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Original Article

# ANTIMICROBIAL AND PHYTOCHEMICAL INVESTIGATION OF *CALOTOROPIS PROCERA* FLOWERS EXTRACTS

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**ABSTRACT**

**Objective:** The present study was planned to screen extracts of different polarities of the flowers of *Calotropis procera* for the detection of different secondary metabolites, estimate the antibacterial activity of the prepared extracts, and study the active extracts by different chromatographic and spectroscopic methods.

**Methods:** The diethyl ether, methanol, and water extracts were phytochemically screened. Petroleum ether, chloroform, and methanol extracts were also tested against two Gram-positive bacteria, namely *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative bacteria, namely *E. coli* and *Pseudomonas aeruginosa* with diffusion method. The methanolic extract was further investigated by column chromatography (CC) and preparative thin-layer chromatography (PTLC). Three pure compounds have been isolated and investigated by IR-spectroscopy.

**Results:** Phytochemical screen showed the presence of various secondary metabolites such as flavonoids, alkaloids, steroids, cardiac glycosides, reducing sugars, and saponins. The antibacterial assay revealed that the methanolic extract was the most active against the tested bacteria, especially against *Pseudomonas aeruginosa* with the high zone of inhibition (23 mm) at 100 mg/ml, and *E. coli* (22 mm) at 100 mg/ml, followed by chloroform extract, while the petroleum ether extract was insignificantly active. Column Chromatography analysis of the methanolic extract separated fifteen fractions. The PTLC of fraction No.14 enabled the isolation of three pure compounds (A, B, and C). The IR-spectroscopy analysis of the three isolated compounds exhibited that they could referred to the alkaloids or cardiac glycosides.

**Conclusion:** The methanolic extract showed significant activity against tested bacteria, especially *E. coli* and *Pseudomonas aeruginosa*. The result also indicates the presence of secondary metabolites in *C. procera* extracts. Subsequently the therapeutic efficacy compounds isolated and purified from *C. procera* could be used as an important source against bacterial ailments in humans and plants.

**Keywords:** Calotropis procera, Anti-microbial, Phytochemical


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## INTRODUCTION

Throughout history, plants have played a substantial role in medicine. For early peoples, they came easily to hand and were complicatedly connected to diet and healing. Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, natural products remain one of the best reservoirs of new structural types [1].

The present study is concerned with *Calotropis procera.* Its common name in Sudan is Ushar. It belongs to the family *Asclepiadaceae* with 180 genera and 2200 species widespread in tropical and subtropical regions of the world such as Africa (Mauritania, Singal), Asia (India, Bangladesh), Australia, and America [2]. This plant used as a medicinal plant: In Southern Blue Nile in Sudan, the fresh flowers are eaten as a digestive [3]. The Milky latex used in leprosy, eczema, inflammation, syphilis, malarial and low hectic fevers, and as an abortifacient. The leaves: in rheumatism, as anti-inflammatory and antimicrobial. The roots: as hepatoprotective agents, against colds and coughs, syphilis, and elephantiasis, as an anti-inflammatory, analgesic, antimalarial, and antimicrobial. Flowers: as abortifacient, antimalarial, in asthma and piles [4]. In Nigeria, leaves are used for the treatment of guinea worm [5].

*Calotropis* is a large shrub or small tree up to 4-10 m, stem erect up to 20 cm in diameter, bark pale gray, latex in all parts. It's flowers are bisexual, regular, 5-numerous, white, cream or purple, with green spongy ovoid fruits which split open to release light brown seed with a white filament [6] (fig. 1a fig. 1b).

**(a)** **(b)**

**Fig. 1: *Calotropis procera* shurb (a), Flowers of *C. procera* (b)**

Studies about the toxicity of *C. procera* revealed that the latex and the crude extracts had a toxic effect against Black Rats and Rattus, Termites, and Goat [7-9]. Also, the extract of leaves of *C. procera* exhibited insecticidal activity against flesh fly, *Sarcophaga haemorrhoidalis* fallen [10]. In the wet season of Northern Australia, the plant was not toxic in the same doses that caused acute poisoning elsewhere, so it was assumed that either the toxin was not present in the northern Australia species or that local conditions did not enhance the production of the toxin [11].

*Calotropis procera* has shown cytotoxic activity [12], and the crud extract produced by the isolated endophytic fungus from its root could be an important source of broad-spectrum antimicrobial metabolites [13].

This study was designed to determine the phytochemicals present in different polarities solvent extracts of the flowers of *C. procera* and to evaluate the antimicrobial activity *of C. procera* extracts.

