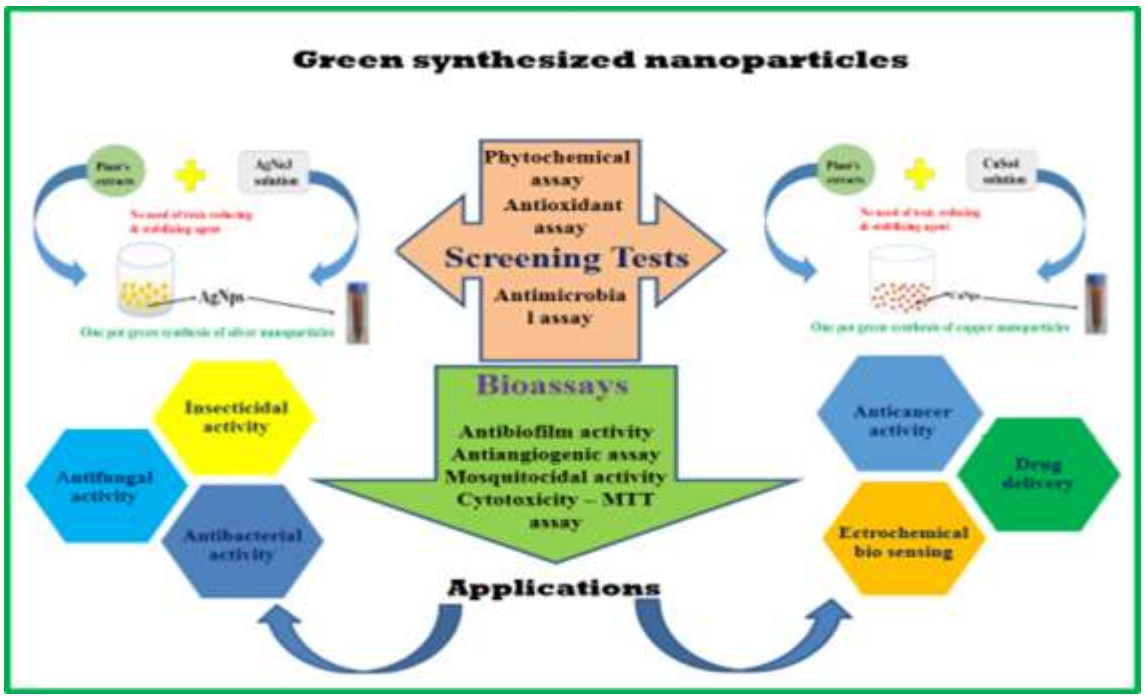
SCHEME OF PROJECT



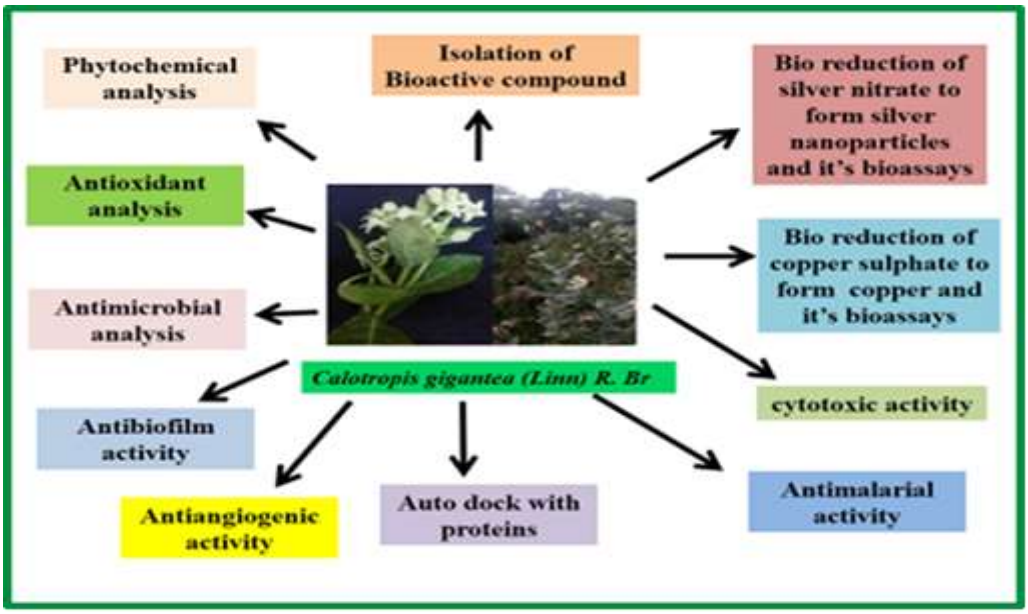


Introduction

Bioactive compounds isolated from medicinal herbs exhibits potent pharmacological actions and are found to be effective in the treatment of various ailments, traditionally. The active phytochemicals including flavonoids, alkaloids, steroids, terpenoids, saponins, phenolics and others are present in abundant in variety of plant components such as leaves, stems, roots, flowers, fruits and seeds **(Kumar *et al*., 2015).**. Therefore, bioactive compounds from medicinal plants could be a valuable source of drugs for the treatment of several diseases with lesser or no side effects. Moreover, herbal medicines were found to be cost-effective with eco-friendly attributes and also provides true relief from disease conditions; hence they deserve detailed studies to employ them in modern medicine **(Ittiyavirah *et al*., 2013).** Medicinal plants naturally synthesize and accumulate some secondary metabolites like; alkaloids, steroids, terpenes, flavonoids, saponins, glycosides, tannins, resins, lactones, quinines, volatile oils etc. Recently, the World Health Organization (WHO) estimated that 80% of the people worldwide rely on medicinal plants partially for their primary health care (**Motaleb *et al*., 2013)**. As science encourage most of the medicinal plants as supreme source of unlimited chemical compounds which are of great interest in pharmacological preparations **(Reddy *et al*., 2018)**.

Nanoparticles possess a wide array of application in the different field's viz. medicine, electronics, therapeutics, and as diagnostic agents. Silver nanoparticles have wide application in biomedical sciences like treatment of burned patients, antimicrobial potential against various pathogenic microorganisms and used the targeted drug delivery **(Singh, Jain *et al*., 2010).** Silver nanoparticles are reported to possess anti-fungal, anti-inflammatory, antiviral, anti-angiogenesis and antiplatelet activity **(Panacek *et al*., 2009, Nadworny *et al*., 2008, Gurunathan *et al*., 2009).