

Original Research Article

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## Antimicrobial Activities Evaluation from the Extracts of Leaves, Flowers, Fruits and Latex of *Calotropis procera* from Taif

Abd-El Aziem Farouk<sup>1,2\*</sup>, N. Thoufeek Ahamed<sup>1</sup>, Othman AlZahrani<sup>1</sup>,  
Khalid H. Alamer<sup>3</sup> and Abdul Aziz Bahobail<sup>3</sup>

<sup>1</sup>Molecular Biotechnology Research Unit, Department of Biotechnology, Faculty of Science, Taif University, Al-Hawiya 888, Kingdom of Saudi Arabia

<sup>2</sup>Tsuno GmbH, Wibiloweg 16, 69123 Heidelberg, Germany

<sup>3</sup>Department of Biology, Faculty of Science, Taif University, Al-Hawiya 888, Kingdom of Saudi Arabia

\*Corresponding author

### ABSTRACT

Antimicrobial activities evaluated from leaves, flowers, fruits and latex of locally obtained *Calotropis procera* from Al-Sharifieya, Taif in different aqueous solutions and solvents such as sterilized Zamzam water and distilled water, ethanol, acetone, isoamyl alcohol and 100 mM Tris-HCl at pH 8.7 by using liquid Nitrogen. *Calotropis procera* was subjected to a standard agar well diffusion method. The ethyl alcohol extract of leaves of *Calotropis procera* showed an activity against both *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 with a concentration of 200 mg/ml clearing zones 10 mm in diameter. The Tris-HCl at pH 8.9 (100 mM) extracts of leaves extracts also showed the highest antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 with same concentrations (diameter 12 mm). The ethyl alcohol extracts of flower and latex showed antimicrobial activity against *Bacillus subtilis* ATCC 6633 with same concentrations (diameter 10 mm and 8 mm). Among the different solvent extracts of *Calotropis procera*, the distilled water and Zamzam water extracts of leaves, flower, fruit, latex and Tris-HCl pH 8.9 (100 mM) extracts of fruits and latex showed activity against *Bacillus subtilis* ATCC6633 with the same concentration (diameter 8 mm). Similar inhibitions (zones of 4-6 mm) for Zamzam and sterilized distilled water extracts of fruit of *Calotropis procera* were seen for the *Escherichia coli* ATCC 8739, *Micrococcus luteus* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853. In isoamyl alcohol and acetone extracts of fruit activity was observed in all four bacteria.

### Keywords

Agar well diffusion method, antimicrobial activities, *Calotropis procera*, medicinal plant extract.

### Article Info

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### Introduction

A small tree like *Calotropis procera* is widely available in Taif, the mild deserted climatic city in the Mecca Province of Saudi Arabia's Sarawat Mountains. The leaves, flowers, latex, roots, and bark of *Calotropis*

*procera* which belongs to the Asclepiadaceae family (Verma *et al.*, 2010) generally grow up to 2.5 - 4 meter (max. 6 meter) high. The evergreen, perennial shrub is a popular medically important plant which deals with many human and animal diseases.

*Calotropis procera* is native to West Africa, Madagascar, the Arabian Peninsula, Southern Asia, India and China with common names: auricula tree, cabbage tree, calotrope, camel tree, dead sea fruit, desert wick in English; dead sea plant, kisher and usar (usher in Arabic). It is also known as sodom apple or French cotton.

Taxonomically, it belongs to the domain: eukaryota; kingdom: plantae; phylum: spermatophyta; subphylum: angiospermae; class: dicotyledonae; order: entiales; family: apocynaceae; genus: *Calotropis*; species: *Calotropis procera*. *Calotropis procera* is very much linked to the ornamental plant *C. gigantea*, which is sometimes misidentified as *Calotropis procera*. It has 10-20 cm long and 4-10 cm wide leaves, multi flowered with 5 sepals that are 4-5 mm long; Fruits are subglobose, ellipsoid or ovoid, with recurved follicles, 7.5-10.0 cm long. Flowering and fruiting take place throughout the year. The plant lives about 12 years (Little *et al.*, 1974; Francis, 2002).

This plant is alexipharmic and cures leprosy, ulcers, and spleen and liver diseases. The juice is anthelmintic, used as a laxative, and cures piles. The flowers are analgesic, astringent and cure inflammations as well as tumors. Mascolo *et al.*, 1988 reported activities of flower extracts against swelling and fever in rats, and also antimicrobial activities. *Calotropis procera* is used for diarrhea, sinus fistula, and skin disease (Alikhan, 2005; Raghubir, 1999). The leaves are used as an antidote for snake bite, sinus fistula, rheumatism, mumps, burn injuries, and body pain. The leaves of *Calotropis procera* are also used to treat jaundice. The flower contains bioactive compounds such as sterols, flavonoids, calotropin, saponins, tannins, phenols, coumarins, polysaccharides and enzymes (Rama, 2012). The spongy fruits consist of brown seeds

which also have antimicrobial activity. The latex of *Calotropis procera* is used in conventional medicine as an antisyphilitic, purgative, and antidontalgic agent and as a cure for verrucas. Extracts from latex, leaves and flowers in Morocco had more effect on yeasts than on fungi (Larhsini *et al.*, 1997). In the Unani system of medicine, *Calotropis procera* is used in treatment for scabies, ringworm of the scalp, piles, asthma, liver, leprosy, and spleen enlargement, and dropsy. Different parts of *Calotropis procera* are used in several Ayurvedic systems of medicinal preparations (Murti, 2010). Extracts from *Calotropis procera* latex have larvicidal activity against mosquitoes (Markouk *et al.*, 2000). Activities against plant pathogenic fungi have also been reported (Singh *et al.*, 1996, Shivpuri *et al.*, 1997). *C. procera*'s usages were also reported (Agharkar, 1991; Ansari, 1999; Dewan *et al.*, 2000; Markouk *et al.*, 2000; Samvatsar, 2000; Sharma *et al.*, 2011). The milky latex is used in traditional medicine to cure skin infections, poison, ulcer, enlargement of spleen, liver, abdominal glands, colic piles, worms and different inflammatory diseases (Lima-Filho *et al.*, 2010). The latex had mild toxic effects on heart, liver and kidneys; that included multi-focal coagulation necrosis of cardiac fibers and vacuolized hepatocytes (Magalhaes *et al.*, 2010).

