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ANTIMICROBIAL, CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF THE EXUDATE OF *CALOTROPIS GIGANTEA*

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ABSTRACT

Plant is one of the important source of drugs that's why we have done this project work to find out some biological activities of the plant exudates of *Calotropis gigantea* where antimicrobial, cytotoxic and antioxidant activity were analyzed. To test of antimicrobial activity, disc diffusion method has been followed. The exudate of the plant has shown antimicrobial activity against four microorganisms such as *Escherichia coli* (15mm), *Vibrio mimicus* (15mm), *Vibrio parahemolytics* (15mm) and *Staphylococcus aureus* (9mm). The exudates of the plant has shown cytotoxic activity which was done by following the brine shrimp lethality bioassay method. The result of the test has demonstrated good cytotoxic activity against Brine Shrimp Nauplii with a LC_{50} value of 12.66 µg ⁄ml. The exudate of the plant has demonstrated good antioxidant activity with a LC_{50} value of 3.301 µg ⁄ml..So from the project we can say that further investigation should be carried out to get the active principles.

INTRODUCTION: A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs.

The plants possess therapeutic properties or exert Beneficial Pharmacological effects on the animal body are generally designated as "Medicinal Plants '' .It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties.

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by export significant role of an economy of a country.

Plants contain useful constituents, including vitamins, minerals, proteins, carbohydrates, essential oils, tannins, alkaloids, bitters and flavonoids. Each part of the plant contains distinct properties and is used for different purposes.

In this project I worked on a medicinal plant named by *Calotropis gigantea*, commonly known as akando in Bangladesh .My experiment was designed to search some biological activities of this plant such as the antimicrobial activity, the cytotoxic effect and the anti antioxidant effect.

Calotropis gigantea R.Br. Asclepiadaceae, commonly known as milkweed or swallow-wort, is a common wasteland weed [1].

Calotropis belongs to Asclepiadaceae or Milkweed or Ak family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Other familiar plants of *Calotropis* are Milk weed or Silk weed (*Asclepias syriaca* L.). Native to India [2], *Calotropis* grows wild up to 900 meters throughout the country on a variety of soils in different climates, sometimes where nothing else grows ditty, the medicinal plant is botanically called Asclepias Gigantea.

Aditya thrives on a variety of soils that is found throughout the country up to an altitude of 1000 metres. This plant can be found from Punjab and Rajasthan to West Bengal and Assam southwards through peninsular India and Bangladesh. It grows in open habitats such as sandy riverbanks, roadsides, cultivated fields, grazing lands and other degraded regions throughout the drier parts of its range. In Bengali, Aditya plant is known as Akand, Gurakand, Akanda and Swe-Takand; while in English it is known as Bowstring Hemp, Madar, Gigantic Swallowwort and Milkweed.

Aditya plant is known Akado in Gujarat; Ag, Ak, Akand, Akaua in Hindi, in Kannada it is called Arka, Lekkedagide and Yekkadagida; while in Konkani, Aditya is known as Rhui. This medicinal plant is known as Bukam, Dinesam, Erikku and Erukka in Malayalam, Akado, Akondo, Bikhortono, Kotuki and Uruk in Oriya. Aditya is extensively used in Ayurvedic practices. This branched, hardy; erect evergreen shrub rise upto 5 metres height.

The stems of Aditya plant are woody with yellowish-white bark; young stems and branches that are covered with soft, loosely appressed, whitish, waxy or sometimes dusty pubescence. Bark is thick, light yellow or ash-grey, corky and deeply fissured. The leaves of Aditya plant are fleshy, obovate or obovate-oblong, apex acute and rarely rounded. The base of the leaves is cordate, 6-20 cms long and 3-8 cms wide, glaucous green and smooth. The petioles of Aditya plant are 0.3-2 cms long and the flowers are lilac, pale rose or purple in color. However, sometimes they are also seen in light greenish-yellow or white shades with spreading corolla lobes, growing on the axillary pedunculate corymbs.

