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Research Article

Antimicrobial Potential and Phytochemical Analysis of Plant Extracts of *Calotropis Procera*

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Abstract

Calotropis procera are widely used traditional medicinal plant to treat various ailments. To provide a scientific basis for traditional uses of *Calotropis procera*, their ethanolic, aqueous, n-Butanol and petroleum ether extracts of various plant parts were tested against human pathogenic microorganisms (*Pseudomonas aeruginosa , Escherichia coli, Cogulase positive staphylococci, Cogulase negative staphylococci , Enterococcus species, Candida albicans* and *Candida parapsilosis)*.The antimicrobial potential of *Calotropis procera* against human pathogenic microorganisms was investigated. Their isolated phytoconstituents were evaluated for their antimicrobial potential. Antimicrobial activity of various plants extracts was compared with commercially available antibiotics. The antimicrobial potential of the above plant extracts was seen against the test organism using agar gel diffusion susceptibility test by standard technique of Opara and Anasa(1993). Phytochemical estimation was carried out according to the methods described by Trease and Evans (1989).Ethanolic and n-Butanol extracts showed considerably good antibacterial activity against all bacteria and fungi. Among all solvents used ethanol extract gave the highest zone of inhibition.

Key words: Antimicrobial activity, Phytochemical analysis, *Calotropis procera*

Introduction

Calotropis procera belongs to the family *Asclepiadaceae* and is a soft wooded, evergreen perennial shrub. It is a xerophytic erect shrub, growing widely throughout the tropical and subtropical regions of Asia and Africa. This plant is popularly known because it produces large quantity of latex. This plant has potential antimicrobial properties against microbial infections. Commercially available antimicrobial agents (antibiotics) are now used to treat diseases arising from microbial infections. A major problem encountered with antibiotics in clinical use is drug resistance, which mostly leads to treatment failure (Mosses *et al*., 2006). Other problems with antibiotics include toxicity high cost, low cost efficacy, etc. This necessitates a continuous search for new antimicrobial agents. Medicinal plants have no doubt remained the major sources of traditional medicine world-wide. Accordingly, attention of scientists and researchers have been attracted towards developing new antibiotics that will curtail the increasing drug resistance among microorganisms (Edith *et al*., 2005). Schimmer *et al*. (1994) reported that plants used for traditional medicine generally contain a number of compounds which may be a potential natural natural antimicrobial combination and which may serve as an alternative, effective, cheap and safe antimicrobial agents for treatment of common microbial infections. This study attempts to determine the antimicrobial effect of *Calotropis procera* .In this report, we provide new information on the antimicrobial activities of *C.procera* using known microbial pathogens as test organisms.

Material and Methods

Method of extraction:- The fresh plant parts were collected, properly washed in tap water,
rinsed in sterile distilled water and then air dried in the hot air oven to remove moisture and
to dry them. They were then grounded soxhlet extracted using 70% ethanol. The extraction
lasted for 24 hours.

Antimicrobial susceptibility testing: -
The antimicrobial potential of the above plant extracts was seen against the test organisms using the agar-gel diffusion susceptibility test. Sterile Mueller – Hinton plates were taken one plate/organism tested. Three wells of about 3.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, with at least 30 mm distance between adjacent wells and the peripherary. According to the standard technique of Opara and Anasa (1993) - 2-4 colonies of the test organisms were inoculated in sterile broth and these inoculums was swabbed using sterile swab on the surface of above punched Mueller - Hinton agar plates. A fixed volume (0.1 ml) of the plant extract was then introduced into the wells in the increasing concentration and then incubated at 37^0C for 24 hours. The resulting zones of inhibition were measured.

Phytochemical estimation: -
This was carried out according to the methods described by Trease and Evans , (1989)

Result and Discussion

All the bacterial and fungal pathogens used in this work demonstrated susceptibility to the Ethanol, n-Butanol, Chloroform, Distilled water and Petroleum ether using extracts of *Calotropis procera*. Ethanol extract gave the highest zone of inhibition (28.2mm) on *Escherichia coli*. With Cogulase (+) *Staphylococci* showed the highest zone of inhibition i.e. 25.2 mm in diameter. Cogulase (-) *Staphylococci* showed the highest zone of inhibition i.e. 23.0mm in diameter. With *Pseudomonas aeruginosa* the highest zone of inhibition was 22.5 mm in diameter. With *Enterococcus sp.* highest zone of inhibition recorded was 23.2 mm in diameter. With *Candida albicans and Candida parapsilosis* it showed the highest zone of inhibition i.e. 23.0mm and 22.5 mm in diameter respectively. The observation that *Calotropis procera* has good inhibition against *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp.*, Cogulase *(+) Staphylococcus sp., Cogulase (-) Staphylococcus sp., Candida albicans and Candida parapsilosis* tends to prove