Antimicrobial activity of *Calotropis procera* was reported earlier (Jain *et al.*, 1996 and Kareem *et al.*, 2008.). Different proteins like laticifer (Souza *et al.*, 2011) and osmotin from the latex of *Calotropis procera* reportedly exhibit potent anti-fungal, antimycoplasmal (Muraina *et al.*, 2010), anti-inflammatory properties (Lima-Filho *et al.*, 2010, Kumar, 2011). Protective effects of proteins derived from the latex of *Calotropis procera* work against inflammatory hyperalgesia in monoarthritic

rats and exhibit anticancer properties (Morsy *et al.*, 2001; Shahi *et al.*, 2010; Ali El-Rabaa 2010; Oliveira *et al.*, 2010). In vitro antimicrobial, antifungal and antiviral screening revealed that the ethyl acetate extracts were effective in suppressing the bacterial pathogens *Pseudomonas aeruginosa*, reported by Subramanian *et al.* *Calotropis procera* is well known for its ability to produce several biologically active compounds (Silva, 2010). The plant parts have exhibited antimicrobial (Ishnava, 2011), antifungal (DeFreitas, 2011), antiviral (Oliveira, 2010), anticancer (Silva, 2010), anti-inflammation (Kumar, 2011), antioxidant (Mst Nazma *et al.*, 2008) and wound healing properties (Romar, 2012, Yesmin *et al.*, 2008). It is emetic (Njama, 2009) and has an anti-inflammatory properties (Soneera, 2005).

In our Lab, the five bacterial strains used in the antimicrobial assay are 1) *gram*-positive bacterium *Bacillus subtilis* (hay bacillus or grass bacillus) (Madigan, 2005) allowing the organism to resist intense environmental conditions; 2) *Micrococcus luteus* (family micrococcaeae), a gram-positive, spherical, saprotrophic bacterium (Madigan, 2005) present in normal flora of the mammalian skin; 3) *Escherichia coli*, a gram-negative, rod-shaped bacterium normally harmless, found in the lower intestine of warm-blooded organisms (Russo, 2003); 4) *Pseudomonas aeruginosa*, (family pseudomonadaceae) a gram-negative, aerobic rod, cocco-bacillus bacterium is commonly seen in soil and water (Lederberg *et al.*, 2000). It is pathogenic to plants found on the surfaces of plants and animals. It causes urinary tract infections, bone & joint infections, gastrointestinal infections, soft tissue and respiratory system infections, dermatitis and a variety of systemic infections, it is also found mainly in patients with brutal burns, with cancer, and sufferers

of immune suppression; 5) *Candida albicans*, a diploid fungus found as both yeast and filamentous cells, causing oral and genital infections. (Ryan, 2004; Enfert, 2007).

This project study on the parts of *Calotropis procera* i.e. “Antimicrobial Activities Evaluation from the Extracts of Leaves, Flowers, Fruits and Latex of *Calotropis procera*” was conducted at Molecular Biotechnology Research Unit, Department of Biotechnology at Taif University.

### Materials and Methods

The *Calotropis procera* parts used in this experiment were collected from *Al-Sharifieya, Taif* in early morning hours. The fresh leaves and flowerers were found with buds and open ones. All flowers were kept in fridge initially for 2 hours to avoid wilting. *Calotropis procera* leaves, flowers, & fruit were weighed individually to 200 mg. The latex (milk) was extracted directly from the plant aseptically. The selected leaves were 6-15cm long and 4.5-8cm broad, broadly ovate or ovate-oblong in shape, elliptical, pubescent when young and glabrous on both sides on maturity.

The *Zamzam* water was obtained from the bottling factory under the Saudi Ministry of “King Abdullah Bin Abdul-Aziz *Zamzam* Project” distribution station in Kuday, Mekkah. The clear, colourless and odourless *Zamzam* water used in this study has a distinct taste due to its pH of 7.9–8.0, (slightly alkaline). It contains Sodium 133mg/l; Calcium 96mg/l; Magnesium 38.88mg/l; Potassium 43.3mg/l; Bicarbonate 195.4mg/l; Chloride 163.3mg/l; Fluoride 0.72mg/l; Nitrate 124.8 mg/l; Sulfate 124.0 mg/l and a total dissolved alkalinity of 835 mg/l (Nour Al Zuhair *et al.*, 2010; Shomar, 2012).

The freshly obtained *Calotropis procera* leaves, flowers & fruit were cleaned with sterilized distilled water and ethyl alcohol. They were taken in mortar and pestle, the liquid nitrogen was poured and homogenized into a fine powder in order to increase its surface area to facilitate the extraction procedure. Fig. 1 shows the flow-chart of the methodology.

The bacterial strains used were *Bacillus subtilis* ATCC 6633; *Candida albicans* ATCC 10231; *Escherichia coli* ATCC 8739; *Micrococcus luteus* ATCC 9341; *Pseudomonas aeruginosa* ATCC 27853 Note: (ATCC: American Type Culture Collection).

The solvents of extraction were *Zamzam* water; distilled water; ethyl alcohol; isoamyl alcohol; acetone and Tris-HCl at pH 8.7, (100 mM). The solvents sterilized water and *Zamzam* water were prepared by autoclaving at 121°C at 15 psig for 20 minutes. The ethyl alcohol, isoamyl alcohol, acetone were purchased from Loba Chemie Pvt. Ltd., Mumbai and Tris-HCl at PH8.7 (100 mM) was prepared in our lab. The chemicals used were liquid nitrogen; nutrient agar; nutrient broth; NaOH was also purchased from Loba Chemie Pvt. Ltd., Mumbai.