The flowers are often seen throughout the year but most commonly from November to March in central India. The fruits of Aditya plants are single or paired, hard, re-curved and 7-10 cm long. They mature mainly between February and August. Numerous seeds are enclosed in the Aditya plant that is broadly ovate, flattened and brown in color. The 2.5-3.2 cms long, seeds have white tuft of silky hair (coma) at the pointed end.

In Ayurvedic practice, the leaves, roots, flowers and latex of this medicinal plant is indispensable. The body parts of Aditya are made into many compound preparations such as Dhanvantaram Ghritam, Kaccoradi oil, Vajrakatailam and Nagaradi tailam. The root bark of Aditya plant is highly valued and commonly used in traditional Indian medicine. It is used as a substitute for it in the treatment of a variety of ailments. In small doses it is diaphoretic and expectorant, acts as a mild stimulant, and prescribed with carminatives to treat dyspepsia.

The powdered root bark of Aditya plant is used to ease diarrhoea and dysentery, syphilis, coughs, asthma and fevers. A paste prepared from the Aditya plant`s root bark and is used externally for the relief in elephantiasis and hydrocele. Among the Kondhs of southwestern Orissa, a paste of the Aditya plant`s roots is applied as a poultice to relieve rheumatic pain. In the Homeopathic system of medicine, Aditya plant is reported to be useful in the treatment of elephantiasis, lupus and chronic rheumatism.

The Bhils of Maharashtra widely use the root extract of this medicinal plant in the form of a pill to kill guinea worms and they also use the latex from the plant as a local application to treat ringworm and other skin diseases. The Paharia and Santhalis of Bihar take a mixture of the Aditya plant`s stem bark and black pepper internally for the treatment of epilepsy. The flowers of the Aditya plant possess digestive and tonic properties, and are used in the form of powder for healing cough, catarrh and asthma [3].

The roots of *Calotropis gigantea* have been used in leprosy, eczema, syphilis, elephantiasis, ulceration, antidiarrhoeal, and cough in the Indian system of traditional medicine. The following activities have been reported for *Calotropis gigantea;* prevention of insulin

resistance [4], hepatoprotective [5], antidiarrhoeal [6], anti-pyretic and analgesic [7, 8] antiinflammatory [9], analgesic activity in Eddy's hot plate, and acetic acid-induced writhings [10] and wound healing activity [11]. The milky juice of *Calotropis gigantea* has been reported as a violent purgative and gastrointestinal irritant and used for inducing abortion [12]. The alcohol extract of the flower of *Calotropis gigantea* reported analgesic activity in chemical and thermal models in mice [13]. The crude latex extract exhibited strong proteolytic activity, hydrolyses casein, human fibrinogen and crude fibrin clot in a dose-dependent manner [14].

Previously isolated classes of constituents include glycosides, proteases, 3'-methylbutanoates of amyrin, and taraxasterol from *Calotropis gigantea* [15], 19-nor- and 18,20-epoxy cardenolides from the leaves of *Calotropis gigantea* [16], stigmasterol and ß-sitosterol from methanol extract of root bark of *Calotropis gigantea*[17] and a new flavonol trisaccharide [18]. Autodigestion of two cystein proteinases, calotropins DI and DII isolated from the latex of *Calotropis gigantea* was studied at 37°C (pH 7.5) in the presence of an activated agent; calotropin DI is more susceptible to autodigestion than calotropin DII [19]. The three-dimensional structure of sulfhydryl protease calotropin DI from *Calotropis gigantea* closely resembled those found in the sulfhydryl proteases papain and actinidin [20].

MATERIALS AND METHODS:

Collection of the Plant Exudates: The exudates of he plant, *Calotropies gigantea* had been collected from Mirpur, Uttara,(Dhaka) Bangladesh in the month of September 2011.A milky exudates had been collected by cutting the plant parts (bark, leaves, etc).