** Nowadays the nanoparticles are coated on the medical appliances, food covering sheets, and cans for storing the beverages and food **(Sharma *et al*., 2009, Prasad *et al*., 2011, Ram *et al*., 2012).** However, there are many problems and toxicity of using metal oxide nanoparticles on the human health. Use of plants for the synthesis of nanoparticles does not require high energy, temperatures, and it is easily scaled up for large scale synthesis, and it is cost effective too **(Ghosh *et al*., 2012, Vankar *et al*., 2012, Mukunthan *et al*., 2011).** Copper-based nanoparticles are of great interest because of low cost, availability and properties possessed are similar to that of other

metallic Nps **(Ahmadi *et al*., 2011, Ramgir *et al*., 2013, Ruparelia J P, *et al*., 2008, Ren *et al*., 2009, Theivasanthi *et al*., 2011).** It finds applications in heat transfer systems as super strong materials, sensors (**Eranna *et al*., 2004, Guo *et al*., 2007, Vinod Vellora, *et al*., 2013),** antimicrobial, bactericidal agents used to coat hospital equipments and also as catalysts **(Stoimenov *et al*., 2002, Gabbay *et al*., 2009, Borkow *et al*., 2009, Borkow *et al*., 2010, AsimUmer, *et al*., 2012, Nasirian *et al*., 2012, Ruth Magaye *et al*., 2009).**



Calotropis gigantea (L.) R. Br commonly known as milkweed or gigantic swallow wort is a common wasteland weed in India. Sanskrit name is 'Alarka', Swetarka. Tamil name is 'Erukku', Vellaierukku. *C. gigantea (L.) R. Br* is a medicinal shrub and sacred plant, belongs to the family Asclepiadaceae. The plant is used in Ayurvedic system, Vedic system and traditional system of medicine for healing various diseases. The plant with many curative principles and other economic values growing in all types of soil, climate etc. In this way its pharmacognostical standardization and phytochemical analysis are very important to establish the uses of the plant more effectively.

