WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 716-721. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING AND ANTIFUNGAL IMPLICATIONS OF *CALOTROPIS PROCERA* LEAF EXTRACTS USING VARIOUS SOLVENTS

H. Zaman*, R. M. Shrivastava, S. Shrivastava and S. Akther

M. V. M. College Bhopal.

Article Received on
26 Feb 2016,

Revised on 17 March 2016,
Accepted on 07 April 2016

DOI: 10.20959/wjpr20165-6021

***Corresponding Author**
Dr. H. Zaman
M. V. M. College Bhopal.

ABSTRACT

Phytochemical screening of leaves of *Calotropis procera* is hereby reported. Antifungal activity of ethanol, chloroform and petroleum ether extracts of leaves of *Calotropis procera* on *Aspergillus niger* and *Candida albicans* were determined by nutrient agar well diffusion method. The results revealed that ethanol was the best extractive solvent for antifungal properties of leaves followed in order by Petroleum Chloroform and Petroleum ($P<0.05$). The ethanolic extracts of leaves of C. procera gave the widest zone of inhibition against Aspergillus niger and Candida. the growth of test fungi were inhibited by ethanolic extract while the chloroform and Petroleum ether was the least effective on the test fungi. The best antifungal activity was recorded in ethanol extract of C. procera than chloroform and Petroleum ether against Aspergillus niger and Candida albicans. This study revealed that the leaves of *C.procera* demonstrated strong inhibitory effect on the test organisms. The results therefore established a good support for the use of C.procera in traditional medicine**.**

KEYWORDS: Antifungal activity, phytochemical ananlysis, *Calotropis procera*.

INTRODUCTION

Calotropis procera (Sodom apple) is a member of the plant family Asclepiadaceae, a shrub about 6m high and is widely distributed in West Africa and other parts of the tropics (Irvine, 1961). The plant is erect, tall, large, much branched and perennial with milky latex throughout. In India, the secretions from the root bark is traditionally used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms (Parrotta, 2001). All parts of the plant are considered to have valuable alternative properties when taken in small doses. Powdered root bark gives relief in dysentery and used as a substitute for

ipecacuanha. In form of paste, root bark is applied in elephantiasis. Dried leaves are used as a remedy for asthma, cough, headache and intermittent fever. Bark of the plant is used as cholagogue, diaphoretic, emetic, alternative and diuretic. The latex is purgative and irritant to the skin and mucous membrane said to causes blindness. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. Due to increasing no. of immuno compromised individuals, fungal infections have increased in the last two decates, affecting million of people worldwide (Wong et al. 1994). Therefore, there is a need to find out more effective and less toxic, new antifungal agents by detection of antifungal compounds in medicinal plants and new antifungal agents are still needed to improve the treatment of superficial fungal infections (Domenico et al. 1999 and Barrett et al. 2002). In continuation of medicinal seek (Mir and Maurya, 2014), the present work has been aimed to work out antibiotic potential of the selected plant extract.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

Fresh leaves of *Calotropis procera* were collected from Bhopal city of Madhya Pradesh state in the month of January. The species were identified by senior taxonomists from botany department of M.V.M. College Bhopal (M.P). The healthy and disease free leaves of *Calotropis procera* was separated and dried in shade so as to avoid decomposition of chemical constituents. The dried leaves were grinded in to fine powder by electric grinder (Sogo, china) at the speed of 500 rpm separately.

Extraction and Preparation of Material for Phytochemical Screening

In present study extraction was performed using continuous hot percolation ‘Soxhlation’. The advantage of this method, compared to previously other methods of extraction, is that large amounts of drug can be extracted with a much smaller of solvent quantity. Powder of leaves of *Calotropis procera* was placed in thimble of Soxhlet apparatus. Extraction was performed at 40°C using petroleum ether as non polar solvent at first. Extraction was continued for a period of 12 hours. The yield of pet ether extract was 4.20grams. Exhausted plant material (marc) was dried and afterward was extracted with Chloroform. The extraction was continued for a period of 13 hours at 40^{o}C, the yield was 6.5 grams. Marc obtained after chloroform extraction was subjected to extraction with ethanol. The extraction was continued for a period

of 14 hours 30 minutes for complete extraction at the same temperature and the yield was 7 grams. The extracts thus obtained was used for pharmacological screening.

