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Cytotoxic and Antibacterial Effects of Aqueous Extract of *Calotropis procera* Latex



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ABSTRACT

Calotropis procera (Asclepiadaceae) is one of known important plant for its high medicinal properties. Aqueous latex extract of *Calotropis procera* was investigated for its cytotoxic and antibacterial activities. The onion bulbs (*Allium cepa* L.) were germinated for evaluating the cytotoxic activity with different concentrations of aqueous extract (0, 5, 10, 15, 20 %) for 72 hours in plastic laboratory tubes. The antibacterial activity of latex extract were tested against three bacterial species including (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) using agar diffusion. The results showed that the mitotic index and root growth rate of onion were decreased in comparison to the control. The latex extract significantly inhibited the growth of roots and mitotic activity in a dose- and time-dependent manner. The EC₅₀ value of latex extract was at 10% concentration after 48 hours and the mitotic index value was (11%) at the same concentration. The maximum antibacterial activity was observed in latex extract against *Staphylococcus aureus* at inhibition zone of about (14.3 ± 0.5 mm) at 15 mg concentration and minimum activity was observed in aqueous extract against *Pseudomonas aeruginosa* at inhibition zone of about (8 ± 1.2 mm) at 5 mg concentration.

INTRODUCTION

In past few years, number of microorganisms increased, that are resistant to chemical antibiotics. This situation has become one of the problems, which worry the workers in primary health care.

Resistance of pathogenic microorganisms to chemical antibiotics as reached unacceptable levels in developing countries and that trends show further increases (1).

The natural constituents of plants are derived from any parts of medicinal plants, such as bark, flowers, roots, fruit and seeds. These may contain active components, as safe remedies for ailments of several diseases. (2-4).

The plant extracts possess antimicrobial activities, which may be of importance in therapeutic treatments, whereas a number of studies have been conducted in different countries to prove such efficiencies (1, 2, 3).

Some plant species are belonging to the Euphorbiaceae, Apocynaceae and Asclepidiaceae families produce natural latex.

Calotropis procera is belonging to Asclepiadaceae family. The plant distributed in tropical and subtropical Africa, Asia, America and Middle East (5, 6, 7). In Yemen, it is called Ushar, as the local name. The latex is produced, when cut or broken any parts of this plant.

Phytochemical studies on the aerial parts of the *Calotropis procera* plant showed the presence of alkaloids, cardiac glycosides, tannins, flavonoids, sterols, cardenolides and/or triterpenes (8, 9).

Cytotoxic chemicals and many other beneficial properties make this plant as a golden gift for humankind. Some important chemicals obtained from the leaves and latex of *C. procera* plant (10).

Latex of *Calotropis procera* contains many biologically active compounds. Its latex possesses different biological activities including: anti-inflammatory, analgesic, antitumor, antiviral, antimicrobial, antidiarrheal, hepatoprotective, antiulcer, anthelmintic, insecticidal, antioxidant, antibacterial, and spasmolytic activities (11, 12, 13, 14).

Evaluation of cytotoxic and anti-mitotic of latex of *Calotropis procera* was studied using *Allium cepa* root, its effect was compared with known anticancer drug cyclophosphamide and non-cytotoxic drugs cyproheptadine and aspirin. The growth of roots and mitotic activity was inhibited by dried latex in a dose- and time-dependent manner.

The present study was carried out to evaluate the cytotoxic and antibacterial effect of aqueous extracts of *Calotropis procera* latex.

MATERIALS AND METHODS:

The Plant Materials

The Plant materials were collected from Faculty of Education/saber at Lahej governorate, Yemen, especially in the dryness seasons and identified by Dr. Alhushabi Othman, at the Biology Department, Faculty of Science, University of Aden.

The plant latex collection was gathered by the stem injury using a sterile surgical blade and collected into tubes then stored in a refrigerator till further use.

Preparation of the Plant Extract:

Extraction of latex of *C. procera* was done with water 100 ml of the latex mixed with 200 ml of distilled H₂O, then the mixture left to stand at room temperature for 24 hr. The extract was filtered through double layered muslin cloth and then filtered through Whatman No.1 filter paper. The extract were concentrated to dryness in an oven at 45°C in order to remove the excess solvent and dissolved in Dimethyl Sulfoxide (DMSO) to give a concentration of 100mg/ml and these were kept in refrigerator till further use for antibacterial activity assay (15).

Isolation of Microorganisms

The bacterial specimens isolated from wound infection of patients and included the following organisms: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The processes of bacterial culturing and the identification of bacterial specimens were done at the bacteriological laboratory of Ibn khaldoon Hospital, Lahj Governorate, Yemen.

Cytotoxic assay:

We chosen healthy equal sized onion bulbs and put them for germination in plastic container with distilled water for 24 hours at 22°C until the root length reached about (0.3-0.4 cm). The onion bulbs divided in to two groups. The first treated group germinated in container containing different concentrations of the fresh *Calotropis procera* latex (5, 10, 15, 20 %) for 72 h. The second group of onion bulbs germinated in distilled water container and served as negative control. After 72 h of germination, the roots length was measured using a millimeter ruler. The inhibition percentage of roots growth was compared to the control for each extract and the result used to calculate the EC₅₀ (16). From every onion, five roots were cutting, washed with distilled water and fixed directly in 3:1 alcohol: acetic acid for 24 h. The roots were taking to 1N HCl for hydrolyzing at 60°C for 5 min, and stained by acetocarmine. About 2 to 3 mm root tips were cutting off by a sharp blade and then placed on a clean slide with acetocarmine drop. The slide was covered with coverslip and was squashed the root tip by applying uniform pressure. The slides were examined under the light microscope (17). The number of cells at dividing phase was recorded in each concentration and mitotic index (MI) was determined by the following formula (18):

$$MI = \text{Total number cells in division} / \text{Total number of cells observed} \times 100$$