Therefore the present research was aimed to study the medicinal plant *Calotropis gigantea (Linn) R. Br*

- Screening for phytochemicals and antioxidant**,** antimicrobial, antibiofilm, antiangiogenic, mosquitocidal and cytotoxicity potential.

- Isolation, identification and characterization of bioactive compounds from the leaves and stems extracts of *Calotropis gigantea (Linn) R. Br* from the ethyl acetate fraction and their analyzed by UV, FTIR, TLC, HPTLC, HPLC, GC, MS, GC-MS and analyzed for various bioassays
- Phytoreduction of precursor salts like silver nitrate and copper sulphate and analyzed through various spectral analysis such as UV–visible, FT-IR, XRD, EDX, and SEM techniques and analyzed for various bioassays

Chapter-I describes the extraction and screening of bioactive compounds from leaves and stems of *Calotropis gigantea (Linn) R. Br* and their bioactivity studies.

Plate:1 *Calotropis gigantea (Linn) R. Br* Plant

Here the plant material - *Calotropis gigantea* (white flower) plant parts were collected from different locations of Azhagar hills (Eastern Ghats), Madurai, Tamil Nadu, India and it was authenticated by Dr. S. John Britto S.J, Rapinat Herbarium, Trichy, Tamil Nadu, India **(MVS OO14 *Calotropis gigantea (Linn) R. Br*)** then the plant parts (leaves and stems) were washed and then shade dried. The dried materials were powdered and extracted sequentially using organic solvents (n- Heptane, ethyl acetate, ethanol and distilled water) of varying polarity was used to screen the presence of various bioactive compounds.

In *C. gigantea (L.) R. Br* leaves and stems extracts were screened by preliminary qualitative phytochemical screening tests, to analyse the radical-scavenging activity against the various free radicals such as DPPH (2, 2'-diphenyl-1-picryl hydrazyl) superoxide, nitric oxide, hydroxyl radicals quantified using a

spectrophotometric assay, to determine the antimicrobial activity against four bacteria(two gram positive bacteria and two gram negative bacteria) and two fungus (*Aspergillus niger* and *Candida albicans*) by disc diffusion assay method, bioassays such as antibiofilm activity*(Pseudomonas aeruginosa* and *Staphylococcus aureus)***,** antiangiogenic assay (eggs of *Gallus domesticus*),mosquitocidal activity (ovicidal with egg and larvicidal with I, II, III and IV instar larvae of *Anopheles stephensi, Aedes aegypti*, and *Culex quinquefasciatus)*, and cytotoxicity – MTT Assay (Hep2- human epithelial larynx cancer and AGS- human gastric cancer cell line) were performed.

The results revealed that the leaves and stems of *C. gigantea (L.) R. Br* solvent extracts contain high quantity of the presence of various phytochemicals such as alkaloids, glycosides, proteins, carbohydrates, tannins, saponins, steroid and terpenoids, significantly showed more effective free radical scavenging activity, bacterial growth inhibition was observed at the concentration of 20µg/ml, fungal growth inhibition was observed at the concentration of 50µg/ml. Out of all the extracts of leaves and stems, ethyl acetate leaves extract showed good efficiency in bioassays such as antibiofilm activity**,** antiangiogenic assay, mosquitocidal activity, and cytotoxicity – MTT assay. Among the four extracts, maximum activity were found in ethyl acetate extracts of leaves and stems and therefore chosen for further studies.

