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ESTIMATION OF PHYTOCHEMICAL ANALYSIS AND INVITRO ANTIOXIDANT ACTIVITY OF *CALOTROPIS GIGANTEA* EXTRACT: WOUND HEALING ACTIVITY AND ITS BIOMEDICAL APPLICATION

Sindhu Radhakrishnan[1], Abdullah A Alarfaj [2] and Gurusamy Annadurai [*1]

Environmental Nanotechnology Division [1], Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627412, Tamil Nadu, India
Department of Botany and Microbiology [2], King Saud University, Riyadh-11451, Saudi Arabia

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Correspondence to Author:
Dr. G. Annadurai

Associate Professor
Sri Paramakalyani Centre for Environmental Sciences Manonmaniam Sundaranar University, Alwarkurichi - 627 412, Tirunelveli, TN, India.

E-mail: annananoteam@gmail.com

ABSTRACT: Wound is unpreventable consequences of life, which originates due to physical damage, chemical injury and microbial pathogenic infections, which extend to loss of cellular and functional continuity of living tissue. *Calotropis gigantea* is a weed plant it used as a wound healing agent. *Calotropis gigantea* is a waste land weed. Antioxidants represent a significant function to assist human, against infections and chronic diseases. The exhibit investigation has been contained to assess the phytochemicals and invitro antioxidant activity *of Calotropis gigantea*. Phytochemicals were analyzed qualitatively and the result sustained the presence of alkaloids, phenols, saponin, and steroids. The invitro antioxidant activity of root was investigated by DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and Super oxide free radical scavenging activity method. In both methods, plant extract possess high antioxidant activity when equated with standard ascorbic acid due to presence of high capacity of various phytochemicals. The pro-wound healing activity of the Calotropis gigantea extract may be due to its high content of glycosides, flavonoids, phenolic compounds, and triterpenoids with antimicrobial and antioxidant properties. From the results obtained, it may be concluded that *Calotropis gigantea* extract has the potential to be developed into new therapeutic agent for wound healing.

INTRODUCTION: *Calotropis gigantea* belongs to *Apocynaceae* family (Tamil name: Erukku) is an improbable shrub reaching 2.4-3m hight. Plant cultivated across India in warm dry places from Punjab to western, central and southern India. *Calotropis gigantea* is considered as a medicinal plant of India.[1] Plants possess stems, branches, and relatively leaves, mostly condensed near the growing tip [2]. The plant contains alkaloids, tannins, phenols and resins.

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From prehistorically times to the modern era in numerous parts of the world and India, plants, animals and the natural objects have profound regulate on culture and civilization of man.

Subsequently the beginning of civilization, human beings have worshiped plants and some plants are preserved as a genetic resource and used as food, fodder, fiber, fertilizer, fuel, febrifuge and in every other way [3], *Calotropis gigantea* is one such plant. These include anti-inflammatory, antiulcer, cytotoxic [4] antiproliferative, larvicidal activity, and antidiabetic effect [5]. An antioxidant is a molecule capable of preventing the oxidation of other molecules. Oxidation is a chemical reaction that Transfer electrons from a substance to an oxidizing agent. Oxidation reactions can develop free radicals which start chain reactions that damage cell. Antioxidants displace these chain reactions by

removing free radical intermediates, and inhibit other oxidation reactions by being oxidized [6]. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved an extremely sophisticated and complex antioxidant protection system. The 95% methanol extract was also subjected to phytochemical screening to determine the presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tanninsand, terpenoids. In this study we analyzed the phytochemicals present in *Calotropis gigantea* and antioxidant activity by DPPH and Super oxide free radical scavenging activity method. The plant has reported as anticonvulsant, antiulcer [7], antioxidant [8], anti-diabetic [9], antifungal, antibacterial [10], larval mortality/repellency, activities.

Wound is one of the major health related problems around the globe[11]. We can differentiate among necrotic wounds (colored in black by dehydration and cell death), sloughy wounds (covered in a thin layer of yellow or gray tissue), granulating wounds (with a dark pink or red appearance), and re-epithelializing wounds (which are superficial and appear at the end of the healing process) [12]. Synthetic drugs presently used for treatment of wounds are not expensive but also pose problems such as allergy and drug resistance, and this situation has forced scientists to seek alternative drugs.