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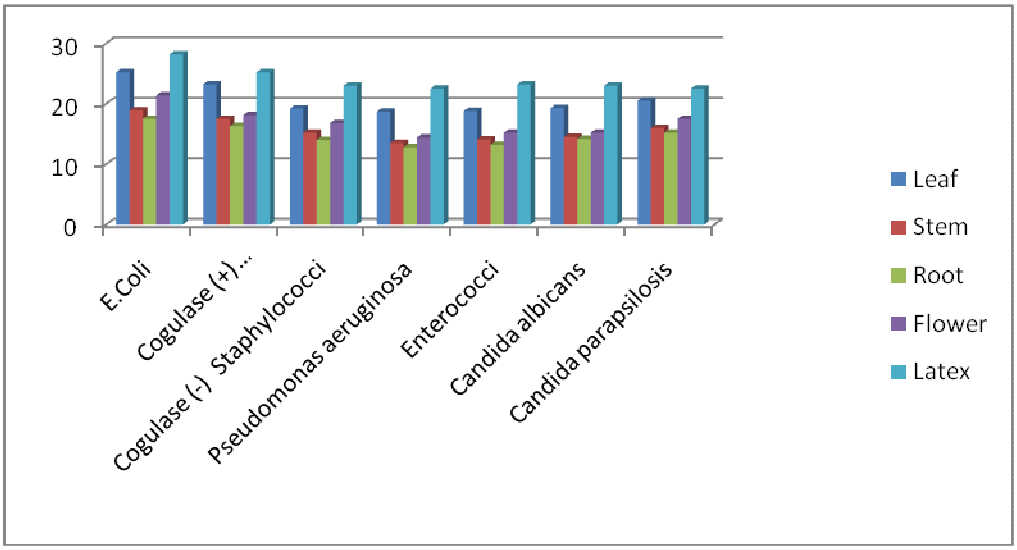
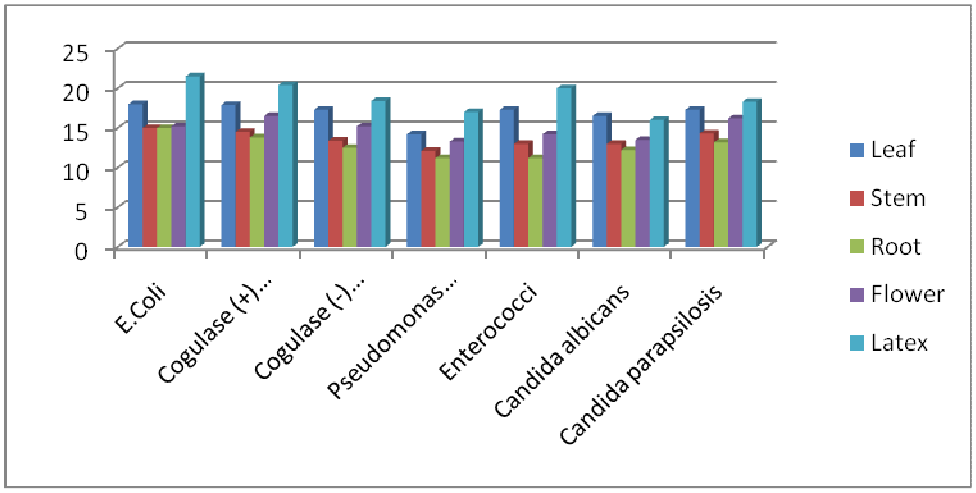
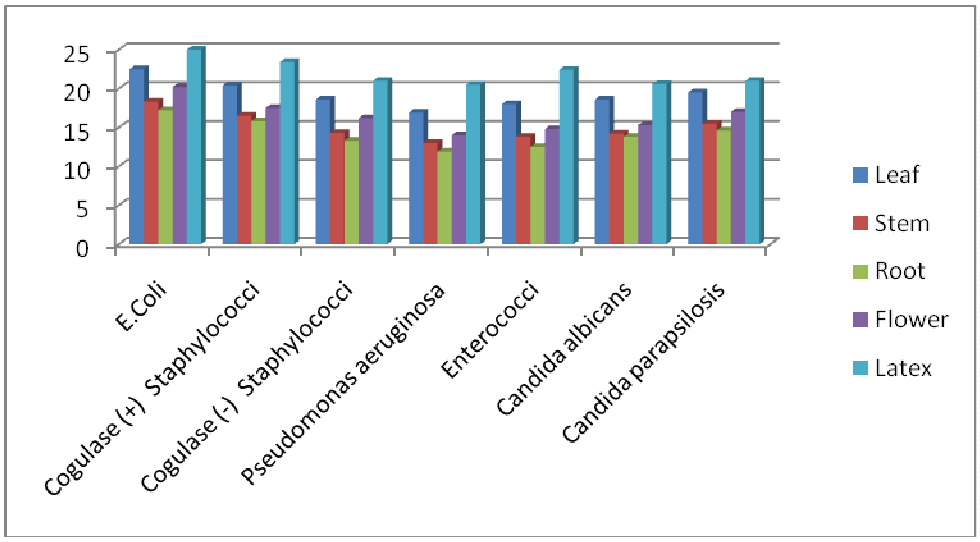
Graph 1: Comparison of *Calotropis procera* ethanolic plant parts extracts effects on test bacteria and fungi