Antimicrobial activity study: Any chemical substance or biological agent that destroys or suppresses the growth of microorganism is called antimicrobial agent. Antimicrobial screening of a crude extract or pure compound isolated from natural sources is essential to ascertain its activity against various types of pathogenic organisms.

The primary assay can be done in three ways as: Diffusion method, Dilution method and Bioautographic method.

Among these methods, the disc diffusion method is widely acceptable for the preliminary evaluation of antimicrobial activity. Disc Diffusion is essentially a qualitative or semi- qualitative test indicating the sensitivity or resistance of microorganisms to the test materials. However no distinction between bacteriostatic or bacteriocidal activity can be made by this method.

Disc Diffusion Method: Diffusion is based on the ability of a drug to a diffuse from a confined source through the nutrient agar medium and creates concentration gradient. If agar is seeded with a sensitive organism, a zone of inhibition will result where the concentration exceeds the minimum concentration (MIC) for that particular organism.

In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile filter paper discs (5 mm diameters) are impregnated with known amounts of the test substances and dried. The dried discs are placed on plates (Petri dishes, 120mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion. A number of events take place simultaneously which includes-the dried discs absorb water from the agar medium and the material under test is dissolved. The test material diffuses from the discs to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel. There is a gradual change of test material concentration on the agar surrounding each disc.

The plates are then kept in an incubator (37°C) for 12-18 hours to allow the growth of microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called “Zone of Inhibition”. The antimicrobial activity of the test agent is determined in term of millimeter by measuring the diameter of the zone of inhibition. The greater the zone of inhibition the greater the activity of the test material against the test organism.

Test Materials: The exudates of the plant, *Calotropies gigantea.*

Test Organisms: Both Gram-positive and Gram-negative stains of bacteria we used

as the test organism to observe the antibacterial activity of the compounds. The bacterial steins used for this investigation are listed in **Table 1**. These organisms were collected from the Microbiology research laboratory, Department of Pharmacy, Southeast University, Dhaka.

Culture Medium: The main requirement for the growth of bacteria was as follows-source of energy such as carbohydrate, protein and nucleic acid. Essential trace elements e.g. Mg, Mn, Fe, and Co. Optimum pH of media and Optimum temperature for incubation

Preparation of the Medium: The instant nutrient agar media was accurately weighted and then reconstituted with distilled water in a conical flask according to specification (4% w/v). It was then heated with water bath to dissolve the agar and a transparent solution was obtained. The prepared media was then transferred in 9ml and 5ml in a number of clean test tubes, respectively to prepare plates and slants. The slants were used for making subculture of microorganism, which in turn use for sensitivity tests. The test tubes were then plugged with cotton and sterilized in an autoclave ate temperature of 126°C and pressure of Ib/sq inch for 15 minutes.

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Preparation of Subculture: With the help of a inoculating lop, the test organisms were transferred from the pure culture to the agar slants in a laminar airflow unit. The incubated slants were then incubated at 37°C for 18-24 hours to ensure the growth of test organisms. This culture was used for sensitivity test.

Preparation of the Test Plate: The test organism was transferred from the subculture to the test tube containing 9ml autoclaved medium with the help of an incubating loop in aseptic area. The test tube was shaken by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile Petri dishes in an aseptic area and were rotated several times, first clockwise and anticlockwise to ensure homogeneous dispersion of the organism into the medium. The depth of media into each Petri dish was approximately 4mm. After plates were cooled to room temperature, it stored in a refrigerator at 4°C.

Preparation of Discs: Three types of discs were used for antibacterial screening. These were-Sample discs, Standard discs, Blank/control discs.

- **Sample discs**: Sterilized filters discs (5 mm in diameter) were taken in a blank Petri dish. Sample solution of the desired concentration was applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of solvent.
- **Standard discs**: these were used to compare to the antibacterial activity of test material. In our investigation Kanamycin (30 µg/disc) was used as a reference.
- **Blank discs**: Only solvent was applied to the disc to determine the antibacterial effects of the solvent used.

