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Research Article

SCREENING FOR ANTIOXIDANT AND FREE RADICAL SCAVENGING POTENTIAL OF EXTRACTS OF LEAVES AND FLOWERS OF *Calotropis gigantea*

SHAZIA USMANI

Assistant professor,Faculty of pharmacy, Integral university, Kursi road, Lko ,Email : Shazia_usmani2001@yahoo.com

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ABSTRACT

Calotropis gigantea L, belonging to family: Asclepiadaceae is also known as Sweat akand, is used in traditional medicine for treatment of various ailments. The surface microscopy of leaf was performed along with powder microscopy. Leaf and flower extracts of *Calotropis gigantea* were prepared by using various solvents like Acetone, Chloroform and Methanol in increasing polarity. The reference drug used was Silymarin. Antioxidant activity was studied by DPPH method.The level of percentage inhibition was found to be more in the Methanolic extract(64%). Since no such work has been investigated and reported in detail earlier, therefore an effort has been made to explore the antioxidant activity in this plant.

Keywords: *Calotropis gigantea;* Silymarin; DPPH; Antioxidant.

INTRODUCTION

Calotropis gigantea L, belonging to family: Asclepiadaceae, also known as sweat akand is found throughout plains and lower hills of India usually near water found growing upto an altitude of 900m throughout India including Andamans (1, 2).

Various chemical constituents have been reported from different parts of the plant (3). Flowers contain waxy matter which has esters of resinols, α-, β-calotropeol, β -amyrin, stigmasterol, giganteol, calotropin, a triterpenoid flavonoid, flavonoid glycoside, wax, acids and alcohols (1, 5). Seeds are rich in aminoacids, major being phenylalanine, lysine and histidine. The leaf contains ascorbic acid, ortho-pyrocatechic acid and also contains β -amyrin, taxasterol, tarasterol and beta-sitosterol (1). Shoot and leaf extracts possess antibacterial activity. Tender fresh leaves have been reported to cure fits and convulsions in children. Extracts of leaf with oil and rock salt warmed are poured into ear for earache (1). Fresh warmed leaves or poultice is bandaged on painful rheumatic (1). Plant is purgative, antihelmintic, antitumor and has been used in diseases of spleen and liver (2). Leaves have been used in enlargement of liver and flowers are also good for liver Paracetamol induced hepatic damage in rats has been reported (7). Aerial parts were collected from medicinal garden of BBDNITM and authenticated by pharmacognostic, phytochemical and other studies while voucher (sample No. N.B.R.I/CIF/Re/08/2008/32) was deposited in taxonomy lab, Ethnopharmacology division, NBRI, Lucknow for future reference. Healthy male wistar rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ±3^{0}C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum.*

MICROSCOPICAL EVALUATION-

Leaf (Surface microscopy)

1. Stomata-Stomata were observed on both the upper and lower epidermis and were of paracytic type. The average number of stomata per square millimeter were found to be between 7 to 10.

Fig 1: *Calotropis gigantea* (leaves)-Stomata.

2. Trichomes- Multicellular uniseriate trichomes were observed on both upper and lower epidermis.The average number of trichomes per square millimeter were found to be 3-7 and length of trichomes were found to be between 0.024 to 0.1mm.

Fig.2: *Calotropis gigantea* (leaves)- trichomes

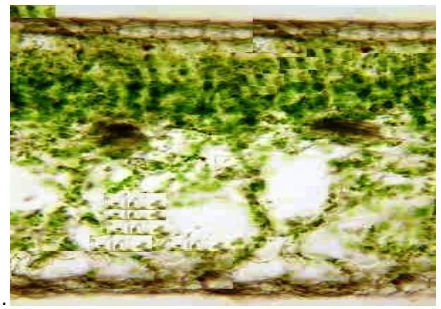


Fig. 2. a

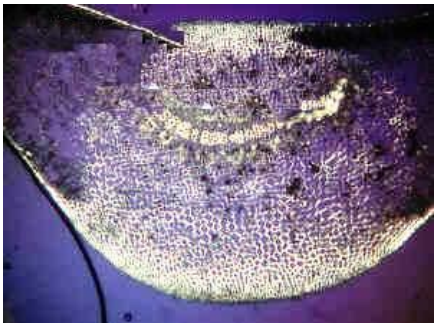


Fig. 2. b

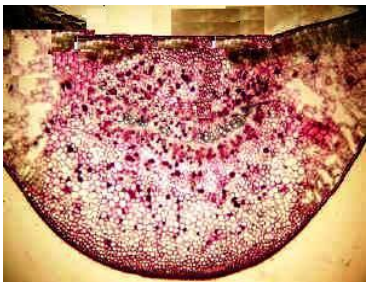


Fig. 2. c

Fig. 2. *Calotropis gigantea* leaves: Transverse section

3. In transverse section, the leaves showed an isobilateral character with lower palisade layer and a mesophyll in between. The midrib showed vascular bundles in a semicircular ring.