More than 80% of the world's population still depends upon conventional medicines for treatment of their ointments [13], especially for wound management as they provide a moist environment to advance the establishment of desirable specifies for wound healing. Skin fibroblast proliferation is important for tissue repair as fibroblasts are involved in the migration, proliferation, contraction and collagen production [14]. Wound healing properties of *Calotropis gigantea* ointment, the present study was expressed to scientifically formalize its use as a world health agent in the light of claims of traditional healers and small-ruminant farmers.

MATERIALS AND METHODS:

Plant collection:

The plant *Calotropis gigantea* was obtained from in and around Alwarkurichi, Tirunelveli district, Tamilnadu. The collected samples were carefully kept in polythene bags for further studies. Habitat and distribution of study plants was observed with the help of available Indian literature.

Processing of plant materials: The disease free and fresh leaf of *Calotropis gigantea* were washed in running water and cut into small pieces to facilitate drying. Methanolic extracts was prepared granting to the methodology of Indian pharmacopoeia. The shady desiccated plant materials were subjected to pulverization to become coarse powder. The coarse powder was used to suxhlet apparatus for extract the sample with methanol. The solvent was removed under reduced pressure. The extract obtained was kept airtight container and stored in refrigerator.

Chemicals:

Ascorbic acid, 2,2' Diphenyl Picryl Hydrazyl, methanol, Riboflavin, EDTA, phosphate buffer, Nitro-blue tetrazolium, 1% HCl, potassium mercuric iodide, potassium bismuth, ammonia solution, H2SO4, 0.1% FeCl3, sabouraud dextrose agar, sabouraud dextrose broth, Nutrient agar, Nutrient broth, Distilled deionized water.

Antioxidant activity:

DPPH Method:

In DPPH method was performed according to the method of Stankovic[15], DPPH (1, 1-Diphenyl-2-picrylhydrazyl) solution was added to methanol and absorbance was taken immediately at 517nm for control reading. Respective concentrations of *Calotropis gigantea* extract as well as standard compound (Ascorbic acid) were accepted and the volume was constituted uniformly to 150 ml using methanol. Each of the samples was then promote diluted with methanol up to 3 ml and to each 150 ml DPPH was added. Absorbance was taken after 15 min at 517nm using methanol as blank on UV-visible spectrometer.

Super oxide free radical scavenging activity:

100 ml of Riboflavin solution [20 mg], 200 ml EDTA solution [12mM], 200 ml methanol and 100 ml NBT [Nitro-blue tetrazolium] solution [0.1mg] were blended and reaction mixture was diluted with phosphate buffer [50mM] [16]. The absorbance of solution was measured at 590nm using phosphate buffer as blank after illumination for 5 min. This is

taken as control. 50 ml of different concentrations of *Calotropis gigantea* extract as well as standard preparation were taken and diluted up to 100 ml with methanol. Absorbance was evaluated after illumination for 5 min at 590nm on UV visible.

Phytochemical analysis:
Phytochemical tests were extended on thermoethanolic leaf extract using standard procedures to identify the phyto constituents. Phytochemical screening for the presence of secondary metabolites such as Saponin, Flavonoids, Tannins, alkaloids, was conducted by the standared procedures[17].

Preparation of *Calotropis gigantea* ointment:
The aqueous extract of *Calotropis gigantea* latex was developed as an ointment using petroleum jelly (melting point 60-65°C) at a concentration of 10% (w/w) using a homogenizer at 1,500 rpm for 45 min, and preserved at 4°C for topical coating to wounds. The consistency of ointment was assessed in terms of physical changes such, phase separation and interchanges in objectionable color and odour, and consistency of the formulation. They were periodically observed for physical changes[18.]

Disc Diffusion Assays:
The sensitivity of different bacterial strains was executed by agar disc diffusion method Kirby-Bauers disc diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) recommendations [19]. The agar diffusion test is a method commonly expended to analyze antimicrobial activity.

