International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 25-05-2021, Accepted: 08-06-2021, Published: 23-06-2021
Volume 6, Issue 3, 2021, Page No. 788-790

Phytochemical and haemolytic activity on Stems of *Calotropis gigantea* Linn

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Abstract
Since ancient times, people have used medicinal plants to treat varied diseases. Medicinal plants are the important source of drugs, and many of them that are currently available in the pharma ceutical market are obtained from plant sources. The objective of present studies deals with the preliminary phytochemical studies on the aqueous extract of stems of *Calotropis gigantea* Linn. And phytochemical test for the identification of active constituents. Saponin was identified by foam test. Since the most characteristic properties of saponin is their ability to cause haemolysis, when added to suspension of blood saponin produced changes in erythrocyte membranes, causes haemoglobin to diffuse into surrounding medium.
The aqueous extract of *Calotropis gigantea* Linn. Produce haemolysis in the test tube 11 (i.e 0.90ml of extract or 0.009gm/ml of extract) and it was calculated by using formula given in the procedure. The haemolytic activity was found to be 722 with reference to standard saponin R i.e. 1000 unit. The result of haemolytic activity is shown in Fig.1.

Keywords: *Calotropis gigantea* Linn, stem and aqueous extract

Introduction
A medicinal plant is any plant which, in one or more of it contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants Posses therapeutic properties or exert Beneficial Pharmacological effects on the animal body are generally designated as "Medicinal Plant". In current scenario of medical and pharmaceutical advancement, microbes involve in the change of their metabolism and genetic structure to acquire resistant against the drugs used in the treatment of common infection disease [1-2]. The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptation by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agent currently in use [3]. Plant have the capacity to produce a large number of organic chemicals called as phytochemicals. The accumulation of phytochemicals in the plant cell cultures had been studied for than thirty years and the generated knowledge had helped in realization of using cell culture for the production of desired phytochemicals [4].
Calotropis belong to Asclepidaceae family. It is also known as Akada, Aak, Mandar, Aakh etc. It has two species procera and gigantea. Here we study about Calotropis Gigantea [5]. The roots and leaves of calotropis gigantea are used traditionally for treatment of abdominal, tumours boils, skin diseases, wound, insect bites. A literature review showed that Calotropis Gigantea contained cardenolide, glucosides, a non-protein, amino acid, flavonoids and steroids. Calotropis gigantea in small dose are also useful in the treatment of cold, cough, asthma inflammatory diseases and loss of digestive and analgesic property of Calotropis Gigantea [6-7].
The objective of present study is to focus on Phyto chemical characteristics and haemolytic activity of stems of *Calotropis gigantea* Linn.

Material and Method
Plant material
The plant specimens for the proposed studies were collected from in the deep forest of Satpuda hills with the help of forest officers of Chopda tahsil, Dist. Jalgaon, Maharshtra (India) in the month of Dec. 2020 care was taken to select healthy plants and for normal organs. The plant was authenticated by Prof. (Dr.) C. R. Jadhav, Scientist, BSI (Botanical Survey of India), Pune (M.S.).

Preliminary Phytochemical Parameters
Preliminary phytochemical test of *Calotropis gigantea* Linn. Were performed and the chemical constituents were detected [7-8].

Haemolytic Activity [9-10]
Many medicinal plant materials, especially those derived from the families Caryophyllaceae, Araliaceae, Sapindaceae, and dioscoreacae contain Saponins. The most characteristic property of Saponins is their ability to cause haemolysis: when added to a suspension of blood, Saponins produce changes in erythrocyte membranes, causes haemoglobin to diffuse into the surrounding medium.
The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, Saponins R, which has haemolytic activity of 1000 units per gm. A suspension of erythrocytes is mixed with equal volumes of serial dilution of the plant material extract. The lowest concentration of effect complete haemolysis is determined after allowing the mixtures to stand for given period of time. A similar test is carried out simultaneously with Saponins R.
Procedures proposed for the determination of haemolytic activity of saponaceous medicinal plant material are based on the same principle although the details many vary, e.g. the source of erythrocytes, methods for the preparation of the erythrocytes suspension and the plant material extract,

the defined haemolytic activity of the reference material of saponin, and the experimental method. In order to obtain reliable result, it is essential to standardize the experimental Conditions, and especially to determine the haemolytic activity by comparison with that of saponin R.

Preparation of Erythrocytes Suspension

To prepared erythrocyte suspension filled a glass stoppered flask to one-tenth of its volume with sodium citrate (36.5g/I) TS, swirling to ensure that the inside of the flask is thoroughly moistened. Introduced a sufficient volume of the of blood freshly collected from healthy ox and shake immediately.

Preparation of reference solution

About 10 mg of Methylene salicylate, accurately weighed, to a volumetric flask and add sufficient phosphate buffer pH 7.4 TS to make 100ml.This solution should be freshly prepared.

Preparation of test solution

1gm of aqueous extract of stems of *Calotropis gigantea* Linn. Was dissolved in 100ml of water to produce concentration of 0.01 gm/ml.

Procedure

Prepared serial dilution of the plant material extract, with phosphate buffer pH 7.4 TS and blood suspension (2%) using 13 test tubes as shown in (Table 1).

Carried out the dilutions and evaluation as mentioned in following table and observed the results after 24 hours. Calculate the amount of medicinal plant material in g, or of the preparation in g or ml, that produce the total haemolysis. To eliminate the effect of individual's variations in resistance of the erythrocyte suspension to saponin solutions, prepare a serial dilutions of saponin R in the same manner as described above for the plant material extract. Calculate the quantity of saponin R that produces total haemolysis (Table 1).

