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ANTIBIOGRAM OF TRADITIONAL MEDICINAL PLANT *CALOTROPSIS GIGANTEA* EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *VIBRIO CHOLERAE*.

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Abstract:

The increasing incidences of microbial infection and gradual rise in resistance in microbes and the available antibiotics had high-lightened the need to find more alternative antifungal and antimicrobial agents from other sources. *Calotropis gigantea* (Asclepiadaceae) a widely used plant that acquires number of medicinal properties and purposes includes 280 genera and 2000 species of worldwide distribution abundant in the sub-tropics and tropics, and rare in cold countries. Classifications of the degree of susceptibility or resistance of the test isolate to each antimicrobial agent (Ofloxacin and NorfloxTZ) were tested. The MIC was evaluated on plant extract, latex and antibiotics that showed antimicrobial activity. The test was performed at four different concentrations (5,10,15,20 µg/ml) of aqueous extract, ethanolic extract, latex and commercial drugs. Phytochemical screening of plant extract was also carried out.

Key Words:

Calotropis gigantea, *Staphylococcus aureus*, *Vibrio cholerae*, Ofloxacin and NorfloxTZ, Ethanolic extracts

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Introduction:

Plants provide abundant resources of antimicrobial compound and have been used for centuries to inhibit microbial growth. The increasing incidences of microbial infection and gradual rise in resistant in microbes and the available antibiotics had high-lightened

the need to find more alternative antifungal and antimicrobial agents from other sources. In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of diseases including cholera and pyogenic infections. Cholera is one of the major problems of causing death in infants. It thus becomes important to identify and evaluate commonly available natural drug as alternative to currently used antibacterial drugs [1].

Calotropis gigantea R.Br Asclepiadaceae a widely used plant that acquires number of medicinal properties and purposes includes 280 genera and 2000 species of worldwide distribution abundant in the sub-tropics and tropics, and rare in cold countries [2]. Traditionally *Calotropis* is used alone or with other medicines [3] to treat diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea [4]. The powdered root is used in asthma, bronchitis and dyspepsia. The leaves are useful in the treatment of paralysis, anthralegia and anthelmintic[5, 6.] Allelopathic effects of *Calotropis* on different agricultural crops have not been well studied. Extract of different plant parts viz. root, stem, leaf and stem with leaf of *Calotropis* affect seedling and germination. The main objective of this study is to determine the antimicrobial activity of leaf extract and latex of *C.gigantea* against pathogenic bacteria and also to have a comparative study by using commercially available drugs.

Materials and Methods

The plant *Calotropis gigantea* was collected and the aerial parts of plant were dried under shade. Each dehydrated plant was powdered to a fine texture and 100 gm of dried plant was used. Cold aqueous extract was prepared following Akueshi[7]. The plant extract was prepared by the modified method of Alade & Irob[8]. Dried powdered plant (15 gm) was soaked in 150 ml of 70% ethanol. The mixture was kept for 72 hours in tightly sealed conical flask at room temperature. The test organisms *Staphylococcus aureus* and *Vibrio cholerae* were obtained from CMC Vellore, India. The commercially available drugs ofloxacin and NorfloxTZ were taken from the medical shop. Muller Hinton Agar was used for the antimicrobial testing. The modified agar well diffusion method of Perez et al[9] and disc diffusion method were employed. The wells were filled with different concentrations of plant extract latex, 5µl (50 mg/ml), 10µl (100mg/ml), 15µl (150mg/ml), 20µl (200mg/ml) and antibiotics ofloxacin (*S.aureus*) and norflox TZ (*V.cholerae*). Plates were incubated at 37º C for 18 h. Strains were characterized as susceptible or resistant based on inhibition zone around the discs. The MIC was evaluated on plant extract, latex and antibiotics that showed antimicrobial activity. The test was performed at four different

concentrations of aqueous extract, ethanolic extract, latex and commercial drugs (5,10,15,20 µg/ml) employing the same modified agar well diffusion method and diameters of zone were measured.