Application of the Test Samples: Standard Kanamycin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample.

Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Determination of Antimicrobial Activity: After 24 hour incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zone of inhibition in millimeter with transparent scale (**Table 1**).

Cytotoxicity study: Brine shrimp lethality bioassay [21] is a rapid general bioassay method for the bioactive compound of the natural and synthetic origin. Bioactive compounds are almost always toxic at high dose. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, anti-microbial and pharmacological activities of natural products and it is a recent development in the bioassay for the bioactive compounds. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their biosphere- activity. Here, in vivo lethality in a simple zoological organism (Brine shrimp nauplii) is used as a convenient monitor for screening and in the discovery of new bioactive natural products. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of the compounds [22].

Brine shrimp eggs are hatched in simulated sea water to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of DMSO. Ten nauplii are taken in vials containing 5 ml of simulated sea water. The samples of different concentrations are added to the pre-marked vials with a micropipette. The assay is performed using three replicates. Survivors are counted after 24 hours. These data are processed in a simple program to estimate LC_{50} values.

Preparation of Simulated Sea Water (Brine water): Since the lethality test involves the culture of brine shrimp nauplii that is the nauplii should be grown in sea water. Sea water contains 3.8% of sodium chloride & hence 3.8% salt solution should be needed for this purpose. Accordingly 3.8% of sodium chloride solution was made by dissolving sodium chloride (38 gm) in distilled water (1000 ml) & was filtered.

Hatching of Shrimps: Sea water was kept in a small tank & shrimps eggs were taken into the divided tank, constant oxygen supply was carried out & constant temperature (37°C) was maintained. Two days were allowed for the shrimp to hatch and mature as nauplii. These nauplii were taken for bioassay.

Preparation of Test Solutions: Measured amount (4.00 mg) of sample was dissolved in100 μl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 50 μl were added to pre-marked glass test tubes containing 5 ml of seawater and 10 shrimp nauplii. So, the final concentration of samples in the test tubes were 4ooμg/ml, 200 μg/ml,100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.125 μg/ml, 1.56 μg/ml, 0.78125μg/ml for 10 dilutions and 100-0.78125μg/ml for 8 dilutions respectively.

Preparation of Controls: Vincristine sulphate served as the positive control. 0.2mg of vincristine sulphate was dissolved in dimethyl sulfoxide (DMSO) to get an initial concentration of 20 μg/ml from which serial dilutions were made using pure DMSO to get 10 μg/ml, 5 μg/ml, 2.5μg/ml, 1.25 μg/ml, 0.625 μg/ml, 0.3125 μg/ml, 0.15625 μg/ml, 0.078125 μg/ml, 0.0390 μg/ml. The control groups containing 10 living brine shrimp nauplii in 5 ml simulated sea water received the positive control solutions. As for negative control, 30 μl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. The test was considered valid as the negative control showed no rapid mortality rate.

Counting of Nauplii and Analysis of data: After 24 hours, the vials were inspected using a magnifying glass and the numbers of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically (**Table 4**). The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

Antioxidant activity study: The free radical scavenging activity (antioxidant capacity) of the plant exudate on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method of Brand-Williams [9]. 2.0 ml of exudate at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20μg/ml). The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of tert-butyl-1-hydroxytoluene (BHT) by UV Spectrophoto-meter (**Table 2**).

DPPH is used to evaluate the free radical scavenging activity (antioxidant potential) of the medicinal plants.[10, 11] DPPH is stable free radicals potentially reactive with substance able to donate a hydrogen atom and thus useful to asses compounds antioxidant activity of specific compounds of extracts [12]. Because of its odd electron, DPPH has a strong absorption band at 517 nm. Since this electron becomes paired in the presence of a free radical scavenger, the absorption decreases stoichiometrically with respect to the number of electrons taken up. This change in absorbance produced by this reaction has been widely used to test the ability of several molecules to act as free radical scavengers [23].