## MATERIALS AND METHODS

### Plant material

The flowers of *Calotropis procera* were collected from the Blue Nile bank in Khartoum, Sudan. The plant was identified at the Medicinal and Aromatic Plants Research Institute, National Center for Research in Khartoum, Sudan, with specimen number (SD-SH-11), by the researcher Yahia Suliman.

### Chemicals and reagents

Chloroform, diethyl ether, distilled water, ethanol 96%, ethyl acetate, methanol, and petroleum ether. Ansildehyde's reagent, Dragendroff's reagent, Kedde's reagent, Molisch's reagent, Mayer's reagent, Fehling's solution, $FeCl_3$ 10%, KOH (alcoholic 25%), $SbCl_3$ 20%, HCl, acetic anhydride, ammonia 25%, sulphuric acid, and vinlline were used for phytochemical screening. Silica gel GLR for PTLC, silica gel 230-400 mesh were used for column chromatography.

### Plant extract preparation

Air-dried and powdered flowers of *Calotropis procera* 300 g were extracted using Soxhlet apparatus at room temperature using three different solvents: petroleum ether, chloroform, and methanol. In a rotary evaporator the extracts were concentrated, then the yields were determined. The extracts were labeled and kept in a refrigerator at 4 ºC for further study.

### Phytochemical screening qualitative analysis

To achieve this analysis, 20 g of powdered flowers were extracted with diethyl ether for 4 h at 30 ºC using a Soxhlet apparatus, and the extract was concentrated up to 50 ml, and was used for the identification of lipophilic constituents. The marc was dried and then extracted with methanol for 2 h at 60 ºC, the extract was concentrated up to 50 ml, and used for identification of an important groups of natural constituents. The marc was dried and extracted with distilled water in conical flask for 24 h, the filtrate solution was concentrated up to 50 ml and used for identification of hydrophilic constituents. The preliminary phytochemicals {fatty substances, sterols, triterpenes, higher fatty acids, alkaloids, flavonoids, anthracene glycosides, coumarins, tannins, reducing compounds, cardiac glycosides/cardenolides/bufadienolides, anthocyanosides, polyuronides, glucides, starch, and saponins} screening at different solvent extracts, were carried out using standard a method as described by Harbone [14].

### Chromatographic analysis

The methanolic extract that exhibited significant activity against the selected bacteria was analyzed by column chromatography using silica gel 230-400 mesh and eluted with petroleum ether, ethyl acetate, methanol, and, at last, distilled water**.** Fraction No.14 was selected and subjected to PTLC to separate pure constituents by utilizing a thick layer of adsorbent silica gel 0.5 mm on glass plates (5×20) cm and (20×20) cm, solvent system: toluene–ethyl acetate–formic acid (5:4:1), and vanillin-sulphuric acid reagent for detection.

### Screening of antimicrobial activity

Four kinds of bacteria were used for the antibacterial test: *Escherichia Coli* (ATCC 25922), *Bacillus subtilis* (NCTC 8236), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923). The standard organisms were obtained from the National Collection of Type Culture (NCTC), Colindale, England and the American Type Culture Collection (ATCC), Rockville, Maryland, USA. For tests, they were obtained from the Microbiology Department of The National Research Center, and the National Health Laboratory, Sudan.

Antibacterial activity of different solvent extracts of *C. procera* was investigated against two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram-negative organisms, *Escherichia coli* and *Pseudomonas aeruginosa*, by well diffusion method that adopted with some minor modifications [15]. The obtained cultures were maintained on nutrient agar and incubated at 37 °C for 18 h and then used for tests. 2 ml of the standardized bacterial stock suspension (108-109) colony-forming units, per ml were thoroughly mixed with about 200 ml of sterile nutrient agar, which was maintained at 45 °C. 20 ml aliquots of the inoculated agar were distributed into sterile Petri dishes. The agar was left to set and cool, then in each of these plates, 4 cups (10 mm in diameter) were cut using a sterile cork borer No 4, while in 4 plates, only 2 cups in the medial of the plates were cut using sterile cork. The agar discs were removed. The cups were then filled with 0.1 ml sample of each extract using an adjustable micropipette and allowed to diffuse at room temperature for about 2 h. The plates were then incubated in the upright position at 37 °C for 18 h. Three replicates were carried out for each extract against each of the tested organisms. Positive control involving the addition of standard antibiotics were carried out, and dimethyl sulphoroxide (DMSO) as the negative control. Diameters of the resultant growth inhibition zone were measured in mm.

