### Antibacterial activity of *Calotropis procera* against some common human pathogens

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**Abstract**

*Calotropis procera* (Oshaar, family Asclepiadaceae) is a species of flowering plant used as a folk medicine for the treatment of various ailments. This study aimed to evaluate antibacterial and antioxidant activities of *C. procera* aerial parts collected from Eastern Desert of Egypt (Wadi Hagul). The different extracts of *C. procera* aerial parts showed a significant antimicrobial inhibitory effect (P < 0.05) on growth of nine pathogenic bacterial using filter paper disc assay. Qualitative and quantitative phytochemical screening was carried out on water and different extracts. The present study indicated that this plant has a good inhibitory activity against *Klebsiella pneumonia* (21.3 mm), *Escherichia coli* (22.5 mm), and *Staphylococcus aureus* (21.6 mm). Moreover, methanol extract of *C. procera* exhibited wide broad spectrum (100%) followed by petroleum ether and water extracts (60%, each). Activity index (AI) illustrated variable antibacterial activities, that support the folkloric usage of medicinal plants for treatment of infectious pathogenic microorganism. The crude extract of *C. procera* showed adequate antioxidant activity, wherein methanol, ethyl acetate and petroleum ether extracts have an IC₅₀ value of 0.81, 0.88 and 1.09 mg mL⁻¹, respectively. Therefore, phytochemicals from *C. procera* could be used as a raw material for producing cheaper pharmaceuticals, pesticides in addition to many more important commercialized products of public use.

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**INTRODUCTION**

Plants and plant extracts were used in ancient times for treatment of diseases. This is because of the diversity of natural products (secondary metabolites) of plant origin that were used in ethnomedicine than that from any other source. The secondary metabolites are very important as they integrated into several industries such as pharmaceutical, cosmetic, agro-industrial and nutritional products (Saroya and Singh, 2018; El-Amier and Aisha, 2019). Around four billion of people living in the developing countries depend on herbal medicine as the main source of their health care. The traditional medical practices using herbs are considered as the main component of the culture in these communities (Jamshidi-Kia et al., 2018).

The genus of *Calotropis* belongs to the family Asclepiadaceae that comprises 180 genera and 2200 species broadly distributed in the tropical and subtropical areas. According to Boulos (2000), this genus is comprised of about three species of shrubs...
distributed in tropical and warm Africa and Asia, which is commonly known as Milkweed. *Calotropis procera* (Oshaar) is a species of flowering plant abundant in North Africa (Morocco-Egypt), Palestine, Arabia, Iraq, Pakistan and India. This species is a large shrub or small tree. Its stems are erect (2-4 m), up to 20 cm in diameter and it is a large broadleaf evergreen plant with a strong odour. In Egypt, it occurs in the Nile Delta, Oases, all the Deserts of Egypt, Gebel Elba and the entire Sinai Peninsula including the coastal Mediterranean strip (Boulos, 2000).

*Calotropis procera* is used as a folk medicine for the treatment of many diseases. It was reported that the plant possess potential anthelmintic, antimicrobial, anticancer, anticoagulant, analgesic, anti-inflammatory, purgative and antipyretic properties and is also used in the treatment of leprosy, leucoderma, liver and abdomen (Morsy et al., 2016; Alzahrani et al., 2019). The plant contains many secondary metabolites with unique structure and obvious biological activities and that could be helpful for generation of new pharmaceutics and therapeutic applications (Zaki et al., 2017). Phytochemicals like alkaloids, phenolics, flavonoids, terpenoids, resin, steroid, glycosides, tannins carbohydrates, aluminum, iron, magnesium, and sodium were reported in literature (Morsy et al., 2016; Alzahrani et al., 2019). In addition, plant secretes important indigenous milky sap (latex) which is a large source of ethnomedicines. The current work determine the chemical constituents of *C. procera* aerial parts collected from Eastern Desert and antimicrobial properties, as well as asses the antioxidant activity.

**MATERIALS AND METHODS**

**Collection of plant material and preparation of extract**

In the flowering stage, the investigated plant, *Calotropis procera* (Willd.) R.Br. (family Asciepiadaceae) in the present study was collected from their different natural habitats in the northern part of the Eastern Desert of Egypt (Wadi Hagul). The samples were identified according to Boulos (2000) by Dr Yasser A, El-Amier, Lecturer of Plant Ecology. Plant sample was handily cleaned, washed several times with distilled water, dried for 24 hours at 55-60 °C in air-forced oven for reduction of moisture content before grinding. 100 grams of the dried plant were soaked separately in water; 95% methanol, petroleum-ether and ethyl-acetate and shake periodically. These extracts were filtered and evaporated and the dried residue was dissolved in dimethyl sulfoxide (DMSO) for further use.