Antifungal screening

Test organisms

The microorganisms used for antifungal activity, were *Aspergillus niger* and *Candida albicans* obtained from Pinnacle Biomedical Research institute Bhopal.

Antifungal activity

The well diffusion were used to determine the growth of inhibition of fungi by plant extracts as described by mohmmad and Dabai (2008), the nutrient agar (Muller Hilton) plates were prepared and seeded with the test organisms. In a plate four wells were prepared at equidistance to each other. Extracts of different concentration 62.5 mg/ml, 125 mg/ml, 187.5mg/ml and 250 mg/ml were instilled into the well at volume of 50 µl in each well. Plates were placed in incubator set to 35°C within 15 minutes. After 1 hour, plates were inverted and again placed in incubator far about 18-24 hrs. The plates were examined for evidence of zone of inhibition which appear as a clear area around the holes (Cheesbrough, 2001). The diameter of such zone of inhibition was measured by vernier caliper and the value was recorded and expressed to the nearest millimeter.

Phytochemical screening

This was carried out according to the methods described by Trease and Evans, (1989).

RESULTS AND DISCUSSION

The results of crude extracts were subjected to phytochemical screening., Tannins were found in Petroleum ether extract. Alkaloids, Tannins and Amino acids compounds were found in Chloroform extract., Flavonoids, Amino acids, Alkaloids, Saponins, Tannins and phenolic compounds were found in ethanolic extract. The results have shown that the main components i.e., flavonoids, alkaloids, tannins and phenolic compounds were present in ethanolic extract. The presence of these compounds were present in *Calotropis procera* (Mamta *et. al.* 2011). Majority of the compounds were present in ethanol extract. These compounds are found to be more usefull for antibacteriall activity (Aliero et al 2011**,** Yesmin et al 2008).

Phytochemical constituents of Calotropis procera.

Phytochemicals	Petroleum ether extract (leaves)	Chloroform extract (leaves)	Ethanol extract(leaves)
Alkaloids	_	+	+
Tannins	+	+	+
Amino acids	_	+	+
Flavonoids	_	_	+
Saponins	_	_	+
Carbohydrates	_	+	_
Glycosides	+	_	_

The results of antifungal activity measured in terms of diameter of zone of inhibition, the antifungal activity of *Calotropis procera* on the test organisms using petroleu ether, chloroform and ethanolic ectracts of leaves of calotropis procera exhibited antifungal activity against Aspergillus niger and Candida albicans. Itwas found that antifungal activity of petroleum ether, chloroform and chloroform against these pathogens at selected concentrations 62.5 mg/ml, 125 mg/ml, 187.5mg/ml and 250 mg/ml. Zone of inhibition was found to be maximum for ethanolic than petroleum ether and chloroform also reported by (Mako et al.2012). The results obtained in the present study shown that the Petroleum ether, Chloroform and ethanol extracts extract of *Calotropis Perocera*. and its purified isolated bioactive compound from ethanolic extracts (Rutin disacharide) displayed significant antibacterial activity. The results provide a use of *Calotropis procera* linn, in traditional medicine and suggest its further advance investigation.

Table 1: inhibition of Aspergillus niger and Candida albicans by Ethanolic extracts of leaves of Calotropis procera.