The diffusion is dependent on the size, shape and polarity of the diffusion material. For antibacterial activity nutrient agar (2.8 g in 100 ml water), nutrient broth (1.3 g in 100 ml water) were prepared and sterilized. Nutrient Broth was employed as growing medium for the bacteria *E. coli*, *pseudomonas* (Lab cultures), *B. subtilis* (MTCC 3053), *K. planticola* (MTCC 2277) Nutrient agar was directed in Petri dish and a loopful of bacterial strain was streak on nutrient agar and incubated at 37°C for 24 hrs. Representative bacteria colony was plucked off used wire loop, placed in pre-sterilized nutrient broth and then incubated overnight at 37°C for 12 hrs. Then bacteria medium was distributed on to agar plate and the sample (*Calotropis gigantea* extract) was placed. The incubation was extended for 12 hrs at 37°C inhibition zone was calculated. For antifungal activity sabouraud dextrose agar, sabouraud dextrose broth was used and activity was checked against *A. niger* as described above. The activity was determined after 48 hrs incubation at 28°C.

UV-Vis spectroscopy:
Ultraviolet-visible (UV-Vis) spectroscopy is to measure pigments of sample (*Calotropis gigantea*) absorbs light at each wavelength. To shine a monochromatic light beam at a sample (*Calotropis gigantea* extract) measure how much of the light is absorbed, in certain wavelength.

FTIR analysis:
Fourier Transform Infrared Spectroscopy (FT-IR) used to detect the functional group of *Calotropis gigantea* compounds. Instrument in diffuse reflectance mode functioned at a resolution of $4cm^{-1}$ in the range between 4000 and 400 cm^{-1}.

Animals:
Male wistar rats were applied in the exhibit study. They were individually housed and asserted in a laboratory environment. All animals were fed with standard pellet diet and water. The experiments were carried under protocols approved by the MSU/Ethical/2013/2.

Wound creation:
The animals were fasted overnight and anesthetized with 1 ml intravenous thiopentone sodium. Each rat was depilated using toothed forceps, sterile pointed scissors, and a scalpel blade. The area was then cleaned with 70% ethanol to maintain aseptic conditions. An excision wound was produced. A full-thickness excision wound of circular area 300 mm and 2 mm depth was inflicted on either side of the depilated dorsum of each rat. Animals were closely observed for any infection. Animals were euthanized after completion of the study.

Experimental design:
The animals were divided into three groups as follows. Excision wound induced rats treated with petroleum jelly, considered as control. Excision

wound induced rats treated with *Calotropis gigantea* extract ointment. Excision wound induced rats treated with standard ointment[20]. The treatment schedule was twice daily with topical application of the developed ointment as well as the standard ointment, while the control group was dressed with ointment base comprising the same amount of petroleum gel. Changes in wound area were calculated, giving an indication of the rate of wound contraction.

Biochemical estimations:

On the 7th and 14th postoperative day, an appreciable amount of granulation tissue formed on the wound, which was excised and its weight recorded. The tissues were dried in an oven at 60°C for 72 hrs, and the dry weight was again observed. The dried tissue was added to 6 N HCl and kept at 110°C for 24 hrs in sealed tubes. The neutralized acid hydrolysate of the dry tissue was used for finding of collagen by estimation of hydroxyproline as described [21]. Hexosamine content was estimated according to the method [22]. Uronic acid content was estimated by the method [23]. The protein content in the tissue extract was estimated [24].

RESULTS AND DISCUSSION:

***In vitro* antioxidant studies:**

Free radical and reactive oxygen species are substantially known inducers of cellular and tissue pathogenesis extending to various human diseases such as cancer, inflammatory disorder, diabets mellitus and aging process [25]. *Calotropis gigantea* species have antioxidant activities which represent as a defensive agent against these diseases. Deliver of this study, the potent antioxidant activity of ethanolic extract of *Calotropis gigantea* was assayed by DPPH and super oxide method. The DPPH methods provide entropy on the reactivity of test compounds with a stable free radical.

Since of its odd electron 2, 2' Diphenyl Picryl Hydrazyl Radical (DPPH) gives secure absorption band at 517nm in visible spectroscopy [26]. The ethanolic extract of *Calotropis gigantea* had corresponding DPPH radical scavenging activity and super oxide scavenging activity with reference to standard ascorbic acid and it was designated on **Fig.1** and **Fig. 2**. The percentage of inhibition was measured at 30 min interval. Free radical scavenging capacity of *Calotropis gigantea* may be due to the flavonoids, which are typical phenolic compounds, act as metal chelators and Free radical scavengers.