An overview of the entire experiment is shown in Figure 1. The water, acetone, ethyl alcohol, isoamyl alcohol and 100 mM Tris-HCl, pH8.7) were used for extraction and the 1.0 ml of extract collected were kept in a shaking incubator at 30°C for 2 hrs.

The *Calotropis procera* leaves, flowers, fruits & latex containing bioactive components were subjected to extraction procedures. 200 mg of the powdered material was dissolved in 1.5 ml of each of the solvents. The organic solvents have a high polarity and were able to dissolve a big amount of the compounds.

The powdered material (200mg) and latex (200mg) were extracted with 1.5 ml of corresponding solvents (The sterilized *Zamzam* water, sterilized distilled water, acetone, ethyl alcohol and 100 mM Tris-HCl, pH8.7) were used for extraction and the 1.0 ml of extract collected was kept in a shaking incubator at 30°C for 2 hours. The organic solvents and water extract was filtered and evaporated until dryness. The extract was stored at 4°C until further use.

The individual fractions were then centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the supernatant was kept at -20°C. Each extract was prepared and named.

Four parts each of 200 mg of *Calotropis procera* was dissolved in six corresponding solvents i.e. a total of 24 samples was used for the study of “Antimicrobial Activities Evaluation from the Extracts of Leaves, Flowers, Fruits and Latex of *Calotropis procera*”. After extraction the tubes were kept in the shaker at 150 rpm at 30°C for 2 hours and then kept in freezer at -80°C.

The agar plates were made by taking 28 grams of nutrient agar obtained from Himedia Lab Pvt. Ltd, Mumbai, India and dissolved in 1l of sterilized distilled deionized water. The composition of nutrient agar was Hivveg peptone 5.00 grams/liter; Hivveg. extract 1.50 grams/liter; yeast extracts 1.50 grams/liter; sodium chloride 5.00 grams/liter and agar 15.00 grams/liter. Final pH (at 25°C) was 7.4. The solutes were shaken until they dissolved and the pH was adjusted to 7.4 using sterilized 1N NaOH (8ml). The volume was adjusted to 1L with deionized water. The solutes were sterilized by autoclaving at 121°C for 20 minutes at 15 psi (1.05kg/cm) on liquid cycle. Autoclaved medium was swirled gently for even distribution of the melted agar in the solution and allowed to cool to



50 to 60°C. Under sterile conditions 20 ml of this medium was poured on to 90 mm Petri dishes and again allowed to cool. After the medium sets completely, inverted Petri dishes were stored at 4°C and removed from storage 1-2 hours prior to use (Sambrook, 2001).

The five bacterial strains were maintained on nutrient agar and freshly prepared sub-cultures in nutrient broth were used during this project. This was done by transferring two or three colonies into a bottle containing 20 ml of liquid nutrient broth medium and grown for 24 hours (or overnight) at 37°C and a small aliquot was poured on plates and dried.

The standard agar-well diffusion method (Collins *et al.*, 1995) was employed to determine the antimicrobial activities for both acetonic and aqueous *Calotropis procera* extracts. The agar was cooled to 50-60°C before adding any thermo-labile substances. The suspension of the bacterial cultures was covered wholly on the agar plates and allowed to dry. Then, in the nutrient agar 18 or 19 wells (6 mm diameter) were made on each plate using sterile yellow tip. Following this, 50µl of the test solution i.e. the supernatant of *Calotropis procera* extract were added inside the laminar flow cabinet for 15-20 minutes to allow the solutions in the wells to diffuse. The agar plates were then inverted and incubated for 24 hours at 37°C. After incubation, clear areas in the region of the wells containing antimicrobial compounds appeared. This diameter of the clear area (called the inhibition zones) around the wells were measured and recorded. Antimicrobial activities of each solvent extract were expressed in terms of average diameter of the inhibition zone (evaluated in milliliter). Each *Calotropis procera* extract was tested in the same manner. The concentration and

solvents that give the optimum result were identified.

## Results and Discussion

Antimicrobial activity of leaves extracts (200 mg/ml) of *Calotropis procera* against different types of pathogenic bacteria: The distilled water and Zamzam water extracts of leaves extracts of *Calotropis procera* showed antimicrobial activity against *Bacillus subtilis* ATCC 6633, the inhibition zones were measured to be 8 mm in their diameter. The water extracts of leaves extracts of *Calotropis procera* showed an almost nil antimicrobial activity in *Escherichia coli* ATCC8739 6633, *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 27853. The ethyl alcohol extracts of leaves extracts of *Calotropis procera* showed the highest antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 with clearing zones 10mm in diameter whereas no antimicrobial activity was measured against *Escherichia coli* ATCC 8739. The isoamyl alcohol extracts of leaves extracts of *Calotropis procera* showed no antimicrobial activity in *Candida albicans* ATCC 10231 and in the other four bacteria's showed activity with clearing zones from 6 to 8 mm in diameter. No antimicrobial activity was measured against *Escherichia coli* ATCC 8739 with distilled water, Zamzam water and ethyl alcohol extracts of leaves extracts of *Calotropis procera*. The acetone extracts of leaves extracts of *Calotropis procera* showed antimicrobial activity in all microorganisms with clearing zones from 4 to 8 mm in diameter whereas no antimicrobial activity was measured against *Candida albicans* ATCC 10231. The Tris-HCl at pH 8.9 (100 mM) extracts of leaves extracts of *Calotropis procera* showed no antimicrobial activity in *Bacillus*

*subtilis* ATCC6633 and *Candida albicans* ATCC 10231 whereas equal activity in *Escherichia coli* ATCC 8739 and *Micrococcus luteus* ATCC 9341 (6 mm). (Table1).