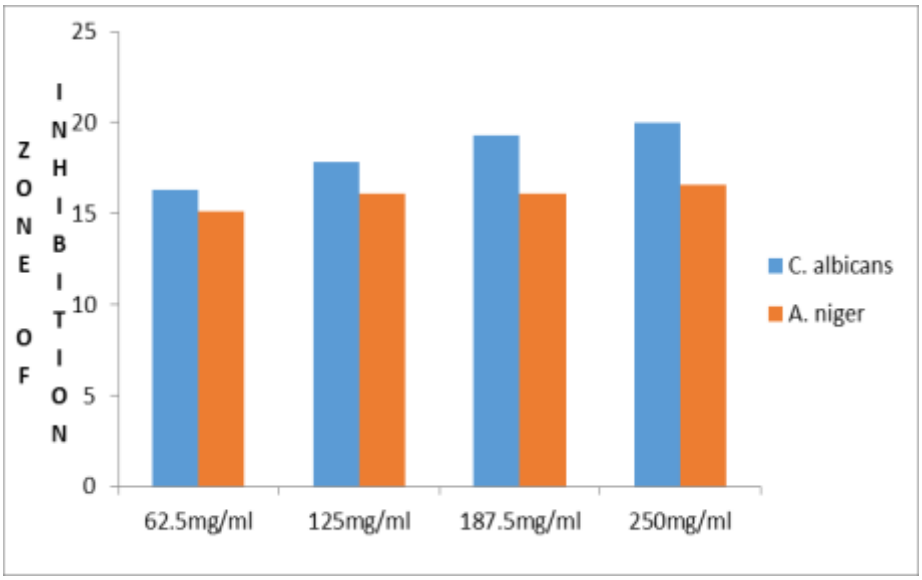
Microorganism	Zone of Inhibition (mm)			
	62.5 Mg/ml	125 Mg/ml	187.5 Mg/ml	250 Mg/ml
C. albicans	16.3±1.527	17.8±2.840	19.**3**±3.690	20.0±1.802
A. niger	15.1±1.040	17.8±1.040	16.5±1.258	16.6±0.763

Table 2: inhibition of Aspergillus niger and Candida albicans by Petroleum ether extracts of leaves of Calotropis procera.

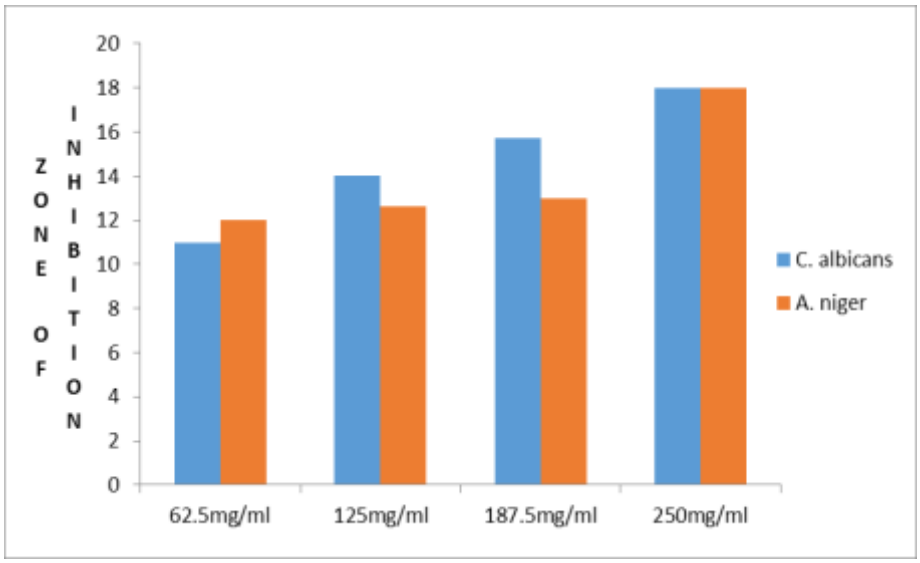
Microorganism	Zone of Inhibition (mm)			
	62.5 Mg/ml	125 Mg/ml	187 Mg/ml	250 Mg/ml
Candida	11.0±1.000	14.0±2.783	15.7±1.530	18.0±2.180
Aspergillis	12.0±1.000	12.6±1.258	13.0±0.500	18.0±1.892

Table 3: inhibition of Aspergillus niger and Candida albicans by Chloroform extracts of leaves of Calotropis procera.