The absorbance was taken by UV-spectrophotometer and methanol was taken for exudate and as a solvent. Ascorbic acid was used as a standard (Table 2). 2.0 ml of a exudate at different concentration (500 to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml).After 30 min reaction period at room temperature in dark place the absorbance was measured against at 517 nm against methanol as blank by UV spetrophotometer. Inhibition of free radical (DPPH) in percent (I%) was calculated as follows:

$$(I\%) = (1 – A\ sample/A\ blank) \times 100$$

where A blank is the absorbance of the control reaction (containing all reagents except the test material) and A sample is the absorbance of the mixture of test sample and DPPH. Exudate concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. BHT was used as positive control.

RESULTS AND DISCUSSION

Result of Antimicrobial Activity: The exudate of *Calotropis gigantea* was tested for the antibacterial activity against a number of Gram positive and Gram negative bacteria. Standard antibiotic discs of Kanamycin (30µg/disc) for bacterial species were used. In this antibacterial screening crude exudate was used at a concentration of 500µg/disc. The results of antibacterial activity of crude extract against a number of Gram positive and Gram negative bacteria are given below (Table 1).

Zone of inhibition is the area around an antibiotic disc that has bacterial growth. The antimicrobial activity of the exudates of *Calotropies gigantea* was examined against a number of Gram positive and Gram negative bacteria and some fungi. The zone of inhibition produced by the *Calotropis gigantea* exudates, was measured at a concentration of 200µg/disc. Standard antibiotic discs of Kanamycin (30µg/disc) for bacterial species were used. The results of antibacterial activity of crude exudate against a number of Gram positive and Gram negative bacteria are given below (Table 1).

TABLE 1: THE ZONE OF INHIBITION (MM) PRODUCED BY THE EXUDATES OF THE PLANT

Group	Name of bacteria	Zone of Inhibition	
		Plant exudates (200µg/disc)	Standard Kanamycin (30µg/disc)
Gram positive	*Staphylococcus aureus*	9±1	33±1.53
Gram negative	*Escherichia coli*	15±1.53	32±0.58
	Vibrio minicus	15±1	30±1.53
	Vibrio parahemolytics	15±0.58	25±1

The test was done in triplicate. Diameter of the zone of inhibitions is given here as mean ± standard deviation.

TABLE 2: RESULT OF CYTOTOXICITY OF THE EXUDATES OF *CALOTROPIS GIGANTEA*

Concentration (µg ⁄ml)	Log C	% of mortality	LC_{50} (µg ⁄ml)
400	2.60206	100±5.78	12.66
200	2.30103	100±0	
100	2	65±5.78	
50	1.69897	75±0	
25	1.397994	60±5.78	
12.5	1.09691	55±5.78	
6.25	0.79588	45±0	
3.125	0.49485	27±5.78	
1.56	0.193125	18±5.78	
0.78125	-0.10721	5±0	

The test was done in triplicate. The % Mortality is given here as mean ± standard deviation

DISCUSSION: The results of antimicrobial screening in term of zone of inhibition are presented in the table. Only four microorganisms have showed activity. In Gram positive bacteria *Staphylococcus aureus* zone of inhibition have showed (9mm). In Gram negative bacteria *Escherichia coli* zone of inhibition have showed (15mm), *Vibrio mimicus* (15mm), *Vibrio parahemolytics* (15mm) zone of inhibition have found. In fungi, zone of inhibition have not showed. In case of other organisms zone of inhibition is not present.

In conclusion, the plant *Calotropis gigantia exudates has shown antibacterial activity*. The highest zone of inhibition above 20mm means strong antimicrobial activity. So *Calotropies gigantea* extract (exudates) zone of inhibition is not present in appropriate range and so exudates of the plant *Calotropies gigantea* has moderate antimicrobial activity.

Result of Cytotoxicity: In this bioassay, methanol extract showed positive results indicating that the compounds are biologically active. From this experiment, it was revealed that each of the test samples showed different mortality rates at different concentrations (Table 2).