Chapter-II describes the screening of bioactive compounds from ethyl acetate extracts of *Calotropis gigantea (Linn) R. Br* leaves and stems with their bioactivity studies. This Chapter-II consists of four phases performed with ethyl acetate extracts of leaves and stems, in the **first phase,** reveals the presences of various phytochemicals, the presence of bioactive compounds were tabulated, the enzymic antioxidants and non enzymic antioxidants were analyzed in the leaves and stems of *C. gigantea (L.) R. Br* was analyzed. From the observed results the presence of the active chemical constituents such as steroids and terpenoids was done on TLC plate, the enzymic and non enzymic antioxidant contents were analyzed in ethyl acetate extract, the activities of all the enzymic and non enzymic antioxidants analyzed were found to be more in the leaves than in the stems.

Encouraged by these results, the antioxidant responses evoked by the leaves and stems homogenate was assessed on several cell-free systems and *in vitro* systems, Free radical generation and scavenging, in the **second phase**, in an effort to understand the nature of the active principles in the C. *gigantea (L.) R. Br* leaves and stems, among the two, leaves evinced the maximum protection compared to the stem. The outcome of the present study clearly demonstrates that the radical scavenging effects and the protective effects on the cellular biomolecules by the leaves and stems extracts of ethyl acetate was potentially high. Thus, the antioxidant protection offered by the *C. gigantea (L.) R. Br* leaves and stems extracts became evident in these cell free and *in vitro* systems.

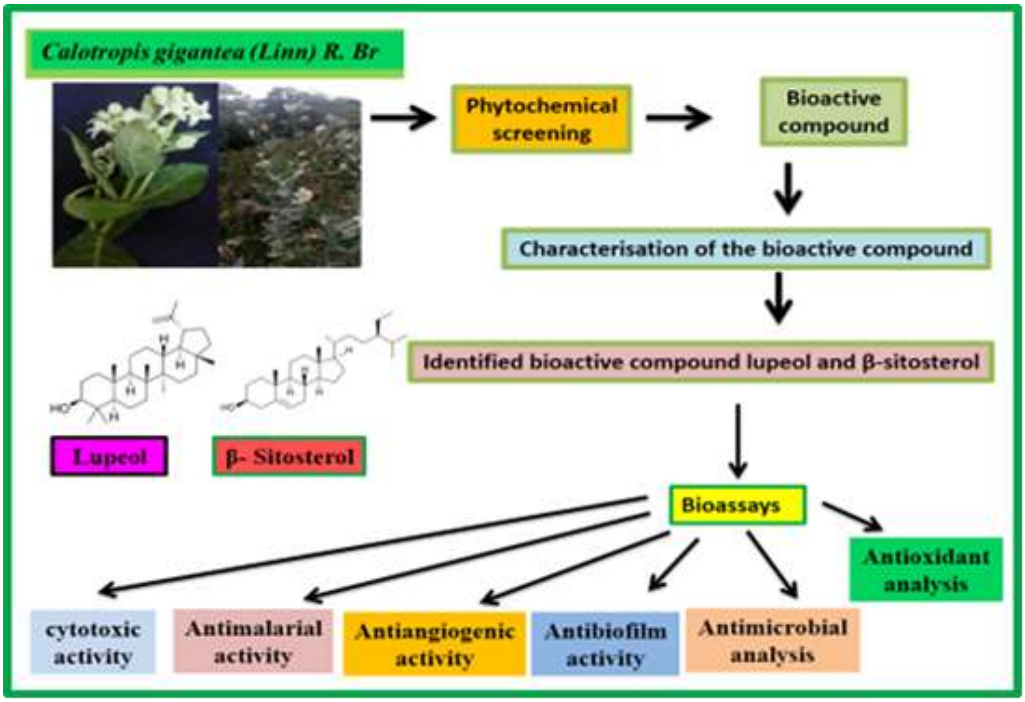
In this **third phase**, in order to study the presences of various phytochemicals in the leaves and stems ethyl acetate extracts (Qualitative analysis) and the presence and absence of phytochemicals the results reveals that the presence of the bio chemical constituents such as steroids and terpenoids in both the *C. gigantea (L.) R. Br* leaves and stems extracts were subjected to TLC. The plate was then sprayed with Liebermann-Burchard and Salkowski reagent, the results of antimicrobial screening (antibacterial activity and antifungal activity) was studied.