**Antibacterial Bioassay**

**Tested bacteria**

The different extracts of *Calotropis procera* were tested against five gram negative bacteria: *Klebsiella pneumonia* (ATCC10031), *Listeria monocytogenes* (ATCC19116), *Escherichia coli* (ATCC10536), *Salmonella typhi* (ATCC25566) and *Pseudomonas aeruginosa* (ATCC9027) and four gram positive bacteria; *Streptococcus epidermis* (EMCC1353), *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (DMS1088) and *Enterobacter cloacae* (DMS30054).

**Antibacterial activity**

Sterilized filter paper discs with five milliliter diameter were immersed overnight in the prepared plant extracts then loaded over the nutrient agar medium seeded with the tested pathogenic microorganisms to be used for the antibacterial assay (Cappuccino and Sherman, 2008). The Petri dishes were incubated at 37 °C for 24 hours then the inhibition zone diameter (mm) was measured. The antibacterial activity of the tested extract was compared with standard antibiotics using the Activity Index (AI) (Neha and Rekha, 2010).

Activity index (AI)= Inhibition Zone of the sample / Inhibition Zone of the standard

**Qualitative phytochemical screening**

Standard procedures were followed for identification of the phytochemical components as described by Trease and Evans (1983).

**Quantitative determination of the chemical constituents**

Total phenolics, flavonoid and alkaloids were estimated according to the assays described by Joshi et al. (2013). Saponins content was determined using the method described by Obadoni and Ochuko (2002), while tannins according to Buren and Robinson (1969).

**Antioxidant activity**

Antioxidant activity was estimated using the free radical DPPH (1,1-diphenyl-2-picyrlhydrazyl) (Miguel, 2010). One milliliter of 0.15x10⁻³ M DPPH was added to one milliliters of the prepared extracts with different concentrations (1000, 800, 600, 400, 200 and 100 ppm). A control was prepared by adding one milliliters of DPPH to one milliliters of the solvent. The mixture was incubated at exclusion of light at the room temperature for thirty minutes and the absorbance was measured at 517 nm. IC₅₀ values were graphically determined and the antioxidant activity was
expressed as,
\[
\% \text{ scavenging activity} = \left[ 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100
\]

**Statistical analysis**

The experiments were done in triplicates and mean ± standard deviation (M±SD) was measured. The data of antimicrobial and antioxidant were analyzed using one-way ANOVA followed by Duncan’s test at probability level of 0.05 using CoStat (version 6.311, Cohort Software, USA, www.cohort.com).

**RESULTS AND DISCUSSION**

**Inhibitory effect of medicinal plant**

The antibacterial activity of *Calotropis procera* was estimated in vitro by agar well diffusion assay using eight different pathogenic strains. The different extracts of *C. procera* aerial parts showed a significant antimicrobial inhibitory effect \((P < 0.05)\) on growth of the tested Gram positive and negative bacteria (Table 1). In this study, methanol extract of *C. procera* expressed wide antimicrobial spectrum (100%) against both Gram-positive bacteria and Gram-negative bacteria (Figure 1), followed by petroleum ether and water extracts (60%, each). Highest antibacterial activity was shown by methanol extract against *Escherichia coli* (22.5 mm), *Staphylococcus aureus* (21.6 mm) and *Klebsiella pneumoniae* (21.3 mm).

The pathogen *Escherichia coli* was the most sensitive bacteria in case of methanol extract (22.5 mm), whereas *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most susceptible in case of methanol and petroleum ether extracts (21.6, 17.3 mm and 21.3, 20 mm, respectively), while the most resistant bacteria were *Listeria monocytogenes* and *Streptococcus epidermidis*. Moreover, it was noted that the antibacterial performance of methanol extract is better than ampicillin and penicillin (Ab), suggesting that methanol extract can be a good alternative for antibiotics (Table 1).

From the obtained results, it could be concluded that Gram negative bacteria are more sensitive to *C. procera* extracts than Gram positive bacteria as previously reported in literature (Chanda et al., 2009; El-Amier et al., 2014).