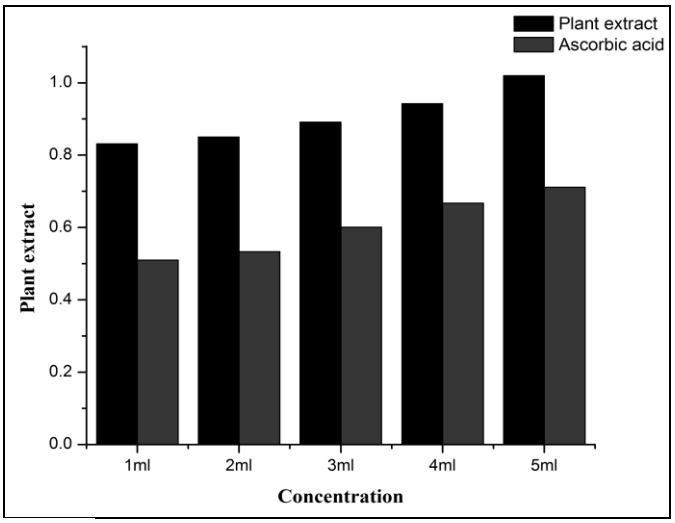

FIG.1: ANTIOXIDANT ACTIVITY OF *CALOTROPIS GIGANTEA* BY DPPH METHOD

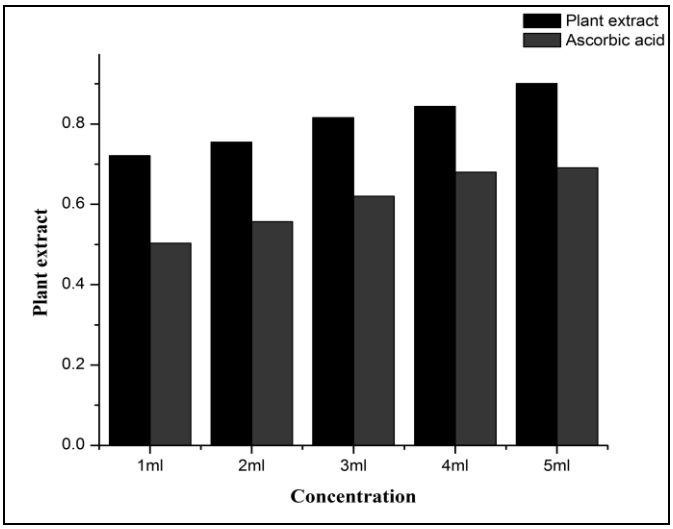

FIG. 2: ANTIOXIDANT ACTIVITY OF *CALOTROPIS GIGANTEA* BY SUPER OXIDE FREE RADICAL SCAVENGING ACTIVITY

Phytochemical Screening:

Phytochemical intensifies were sieved in *Calotropis gigantea* through qualitative method. The results pointed the Presence of steroids, Terpenoids, alkaloids, Acid compounds, sterols, flavonoids, resins and absence of Saponin, tannins and cardiac glycosides which was shown on **Table. 1**. Phytochemical screening exposed the presence of flavonoids which could be responsible for its anti-mutagenic, antioxidant and anti-inflammatory activity.

TABLE 1: PHYTOCHEMICAL STUDIES OF *CALOTROPIS GIGANTEA*

S. No	Phytochemical screen	plant extract
1	Tannins	-
2	Saponin	-
3	Flavonoids	+
4	Steroids	+
5	Terpenoids	+
6	Alkaloids	–
7	Cardiac glycosides	+
8	Acid compounds	+
9	Sterols	+

+ Presence,−Absence

Disc Diffusion Assays:

The antibacterial and antifungal activities of the extracts de from the test samples in terms of minimum inhibitory concentrations and diameters of inhibition zones are reported in **Fig. 3** and **4**. The crude extracts from *Calotropis gigantea* were detected to be conspicuously active against the tested microorganisms at the different concentrations. Among the bacteria *Serratia* spp in **Fig.3** forms high zone of inhibition which shows the *Calotropis gigantea* has high antibacterial activity against *Serratia* spp.

The extracts showed antimicrobial activity against both Gram-positive and Gram-negative bacteria. It is concerning to note that extracts were more effective against bacteria and fungi. The extract of *Calotropis gigantea* was the most effective against the fungi *A. niger* and *A. flavus* in **Fig. 4**.