In this **fourth phase**, bioassays were performed with the exudates of *C. gigantea (L.) R.Br* ethyl acetate extracts of leaves and stems such as antibiofilm activity**,** antiangiogenic assay, mosquitocidal activity, and cytotoxicity – MTT assay.

Chapter-III describes the isolation, identification and characterization of lupeol from leaves and β sitosterol from stems of *Calotropis gigantea (L.) R. Br* and their bioactivity studies. *Calotropis gigantea (L.) R. Br* is one of the important medicinal plants having many therapeutic uses, thus the ethyl acetate plant extracts were subjected to HPTLC. The ethyl acetate leaves and stems extracts of *C. gigantea (L.) R. Br* were subjected to GCMS, Column chromatographic method and then fractions scrutinized with TLC, the results reveals that the presence of the bio chemical constituents such as steroids and terpenoids in both the *C. gigantea (L.) R. Br* leaves and stems extracts among various solvent systems, ethylacteate: toluene (8:4) solvent system was found be the best for the isolation of the desired bioactive compound steroids and terpenoids were found to be maximum, which isolated compounds were characterized by spectral studies to analyse the presence of

interested bioactive compound. After the isolation of the bioactive compounds were subjected to various characterisation analysis such as UV, FTIR, NMR (H^1 and C^{13}), HPLC, GC, MS, from which they obtained compound was to be similar to lupeol and β- sitosterol and these two compounds are commercially procured and they were used as standard to evaluate the efficiency of the isolated compounds.

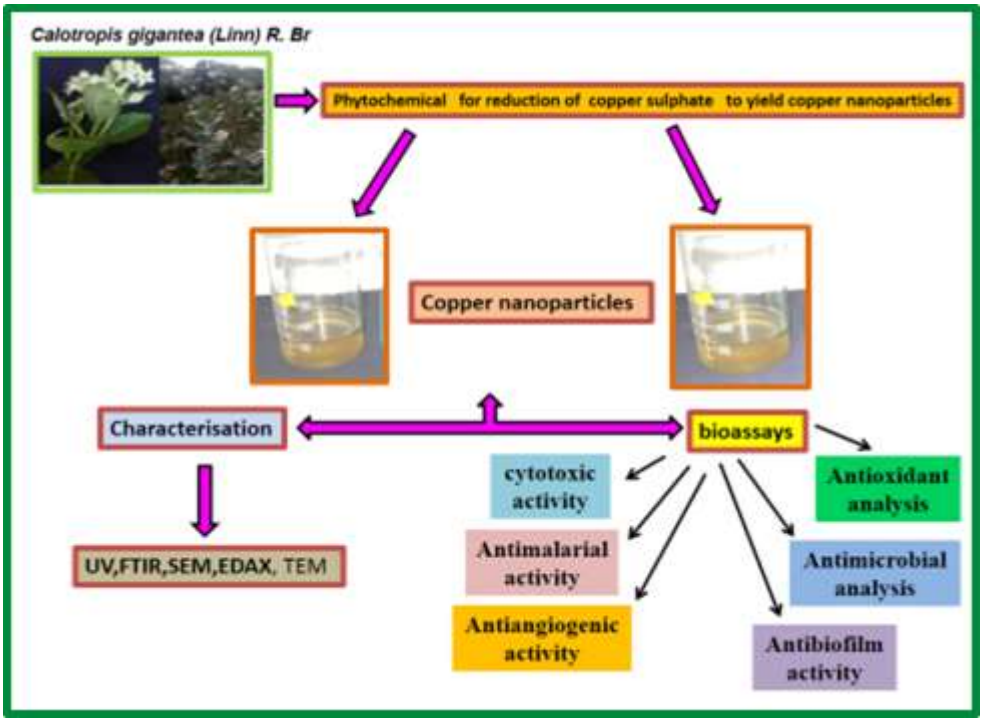
For all the standard compounds were procured and compared with the isolated once and the results were compared and the similarities has been observed. The above results revealed that the isolated bioactive compounds from *C. gigantea (L.) R. Br* ethylacteate extract fractions leaves and stems were reported the presence of various phytochemicals especially lupeol was isolated from the leaves extracts, β sitosterol was isolated from the stems and phthalate was isolated from leaves and stems and significantly showed more effective free radical scavenging activity and antimicrobial (antibacterial and antifungal) activity, isolated bioactive compounds from leaves and stems showed good efficiency in bioassays such as antibiofilm activity**,** antiangiogenic assay, mosquitocidal activity, and cytotoxicity – MTT assay. Lupeol from leaves, β sitosterol from stems subjected to perform auto docking model.