## RESULTS AND DISCUSSION

Exhaustive extraction of the *Calotropis procera* flowers was performed in a Soxhlet apparatus with solvents increasing in polarity. The percentage yield and the physical properties were summarized in (table 1).

**Table 1: Percentage yield and physical properties of *C. procera* flowers**

| Extract | Percentage yield | Physical properties |
|---|---|---|
| Petroleum ether | 1.29 %±0.00 | Waxy yellow |
| Chloroform | 1.039%±0.00 | Waxy dark green |
| Methanol | 21.20 %±0.00 | Sticky brown |

Number of experiments: 3,'**±**'SD

### Phytochemical screening of the flowers of *Calotropis procera*

Results of general phytochemical screening of diethyl ether, methanol, and water extracts of the flowers of *Calotropis procera* were presented in (table 2, table 3, and table 4) respectively. Qualitative analysis of the flowers extracts revealed the presence of alkaloids, flavonoids, carotenoids, sterols/triterpenes, higher fatty acids, coumarins, tannins, reducing compounds, cardiac glycosides, polyuronides, glucides, and saponins. The previous study on the aerial parts of *C. procera* also supported the presence of different phytochemicals such as alkaloids, cardiac glycosides, tannins, flavonoids, sterols, and triterpenes [16].

**Table 2: Qualitative analysis of diethyl ether extract of *C. procera* flowers**

| Test for | Result |
|---|---|
| Sterols and Triterpenes | + |
| Carotenoids | + |
| Higher fatty acids | + |
| Basic alkaloids | - |
| Flavonoids | + |
| Anthracen-glycosides | - |
| Coumarins | + |

'+'-Present, '-'-Absent

**Table 3: Qualitative analysis of methanol extract of *C. procera* flowers**

| Test for | Result |
|---|---|
| Tannins | + |
| Reducing reagent compounds | + |
| Alkaloids | + |
| Anthracene-glycosides | - |
| Sterols and triterpenes | + |
| Coumarins | + |
| Flavonoids | + |
| Cardiac glycosides:- | |
| Kedde test | + |
| Liebermann test | + |
| Antimony trichloride | + |

'+'-Present, '-'-Absent

**Table 4: Qualitative analysis of aqueous extract of *C. procera* flowers**

| Test for | Result |
|---|---|
| Polyuronides | + |
| Reducing reagent compounds | + |
| Glucides | + |
| Starch | - |
| Saponins | + |
| Tannins | + |
| Alkaloid salts | - |

'+'-Present, '-'-Absent

**Antibacterial activity of extracts of *C. procera* flowers**

The extracts of petroleum ether, chloroform, and methanol (100 mg/ml), were tested against four kinds of bacteria: *Escherichia Coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The inhibition zones (mm) were shown in (table 5). The methanolic extract exhibited antibacterial activity against both (G-positive) and (G-negative) bacteria. Significant activity was observed against *pseudomonas aeruginosa* and *E. coli* (G-negative). On the other hand, the petroleum ether and chloroform extracts showed insignificant activity against the tested bacteria (fig. 2, fig. 3). Various active components present in *C. procera* extract like alkaloids, tannins, saponins, terpenoids, glycosides, and flavonoids, are secondary metabolites which serve a protective mechanism against many microorganisms [17]. A previous study has shown that flavonoids have been identified as polyphenolic compounds capable of exerting antibacterial activities via various mechanisms of action [18]. Similarly, Larhsini M *et al.* reported the potent antibacterial activity of the n-butanol extract of *Calotropis procera* flowers against eight pathogenic bacteria [19]. Another study revealed that the flower extracts of *C. procera* have the potential to be used as an antibacterial agent against pathogenic organisms like *Salmonella typhi*, *Escherichia coli*, *Micrococcus luteus*, and methicillin-resistant *Staphylococcus aureus* [20].

The number and physical properties of fractions obtained from the methanol extract by column chromatography were presented in (table 6).