Many wild plants exhibited inhibition zones of different diameters due to several parameters like ability of substances in the extract to diffuse through the agar, the antimicrobial potential of the diffused substances and the growth and metabolic activity of the examined microorganisms (Chanda et al., 2009). The phytochemicals may be related with its ethnomedicinal use in the treatment of various diseases. Generally, alkaloids are known to have antimicrobial and antiinflammatory effects (Patel et al., 2012). Flavonoids are beneficial for human health. They possess biological and pharmacological activities such as antioxidant and antimicrobial activity. Alkaloids and saponins have been ability to cause protein leakage as well as certain enzymes from the cell (Mantle et al., 2000).

**Activity index (AI) of extracts**

The significance of using the prepared methanol, petroleum ether and water extracts with standard antibiotics was calculated using activity index (Table 2). Four antibiotics were used against all tested pathogenic strains. The maximum inhibition was recorded by chloramphenicol and Gentamicin against *Klebsiella pneumoniae, Streptococcus epidermidis* and *Staphylococcus aureus*. On the other hand, ampicillin and penicillin expressed both moderate and low effects against all tested pathogens as mentioned in Table 1. The sensitivity of different *C. procera* extracts was compared with antibiotics using activity index (Table 2). Activity
agrees with the results of The phytochemical analysis of (13.27, 11.24 and 9.31 mg g⁻¹ tannins, flavonoids and saponins attained values 20.62 mg g⁻¹ been showed in Figure 2. The results show that the aerial parts contained mainly alkaloids compound (20.62 mg g⁻¹) and phenolics (17.64 mg g⁻¹). While tannins, flavonoids and saponins attained values (13.27, 11.24 and 9.31 mg g⁻¹ dry weight, respectively).

The phytochemical analysis of C. procera relatively agrees with the results of Shobowale et al. (2013); Morsy et al. (2016) on same species from Nigeria and Kingdom of Saudi Arabia, respectively and agrees with the results of El-Amier et al. (2014) on Senecio glaucus present in the arid regions.

**Phytochemical constituents**

The powder and crude extract of C. procera aerial parts was subjected to phytochemical screening and the results were recorded in Table 3. In this study, the use of different solvents showed different response for the presence of phytoconstituents and according to the intensity of colour or precipitates produced the terms of scores are using as -, +, ++, ++++. In case of methanol solvent, the extract showed the presence of all tested chemical constituents except coumarins not detected. While traces or absence of phytoconstituents were in petroleum ether and water solvents (Table 3). Extraction efficiency of biologically active compounds is affected by the properties of phytochemicals, the extraction method, the solvent used, time and temperature of extraction (Mostafa et al., 2018). The quantitative analysis of aerial parts of wild C. procera has been showed in Figure 2. The results show that the aerial parts contained mainly alkaloids compound (20.62 mg g⁻¹) and phenolics (17.64 mg g⁻¹). While tannins, flavonoids and saponins attained values (13.27, 11.24 and 9.31 mg g⁻¹ dry weight, respectively).

The powder and crude extract of C. procera aerial parts have a high content of flavonoid and phenolic compounds (Figure 2), this index (AI) values above one expressed the reasonable role of herbal extracts while those below zero expressed higher antibiotics potential against tested pathogenic strains (Neha and Rekha, 2010). Results confirmed the potential use of methanolic extract as compared to antibiotics except chloramphenicol.