Chapter-IV describes the green synthesis of copper nanoparticles using *Calotropis gigantea (L.)R.Br* leaves and stems extracts and their bioactivity studies. Due to the presence of various phytoconstituents in *C. gigantea (L.) R. Br*leaves and

stems extracts used for the reduction of copper sulphate solution to yield copper nanoparticles. Under the optimised experimental conditional further the obtained copper nanoparticles were characterised with various spectral analysis and the copper nanoparticles were analyzed for their potential activities via various bioassays.

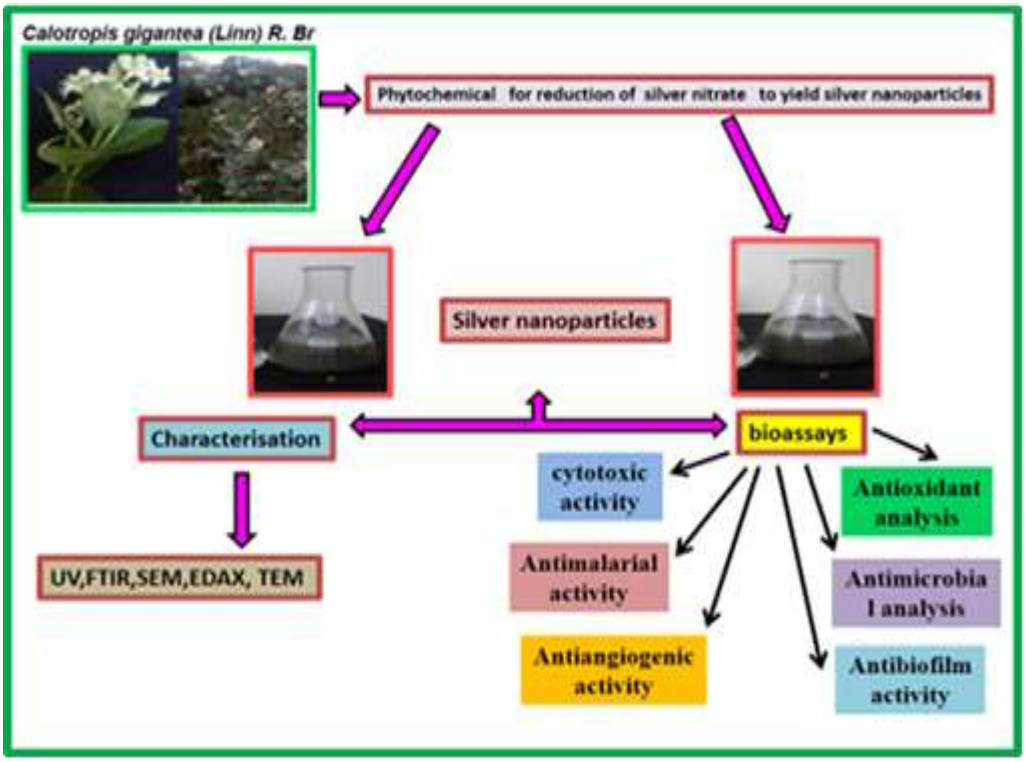
From the observed results green synthesised copper nanoparticles, *C. gigantea (L.) R. Br*leaves and stems CuNPs showed potential effects, among that leaves CuNPs shows highest activity. The study reveals that the traditional medicinal plants has potential phytoconstituents which triggers their biological activities and also for various applications.