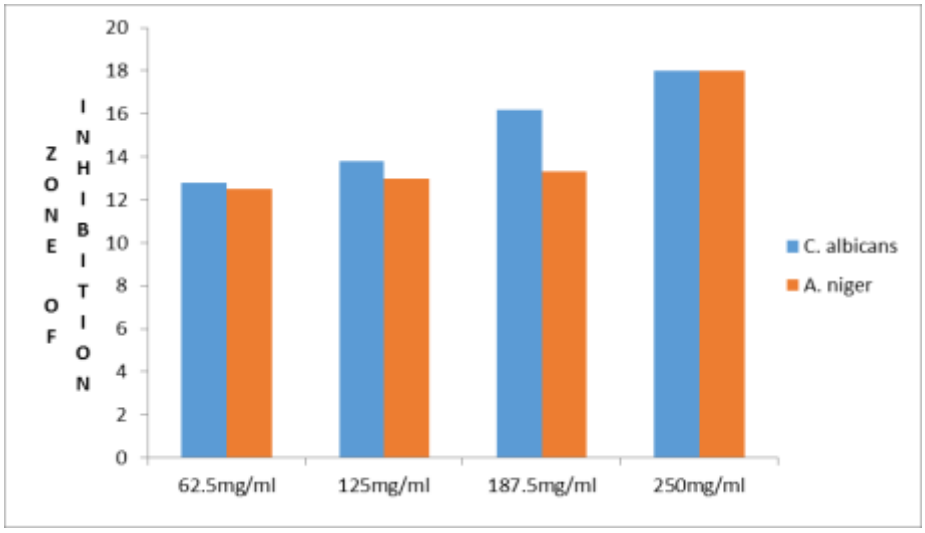
Microorganism	Zone of Inhibition (mm)			
	62.5 Mg/ml	125 Mg/ml	187.5 Mg/ml	250 Mg/ml
C.albicans	12.8±1.6017	13.8±1.607	16.2±1.040	18.0±1.260
A. niger	12.5±0.500	13.0±0.500	13.3±1.040	18.0±1.040



Graph 1. Antifungal activirtyof *Calotropis procera.*



Graph 2. Antifungal activirtyof *Calotropis procera.*



Graph 3. Antifungal activirty of *Calotropis procera*

CONCLUSION

The analysis of antifungal activity of ethanolic, Petroleum ether and chlorofom of leaves of Calotropis procera against *Aspergillus niger* and *Candida albicans* reveals in well diffusion method, the zone of inhibition produced by the above extracts showed inhibitory effects on the growth of isolates. The effect exhibited by ethanolic extract of leaves of Calotropis procera was significantly greater than Chloroform and Petroleum ether extract of leaves. The results provide a use of *Calotropis procera* linn, in traditional medicine and suggest its further advance investigation.

REFRENCE

1. Irvine, F. R. Woody plants of Ghana. Oxford University Press, London., 1961; 48-50.
2. Parrotta, J. A. Healing plants of peninsular India. (AB International Wallingford, UK., 2001; 944.
3. Maurya RC and Mir JM. *Int. J. Sci. & Eng. Res.*, 2014; 5: 305
4. Domenico B. Novel antifungal drugs. Curr Opin Microbial., 1999; 2: 509-15.
5. Trease GE, Evans WC. Pharmacognosy. Brailliar Tiridel Can. Acmillian Publishers., 1989; 13.
6. Mamta Goyal and Rashmi Mathur, International Journal Of drug Discovery And Herbal Researach, July-September 2011; 1(3): 138-143.
7. Cheesbrough, M.M. District laboratory practice in Tropical countries, part I Cambridge university press. London., 1991; 157-206.