Antimicrobial activity of flower extracts (200 mg/ml) of *Calotropis procera* against different types of pathogenic bacteria: The Zamzam water and distilled water extracts of flower extracts of *Calotropis procera* showed antimicrobial activity against *Bacillus subtilis* ATCC 6633, the inhibition zones were measured to be 8 mm in their diameter and nil antimicrobial activity in *Candida albicans* ATCC. The *Escherichia coli* ATCC 8739, *Micrococcus luteus* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853 showed similar inhibitions zones from 4 to 6 mm for both solvents extracts of flower extracts of *Calotropis procera*. Like the leaf extracts, the ethyl alcohol extracts of flower extracts of *Calotropis procera* showed antimicrobial activity against *Bacillus subtilis* ATCC6633 with clearing zone each with 10 mm. in their diameter. Also no antimicrobial activity was measured against *Candida albicans* ATCC 10231. *Pseudomonas aeruginosa* ATCC 27853 and *Micrococcus luteus* ATCC 9341 showed similar activity i.e. 6 mm. The distilled water, ethyl alcohol, isoamyl alcohol and acetone extracts of flower extracts of *Calotropis procera* showed almost equal antimicrobial activity in *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with inhibitions zones of 6 mm whereas no antimicrobial activity against *Candida albicans* ATCC 10231. Though the Tris-HCl extracts of flower showed antimicrobial activity with inhibition zones measured 8 mm in their diameter against *Escherichia coli* ATCC 25922 and *Micrococcus luteus* ATCC 9341, the other three bacteria

(*Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 27853) showed nil activity (Table1).

Antimicrobial activity of fruit extracts (200 mg/ml) of *Calotropis procera* against different types of pathogenic bacteria: All the five solvent extracts of fruit extracts of *Calotropis procera* showed antimicrobial activity against *Bacillus subtilis* ATCC6633, the inhibition zones were measured as 8 mm in their diameter except Tris-HCl solvent (6 mm) and nil activity against *Candida albicans* ATCC 10231. The Zamzam water, water, ethyl alcohol, isoamyl alcohol and acetone extracts of fruit extracts of *Calotropis procera* showed almost equal antimicrobial activity in *Micrococcus luteus* ATCC 9341, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and the inhibition zones were measured positively as 6 mm clearances in diameter with no changes observed with Tris-HCl as solvent (Table1).

Antimicrobial activity of latex extracts (200 mg/ml) of *Calotropis procera* against different types of pathogenic bacteria: The Zamzam water, distilled water, ethyl alcohol and amyl alcohol extracts of latex showed antimicrobial activity against *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853, the inhibition zones were measured from 6 to 8 mm in diameter. The antimicrobial activity of latex extracts of *Calotropis procera* against *Bacillus subtilis* ATCC 6633 with Tris-HCl with the inhibition zones were measured as 14 mm in their diameter. All the solvent extracts of latex of *Calotropis procera* had no antimicrobial activity against *Candida albicans* ATCC 10231 was shown (Table1). Antimicrobial activities (inhibition zones mm/ml) of different

microorganisms were evaluated against standard oxytetracycline was shown in Table 2. The inhibition zones indicated with the numbers and their corresponding order of extracts of leaves, flowers, fruits and latex of *Calotropis procera* extracted with 6 Different solvents used were shown in Table 3. The order of extracts were denoted as LD, LZ, LE, LA, LI, LT where L represented Leaf extract; FD, FZ, FE, FA, FI, FT where F Flower extract; BD, BZ, BE, BA, BI, BT where B represented Fruit extract and MD, MZ, ME, MA, MI, MT where M represented Latex extract; The Solvents were denoted as Distilled water (D), Zamzam water (Z), ethanol (E), acetone (A), isoamyl alcohol (I) and Tris-HCl (T).

In this study of the leaves, flowers, fruits and latex extracts of *Calotropis procera*, the evaluation of the antimicrobial activities of the extracts of *Calotropis procera* against above mentioned five microorganisms was carried out by standard agar well diffusion clearing zone method showed different results. The ethyl alcohol extract of leaves of *Calotropis procera* had shown considerable activity against both *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 in concentrations of 200 mg/ml with clearing zones 10 mm. in their diameter. The ethanolic extracts of leaves and flowers showed significant activity was earlier reported [13]. The Tris-HCl at pH 8.9 (100 mM) leaves extracts of *Calotropis procera* also showed significant antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 with the same concentration (diameter 12 mm). The previous antimicrobial studies with inherent isolated bacteria from sea cucumber documented moderate antimicrobial activity against *Pseudomonas aeruginosa*. (Farouk *et al.*, 2007) and also the ethyl alcohol extracts of rose petals also showed

antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 was reported Farouk *et al.*, 2014).

Wider studies pertaining to the use of plants (about 14 mm as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes). The results obtained also showed that the ethyl alcohol extracts of flower and latex of *Calotropis procera* showed moderate antimicrobial activity against *Bacillus subtilis* ATCC6633 in same concentrations (diameter 10 mm. and 8 mm.). The ethanolic extracts of roots of *Calotropis procera* has potential activity against the tested *pseudomonas aeruginosa* was also already reported (Swapnali *et al.*, 2012). In isoamyl alcohol and acetone extracts of fruit showed an activity in all four bacteria except *Candida albicans* ATCC 10231. The alcoholic and acetone extracts of the leaves and stem extracts of from *Eurycoma longifolia* (Tongkat Ali) and *Labisia pumila* (Kacip Fatimah) and their purified peptides were active against both Gram-positive and Gram-negative bacteria except against 2 strains of Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) (Farouk, *et al.*, 2007 & 2008).

Though the distilled water and Zamzam water extracts of leaves, flower, fruit, latex and Tris-HCl at pH 8.9 (100 mM) extracts of fruit and latex of *Calotropis procera* had shown an activity against *Bacillus subtilis* ATCC6633 in same concentrations (diameter 8 mm), no activity was found for Tris-HCl at pH 8.9 (100mM) extracts of leaves and flowers of *Calotropis procera* against *Bacillus subtilis* ATCC6633. Water extracts of leaves, flower and fruit showed significant activity against both Gram positive and Gram negative strains as reported earlier (Yesmin *et al.*, 2008, Mainasara *et al.*, 2011). The bacteria

isolated from flowers of *Rosa damascena* cv. Taifi also showed antimicrobial activity and enzymatic activities were reported by Farouk *et al.*, (Farouk *et al.*, 2014).