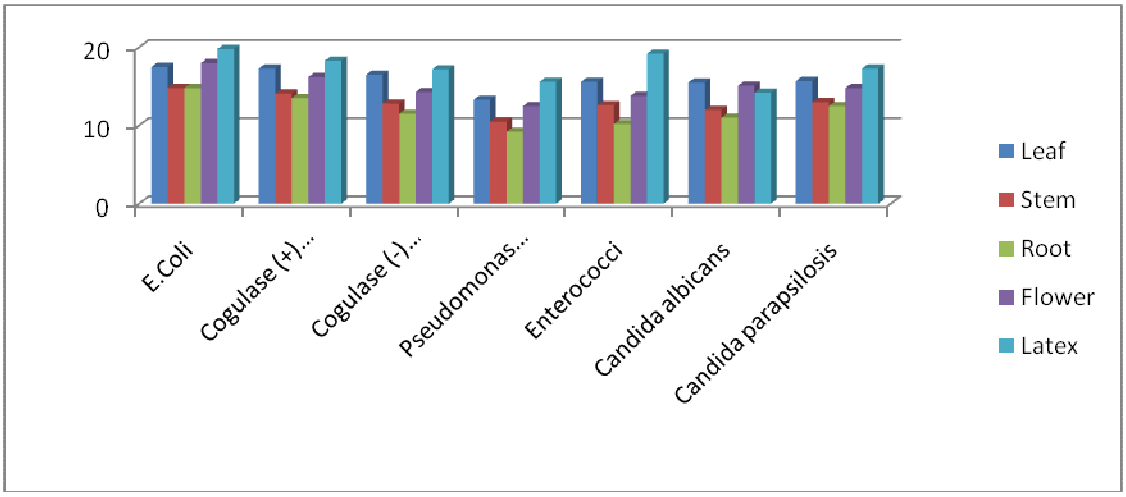
Graph 2: Comparison of *Calotropis procera* distilled water plant parts extracts effects on test bacteria and fungi



