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PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTI-DIABETIC ACTIVITY OF STEM BARK EXTRACT OF *CALOTROPIS GIGANTEA* LINN

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ABSTRACT

The present study aims to screen the preliminary photochemical present in the stem bark extracts of *Calotropis gigantea*. *Calotropis gigantea* Linn. is prevalently known as the aak, swallow-wort or milkweed. In the traditional system of medicine, the plant is used as one of the most important drugs to treat different diseases. The results showed the presence of phytochemical active compounds of alkaloids, flavanoids, glycosides, phenolics compounds, tannins, saponins, sterols, proteins, and amino acids in the stem bark extracts. Medicinal plants are the key source of bioactive compounds such as phenolics, tannins, alkaloids, and flavonoids which have been demonstrated *in vitro* to show anti-diabetic properties.

Keywords: Phytochemical screening, anti-diabetic activity, *Calotropis gigantea*

INTRODUCTION

Calotropis is a diminutive genus and having 6 different species of shrubs or small trees, scattered in arid, dry, tropical and subtropical region of Africa, Asia and central, South America(Kirtikar *et al.,* 1994) and known as Akada, Aak, Mandar, Aakh, etc (Kori and Alawa 2014). *Calotropis gigantea* is one of the noteworthy herbal plant which play a key role and is useful in preventing and treatment of various diseases of the human being (Chattopadhyay *et al.,* 2004; Ahmad 2020). It is laticiferous shrubs, glabrous or hoary, about 6-10 feet in height (3-4 m) commonly known as the aak, swallow-wort or milkweed. The diameter of the stem up to 20 cm and in erect form (Carol *et al.,* 2012; Dhivya and Manimegalai *et al.,* 2013; Singh *et al.,* 2014). The plant has oval, light green leaves, and milky stems. *Calotropis gigantea* has marvelous anti-inflammatory activity. It show tremendous wound healing activity and considered as usual cleansing and potent astringent properties help in the early healing of the wound, itching, healing skin diseases and splenic disorders (Ranjit *et al.,* 2012; Alluri and Majumdar 2014; Rajamohan *et al.,* 2014; Kumar *et al.,* 2010; Ahmad 2020). Plant also used as a digestive stimulant that cures the various digestive disorders and also considered for the normal functioning of body organs. Various parts of the plant are used from ancient Indian time for the treatment of various ailments of human beings. It is utilized in different polyherbal preparations, formulations are used alone or in formulation with other herbs to cure a variety of human and animals ailments (Kumar *et al.,* 2013; Singh *et al.,* 2014).

The key role the present investigations were to estimating the phytochemical and antidiabetic activity of *Calotropis gigantea* Linn. stem bark extracts. In the anti-diabetic study, we additionally check the effect of the α-amylase test on stem bark extracts.

MATERIALS AND METHODS

Plant Collection and Authentication

The medicinal plant was collected from dry and arid local area of Hisar, Haryana. The plant collected was identified botanically in department of Botany, Maharshi Dayanand University Rohtak Haryana by Dr. S.S Yadav, Assistant Professor. The stem bark of the selected plant was collected and then the dust of the plant was removed by running fresh water. The sample was dried for a few days in the shade after drying the plant material was grinded into powered form and stored into bags (polythene) for further studies.

Evaluation Parameters

Evaluation of significant parameter including total ash, swelling index, loss of drying, crude fiber content, foaming index, bitterness value, foreign organic matter, were studied (Anonymous 1996; WHO 2000; WHO 2002; Singh and Devi 2020)

Preparation of plant extracts

Soxhlet extraction method was employed for the extraction process of crude plant. 100 g of powdered plant material (stem bark) was consistently crammed into a thimble and extracted by using 500 ml of various solvent systems based on increasing polarity of solvents. Rotary evaporator apparatus was used for evaporation of solvents after extraction and recorded the percentage yield. Dried extracts are placed in desiccators and then transferred and stored in airtight containers for further research investigations.

Phytochemical Screening

The concentrated extract of selected plant was subjected to different chemical tests for the detection of different Phytoconstituents using standard methods. (Harborne 1973; Trease and Evans 1989; Singh *et al.,* 2012; Singh

Table 1: Different evaluation parameters of *Calotropis gigantea* Linn. stem bark

S. No	Parameters	*Calotropis gigantea* Linn. stem bark
1.	Total ash (%)	6.04 ± 0.01
2.	Swelling Index	Absent
3.	Loss on drying (%)	5.3 ± 0.01 % w/w
4.	Crude fiber content (%)	2.25
5.	Foaming index	Less than 100
6.	Bitterness value	Bitter
7.	Foreign organic matter (%)	0.45 ± 0.03 %w/w

Table 2: Preliminary phytochemical screening of *Calotropis gigantea* Linn. Stem extracts