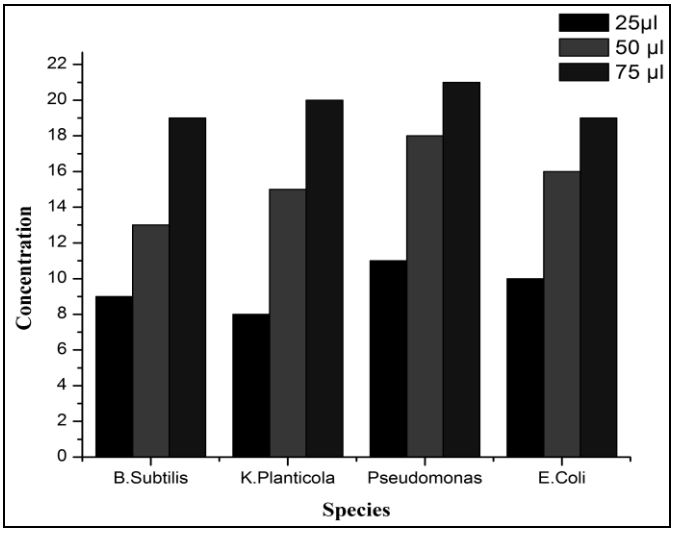

FIG.3: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *CALOTROPIS GIGANTEA* EXTRACT

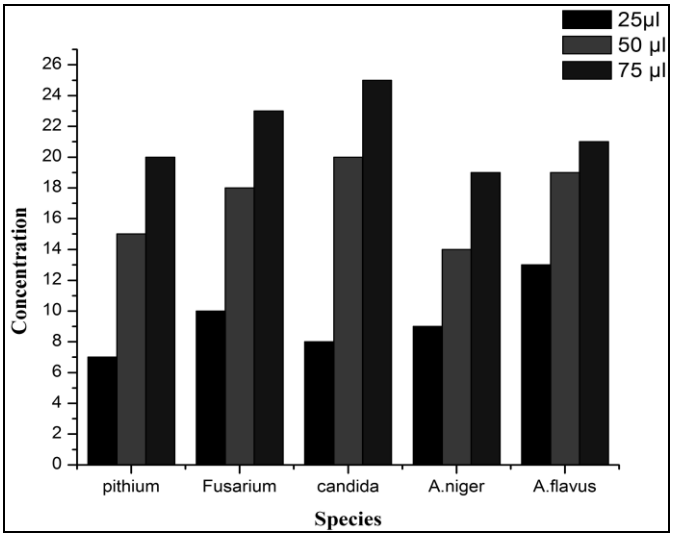

FIG.4: ANTIFUNGAL ACTIVITY OF *CALOTROPIS GIGANTEA* EXTRACT

Calotropis gigantea visible spectrum:

Determine the existence and quantity of screening pigments in *Calotropis gigantea* of ethanol extract, *in vivo* absorption spectra were recorded in **Fig. 5.** The maximum absorption spectrum of *Calotropis gigantea* which shows chlorophyll (435 and 675nm) as well as the peaks related to carotenoid (490nm) and phycoerythrin (560nm) absorption. There is a remarkable absorption in the range of 350nm indicative of the presence 356 of UV screening pigments.

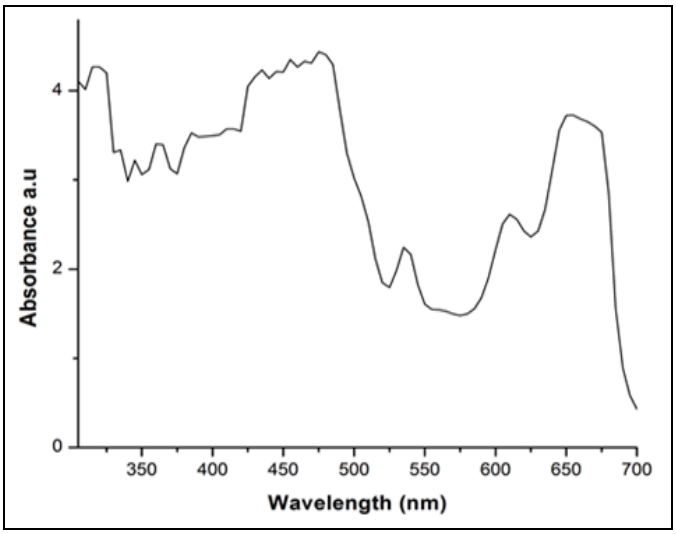

FIG.5: UV VISIBLE SPECTRUM OF *CALOTROPIS GIGANTEAN*

FTIR spectrum:

The main experimental infrared spectroscopic data on **Fig. 6** were assigned based on the theoretical Infrared band with the ranges of strong and broad,

3351cm-1 attributed to the O–H stretch, H–bonded agreement with the alcohols and phenols, assignment shows a medium band 2975cm-1 attributed to the C–H stretch alkanes, corresponding peak at 1650cm-1 shows the strong band C=O stretch as functional group as carbonyls, medium band 1044 cm-1 attributed to the aliphatic amines C–N stretching mode, shows the strong band of 879 cm-1 attributed to the C–H "oop" aromatics.

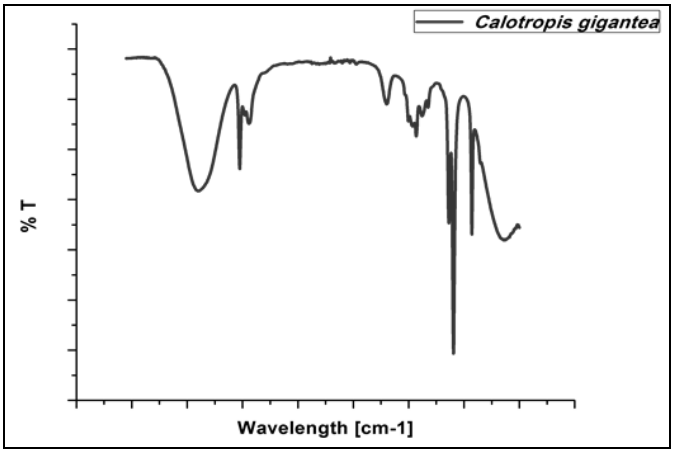

FIG.6: FTIR SPECTRUM OF *CALOTROPIS GIGANTEA* ETHANOL EXTRACT

Biochemical estimations:

The results of the present study are in line with the above data. It was observed that the control rats had hard and crusty wounds. By comparison, wounds treated with the *Calotropis gigantea* extract ointment as well as standard ointment. Uronic acid levels in **Fig. 8** also increased significantly when compared with control. Hexosamine level was significantly increased in groups treated with both *Calotropis gigantea* latex ointment as well as standard ointment when compared with control. Hexosamine and uronic acid, the ground substratum for collagen synthesis, are significantly increased during early stages of wound healing, and their degree of elevation is decreased thereafter in **Fig. 7**.

The relative decrease in hexosamine content was highest synthesis of hexosamine was noticed on 7th postoperative day in all groups, thereafter, the degree of elevation was decreased at 14th postoperative day when compared with the control group. The increase in collagen content in the *Calotropis gigantea* extract ointment treated group agrees with the increase in the levels of protein content in **Fig. 9**, which is predominantly due to enhanced collagen synthesis.