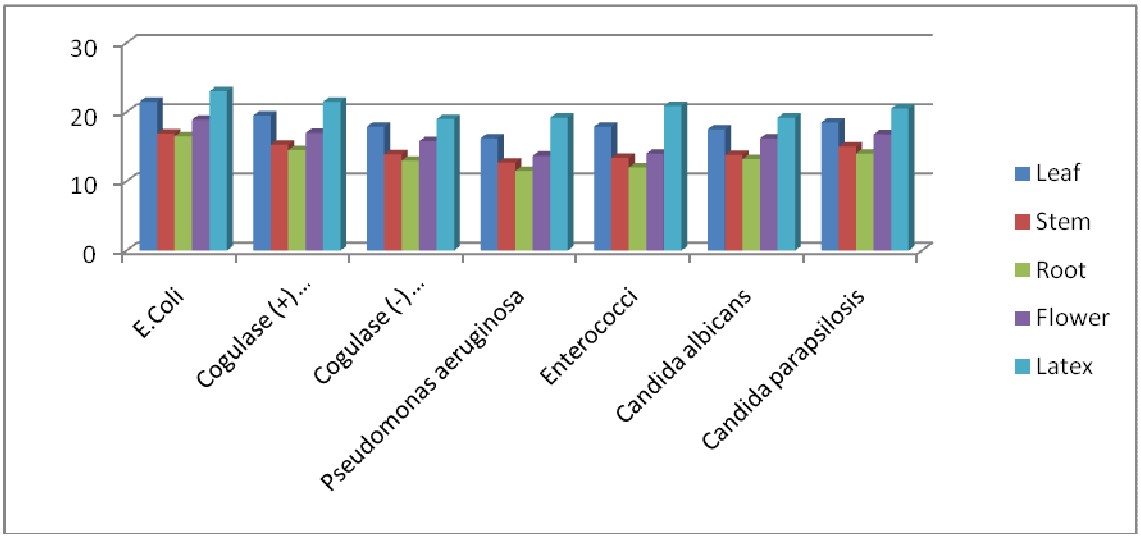
Graph 3: Comparison of *Calotropis procera* n- Butanol plant parts extracts effects on test fungi and bacteria



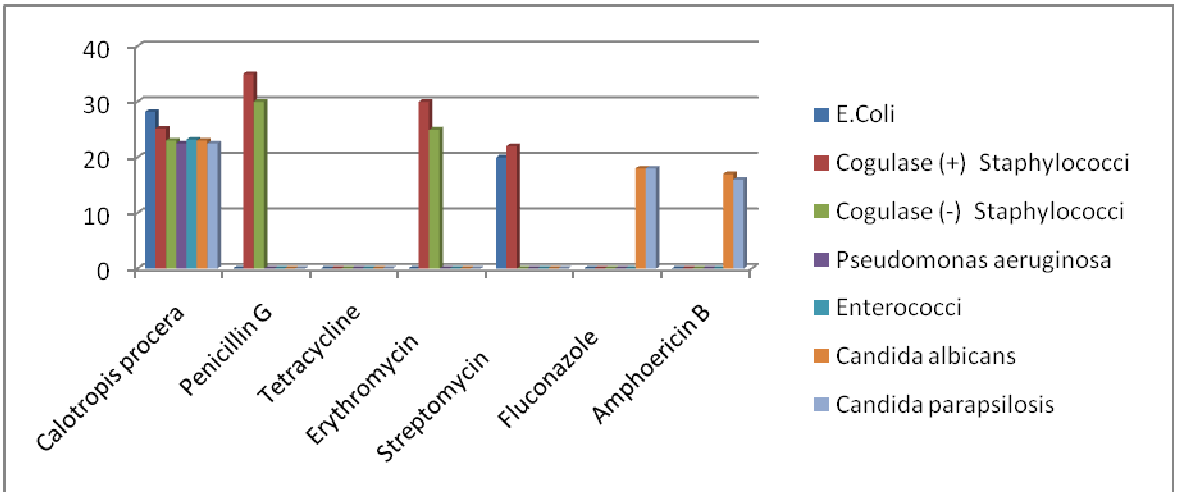
Graph 4: Comparison of *Calotropis procera* petroleum ether plant parts extracts effects on test bacteria and fungi



Graph 5: Comparison of *Calotropis procera* chloroform plant parts extracts effects on test fungi and bacteria



Graph 6: A Comparison of commercially available antibiotics with maximum zone found in *Calotropis procera*



worthy remedy to the problem of drug resistance against these pathogens which are already known to be resistant to the most of the standard antibiotics (Penicillin, Tetracycline, Erythromycin, Streptomycin, Fluconazole and Amphotericin B) Tetracycline showed no zone of inhibition against all tested microorganisms. *E.coli* developed resistance against most of the antibiotics except streptomycin. *Pseudomonas aeruginosa* and *Enterococci* showed resistance against all tested antibiotics. But *Calotropis procera* showed effective results against most of the resistant organisms. Bacteria developed resistance against most of the tested antibiotics. Latex and Leaves showed very effective results as compared to flower, root and stem of *Calotropis procera*..Graph 1,2,3,4 and 5 showed Comparison of *Calotropis procera* ethanolic, distilled water, n-Butanol, petroleum ether, chloroform plant parts extracts effects on test bacteria and fungi. Graph 6 showed Comparison of commercially available antibiotics with maximum zone found in *Calotropis procera*.Table1 showed Preliminary phytochemical screening of plant parts of *Calotropis procera*..