Table 1: Haemolytic activity determination procedure

	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant material extract (diluted if necessary) (ml)	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
Phosphate buffer PH 7. 4 TS (ml)	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.5	--
Blood suspension (2%) (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Results and Discussion

Preliminary Phytochemical Studies

Aqueous extract of stem of *Calotropis gigantea* Linn. Showed the presence of various Phytoconstituents showed in (Table 2).

Table 2: Preliminary phytochemical screening of various extracts of stems of *Calotropis gigantea* L.

Sr. No.	Constituents	Tests	Methanol	Aqueous
1.	Alkaloids	Mayer's test	-	-
		Dragordraff's test	-	-
		Hager's test	-	-
		Wagner's test	-	-
2.	Sterols	Libermann's Burchard test	+	+
		Salkowski's	+	+
3.	Carbohydrates and Glycosides	Molisch's test	+	+
		Fehling's test	+	+
		Benedict's test	+	+
		Borntrager's test	+	+
4.	Fixed Oils and Fats	Spot test	-	-
		Saponification test	-	-
5.	Phenolic compound	$FeCl_3$ test	-	+
6.	Protein and Amino acids	Biuret test	-	-
		Ninhydrin test	-	-
		Xanthoprotein test	-	-
		Millon's test	-	-
7.	Triterpinoid and Saponins	Foam test	-	+
		Haemolysis test	-	+
8.	Tannins	Gelatin test	-	-
		$FeCl_3$ test	-	-
9.	Gums and Mucilage	Ppt. with 90% alcohol	-	-
10.	Flavonoids	Aqueous NaOH	+	+
		Conc. H_2SO_4	+	+

Haemolytic activity

Since the most characteristic properties of saponin is their ability to cause haemolysis, when added to suspension of blood saponin produced changes in erythrocyte membranes, causes haemoglobin to diffuse into surrounding medium.

The aqueous extract of stem of *Calotropis gigantea* Linn. Produce haemolysis in the test tube no.11 (i.e. 0.90ml of extract or 0.009gm/ml of extract) and it was calculated by using formula given in the procedure. The haemolytic activity was found to be 722 with reference to standard

saponin R i.e. 1000 unit. The result of haemolytic activity is shown in (Figure 1).

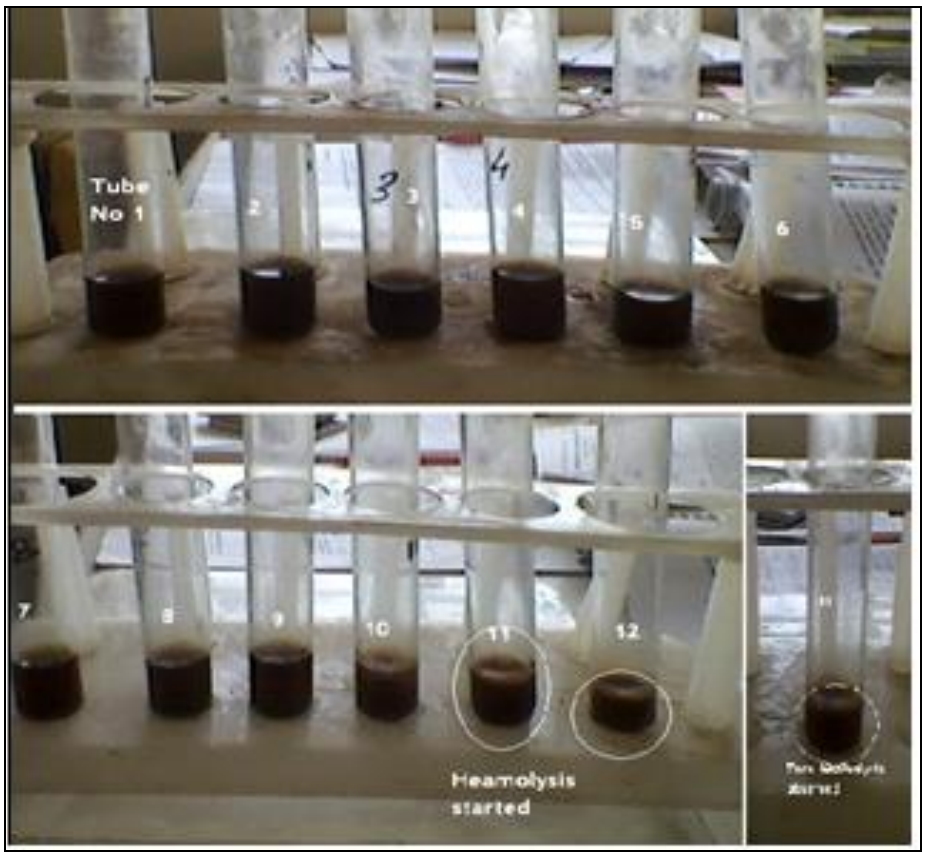

Fig 1: Result of haemolytic activity

Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 * a/b$$

Where 1000= the defined haemolytic activity of saponin R relation to OX blood,
a= quantity of saponin R that produces total haemolysis (g),
b= quantity of plant material that produces total haemolysis (g).
= 1000*0.65/0.90
= 722

Conclusion

The present phytochemical studies on stems of *Calotropis gigantea* Linn. Might be useful to supplement assumed significantly in the way of acceptability of herbal drugs in present scenario that lacks regulatory laws to control quality of herbal drugs.

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