The acetonic extracts of leaves, flowers, fruits and latex extracts of *Calotropis procera* had varying inhibitory effects (6 to

8 mm) on most of the four tested microorganisms as represented in Table 1 except *Candida albicans* ATCC 10231 with nil antimicrobial activity. The acetonic extracts of various leaves showed considerable activity against tested microorganisms (Al-Kahtani *et al.*, 2000).

**Table.1** Antimicrobial activities (inhibition zones mm/ml) of leaves, flowers, fruits and latex of *Calotropis procera* extracted with 6 Different solvents VS 5 microorganisms.

Bacteria	<i>Calotropis procera</i> (Parts)	Antimicrobial activities of <i>Calotropis procera</i> in 200 mg/ml Inhibition zones (mm) with different solvents.					
		Distilled water (mm)	Zamza m (mm)	Ethyl alcohol 1 (mm)	Acetone (mm)	Isoamyl alcohol (mm)	Tris-HCl (mm)
<i>Bacillus subtilis</i>	Leaves	6±2	6±2	6±2	6±2	6±2	--
<i>Escherichia coli</i>		4±2	4±2	4±2	4±2	4±2	4±2
<i>Candida albicans</i>		--	--	8±2	--	--	--
<i>Micrococcus leutus</i>		4±2	4±2	4±2	4±2	4±2	6±2
<i>Pseudomonas aeruginosa</i>		2±2	4±2	8±2	4±2	2±2	2±2
<i>Bacillus subtilis</i>	Flowers	6±2	6±2	8±2	6±2	6±2	--
<i>Escherichia coli</i>		4±2	4±2	4±2	4±2	4±2	4±2
<i>Candida albicans</i>		--	--	--	--	--	--
<i>Micrococcus leutus</i>		4±2	4±2	4±2	4±2	4±2	4±2
<i>Pseudomonas asaeruginosa</i>		4±2	2±2	4±2	4±2	--	--
<i>Bacillus subtilis</i>	Fruit	6±2	6±2	6±2	6±2	6±2	4±2
<i>Escherichia coli</i>		4±2	4±2	4±2	4±2	4±2	--
<i>Candida albicans</i>		--	--	--	--	--	--
<i>Micrococcus leutus</i>		4±2	4±2	4±2	--	4±2	4±2
<i>Pseudomonas aeruginosa</i>		4±2	4±2	4±2	4±2	4±2	--
<i>Bacillus subtilis</i>	latex	6±2	6±2	6±2	6±2	6±2	--
<i>Escherichia coli</i>		4±2	4±2	4±2	4±2	4±2	4±2
<i>Candida albicans</i>		--	--	--	--	--	--
<i>Micrococcus leutus</i>		--	--	--	4±2	--	--
<i>Pseudomonas aeruginosa</i>		--	4±2	--	4±2	4±2	8±2



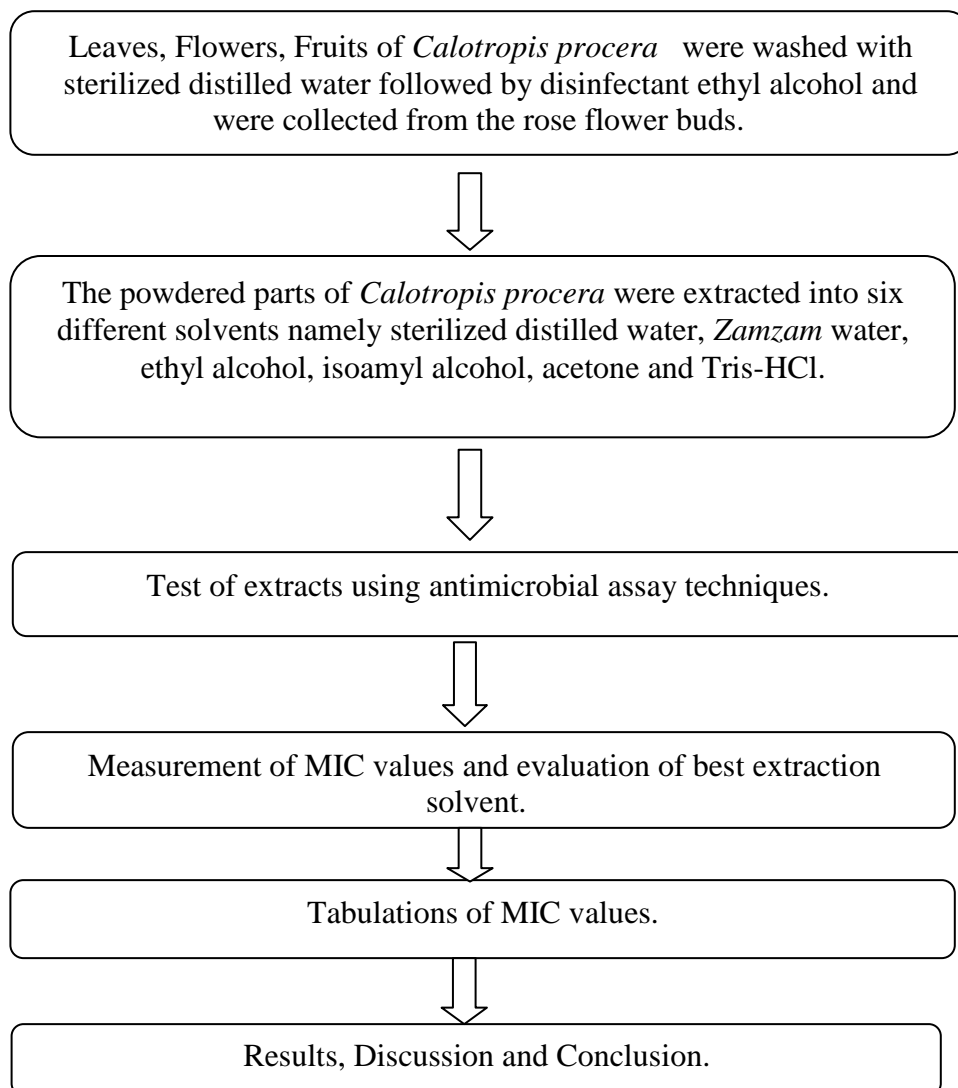
**Table.2** Antimicrobial activities (inhibition zones mm/ml) of different microorganisms VS oxytetracycline