The results of these investigation showed that plant extracts of *Calotropis procera* possess appreciable and potential antimicrobial activity against commonly encountered microorganisms in humans. It is interesting to note that the action of the extracts of *Calotropis procera* is non toxic. Studies have shown that the alcohol and water based extracts of the plant have very low toxicity in mammals.

Discussion and conclusions:

The effect of *Calotropis procera* extracts agrees with the work of Filgona *et al*., (2005) that showed the antimicrobial activity of the root extract of *Calotropis procera and Moringa oleifera over Neisseria gonorrhoeae, Staphylococcus aureus and Escherichia coli.* Studies were conducted on every plant parts of *Calotropis procera* which showed effective antimicrobial activity against all tested microorganisms. These results support the findings of Kareem *et al*., (2008) that showed antimicrobial activity over *Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Streptococcus pneumoniae* and few fungi namely *Aspergillus niger, Aspergillus flavus and Microsporium boulardii* and a yeast called *Candida albicans* . The results of their study revealed that ethanol was the best extractive solvent for antimicrobial properties of leaf and latex of *C. procera* followed in order by chloroform and water. Their experiments concluded that the ethanolic extracts of *C. procera* latex gave the widest zone of inhibition. The effect of *Calotropis procera* extracts agrees with the work of Nenaah *et al*., (2011) who evaluated the antimicrobial activity of extracts and latex of *Calotropis procera* and also examined the synergistic interaction between *C. procera* latex and antibiotics. Results of the study revealed that *C. procera* extracts showed considerable antibacterial and antifungal activities against the tested microorganisms. They concluded that extract showed promising antifungal activities against *C. albicans* and *P. chrysogenum* and the leaf and latex extracts showed the strongest activities, where *Escherichia coli, Staphylococcus epidermides,* and *Bacillus* spp. were the most sensitive. Similar conclusion were drawn with the present work. The effect of *Calotropis procera* also agrees with the work of Yesmin *et al*., (2008) which showed both antioxidant and antibacterial activities. They examined that *Calotropis procera* have significant antibacterial properties against few gram positive & gram negative bacterial strains. They not only have good antibacterial activity but also good antioxidant activity. The obtained results provide a support for the use of this plant in traditional medicine and suggest its further advance investigation. Phytochemical estimation of *Calotropis procera* agrees with the work of Kuta (2008) who evaluated phytochemical and antifungal effect of *Calotropis procera* stem bark on *Epidermophyton flocosum* and *Trichophyton gypseum.* Phytochemical screening of the crude extract revealed the presence of saponin, tannins, sequiterpene and alkaloids. Other phytoconstituents
which were reported during present investigation were cardiac glycosides, flavonoids, glycosides, steroid, terpens and tannins. The results of Kuta (2008) suggested that *C. procera* stem could be a potential source of chemotherapeutic drugs for the treatment of tinea associated with *E.flocosum* and *T. gypseum.* Okiei *et al*., (2009) observed analysis of essential oil constituents in hydro-distillates of *Calotropis procera*. Sharma *et al.*, (2011) studied the pharmacognostical aspects of *Calotropis procera.* Murti *et al.*, (2010) analysed pharmacognostic standardization of leaves of *Calotropis procera*. Phytochemical and antimicrobial evaluation has been carried out on other species of *Calotropis.* Sharma *et al.*, (2002) worked on screening of fresh leaf extracts of *Datura stramonium, Calotropis procera, Verbesena enceloides, Parthenium hysterophorus, Morus alba, Phyllanthus amarus, Eichhornea crassipes, Ricinus communis, Jatropha curcas, Azadirachta indica,Tinospora cordifolia, Clerodendron multiflorum, Catharanthus roseus and Adhatoda vesica* for their Nematicidal and Fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. The result of their investigation showed that *Calotropis procera* and *Ricinus communis* gave best results against the nematode and *Datura stramonium* and *Calotropis procera* showed maximum antifungal activity against *Fusarium oxysporum* .Varahalarao *et al*., (2010) examined bioassays for antimicrobial activities using stem, leaves and flowers of *Calotropis procera*. The antimicrobial activities of the organic solvent extracts are tested on the various test organisms *Alternaria alternata, Aspergillus flavus, Asperigellus niger, Bipolaris bicolor, Curvularia lunata, Pencillium expansum, Pseudomaonas marginales, Rhizoctonia solani* and *Ustilago maydis.* Dissimilar results were obtained using genus *Pseudomonas* in the present study. The results of *C. procera* shown highest antimicrobial activity against *A. alternata* with all concentrations where as no activities were found against *B.bicolor, P.marginales* and *P. syringae.* Whereas our work was conducted on *Calotropis procera* , Subramanian *et al.*, (2010) evaluated the antibacterial activity of *Calotropis gigantea* latex extract on selected pathogenic bacteria and it was concluded that the latex extract possesses potent bactericidal activity which may be due to the presence of biologically active ingredients with antimicrobial activity in the ethanolic extract of *C.gigantea*. The present study would also contribute to the acceptance of *C.gigantea* latex in traditional medicine and to the solution of growing problems of drug resistance by microorganisms. The effect of *Calotropis procera* against *Candida albicans* agrees with the work of Sehagal *et al*., (2005,2008) which analyzed the inhibitory effect of extracts of latex of *Calotropis procera* against *Candida albicans* .Their studies demonstrated the anticandidial activity of the extracts of *Calotropis procera*. This property is related to the presence of enzymes and stable cysteine proteases in the latex. The anticandidial activity of latex suggested that it might be effective against other fungal strains as well. The present investigation was disagree with the work of Sukanya *et al.*, (2009) who