The mortality rate of brine shrimp was found to be decreased with the increase of concentration of samples, and a plot of log of concentration versus percent morality on the graph produced an approximate linear correlation between them (**Figure 3**). From the graph the concentration at which 50% mortality (LC_{50}) of the brine shrimp nauplii occurred was determined and the LC_{50} for the crude methanol extract of the bark of *Calotropis gigantia* was found to be 6.163µg/ml.

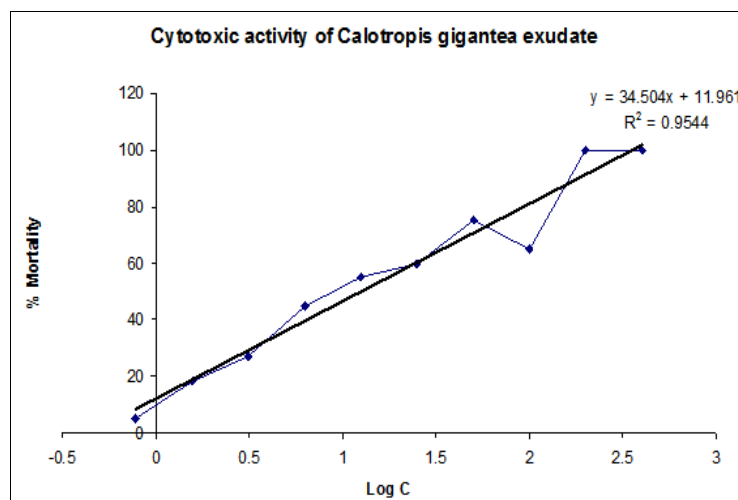

FIG 1: EFFECT OF THE EXUDATES OF *CALOTROPIS GIGANTEA* ON BRINE SHRIMP NAUPLII

TABLE 3: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

Conc.(C) (µg/ml)	Log C	% Mortality (Vincristine Sulphate)	LC_{50} (µg/ml)
20	1.3010	100±0	
10	1	90±0	
5	0.6989	86.67±5.78	
2.5	0.3979	83.33±5.78	
1.25	0.0969	73.33±5.78	
0.625	-0.2014	60±0	0.339
0.312	- 0.5051	46.67±5.78	
0.156	- 0.8061	36.67±5.78	
0.078	- 1.1072	30±0	
0.039	-1.4089	20±0	

The test was done in triplicate. The % Mortality is given here as mean ± standard deviation.

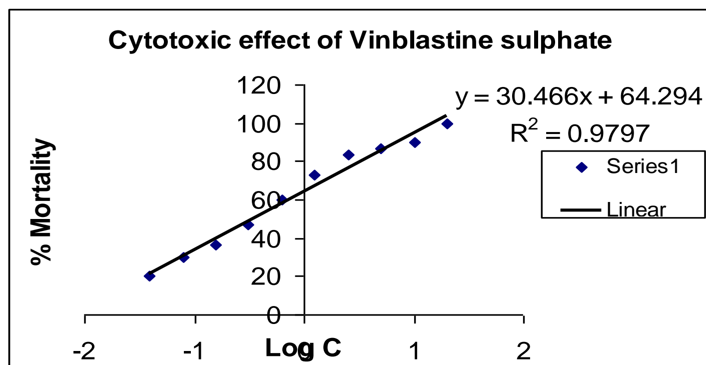

FIG. 2: EFFECT OF VINCRISTINE SULPHATE ON BRINE SHRIMP NAUPLII

DISCUSSION: In brine shrimp lethality bioassay, the exudates of *Calotropis gigantea* exhibited significant cytotoxic activity with the LC_{50} value of 12.660µg/ml.So from this result we can say that the exudates of the plant contains some compounds which are cytotoxic up to a certain level of concentration.