**Table 1: Inhibitory activity of Calotropis procera extract against the tested organisms (inhibition zone = mm).**

| Bacterial test | Plant extract | Standard antibiotic (Ab) | LSD0.05 |
|---------------|---------------|--------------------------|---------|
|               | Mehanol       | Petroleum ether | Water | Amp. | Gen. | Chl. |
| **Gram-negative bacteria** | | | | | | |
| KP            | 21.3±0.58     | 20±0.54         | 9±0.24 | 17±0.46 | 22±0.39 | 33±0.89 | 0.57*** |
| LM            | 12.5±0.34     | n.a             | n.a   | 23±0.62 | n.a   | n.a   | 0.58*** |
| EC            | 22.5±0.61     | 15.6±0.42       | 8±0.21 | 11±0.30 | 21±0.57 | 23±0.42 | 0.62*** |
| ST            | 13.8±0.37     | n.a             | 7±0.19 | n.a   | 25±0.68 | 22±0.59 | 0.23*** |
| PA            | 14.2±0.31     | 10±0.27         | 9d±0.14 | 10±0.27 | 19±0.51 | 25±0.68 | 1.18*** |
| **Gram-positive bacteria** | | | | | | |
| SE            | 14.5±0.39     | n.a             | n.a   | 31±0.84 | 30±0.81 | 0.42*** |
| SA            | 21.6±0.48     | 17.3±0.47       | n.a   | 13±0.35 | 27±0.73 | 28±0.76 | 0.57*** |
| BS            | 17.5±0.37     | 10.3±0.28       | 8±0.22 | n.a   | 10±0.27 | 19±0.51 | 1.19*** |
| EC            | 14.5±0.39     | 12±0.32         | 8±0.10 | 17±0.46 | 14±0.38 | 23±0.32 | 0.36*** |

- n.a: Not active, Values are means ± standard error of triplicates. DMSO: Not active against plant extract and antibiotics
- - Amp.: Ampicillin, Gen.:Gentamicin, Chl.: Chloramphenicol
- KP: K. pneumoniae, LM: L. monocytogenes, EC: E.coli, ST: S. typhi, PA: P. aeroginosa, SE: S. epidermis, SA: S. aureus, BS: B. subtilis, EC: E. cloeoe

Antioxidant activity

The crude extract from the aerial parts of C. procera expressed adequate radical scavenging activity of the DPPH in a concentration-dependent manner (Table 4). In case of methanol, petroleum ether and ethyl acetate extracts the increase was up to 1000 μg/mL where the scavenging activity was 55.75%, 46.55% and 52.34, respectively. The crude extract from methanol solvent of C. procera revealed more antioxidant activity than that of the other solvents, where IC₅₀ values of methanol, ethyl acetate and petroleum ether extract 0.81, 0.88 and 1.09 mg mL⁻¹, respectively.

However, the IC₅₀ value for catechol (standard antioxidant) was 0.29 mg mL⁻¹ (Table 3). The significant variation in the antioxidant activity of C. procera extracts could be ascribed to the variation in the solvent used. Plant secondary metabolites are not only a useful array of natural products but helping plants to acclimatize to stress environments (salinity, drought, heavy metals, pesticides, temperature, etc.).

Severe stress conditions stimulate the production of the reactive oxygen species (ROS) plant cells, and consequently triggering the antioxidant defense systems (enzymatic and non-enzymatic) (Bujor et al., 2015). C. procera aerial parts have a high content of flavonoid and phenolic compounds (Figure 2), this

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| Pathogens       | Solvents          | Antibiotics               |
|----------------|-------------------|---------------------------|
|                | Methanol          | Petroleum ether          | Water         | Ampicillin (17) |
|                | 1.25              | 1.18                      | 0.53          |                |
|                | E>A               | E>A                       | E>A           |                |
| *K. pneumoniae*| 0.97              | 0.91                      | 0.41          | Penicillin (0) |
|                | 0.65              | 0.61                      | 0.27          | Gentamicin (22)|
| *L. monocytoyenes*| E>A              | -                         | -             | Ampicillin (0) |
|                | 1.56              | -                         | -             | Penicillin (8) |
| *E. coli*      | 2.05              | 1.42                      | 0.73          | Ampicillin (11)|
|                | 1.41              | 0.98                      | 0.50          | Penicillin (16)|
|                | 1.07              | 0.74                      | 0.38          | Gentamicin (21)|
|                | 0.98              | 0.68                      | 0.35          | Chloramphenicol (23)|
| *S. typhi*     | E>A               | -                         | E>A           | Ampicillin (0) |
|                | 1.15              | -                         | 0.58          | Penicillin (12)|
|                | 0.55              | -                         | 0.28          | Gentamicin (25)|
|                | 0.63              | -                         | 0.32          | Chloramphenicol (22)|
| *P. aeruginosa*| 1.42              | 1.00                      | 0.90          | Ampicillin (10)|
|                | 1.78              | 1.25                      | 1.13          | Penicillin (8) |
|                | 0.75              | 0.53                      | 0.47          | Gentamicin (19)|
|                | 0.57              | 0.40                      | 0.36          | Chloramphenicol (25)|
| *S. epidermis* | E>A               | -                         | -             | Ampicillin (0) |
|                | E>A               | -                         | -             | Penicillin (0) |
|                | 0.47              | -                         | -             | Gentamicin (31)|
|                | 0.48              | -                         | -             | Chloramphenicol (30)|
| *S. aureus*    | 1.66              | 1.33                      | -             | Ampicillin (13)|
|                | 1.20              | 0.96                      | -             | Penicillin (18)|
|                | 0.80              | 0.64                      | -             | Gentamicin (27)|
|                | 0.77              | 0.62                      | -             | Chloramphenicol (28)|
| *B. subtilis*  | E>A               | E>A                       | E>A           | Ampicillin (0) |
|                | E>A               | E>A                       | E>A           | Penicillin (0) |
|                | 1.75              | 1.03                      | 0.80          | Gentamicin (10)|
|                | 0.92              | 0.54                      | 0.42          | Chloramphenicol (19)|
| *E. cloeae*    | 0.85              | 0.71                      | 0.47          | Ampicillin (17)|
|                | 1.12              | 0.92                      | 0.62          | Penicillin (13)|
|                | 1.04              | 0.86                      | 0.57          | Gentamicin (14)|
|                | 0.63              | 0.52                      | 0.35          | Chloramphenicol (23)|