Standard ( $\mu\text{g/ml}$ )	<i>Bacillus subtilis</i> (mm)	<i>Escherichia coli</i> (mm)	<i>Candida albicans</i> (mm)	<i>Micrococcus Leutus</i> (mm)	<i>Pseudomonas aeruginosa</i> (mm)
oxytetracycline at 1.0 $\mu\text{g/ml}$	3.5 $\pm$ 0.2	6 $\pm$ 0.1	6.5 $\pm$ 0.2	4 $\pm$ 0.2	2.9 $\pm$ 0.1

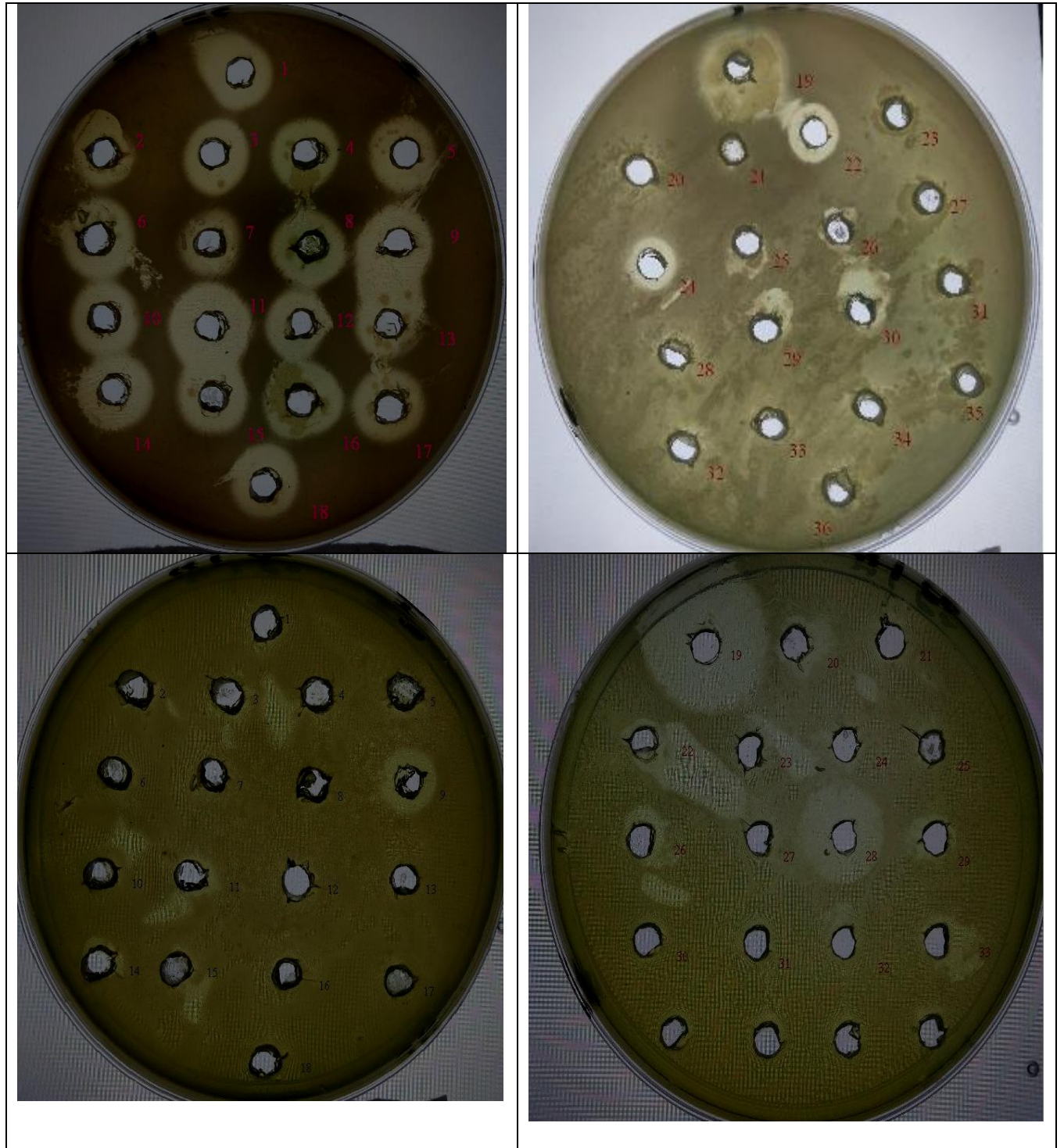
**Table.3** Inhibition zones indicated with the numbers (Fig 2 & Fig 3) and their corresponding order of extracts of leaves, flowers, fruits and latex of *Calotropis procera* extracted with 6 Different solvents used