**Fig. 2: Antibacterial activity of the extracts of *C. procera* flowers against *E. coli***

**Fig. 3: Antibacterial activity of the extracts of *C. procera* flowers against *Staphylococcus aureus***

**Table 5: Antibacterial screening of the extracts of *C. porcera* flowers**

| Extracts | Inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Organism | | | |
| | *S. A* | *Ps. a* | *B. a* | *E. coli* |
| Petroleum ether | - | 12±0.05 | - | - |
| Chloroform | - | 14±0.05 | 13±0.11 | 06±0.05 |
| Methanol | 15±0.10 | 23±0.25 | 18±0.15 | 22±0.10 |

Number of experiments (ne):3, '±'Standard Deviation, '-'-Absent

**Table 6: Numbers of fractions and colours of fractions obtained from column chromatography**

| Number of fraction | Colour |
|---|---|
| 1 | Colourless |
| 2 | Colourless |
| 3 | Yellow |
| 4 | Yellow |
| 5 | Yellow |
| 6 | Green |
| 7 | Green |
| 8 | Colourless |
| 9 | Colourless |
| 10 | Yellow |
| 11 | Brown |
| 12 | Brown |
| 13 | Brown |
| 14 | Brown |
| *15 | Brown |

Solvents of elution: -petroleum ether, ethyl acetate, methanol and distilled water. *Fraction No. 15 contained a crystalline compound with melting point = 150-160 °C

**Isolation of three pure constituents of *C. procera* flowers**

Fraction No. 14 was further selected for the separation of compounds by PTLC and ended up with the isolation of three pure compounds. The three compounds (A, B, and C) were examined by IR-spectroscopy (Thermo Nicolet Mattson 300IR) to determine their functional groups in an attempt to spot more light on their structure (fig. 4, fig. 5, fig. 6) respectively. Comparing the type of vibration, frequency, and intensity of IR-spectra of compounds A, B, and C with data available in the literature, one could conclude that the three compounds were from one class, either Cardenolides or Nitrogenous compounds. Both types were detected in the extracts of the flowers and confirmed upon comparison with published data in the current literature.

**Table 7: Assignments of IR-spectrum of compound A**

| Peak (cm-1) | Bond | Type of compound | Intensity |
|---|---|---|---|
| 3850.14 | | | |
| 3442.19 | O-H<br>N-H | Monomeric alcohols, phenols, hydrogen-bonded alcohols, phenol, amine, amide | 67.860 |
| 2923.97 | C-H | Alkanes | 63.108 |
| 2852.90 | C-H | Alkanes | 67.818 |
| 2358.86 | CΞ N | Nitriles | 76.144 |
| 1728.94 | C= O | Aldehydes, ketones, carboxylic acid, ester | 75.093 |
| 1600.78 | C=C | Aromatic ring | |
| 1463.04 | C-H | Alkanes | |
| 1383.32 | C-H | Alkanes | |
| 1276.27 | C-N | Amine,amide | |
| 1036.19 | C-O | Alcohols, ether, carboxylic acids, esters | 74.020 |

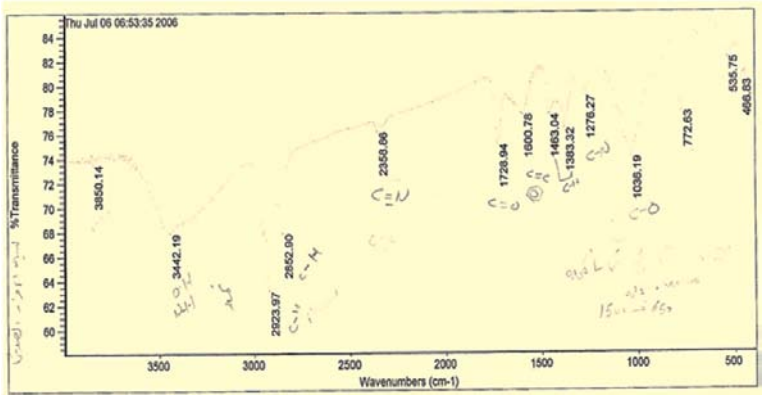


**Fig. 4: IR-spectrum of compound A**

**Table 8: Assignments of IR-spectrum of compound B**

| Peak (cm-1) | Bond | Type of compound | Intensity |
|---|---|---|---|
| 3901.42 | | | |
| 3734.02 | | | |
| 3565.79 | O-H | Monomeric alcohols, phenols, hydrogen- | 65.182 |
| | N-H | bonded alcohols, phenol. Amine, amide | |
| 3445.23 | O-H | Monomeric alcohols, phenols, hydrogen- | 64.114 |
| | N-H | bonded alcohols, phenol, Amine amide | |
| 2924.08 | C-H | Alkanes | 62.548 |
| 2853.20 | C-H | Alkanes | 65.032 |
| 2360.19 | C≡ N | Nitriles | 61.946 |
| 2341.29 | C≡ N | // | 64.039 |
| 1747.09 | C= O | Aldehydes, ketones, carboxylic acids, esters. | |
| 1616.96 | C=C | Aromatic ring | |
| 1456.76 | C-H | Alkanes | |
| 1396.47 | C-H | // | |
| 1031.74 | C-O | alcohols, ether, carboxylic acids, esters | |