E: extract; A: antibiotics
E > A and > 1 values indicate extracts have higher effect against bacterial pathogens compared to antibiotics.
E < A and < 1 values indicate antibiotics have higher effect against bacterial pathogens compared to extracts.
Table 3: Phytochemical screening for the active constituents of aerial parts *Calotropis procera*.

| Screening test       | Methanol | Petroleum ether | Aqueous |
|----------------------|----------|-----------------|---------|
| Alkaloids            | ++       | -               | -       |
| Flavonoids           | +++      | -               | +       |
| Saponins             | ++       | +               |         |
| Tannins              | ++       | -               | -       |
| Phenols              | ++       | -               | -       |
| Sterol               | ++       | -               | -       |
| Triterpenoids        | +++      | -               | +       |
| Coumarins            | -        | -               | -       |
| Anthraquinones       | +        | -               | -       |
| Cardiac glycosides   | +++      | +               | -       |

Positive mark (+) indicates the presence of the phytochemical. Negative mark (−) indicates the absence of the phytochemical.

Table 4: Percentage of DPPH radical scavenging activity and IC<sub>50</sub> values of different extracts of *Calotropis procera*

| Concentration μg mL<sup>−1</sup> | % of scavenging activity | Methanol | Petroleum ether | Ethyl acetate | Catechol |
|---------------------------------|--------------------------|----------|-----------------|---------------|----------|
| 1000                            | 55.75±1.69               | 46.55±1.41| 52.34±1.59      | 83.11±2.52    |
| 800                             | 51.07±1.55               | 37.68±1.14| 48.66±1.47      | 80.56±2.44    |
| 600                             | 44.95±1.36               | 31.64±0.96| 41.54±1.26      | 73.54±2.23    |
| 400                             | 30.56±0.93               | 20.27±0.61| 27.15±0.82      | 62.22±1.89    |
| 200                             | 23.99±0.73               | 14.35±0.43| 20.58±0.62      | 48.56±1.47    |
| 100                             | 21.99±0.67               | 10.21±0.31| 18.58±0.56      | 28.33±0.86    |
| LSD<sub>0.05</sub>              | 4.23                     |          |                 |             |
| IC<sub>50</sub> (mg mL<sup>−1</sup>) | 0.81               | 1.09                   | 0.88                   | 0.29                   |

Values are means ± standard error of triplicates. LSD<sub>0.05</sub>: least significant difference at 0.05 probability level.

explains the more antioxidant activity of *C. procera* extracts.

**CONCLUSIONS**

*Calotropis procera* (Oshaar) is an important wild plant, used in traditional medicine for the treatment of many diseases. The present study indicated that this plant has a good inhibitory activity against *Klebsiella pneumonia*, *Escherichia coli* and *Staphylococcus aureus*. Activity index (AI) expressed a reasonable antibacterial activity, which helps in the folkloric usage of medicinal plants for treatment of infectious pathogens. In addition, phytochemicals of *C. procera* can be used as a raw material for production of cheaper pharmaceuticals, pesticides and many more important commercialized products of public use.

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**Conflict of interest**

The authors declare that they have no conflict of interest for this study.

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