Inhibition zones indicated with the numbers	Petri dish BS1 containing <i>Bacillus Subtilis</i> in Fig 2 OR	Second Petri dish BS2 containing <i>Bacillus Subtilis</i> in Fig2 OR
	Petri dish PA1 containing <i>P.aeruginosa</i> in Fig 2 OR	Second Petri dish PA2 containing <i>P.aeruginosa</i> in Fig2 OR
	Petri dish EC1 containing <i>Escherichia coli</i> in Fig 3 OR	Second Petri dish EC 2 containing <i>Escherichia coli</i> in Fig 3 OR
	Petri dish ML1 containing <i>Micrococcus leutus</i> in Fig 3 OR	Second Petri dish ML2 containing <i>Micrococcus leutus</i> in Fig 3 OR
	Petri dish CA1 containing <i>Candida albicans</i> in Fig 3 OR	Second Petri dish CA2 containing <i>Candida albicans</i> in Fig 3
<b>2 to 7</b>	L (Order LD, LZ, LE, LA , LI, LT)	Nil
<b>8 to 13</b>	F (Order FD, FZ, FE, FA , FI, FT);	Nil
<b>14 to 16</b>	B (Order BD, BZ, BE);	Nil
<b>17</b>	Ethyl alcohol	Nil
<b>18</b>	Acetone	Nil
<b>20 to 22</b>	Nil	B (order: BA, BI, BT)
<b>23 to 28</b>	Nil	M (order MD MZ,ME,MA,MI,MT)
<b>29</b>	Nil	Ethyl alcohol
<b>30</b>	Nil	Acetone

**Fig.1** Flowchart of the methodology

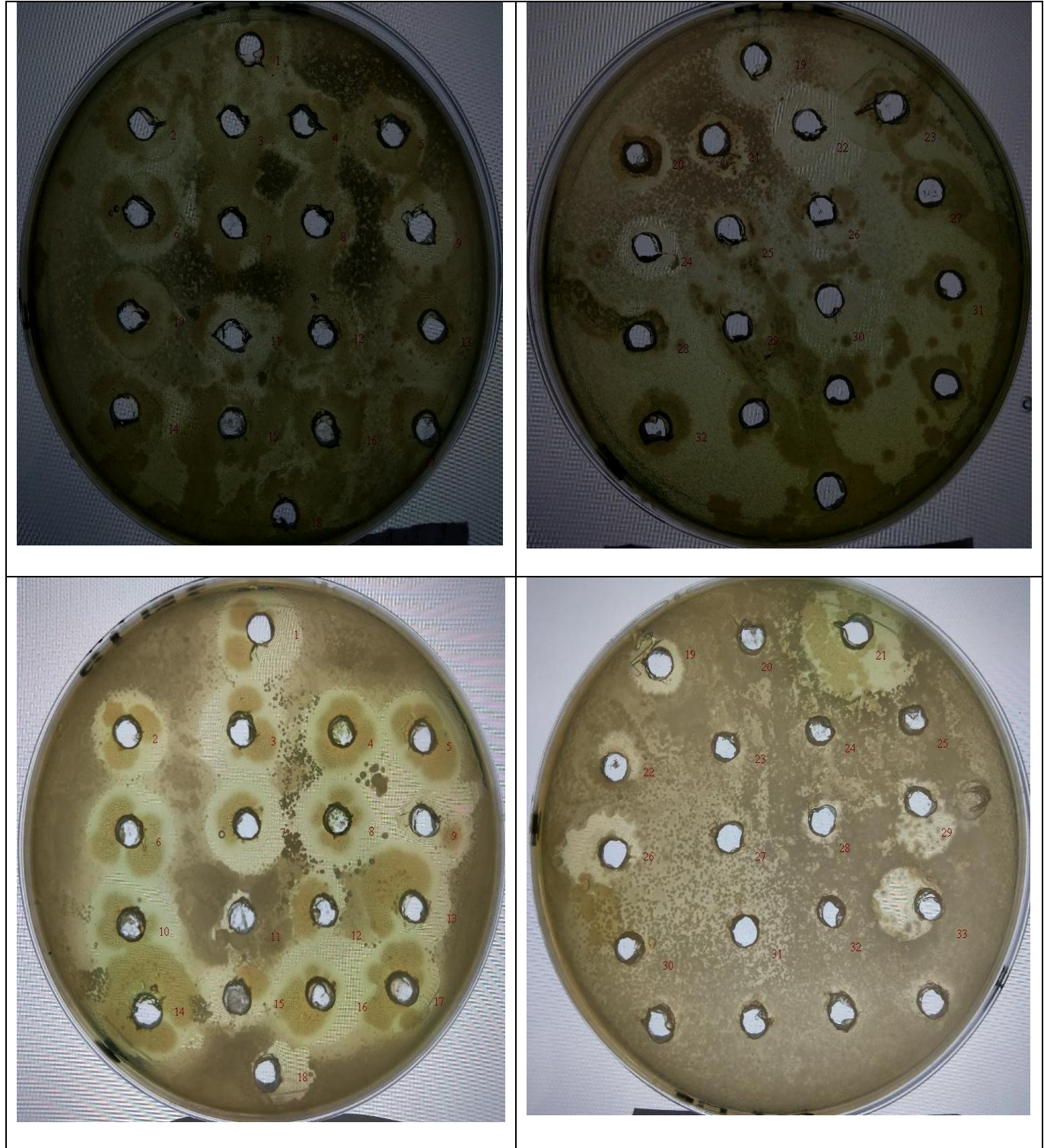


**Fig.2** The antimicrobial activity of leaves, flowers , fruits, latex of *Calotropis procerain* different aqueous solutions and solvents such as sterilized Zamzam water, distilled water, ethanol, acetone , isoamyl alcohol and 100 mM Tris-HCl at pH 8.7 using liquid Nitrogen with *Bacillus subtilis* and *Pseudomonas aeruginosa*.