Phytochemical screening of plant extract

Phytochemical screening of plant extract was carried out by modified procedure of Milton Z Nichaman[10]. The ethanolic plant extract was analysed for the presence of carbohydrate, fatty acid and protein. the test for fatty acid was conducted by preparing of silica gel plate in 40 % aqueous $FeCl_3$, spotted the sample on plate and developed in mixture of solution (70:30:1) of petroleum ether, diethyl ether, and glacial acetic acid and visualized by spraying with 10% H_2SO_4 .For carbohydrate spotted the sample then developed in the same above given medium and visualized by keeping in iodine vapour. The test for amino acid was conducted by spotting the sample on silica gel (1:1.5 water),developed in acetic acid and acetonitrile (50:50) and visualized by spraying with ninhydrin (1.5gm ninhydrin in 40 ml n-butanol and 3ml acetic acid). Retardation factor was calculated by using this formula:

$$R_f = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

Results

Antibiogram of some common antibiotics against test microorganism revealed that the bacterial strain were resistance to widely used board spectrum commercially used drugs. The antimicrobial screening of leaf extract of plant and latex of *C.gigantea* on *S.aureus* and *V.cholerae* revealed that the ethanolic leaf extract were found to be more effective than of cold aqueous extract. Plant Latex has no effect on bacterial strains. Table 1 showed that *S.aureus* exhibited higher MIC value for 200mg/ml concentration against ofloxacin among four different concentrations (50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml) used and zone of diameter 15 mm was measured. Table 1 showed that *V.cholerae* exhibited higher MIC value for concentration 100mg/ml against commercially used drug Norflox TZ and diameter of zone was higher for 200mg/ml. For both the organisms ethanolic extract showed higher MIC value.

Phytochemical screening of compound present in ethanolic plant extract revealed that carbohydrate, fatty acid and amino acids could be responsible for the antimicrobial activity. Table 2 showed that the Rf value for carbohydrate (0.866) was higher than fatty acid (0.692) and amino acid (0.671).

Discussions

The ethanolic extract of the leaves of *C.gigantea* showed greater antibacterial effect than the cold-water extract. The finding was interesting, because in the traditional method of treating a bacterial infection, decoction of plant part or boiling the plant in water was employed. Whereas, according to present study preparing an extract with organic solvent was shown to provide a better antibacterial activity, in accordance with the result obtained by *Nair et al.*[11].

These observations may be attributed by two reasons: Firstly, the nature of biological active component can be enhanced in the presence of ethanol. Secondly, the stronger extraction capacity of ethanol could have produced greater number of active constituents responsible for antibacterial activity. The plant also exhibited different kind of secondary metabolites like amino acid, cholesterol and carbohydrate. These compounds could be responsible for the antimicrobial activity. The latex of this plant has anticoagulant property and also used as a remedy for the snake bite and scorpion sting but it did not show any antibacterial activity. *C.gigantea* plant can be used to discover natural products that will lead to the development of new pharmaceuticals. Such screening of various natural organic compounds and identification of active agents must be considered as a fruitful approach in the search of new herbal drugs.

Moreover, due to effectiveness of extract, *in vivo* study of this medicinal plant is necessary to determine toxicity of the active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. Antimicrobial activity can be enhanced if the active components are purified and adequate dosages determined for proper administration. Ahmad et al.,[12] and Desta[13] reported that aqueous and alcoholic extracts from roots of *Plumbagin zeylanica* showed antibacterial activity against *Staphylococcus* sp, *Pseudomonas aeruginosa* and *Proteus vulgaris.* The result from the current study revealed that the plant *Calotropis gigantea* showed the MIC against *Vibrio cholerae* and *S.aureus.*

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Table 1. Comparison of *C.gigantea* extracts against human pathogens

Concentration (mg/ml)	*Staphylococcus aureus*				*Vibrio cholerae*			
	Diameter of Zone (mm)							
	Ofloxacin	Leaf Aqueous Extract	Leaf Ethanolic Extract	Latex	Norflox TZ	Leaf Aqueous Extract	Leaf Ethanolic Extract	Latex
50	19	7	11	-	8	9	13	-
100	11	7	14	-	15	-	14	-
150	8	8	14	-	12	-	16	-
200	10	11	17	-	7	10	19	-

Table 2. Phytochemical analysis of *C.gigantea* extract

Sl No	Component	R_f Value
1	Amino Acid	0.671
2	Fatty Acid	0.692
3	Carbohydrate	0.66