4. Vein islet number-An average of 4-5 vein islet no. was observed after five observations.

Powder microscopy-

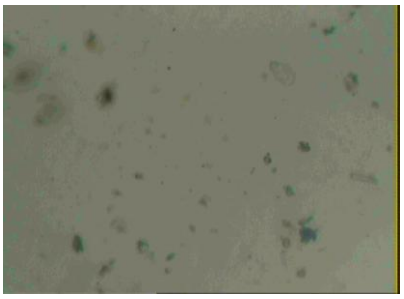


Fig. 3.5 a : IODINE

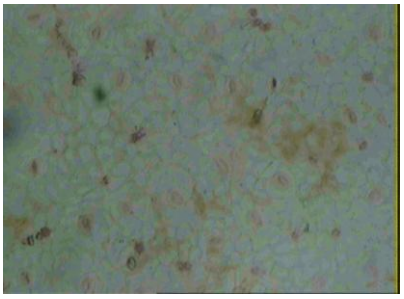


Fig. 3.5 b : SUDAN RED

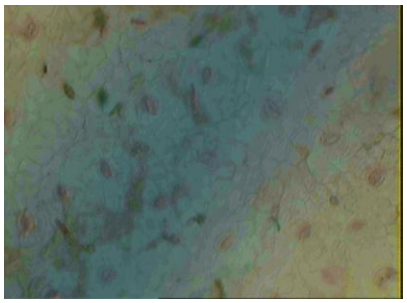


Fig.3.5c: SAFRANIN

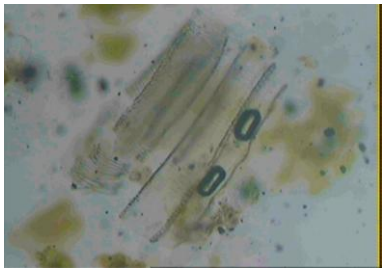
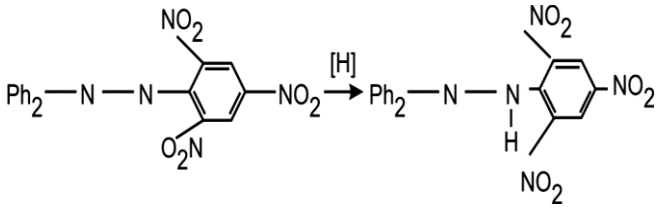


Fig.3.5d :PHLOROGLUCINOL & DIL HCL

SCREENING FOR ANTIOXIDANT ACTIVITY

DPPH METHOD

DPPH is stable nitrogen centered free radical and has been extensively used to characterize an antioxidant. The reduction of DPPH radical serves as a quick and simple method to detect the antioxidant potential of compounds especially those with phenol group. It is known that DPPH react rapidly with compound containing weak N – H or O – H bonds. Electron transport is also an important mechanism for its reduction. It is reversible, reduced and due to its unpaired electron, densely colored. This property makes it suitable for spectrophotometric studies.



Reduction of 1,1 diphenyl 2 Picryl

Procedure

The free radical scavenging effect of fractions was assessed by the decoloration of a methanolic solution of DPPH with minor modifications. Samples for Screening of antioxidant and free radical scavenging potential of various extracts of leaves were dissolved in 0.1 ml DMSO and added to 0.1 ml of 0.1 m M DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature in the dark. The absorbance at 565 nm by DPPH was measured by a spectrophotometer. BHT was used as a positive control.

Statistical analysis:

(Anova) followed by Newmann Keuls test, was carried out & $p < 0.005$ was considered as significant . Groups were compared with control group.

Screening for antioxidant and free radical scavenging potential of extracts of leaves.