Chapter-V describes the green synthesis of silver nanoparticles using *Calotropis gigantea (L.) R.Br* leaves and stems extracts and their bioactivity studies**.** Due to the presence of various phytoconstituents in *C. gigantea (L.) R. Br* leaves and stems extracts used for the reduction of silver nitrate solution to yield silver nanoparticles. Under the optimised experimental conditional further the obtained silver nanoparticles were characterised with various spectral analysis and the silver nanoparticles were analyzed for their potential activities via various bioassays.

From the observed results green synthesised silver nanoparticles, *C. gigantea (L.) R. Br* leaves and stems AgNPs showed potential effects, among that leaves AgNPs shows highest activity. The study reveals that the traditional medicinal plants

has potential phytoconstituents which triggers their biological activities and also for various applications.



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Publications:

1. **M. Vijaya Subhashini and S. Dinakaran;** (2018), Screening of Bioactive efficiency on *Calotropis gigantea (Linn) R. Br* Leaves and Stems, Indo American Journal of Pharmaceutical Research, 8(10), (impact factor 0.85).
2. **M. Vijaya Subhashini and S. Dinakaran ;**(2018), Bioactivity of Green Synthesized Silver Nanoparticles from *Calotropis Gigantea (L.) R.Br* Leaves and Stems, Indo American Journal of Pharmaceutical Science, 05(11), (impact factor 3.6).

Manuscripts to be shortly communicated

1. **M. Vijaya Subhashini and S. Dinakaran;** (2018), Bioeffieciency of phytosynthesized copper Nanoparticles from *Calotropis Gigantea (L.) R.Br* Leaves and Stems.
2. **M. Vijaya Subhashini and S. Dinakaran;** (2018), Study of Bioactive lupeol efficiency from Calotropis *gigantea (Linn) R. Br* leaves.

Manuscript under preparation

1. **M. Vijaya Subhashini and S. Dinakaran;** (2018), Bio efficacy of lupeol and β- sitosterol from Calotropis gigantea (Linn) R. Br.

Seminars and Conferences attended:

1. Presented a paper entitled **"Screening of bioactive efficiency on *Calotropis gigantea (L.) R. Br*"** in the International Conference on Environment, Genes, Health & Diseases held on 22nd - 24th August 2017 organized by the Department of Human Genetics and Molecular Biology, at Bharathiyar University, in Coimbatore, India.
2. Presented a Oral entitled "**Study on Biodiversity of Medicinal Plants with Conservation among Weed Management**" in the National Conference on Emerging Trends in Environmental Biotechnology- Approach to Conserve Biodiversity Organized by Department of Life Sciences, at Garden City University, on September 21st & 22nd, 2017 in association with Karnataka State Pollution Control Board, in Bangalore.
3. Presented an Oral entitled **"Study on Nanoparticles efficiency with Selected Medicinal Plants"** in the National Seminar on Recent Advances in Biological Sciences, held on 22nd September 2017 organized by the Department of Biochemistry and Microbiology, at Sourashtra College, in Madurai, Tamil Nadu.
4. Presented a paper entitled **"Ecofriendly Phytomedicine of *Calotropis gigantea (Linn.) R.Br* leaves to Future Natural products for Human health and Environment"** in the International Conference on Advanced Functional Materials for Energy, Environment, and Biomedical Applications (AFMEEB-2017) held on 11th- 12th December 2017 organized at Madurai Kamaraj University, Madurai, Tamilnadu, India.