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TO STUDY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF CALOTROPIS *PROCERA* LEAF EXTRACTS

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ABSTRACT

The plants are an important medicinal value and its different parts are used in ayurvedic medicines. The present study was an attempt to study Anatomy, phytochemical and antimicrobial activity of methanol, ethanol and water extracts obtained from the leaves of *calotropis procera* to understand the cellular organization and an attempt to evaluate the medicinal potentials. The phytochemical screening revealed the presence of alkaloids, flavenoids, tannins, saponins, and cardiac glycoside with a very high content in methanol, water and then in ethanol. The methanol, ethanol and water extracts of *Calatropis* procera leaves were evaluated for antimicrobial activity against gram+ve, gram-ve bacteria and fungi. Zone of inhibition diameters were observed. 100µl methanol extract showed the inhibition for the above organism. But ethanol and water extract did not have maximum impact in inhibition. The result of this study validates the use of methanol extract of this species in ethanomedicine providing lead for antifungal and antimicrobial for above strains.

KEYWORDS: *Calotropis procera*, Phytochemical, Antimicrobial, Leaf extract.

1. INTRODUCTION

On earth the plant produce natural products which are used as traditional medicine for various diseases from thousand of year. Plants naturally synthesized and accumulate different types of valuable secondary metabolites like alkaloids, glycosides, tannins, minerals and vitamin etc.[1] *Calotropis* is a spreading shrub or small tree belongs to the family of Asclepiadaceae. It is found throughout the tropical world and they are native to the tropical and sub tropical part

of Asia.[2] Leaves opposite grey, green large up to 50 cm long and 10cm broad with a pointed tip to rounded basal lobes. Flower waxy white petals 5, purple tipped inside and with a central purplish crown. The *Calotropis* plant produced large quantity of latex, which is milky liquid consisting of several biologically active compound like proteins, amino acids, carbohydrates, lipids, vitamins, alkaloids, resins and tannins.[3] The latex is used as an abortifacient, spasmogenic and carminative properties, antidysentric, antisyphilitic, antirheumatic, antifungal, mullusccide, diaphoretic and for the treatment of leprosy, bronchial asthma and skin infection. The root and leaves of *Calotropis* are used traditionally for treatment of abdominal, tumours boils, skin diseases, wound and insect bites.[4] Different parts of the plant have been reported to possess a number of biological activities such as proteolytic, antimicrobial, larvicidal, nematocidal, anticancer, anti-inflammatory. Its flowers possess digestive and tonic properties.[5] The root of the plant is used as a carminative in the treatment of dyspepsia. The leaves of *Calotropis procera* are used by various tribes of central India as a curative agent for jaundice. The present work report the phytochemical properties and antimicrobial activity of leaf extracts of *C.procera*.

2. MATERIALS AND METHODS

2.1. Plant material collection

Fresh leaves of *Calotropis* was collected from near college campus and identified with help of flora. The collected leaves cleaned for debris using tap water and rinsed in sterile distilled water. The leaves were shed dried and grinded in powder and stored in air tight bottles for further use.

2.2. Preparation of plant extract

The stored powder weighs exactly 10 gm for each extraction and was packed in soxhlet apparatus and extracted with methanol, ethanol and water separately for 8 hours.

2.3. Preliminary Phytochemical Screening

Phytochemical screening was carried out using standard procedure. The tests are briefly described below.

2.3.1. Detection of Alkaloids: A 1 ml of filtrate taken into the test tube and a drop of Mayer's reagent (**Mayer's test**[6]**)** was added by the side of test tube to produce white or creamy precipitate indicates the test is positive. In second test tube take 1 ml filtrate and add

few drops of Wagner's reagent and appearance of reddish-brown precipitate confirms the test is positive.

2.3.2. Detection of Carbohydrates and Glycosides: Take 1 ml of filtrate and few drops of Barrfoed's reagent **(Barrfoed's test)** was added and heated in a boiling water bath for 2 min; a red precipitate indicates presence of sugars.