GROUP	TREATMENT	CONC µg/ml	DPPH RADICAL SCAVENGING ACTIVITY	
			ABSORBANCE	%
I	Control Blank	----	0.892	-----
II	Ref (BHT)	1000	0.175	80***
III	Acetone (sample-1)	15	0.607	39.2*
IV	Chloroform (sample-2)	30	0.570	39.7*
V	Methanol (sample-3)	45	0.565	37*

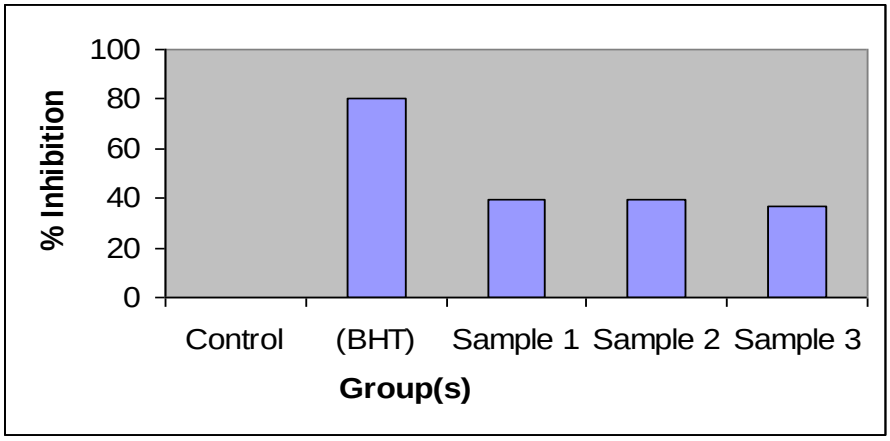


Fig 4(a). Screening for antioxidant and free radical scavenging potential of extracts of leaves.

Table 4. b Screening for antioxidant and free radical scavenging potential of extracts of flowers.

GROUP	TREATMENT	CONC µg/ml	DPPH RADICAL SCAVENGING ACTIVITY	
			ABSORBANCE	%
I	Control Blank	----------	0.892	-----
II	Ref (BHT)	1000	0.175	80***
III	Acetone(sample-1)	15	0.690	23*
IV	Chloroform(sample-2)	30	0.670	25*
V	Methanol(sample-3)	45	0.560	33*

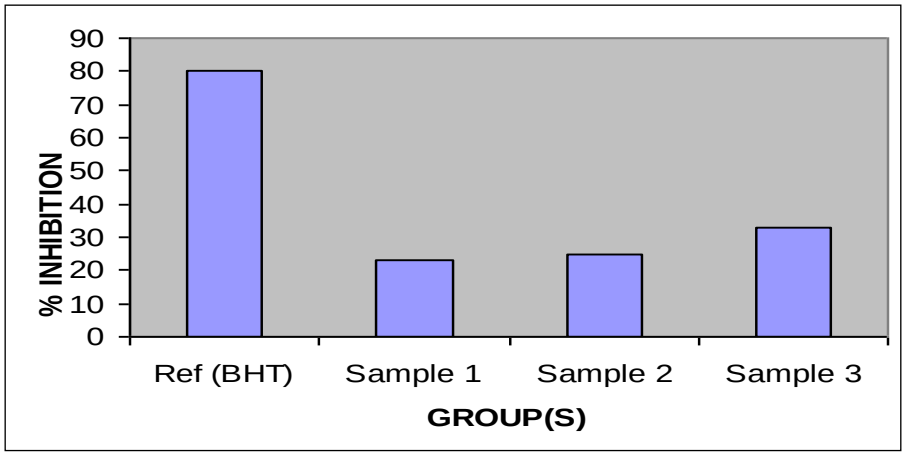


Fig.4(b) Screening for antioxidant and free radical scavenging potential of extracts of flowers.

CONCLUSION

Free radicals are a major cause of initiation of cellular injury and may cause oxidative stress which may lead to diseases like cancer, tissue injury, neuro-degenerative diseases, arthritis etc. The free radicals initiate a long chain of cellular process causing changes in intracellular calcium ion balance ultimately causing gradual destruction of calcium skeleton of the cells leading to necrotic changes.

While investigating the antioxidant potential of leaves and flowers of Calotropis gigantean, it was found that the acetone and chloroform extracts showed nominal free radical scavenging activity of 30% and 37% respectively whereas methanolic extract showed considerable significant free radical scavenging activity of 64% ($p<0.001$). The standard BHT showed considerable significant free radical scavenging activity of 80%.

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