Result of Antioxidant activity: The antioxidant activity (DPPH radical scavenging activity) of *Calotropis gigantea* is depicted in **Figure 1**. This activity was

increased by increasing concentration of the sample. The IC_{50} value of the crude exudate of the plant was found to be 3.301 µg/ml (Table 2) while the IC_{50} value of the reference standard ascorbic acid was found to be 0.365 µg/ml (Table 4 & 5).

TABLE 4: DPPH RADICAL SCAVENGING ACTIVITY OF *CALOTROPIS GIGANTEA*

Plant	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ ml)
Calotropis gigantea (Plant exudates)	1	34	3.3012
	5	52	
	10	63	
	20	74	
	30	78	
	50	83	

TABLE 5: DPPH RADICAL SCAVENGING ACTIVITY OF ASCORBIC ACID

Standard	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ ml)
Ascorbic acid	1	37	0.3652
	5	55	
	10	69	
	20	80	
	30	90	
	50	98	

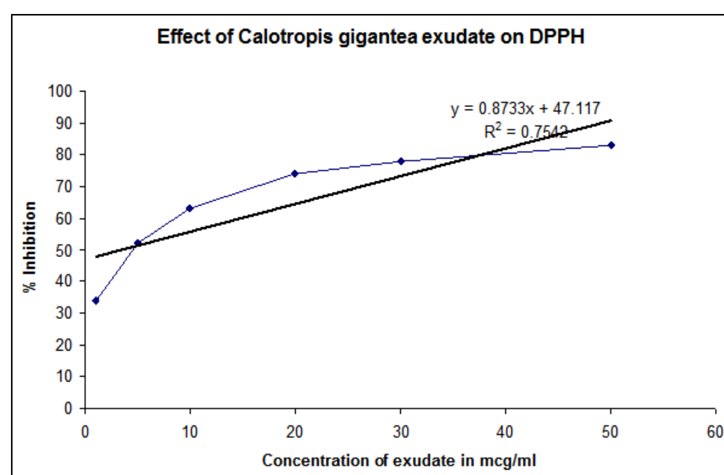

FIGURE 3: GRAPHICAL REPRESENTATION OF ANTIOXIDANT ACTIVITY OF PLANT EXUDATES *CALOTROPIS GIGANTEAN*

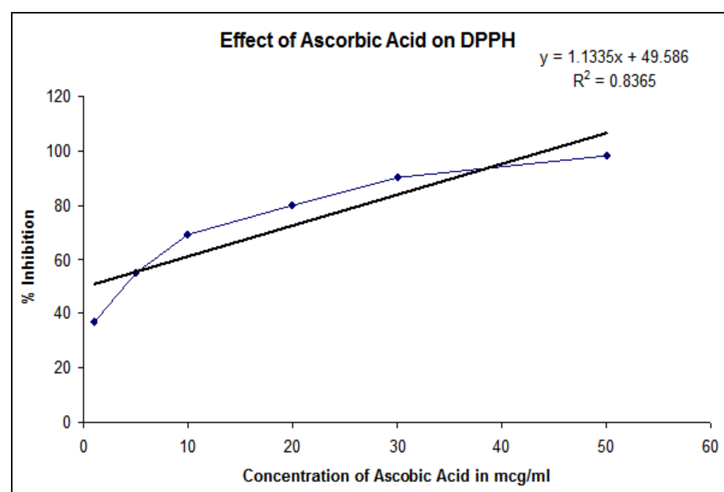

FIGURE 4: GRAPHICAL REPRESENTATION OF ANTIOXIDANT ACTIVITY OF ASCORBIC ACID

DISCUSSION: The result of the tests is present in the following figure. The exudates of the plant *Calotropis gigantea* has shown antioxidant activity. The exudate of the plant has demonstrated good antioxidant activity with a $\mathbf{LC}_{50}$ value of 3.301 µg ⁄ml.

CONCLUSION: The present study indicates that the exudate of *Calotropis gigantea* has got intense antimicrobial, antioxidant and cytotoxic activity and may have potential use in medicine. From the previous studies and our current investigation it may be concluded that further study can be carried out to investigate the individual bioactive principles.

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