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ANTIBACTERIAL ACTIVITY OF NATURAL COMPOUNDS EXTRACTED WITH DIFFERENT SOLVENTS FROM *CALOTROPIS PROCERA*

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ABSTRACT

Antibiotics resistance effects become an ever-increasing therapeutic problems. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action. *Calotropis Procera* shows limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, These substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. When these substances isolate with four different solvents(Hexane, Chloroform, Butanol, Methanol) one by one ,then some extracts showed great potential to inhibit the growth of four different bacterial strains like: *Pectobacterium Carotovorum, Xenthomonas Compestris, E. Coli and Stephylococcus aureus*

KEY WORDS : Antibacterial activity, Calotropis Procera, Hexane extract, Chloroform extract, Methanol extract etc.

INTRODUCTION

The use of and search for drugs and dietary supplements derived from plants have acelerated in recent years. While 25 to 50%of current pharmaceuticals are derived plants ,none are used as antimicrobials. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. (Geissman 1963). Most are secondary metabolites. These substances serve in plant defense mechanisms against predation by microorganisms, insects and herbivores. Some others compounds like terpenoids give plants their odors, while quinones and tannins are responsible for plant Pigment (Marjorie Murphy cowan ,1999).

Plants of the milkweed family are herbs, shrubs with thick, milky juice. They have sometimes been used for laxative, expectorant, diuretic, emetic purposes, and for wart removal. All members of this family are toxic. The plant has been taken to study was *Calotropis procera*. Hole plant is rich to contain medicinal property(Kumar and Arya 2007), such as:-secretion of the root bark of Calotropis Procera is used to treat skin diseases, enlargements of abdominal viscera, intestinal worms, cough, ascites, anasarca, elephantiasis. Hole plant is used to treat eczema , diarrohea (Kew, 1985) and Jaundice (Jan et al. 2009). Flowers are considered to improve digestion , catarrh. The leaf ash is used to treat ascites and enlargements of abdominal viscera. Stem is used as tooth brush having the property of curing toothache (Zabihullah et al. 2006, Jan et al. 2008). Smokes of leaves with latex used to treat cough, leprosy, elephantiasis, asthma and paralysis (Bhogaonkar et al. 2007). A powerful bacteriolytic enzyme (Shukla et al. 1961), a very toxic glycoside Calactin, Calotropin D 1, Calotropin D 2, Calotropin F1, Calotropin F 2 and a non toxic protealytic enzyme Calotropin has been identified in latex of this plant. Toxic glycoside Calactin takes part in defense mechanism of plant to protect from insects and grasshopper attack. The alcoholic extract of leaves and roots were found to have anticancer activity against human epidermal carcinoma of the nasopharynx in tissue culture (Dhar et al. 1969).

MATERIALS AND METHOD

Fresh stems of plant Calotropis Procera were collected from Naini area of the Allahabad region. The plant was identified by the experts of the S.H.I.A.T.S., Allahabad. Plant stems were dried at 20-25^{0}C in shed and ground to the fine powder using with grinding machine and followed two steps of the extraction :-

(1) 100 gm. Powdered plant material were socked in Hexane for 3 days, then filtered it. This plant material were socked again in Chloroform for 3 days, then filtered. Socking were continued with butanol and then methanol for 3-3 days again, followed with their increasing polarity. All extracts were concentrated in vacuum desicator.

(2) 100 gm. Powdered plant material was taken in to the soxhlet and treated with four different solvents (Hexane, Chloroform, Butanol, Methanol) one by one followed by increasing their polarity, each extract was collected after 3 days.

Finally four cold (from socking) and four hot (from soxhlet) extracts were obtained.

Test microorganisms :-

Bacterial strains were supplied by Microbial Type Culture Collection (MTCC), Chandigarh, India. The bacterial strains were *Xenthomonas Compestris*, *Pectobecterium*

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Carotovorum, *Escheric*hia coli, *Staphylococcus aureus.*

Preparation of inoculums:-

Stock culture were maintained at 4 °C on slopes of nutrient agar. Active culture for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Mueller- Hinton broth (MHB), that were incubated without agitation for 24 hr. at 37 °C. To 5 ml of MHB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 Mc farland solution **(Mcfarland *et al* 1907).**

Antimicrobial Testing :-

Kirby-Bauer method was followed for disc diffusion assay (Baur *et al*, 1966) In vitro antimicrobial activity was screened by using Mueller –Hinton Agar (MHA). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min. and 0.1 % inoculums of each bacterial suspension was swabbed uniformly in separate MHA plates and it was allowed to dry for 5 min.

Four cold and four hot extracts were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of the medium and the extracts were allowed to diffuse for 5 min. and the plates were kept for incubation at 37°C for 24 hrs. Negative control was prepared using respective solvent. At the end of incubation inhibition zone formed around the disc were measured in millimeter

RESULTS AND DISCUSSION

Infectious diseases account for high proportion of health problems in the developing country es. results of the research showed that the plant *Calotropis procera* belongs to the family *Ascelepiadaceae* is rich as source of phenolic compounds and their oxygen substituted derivatives, which are responsible for antibacterial activity in different extracts. Antibacterial activity of different extracts against four bacterial strains shows in table 1:-

Table 1:- Antibacterial activity of extracts with different solvents against 4 bacterial strains.

Extracts	P. Caroto-vorum	X. compe-stris	Escherichia Coli	Staphy-lococcus aureus
Hexane(cold)	-	-	-	-
Hexane(hot)	+	-	-	-
$CHcl_3$(cold)	+	+	-	+
$CHcl_3$(hot)	-	-	+	+
Butanol(cold)	-	-	-	-
Butanol(hot)	-	-	-	-
Methanol(cold)	+	+	+	+
Methnol(hot)	+	+	+	+

+ Showing inhibition, - Not showing inhibition

Largest potential to give antibacterial activity showed by methnolic extract of the plant, MeOH extract shows higher inhibition zones against *Pectobacterium carotovorum*, *Xenthomonas Compestris*, *Escherichia Coli and Staphylococcus aureus* while butanol extract did not show any activity against any bacterial strain used in the research.

Chloroform extract in cold (socked) condition is also showed great potential to inhibit the growth of *Pectobacterium Carot*ovorum , *Xenthomonas Compestris and Staphylococcus aureus.* But it was not effective for the strain of E. Coli.

Table 2:- Presentation of zone of Inhibition in mm. on petry plates through disc diffusion method.

Extracts	P. Caroto-vorum	X. compe-stris	Escherichia Coli	Staphy-lococcus aureus
Hexane(cold	-	-	-	-
Hexane(hot)	2.5 mm	-	-	-
$CHcl_3$(cold)	5 mm	3 mm	-	7 mm
$CHcl_3$(hot)	-	-	2 mm	7 mm
Butanol(cold)	-	-	-	-
Butanol(hot)	-	-	-	-
Methanol(cold)	15 mm	8 mm	8 mm	12 mm
Methnol(hot)	12 mm	10 mm	8 mm	11 mm

Chloroform extract in hot (soxhlet) condition did not effected the growth of two plants pathogenic strains like *Pactobacterium Carotovorum* and *X. Compestris*, but it have potential against *E. Coli* and *S. aureus* . Hexane extract (through soxhlet) showed inhibition zone against *P. Carotovorum* and other bacterial strains was not effected.

It is concluded that, the medicinal plant *Calotropis Procera* have large possibilities to use it as antimicrobial pouch ,due to its toxicity against many infectious micro organisms.

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