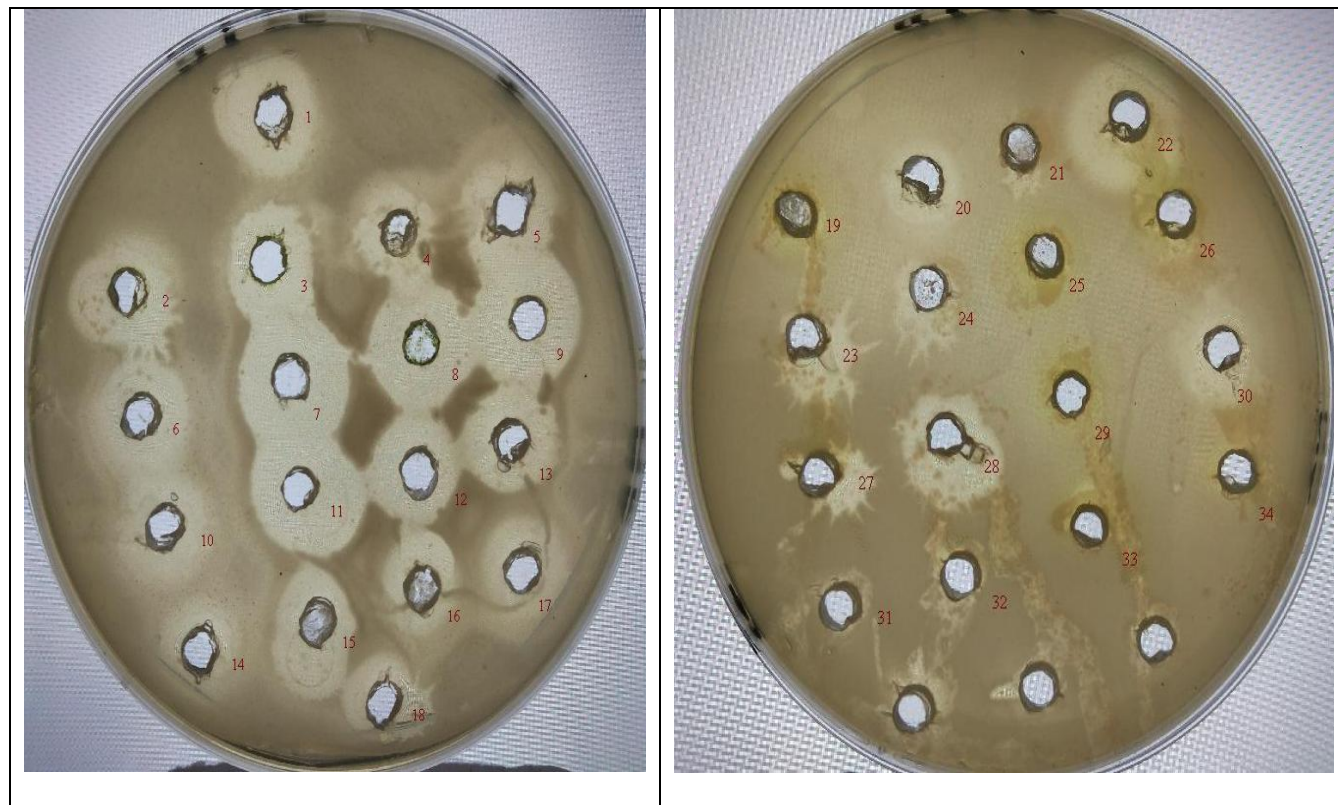




**Fig.3** The antimicrobial activity of leaves, flowers, fruits, latex of *Calotropis procera* in different aqueous solutions and solvents such as sterilized Zamzam water, distilled water, ethanol, acetone, isoamyl alcohol and 100 mM Tris-HCl at pH 8.7 using liquid Nitrogen with *Escherichia coli* and *Micrococcus leutus*



**Fig.4** The antimicrobial activity of leaves, flowers, fruits, latex of *Calotropis procera* in different aqueous solutions and solvents such as sterilized Zamzam water, distilled water, ethanol, acetone, isoamyl alcohol and 100 mM Tris-HCl at pH 8.7 using liquid Nitrogen with *Candida albicans*



Inhibitions zones from 4 to 6 mm for Zamzam and sterilized distilled water extracts of fruit of *Calotropis procera* were observed for the *Escherichia coli* ATCC 8739, *Micrococcus luteus* ATCC 9341 and *Pseudomonas aeruginosa* ATCC 27853. Water and ethanolic extracts of fruit showed antimicrobial activity against pseudomonas was also reported (Mainasara *et al.*, 2011). In isoamyl alcohol and acetone extracts of fruit of *Calotropis procera*, antimicrobial activity found in all four bacteria except *Candida albicans* ATCC 10231.

The Tris-HCl at pH 8.9 (100 mM) extracts of latex extracts of *Calotropis procera* showed considerable (14 mm) antimicrobial activity against *Bacillus subtilis* ATCC 6633, moderate (8 mm) antimicrobial

activity against *Micrococcus luteus* ATCC 9341 and good (6 mm) antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Also the Tris-HCl pH 8.9 (100 mM) extracts of leaves of *Calotropis procera* had shown best result (10 mm) which is similar to the ethyl alcohol extract of leaves extracts of *Calotropis procera* against *Pseudomonas aeruginosa* ATCC 27853.

200mg/ml fruit extracts of *Calotropis procera* in all solvents showed activity against tested organisms except nil activity in *Candida albicans* ATCC 10231. The Tris-HCl at pH 8.9 (100 mM) extracts of fruit extracts of *Calotropis procera* showed activity for *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341.



In this study, the ethyl alcohol and acetone extracts of leaves extracts of *Calotropis procera* showed excellent results against *Pseudomonas aeruginosa* and *Candida albicans* ATCC 10231. The ethyl alcohol extracts of flower and latex showed moderate antimicrobial activity against *Bacillus subtilis* ATCC6633 in same concentrations (diameter 10 mm. and 8 mm.).

The distilled water and Zamzam water extracts of leaves, flower, fruit, latex and Tris-HCl at pH 8.9 (100 mM) had shown activity against *Bacillus subtilis* ATCC6633 in same concentrations (diameter from 8 to 12 mm). In isoamyl alcohol and acetone, extracts of fruit showed activity in all four bacteria except *Candida albicans* ATCC 10231. The resourceful *Calotropis procera* which contains rich antimicrobial sources needs to be further studied in order to confirm the purification of the antimicrobial compounds. The *Calotropis procera* should be investigated to better understand their antimicrobial properties, safety and efficiency. The present study revealed that there is a possibility for developing novel eco-friendly antimicrobial or antiviral drugs from *Calotropis procera* against aquatic vital pathogens. In this study, different solvent extracts proved that *Calotropis procera* has medicinal values.

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