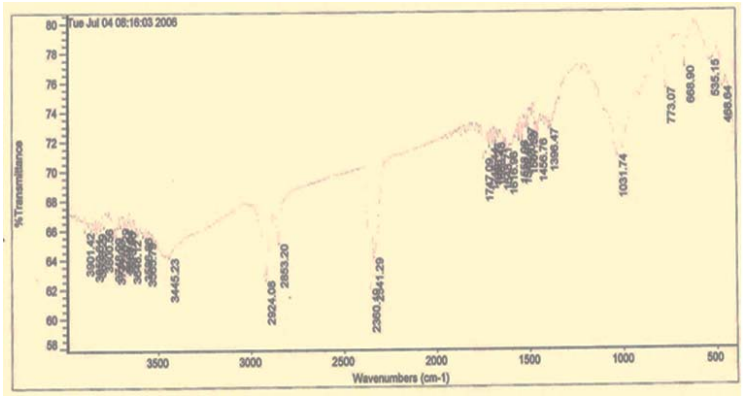


**Fig. 5: IR-spectrum of compound B**

**Table 9: Assignments of IR-spectrum of compound C**

| Peak (cm-1) | Bond | Type of compound | Intensity |
|---|---|---|---|
| 3735.96 | | | 74.980 |
| 3461.70 | O-H | Monomeric alcohols, phenols, hydrogen- | |
| | N-H | bonded alcohols, phenol, amine, amide | |
| 2924.43 | C-H | Alkanes | 69.786 |
| 2853.49 | C-H | Alkanes | 72.65 |
| 2360.29 | C≡ N | Nitriles | 71.996 |
| 2341.37 | C≡ N | Nitriles | 73.310 |
| 1734.40 | C=O | Aldehydes, ketones, carboxylic acid, esters | 76.434 |
| 1619.16 | C=C | Aromatic ring | |
| 1458.87 | C-H | alkanes | |
| 1383.44 | C-H | // | |
| 1273.74 | C-N | Amines,amides | |
| 1219.47 | C-N | // | |
| 1037.57 | C-O | alcohols, ether, carboxylic acids, esters | |

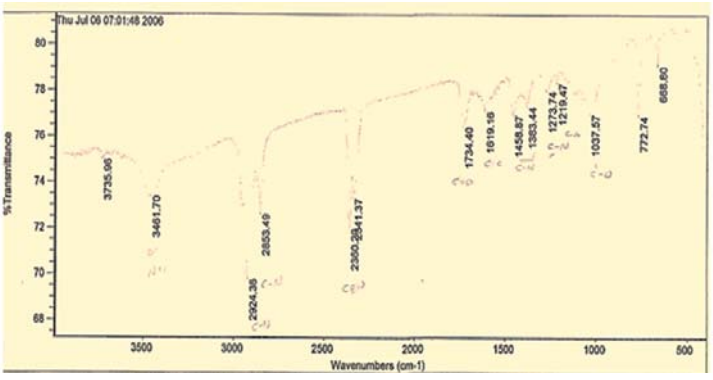


**Fig. 6: IR-spectrum of compound C**

## CONCLUSION

The phytochemical screen of the extracts of flowers of *C. procera* revealed the presence of secondary metabolites, namely alkaloids, flavonoids, fatty acids, sterols/triterpenes, cardiac glycosides, coumarins, saponins, tannins, reducing sugars and polyuronides. The analysis of antimicrobial activity showed that the petroleum ether and chloroform extracts had insignificant activity against the tested bacteria, while the methanolic extract was significantly active, there for the methanolic extract was selected for more detailed studies using different chromatographic methods such as CC, and PTLC. Three pure compounds designated as A, B, and C have been isolated in a pure form and studied by IR-spectroscopy to spot more light on their structures. Interpretation of results could give grounds to suggest that the compounds were probably alkaloids or cardiac glycosides. further investigation into this plant could be more valuable in the availability of advanced technology.

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Nil

## AUTHORS CONTRIBUTIONS

Sara H. Altayeb performed the experiments and edited the document, Asaad Kh. designed and edited the document. Both authors have read and approved the final manuscript of this article.

## CONFLICTS OF INTERESTS

Declared none

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