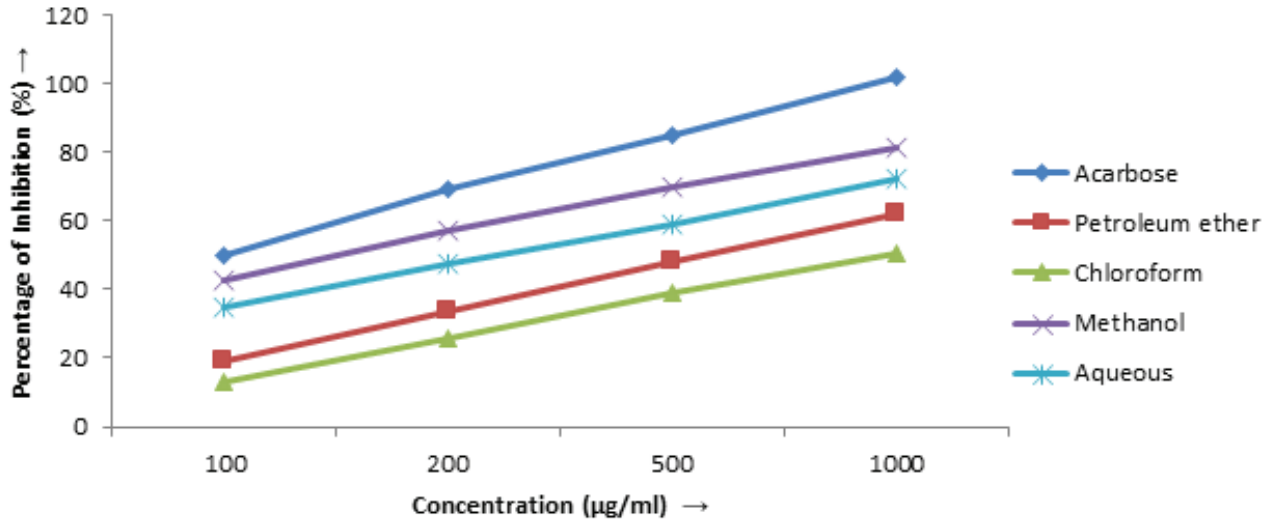
S. NO	Plant constituents	STEM BARK EXTRACTS			
		PE	C	M	A
1	Alkaloids	+	+	+	+
2	Carbohydrate	-	-	-	-
3	Flavonoids	+	+	+	+
4	Glycosides	+	-	+	+
5	Phenolics compounds and tannins	+	-	+	+
6	Proteins and amino acids	+	+	+	+
7	Saponins	-	-	-	+
8	Sterols	+	+	+	+

+ Present; -Absent; PE-Petroleum ether; C- Chloroform; M- Methanol; A- Aqueous

Table 3: Inhibition of *In vitro* α-amylase enzyme activity of *Calotropis gigantea* Linn. stem extracts

Conc (µg/ml)	Percentage inhibition				
	Acarbose	Petroleum ether	Chloroform	Methanol	Aqueous
100	50.23±0.7	19.21±0.8	13.05±0.76	42.87±0.9	35.03±0.2
200	63.42±0.8	33.53±0.4	25.86±0.23	57.03±0.2	47.32±0.1
500	89.04±0.9	48.07±0.5	39.62±0.76	69.76±0.3	59.31±0.2
1000	100.08±0.2	62.04±0.4	50.39±0.06	81.52±0.4	72.05±0.4
IC50	100.81±0.7	590.05±0.4	1000±0.2	140±0.3	250±0.7
6	Proteins and amino acids	+	+	+	+
7	Saponins	-	-	-	+
8	Sterols	+	+	+	+

*All values are expressed as MEAN±SEM (n=3), Extracts groups was compared by One way ANNOVA followed by Dunnett's test *** P < 0.001, ** P < 0.01, *P < 0.05.*



All the values are expressed in terms of mean±SEM (n=3), One way ANNOVA followed by Dunnett's test

and Devi 2018). They were screened for the presence of

Alkaloids, carbohydrates, flavonoids, glycosides, phenolics compounds, tannins, saponins, sterols, proteins and amino acids

***In vitro* Anti Diabetic Activity**

Measurement of α-amylase inhibitory activity

The activity was determined using micro-plate reader according to the method reported by Rani *et al.,* 2012 with slight modifications. The concentrations of the plant extract used were 100µg/ml, 200 µg/ml, 500µg/ml and 1000µg/ml. 500mg of soluble starch was dissolved in 25ml of 0.4M NaOH solution and heated at 100ºC for 5minutes to prepare the substrate solution. The pH of the solution was adjusted to 7.0 with 2M hydrochloric acid after cooling in ice water and the volume was adjusted to 100ml with water. The sample solutions were prepared by dissolving each sample in phosphate buffer (pH 7.0) to make different concentrations (100µg/ml, 200 µg/ml, 500µg/ml and 1000µg/ml). 40µL of substrate solution and 20µL of sample solutions were mixed in a microplate well and incubated for 3minutes at 37ºC. Then to each well, 20µL of α-amylase solution (50µg/ml)was added and incubated for 15minutes. To terminate the reaction, 80µL of 0.1M hydrochloric acid and 200µL of 1mM iodine solution was added and the absorbance was measured at 650nm.

Inhibitory activity (%) was calculated as follows:

Percentage of Inhibition=]× 100

where, Abs1 is the absorbance of incubated solution containing extract, starch and amylase; Abs2 is the absorbance of incubated solution containing extract and starch; Abs3 is the absorbance of incubated solution containing starch and amylase; Abs 4 is the absorbance of incubated solution containing starch.

RESULTS AND DISCUSSION

Results of different evaluation parameters are shown in table 1.

Preliminary phytochemical study

The aqueous extracts, chloroform, methanol, petroleum ether extracts of stem bark were implanted for the preliminary phytochemical screening using standard analytical method. The results of tests for alkaloids, carbohydrates, flavonoids, glycosides, phenolic, tannins, saponins, sterols, proteins and amino acids are shown in table 2.

CONCLUSION

The aqueous and alcoholic extracts showed presence of alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, sterols, proteins and amino acids, so that it might be utilized for further development of herbal based medicines and further research investigation is needed to find out the potent novel active compounds from the medicinal plants which may develops a new treatment strategy for many incurable diseases. The presence of these phytoconstituents reveals that *Calotropis gigantea* Linn. stem bark extracts can further study for its different medicinal uses.

Conflict of interest – Nil

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