showed the antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. They analyzed that the extracts of *Achyranthes aspera, Artemisia parviflora, Calotropis gigantean, Lawsonia inermis, Mimosa pudica, Ixora coccinea* were found to be ineffective or showed poor inhibition on tested human and phytopathogenic bacteria .They concluded that the extract of *Azadirachta indica, Parthenium hysterophorus and Chromolaena odorata* were found to be effective against phytopathogenic bacteria.

Alam *et al.*, (2008) worked on antimicrobial activity of *Calotropis gigantea* on some pathogenic bacteria. The results of their studies showed that among the tested materials, methanol extract and its chloroform fraction showed comparatively better results than petroleum ether and ethyl acetate fractions.

In the end on the basis of various investigation conducted so far it was concluded that *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp.,* Cogulase *(+) Staphylococcus sp., Cogulase (-) Staphylococcus sp., Candida albicans and Candida parapsilosis* produced significant susceptibility with the plant extracts. The consequences of this work has clarified that many active bioconstituents of *Calotropis procera* consist effective qualities in its tending action. Thus it must be exploited upon by scientists in the development of human medicines and drugs. *Calotropis procera* research for human gastroenteritis, neonatal meningitis ,pneumonia, septicemia, urinary tract infection ,gastrointestinal infection, bactermia, bacterial endocarditis, diverticulitis , meningitis and candidiasis has thus given a path for the possibility of finding cures for many strains of enteric microorganisms which are now resistant to many usable and common antibiotics in our countries. *Calotropis procera* is a reliable cure for human diseases as have been performed in this work .This research has been proved as a path giver to many scientists who may implement the result of the present work in developing drugs from *Calotropis procera* against human pathogenic microorganisms.

Phyto chemicals	Petroleum Ether					Distilled Water					Ethanol					Chloroform					n-Butanol				
	L	S	F	R	LA	L	S	F	R	LA	L	S	F	R	LA	L	S	F	R	LA	L	S	F	R	LA
Alkaloids	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+
Glycosides	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Saponins	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Terpenoids	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Phenols	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+
Tannins	+	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Flavonoids	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Tritepenoids	-	+	+	+	+	-	+	+	+	+	_	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Steroids	+	-	+	+	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+
Redusing sugar	-	-	+	+	+	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+

'+' = Presence Of The Compound; '-'= Absence Of Compound 'L' = Leaves , 'S'= Stem, 'F'=Flower, 'R'=Root, 'LA'=Latex,

Table1: **Preliminary phytochemical screening of plant parts of *Calotropis procera***

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