ANTIMICROBIAL ACTIVITY ASSAY

The Antibacterial activity of the aqueous latex extract of the *Calotropis procera* were individually tested against studied microorganisms using well Agar diffusion test on Mullar Hinton agar (Himedia, India) with different diluted extract concentrations (5 mg, 10 mg and 15 mg). The DMSO solvent was served as a negative control and the Amikacin (30 µg) (Himedia, India) as a positive control for bacteria. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Cytotoxic assay:

The cytotoxic effects of fresh *Calotropis procera* latex were determined by the change rate (%) of the root length and mitotic index (MI).

LD₅₀ was calculated as the concentration, where the growth of root reduced to 50% compared to the root length of control group. The control group change rate (%) was accepted 100%.

The LD₅₀ value was noticed at 10% latex concentration, where the root length reduced from 2.66 ± 0.3 cm (100%) at control group to 1.22 ± 0.19 cm (46 %) after 48 hr (Table 1). The root length gradually decreased in all concentration of latex compared to the root length of control group (2.66 ± 0.3 cm), and the lowest root length (0.88 ± 0.13 cm) was recorded at 15% latex concentration.

The result showed different effect on mitotic index. We observed number differences in mitotic index percentage in all concentration after 24 hr. It was the highest value (21%) in control group and the lowest value (9.2%) was in the treated group at 15% concentration of latex (Table 1).

Allium cepa root tip one of the widely used models to study the cytotoxic and antimutagenic activity of plant extracts or various compounds (17, 19, 20).

According to the results of our study, the fresh latex of *C. procera* showed considerable cytotoxic effect on the root growth of onion. We observed significant decreasing in root length and mitotic index of treated group compared to control group (Table 1).

In previous studies, they reported that the extracts of flowers, leaves, roots and latex of *C. procera* exhibited potent cytotoxic activity (20, 21). The cytotoxic activity of latex of *C. procera* is due to the considerable cardenolide calotropin that present in the latex (22). The latex of *calotropis procera* might be inhibiting growth factor mediated mitogenic signaling pathway (20).

The root extract of *C. procera* was studied on COLO 320 tumor cells, where the extract was produced a strong cytotoxic effect on cells. The dried latex (DL) of plant showed protection against hepatocarcinogenesis in mice. The latex of *C. procera* induced a decrease of cell growth and values of IC₅₀ obtained after 24h of exposure for LP was 88.33µg/ml. The studies demonstrated the cytotoxic activity of LP is due, the inhibition of the synthesis of DNA [23].

Other studies have demonstrated DL (dried latex) significantly inhibited the growth of roots and mitotic activity in *Allium cepa* root meristem and the effect was compared with standard anticancer drug cyclophosphamide and non-cytotoxic drugs cyproheptadine and aspirin [20, 24].

Antibacterial activity assay

The antibacterial activity of the *C. porcera* showed that the aqueous latex extract was effective against all of the tested bacteria. Table (2) shows diameter zones of inhibition of the bacterial growth at different concentration of the aqueous latex extract.

The highest activity were demonstrated against *staphylococcus aureus*, followed by *E. coli* and the lowest activity against *Pseudomonas aeruginosa*, the respective diameter zones of inhibition were 14.3 ± 0.7 , $12. \pm 1.2$, 11.3 ± 0.5 mm for *S. aureus*; and 12 ± 1 , 11.3 ± 0.7 , 10 ± 0.5 mm for *E. coli* respectively by the different concentration aqueous latex extract. The aqueous extract was effective against *staphylococcus aureus* than the other two ones.

In this study, the latex extract exhibited lower antibacterial activity against tested bacterial species compared to the Amikacin (30 µg) antibiotic.

Our results were similar to previous studies of *C. porcera* extract on antibacterial activity. They have suggested, all the aqueous extract fractions of the *C. porcera* plant parts showed complete growth inhibition of all the tested organisms. The antimicrobial activity was evaluated against some of the tested microorganisms (pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and one pathogenic fungus, *Candida albicans*) from the extracts of *Calotropis procera* (25,26).

Table No. (1): Effect of different *Calotropis procera* latex concentrations (%) on *Allium cepa* root growth and mitotic index.

Concentration (%)	Mean root length (cm \pm S.D.) at time (hour):		Change rate (%) at 48 hr.	Mitotic index (%) at 24 hr
	24	48		
Control	2.24 ± 0.29	2.66 ± 0.3	100	21
5	1.62 ± 0.13	1.8 ± 0.23	68	16
10	0.98 ± 0.18	1.22 ± 0.19	46	11
15	0.86 ± 0.15	0.88 ± 0.13	33	9.2

Table No. (2): Antibacterial activity of *Calotropis procera* aqueous latex extract against some bacterial Pathogens

Microorganisms	Zone of inhibition (mm) (Mean \pm SD)			
	aqueous latex extract			Amikacin
	5 mg	10mg	15 mg	30 μ g
<i>Staphylococcus aureus</i>	11.3 \pm 0.5	12. \pm 1.2	14.3 \pm 0.5	17
<i>Pseudomonas aeruginosa</i>	8 \pm 1.2	9 \pm 00	10 \pm 1.6	15
<i>Escherichia coli</i>	10 \pm 0.5	11.3 \pm 0.7	12 \pm 1	16

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