2.3.3. Carbohydrate: 1 ml of the filtrate was added about 1 ml of iodine solution; a purple colour at the interphase indicates the presence of carbohydrate.

2.3.4. Detection of Saponins: The 1 ml extract was diluted with distilled water and made up to 20ml. The suspension is shaken in a graduated cylinder for 15 min and there is formation of two cm layer of foam indicates the presence of Saponin.

2.3.5. Detection of Proteins and Amino Acids: In 2 ml of filtrate adds a few drops of Million's reagent and a white precipitate indicate the presence of proteins and amino acid. For second test mix filtrate and few drops of ninhydrin solution in test tube and added 2 ml of aqueous filtrate; a characteristic purple colour indicates presence of amino acids.

2.3.6. Detection of Phenolic Compounds: The extract is dissolved in 5 ml of D.W; a few drops of neutral 5% ferric chloride solution are added; a dark green colour indicates the presence of phenolic compound.

2.3.7. Detection of Gum and Mucilage: The extract (100 mg) is dissolved in 10 ml of D.W. and to this 25ml of absolute alcohol is added with constant stirring; white cloudy precipitate indicates the presence of gums and mucilage.

2.3.8. Terpenoids and Steroids: 4mg of extract was treated with 0.5 ml of chloroform; then concentrated solution of sulphuric acid was added slowly and red violet colour for steroids.

2.3.9. Tannins: - In a test tube 0.5 ml extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added; blue colour was observed for gallic tannins and black colour for catecholic tannins.

2.3.10. Fixed Oils and fats: A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture is

heated on water bath for two hours. Formation of soap partial neutralization of alkali indicates the presence of mixed oil and fats.

2.4. TEST ORGANISMS

Five pathogenic microorganisms in total, including three bacteria, viz., *Staphylococcus aureus, E. coli and Pseudomonas* and two fungal strains, viz., *Aspergillus niger and Aspergillus flavous* were procured from microbiology department HPT Arts and RYK Science College, Nashik.

2.5. ANTIMICROBIAL STUDIES

Bacterial strains were grown and maintained on Nutrient Agar medium, while fungi were maintained on Potato dextrose agar (PDA) medium. The antibacterial activity of different extracts was tested using agar disc diffusion method. The bacterial and fungal isolates collected in prepared slants of nutrients gar were sub cultured in to prepared nutrient broth and incubated at 37^{o}C for 24 h.The well plate diffusion method and disk diffusion assay were used to determine the growth inhibition of bacteria and fungi by plant extract. The nutrient agar plates and P.D.A plates were seeded with the bacterial and fungal strain. Five holes of 6.0 mm diameter each were made with sterile cock borer and filled with 25%, 50%, 75%, 100% plant extract & pure solvent as control for about 100 micro litters. The inoculated plates were allowed to congeal for 1 hour and then were incubated at 37^{o}C for 24 hours. After 24 h incubation, the plates were observed for zones of inhibition which were recorded in centimetres (cm).

3. RESULTS AND DISCUSSION

Physical characteristic of the extracts indicate that there were variations in the colour, odour and texture of the extracts (Table.1). Table 2 presents the phytochemical compound recovered from the extract of the plant part screened in which presence of alkaloids, saponins, proteins, amino acids, phenols, terpenoid and steroids, mixed oil and fats. This support the earlier finding of Mainasara et al,[7] Shobowale et al,[8] Kawo et al,[9] Korii and Alawa,[4] Swapnali et al,[2] Kumar et al,[5] Shrivastava et al,[10] Morsy et al.[11] Among phytochemicals, alkaloids are one of the largest groups in plants having amazing effect on human and this led to the development of powerful painkiller medicine. Subramanian & Saratha.[12] Table 3 & 4 present the antifungal and antibacterial activity pattern of the extracts. The results shows that methanol and ethanol leaf extract of *calatropis* have antifungal activities on *Aspergilus niger* at all concentration. But there is no activity against *Aspergilus flavus* at all concentration