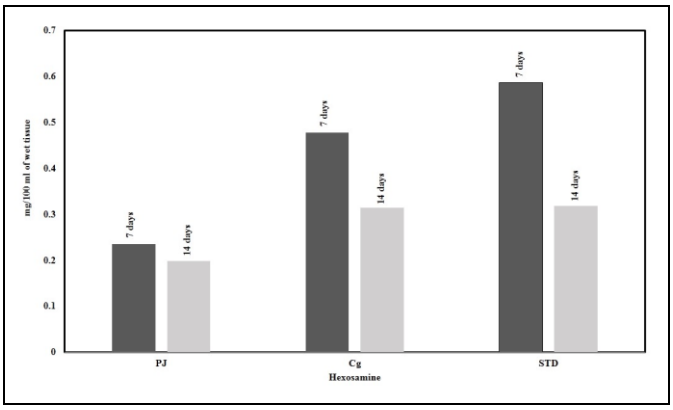

FIG. 7: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT ON THE LEVEL OF HEXOSAMINE IN EXCISION WOUND MODEL

PJ- Petroleum Jelly, Cg- *Calotropis gigantea*, STD- Standard ointment

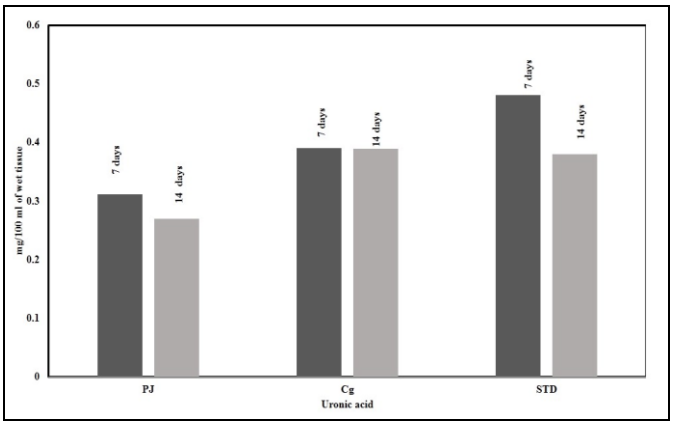

FIG. 8: EFFECT OF *CALOTROPIS GIGANTEA* EXTRACT ON THE LEVEL OF URONIC ACID IN EXCISION WOUND MODEL

PJ- Petroleum Jelly, Cg- *Calotropis gigantea*, STD- Standard ointment

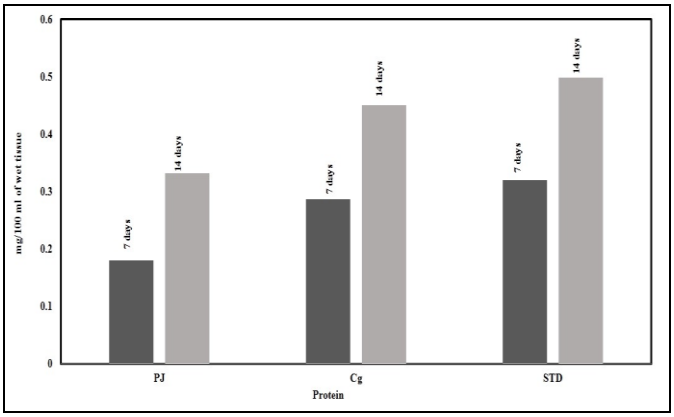

FIG. 9: EFFECT OF *CALOTROPIS GIGANTEA* LATEX EXTRACT ON THE LEVEL OF PROTEIN IN EXCISION WOUND MODEL

PJ- Petroleum Jelly, Cg- *Calotropis gigantea*, STD- Standard ointment

CONCLUSION: Hydroalcohlic extracts of *Calotropis gigantea* leaves had significant antioxidant activity. The results pointed the Presence of steroids, Terpenoids, alkaloids, Acid compounds, sterols, flavonoids, resins and absence of Saponin, tannins and cardiac glycosides. Phytochemical screening exposed the presence of flavonoids which could be responsible for its wound healing activity. *Calotropis gigantea* exhibit better antimicrobial agent against bacterial and fungal. It may be concluded that *Calotropis gigantea* latex has the potential to satisfy all the requirements for an ideal topical ointment in that it provides an environment at the surface of the wound in which healing takes place at the maximum rate consistent with the formation of granulation tissue. The present study also provides a rationale for the use of *Calotropis gigantea* latex in the traditional system of medicine to promote wound healing. Further studies are in progress to isolate, characterize, and identify the specific active compounds responsible for wound healing activity.

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REFERENCES:

1. Joshi H, Gururaja MP and Suares D: Calotropis gigantea R. Br Asclepiadacae): A review. International journal of pharmaceutical Research 2011; 3(1): 10-14.
2. Sharma PC, Yelne MB, Dennis J and Lodhra T: Data base on medicinal plants used in ayueveda. Central council for research in ayurveda and siddha New Delhi 2000; 164-170.
3. Sureshkumar P, Chezhian A, Senthil Raja P and Sathiyapriya J: Computational selections of terpenes present in the plant *Calotropis gigantea* as mosquito larvicides by blocking the sterol carrying protein. Bangladesh J Pharmacol 2012; 7: 1-5.
4. Lee KH, Lin YH, Wu ST, Zhang DC, Yamagishi T, Hayash T,Chang JJ, Yim EK, Lee MJ, Lee KH, Um SJ and Park JS: Antiproliferative and antiviral mechanisms of ursolic acid and dexamethasone in cervical carcinoma cell lines. Int J Gynecol Cancer 2006; 16:2023-231.
5. Kazmi I, Rahman M, Afzal M, Gupta G, Saleem S, Afzal O, Shaharyar MA, Nautiyal U, Ahmed S and Anwar F: Anti-diabetic potential of ursolic acid stearoyl glucoside: a new triterpenic gycosidic ester from *Lantana camara*. Fitoterapia 2012; 83:142–146.
6. Elakkiya P and Prasanna G: A Study on Phytochemical Screening and invitro antioxidant Activity of *Calotropis gigantean*. International Journal of PharmTech Research 2012; 1428-1431.
7. Sathish R, Vyawahare B and Natarajan K: Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. J. Ethnopharmacol 2011; 134: 195–207.
8. Gomes de Melo J, de Sousa Araujo TA, Thijan Nobre de Almeida e Castro V, Lyra de Vasconcelos Cabral D, do Desterro Rodrigues M, Carneiro do Nascimento S, Cavalcanti de Amorim EL and de Albuquerque UP: Antiproliferative activity, antioxidant capacity and tannin content in plants of semi-arid northeastern Brazil. Molecules 2010; 15:8534–8542.
9. Venkatachalam T, Kumar VK, Selvi PK, Maske AO, Anbarasan V and Kumar PS: Antidiabetic activity of *Lantana camara* Linn fruits in normal and streptozotocin-induced diabetic rats. J Pharm Res 2011; 4:1550–1552.
10. Barreto F, Sousa E, Campos A, Costa J and Rodrigues F: Antibacterial Activity of *Lantana camara* Linn and Lantana montevidensis Brig Extracts from Cariri-Ceara, Brazil. J. Young Pharm. 2010; 2:42–4.
11. Sudha S, Sankar R and Saradhai P. Antibiogram pattern of bacterial pathogens isolated from post operative surgical site wound infections. Drug Invent Today 2012; 4: 575–8.
12. Abdelrahman T and Newton H. Wound dressing: principles and practice. Surgery 2011; 29: 491–495.
13. WHO: General guidelines for methodologies on research and evaluation of traditional medicine. Geneva Switzerland WHO/EDM/TRM 2000; pp 1–80.
14. Stankovic MS, Niciforovic N, Mihailovic V, Topuzovic M and Solujic S: Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium L*. subsp, *polium*. Acta Societatis Botanicorum Poloniae 2012; 81:117-122.
15. Patel Rajesh M and Patel Natvar J: *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of Advanced Pharmacy Education & Research 2011; 1:52-68.
16. Adetutu A, Morgan W and Corcoran O: Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria. J. Ethnopharmacol 2011; 137:50–56.
17. Kokate CK: Practical Pharmocognosy, Vallabh Prakashan, Kakatiya University, Warangal, A.P. India. 2001; 107-111.
18. Saratha V, Subramanian S and Sivakumar S :Evaluation of wound healing potential of *Calotropis gigantea* latex studied on excision wounds in experimental rats Med Chem Res 2010; 19:936–947.
19. LSI: Clinical and Laboratory Standards Institute, 2003*;* Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. approved standard M7-A6. Wayne. Pa.
20. Perez Gutierrez RM and Vargas SR: Evaluation of the wound healing properties of *Acalypha langiana* in diabetic rats. Fitoterapia 2006; 77:286–289.
21. Harish BG, Krishna V, Santosh Kumar HS, Khadeer Ahamed BM, Sharath R and Kumara Swamy HM: Wound healing activity and docking of glycogen-synthase-kinase-3-beta-protein with isolated triterpenoid lupeol in rats. Phytomedicine2008; 15:763–776.
22. Woessner JF: The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961; 93:440–447.
23. Adamsons RJ, Musco F and Enquist IF: The relationship of collagen content to wound strength in normal and scorbutic animals. Surg Gynecol Obstet 1964; 119:323–329.

24. Bitter T and Muir HM: A modified uronic acid carbazole reaction. Anal Biochem 1962; 4:330–334.
25. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.
26. Aviram M: Review of human studies on oxidative damage and antioxidant protection related to cardiovascular disease. Free Rad 2000; 585-87.

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