except 75 µl and 100 µl concentrations of methanol. However the antifungal effect was more pronounced against *Aspergilus niger*, which was seen to be more sensitive to the leaf methanolic extract at a concentration of 100 µl with zones of inhibition of 1.2 cm respectively. The effect of *Calatropis procera* agrees with the work of Manoorkar et al[3] and Shobowale et al,[8] which showed antifungal activity against *A.niger*. Antibacterial efficacy of different solvent extracts of *C. procera* is shown in table 3.methanol and D.W. have shown better activity against these pathogenic organisms. In case of antibacterial activity, it was only inhibited by leaf methanol and distilled water extract of highest recorded zone of inhibition at (0.9 cm) 100 µl against *Pseudomonas,* this result is similar to that of Kumar et al, [5] Mainasara et al.[7] However kawo et al[9] showed there was no activity of ethanol leaf extract of *C. procera* against *pseudomonas* at all concentration.

Table 1: Physical characteristics of different extracts of *Calotropis.*

Sr. No	Initial Weight of Powder(gm)	Final Weight Of powder(gm)	Weight Of Crude Extract(gm)	Color Of Extract	Odour	Texture
Distilled Water	10	7.82	2.18	Golden Brown	Pleasant Fruity	Soft
Ethanol	10	8.93	1.07	Dark Green	Slightly repulsive	Oily
Methanol	10	8.24	1.76	Dark Green	Slightly repulsive	Oily

Table 2: Preliminary phytochemical analysis of crude extract of *Calotropis procera.*

Sr. No.	Phytochemical Test	Distilled Water	Ethanol	Methanol
1.	Alkaloids Test			
	a. Mayer's Reagent	++	++	++
	b. Wagner's Reagent	+	+	+
2.	Carbohydrate			
	a.Barrfoed's Test	-	-	-
	b. Iodine Test	-	-	-
3.	Detection of Saponin	+	+	+
4.	Detection of protein And Amino Acids			
	a.Million's Test	+	+	+
	b. Ninhydrin Test	+	+	+
5.	Detection of Phenolic Compound and Tannins			
	a.Ferric chloride Test	+	-	+
6.	Detection Of Gum And Mucilage	-	-	-
7.	Terpenoids and Steroids	+	+	+
8.	Mixed Oils and Fats			
	a.Saponification Test	+	+	+

Table 3: Antifungal activity of *Calotropis.*

Sr. No.	Microorganisms strain	Extract	zone observed in that extract				
		Conc. of extract in µl	25	50	75	100	control
1.	*Aspergillus niger*	D.W.	Nil	Nil	Nil	0.4	Nil
		Methanol	0.3	0.4	0.7	1.2	Nil
		Ethanol	0.4	0.5	0.7	0.9	Nil
2.	*Aspergillus flavous*	D.W.	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	0.3	0.5	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil

Table 4: Antimicrobial activity of *Calotropis.*

Sr.No	Micro organism strain	Extract	Zone of inhibition in cm				
		Conc. of extract in µl	25	50	75	100	control
1.	*Staphylococcus. aureus*	D.W.	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil
2.	*E.coli*	D.W.	Nil	Nil	Nil	Nil	Nil
		Methanol	0.1	0.4	0.5	0.9	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil
3.	*Pseudomonas*	D.W.	0.3	0.4	0.6	0.9	Nil
		methanol	Nil	0.2	0.5	0.6	Nil
		Ethanol	Nil	Nil	Nil	Nils	Nil

4. CONCLUSION

The remarkable phytochemical, fungicidal and bactericidal effects of *C. procera* leaf extract suggest that the leaf extract may be useful source for the development of antifungal and antibacterial agent against pathogenic fungi and bacteria as well as used as phytomedicine.

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