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PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS AND FRACTIONS OF LEAVES OF *CALOTROPIS PROCERA* (AIT) R.BR.

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ABSTRACT: Aim: *Calotropis procera* is a perennial shrub belonging to the family Asclepiadaceae. The aim and objective of the study are to screen the ethanolic, methanolic, and chloroform extract of leaves for their phytochemical constituents and the comparison of the phytochemical components. Further evaluation of fractions of methanolic extracts of *C. procera* leaf. **Methods:** The various extracts *viz.* ethanolic, methanolic, and chloroform of leaves of *Calotropis procera* screened for the various secondary metabolites like alkaloids, flavonoids, tannins, glycosides, saponin, proteins, *etc.* the methanolic extract of *C. procera* was fractionated by using Column Chromatography with ethyl acetate and acetone. **Results:** The methanolic and chloroform extract of *Calotropis procera* shows the presence of alkaloids, glycosides, saponins, flavonoids, and proteins. While the ethanolic extract of *C. procera* shows the presence of alkaloids, tannins, saponins, protein, and flavonoids. The ethyl acetate fractions mainly contain the flavonoids and glycosides.

Keywords: *Calotropis procera*, Asclepiadaceae

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INTRODUCTION: *Calotropis procera* is a small shrub of family Asclepiadaceae, commonly known as "Giant milkweed" or "Sodom apple" in English, "Madar" in Hindi, and "Rui" in Marathi. *C. procera* is a small tree or shrubs up to 2.5 to 5 meter height, usually stem simple, rarely branched, woody at the base and covered with fissured, corky bark. Branches somewhat succulent and densely, white tomentose [1].

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All parts of the plant exude white latex when cut or broken. The leaves are sub-sessile, opposite, broadly ovate-oblong, elliptic, acute, thick, glaucous, green, and covered with fine cottony pubescent hairs [1].

It found in most parts of the world with a warm climate, sandy and alkaline soils. It frequently occurs in Indonesia, Malaysia, China, and India as wasteland weed. In India, it is found from Panjab and Rajasthan to Assam and Kanyakumari up to an altitude of 1050 meter [2]. The plant contains the cardenolide, proceragenin while the bark contains benzoylinesolone, benzoylisolinelone. The leaves and stalk contain calotropin and calotropagenin while flower contains calotropenyl acetate and multi-flavonol, and the latex contains uzarigenin

and terpenol ester [3]. In leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and three toxic glycosides calotropin, uscharin, calotoxin [4]. All parts *viz*., root, stem, leaf, and flowers of *Calotropis* are in common use in indigenous system of medicine. Latex has been used in leprosy, eczema, inflammation, cutaneous infection, syphilis, malaria, and low hepatic fever [5].

FIG. 1: IMAGE OF *CALOTROPIS PROCERA* (AIT)

The leaf extract has been shown to have analgesic, anti-inflammatory [6], hypoglycaemic [7], larvicidal [8], antibacterial [9], antiangiogenic [10,] and antioxidant activities [11].

MATERIALS AND METHODS:

Collection of Plant: The plant was collected from the campus area of Swami Ramanand Teerth Marathwada University, Nanded. The authentication of the plant was done by Prof. Kadam Sir, Head of Ausa College where the voucher specimen prepared and deposited (Voucher specimen no.1/12/2015). The leaves of the plant were collected and shade dried. Shade-dried plant leaves were powdered by using an electrical blender. Coarse powder of leaves was used for extraction. The powder was stored in polythene airtight bag until the extraction process.

Extraction and Fractionation of Leaves: The coarse powder of leaves was separately extracted with chloroform, methanol, and ethanol by using Soxhlet extractor. 100 gm of leaf powder was extracted by using 500 ml of each methanol, ethanol, and chloroform. The extract was filtered with Whitman filter paper and concentrated by using a water bath. The concentrated extract was weighed and stored in an airtight bottle the yields were 3.71%, 8.07%, and 15.35% respectively. Most active extract (methanolic) was further fractionated with ethyl acetate and acetone by using solid-liquid partition technique. Fractions were dried using rotary evaporator and were equivalent to 7.95% and 4.1% respectively of the dry methanolic extract. The extracts and fractions were preserved in vacuum desiccators at 4 °C until further use.

Phytochemical Screening: Phytochemical screening was carried out using standard methods to detect bioactive compounds like alkaloids, tannins, flavonoids, saponins, steroids, phenols, *etc.*

Test for Flavonoids: Take 500 mg of plant sample and dissolve in 5 ml of ethanol. Gently warm the solution and then filter. Add a few pieces of magnesium chips the filtrate and then add a few drops of conc HCl. Observe for the change in coloration. Formation of pink, orange, or red to purple coloration is a positive confirmation test for the presence of flavonoids [12].

Test for Tannins: Take 0.50 gm of the dried powdered sample of plant. Boil dried powdered samples in 20 ml of water in a test tube and then filtered. Add a few drops of 0.1% ferric chloride ($FeCl_3$) and observe for brownish green or blue-black coloration. The appearance of brownish green or blue-black color is considered a positive test for tannins [12].

Test for Glycosides: An aliquot of 5 ml of extract and its various fractions (10 mg/ml in methanol) were added in the sequence of glacial acetic acid (2 ml) and $FeCl_3$ solution (one drop). Concentrated H_2SO_4 (1 ml) was added to it. Formation of the brown ring at the interface confirmed the presence of cardiac glycosides [12].

Test for Alkaloids: 0.5 g of the plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate was treated separately with both reagents (Maeyer's and Dragendorff's) After which it was observed whether the alkaloids were present or absent in the form of turbidity or precipitated formation [13].

Test for Steroids and Triterpenoids: Take 5 ml of plant extract and mix with few drops of acetic anhydride and boil. Then carefully add 3 ml of concentrated H_2SO_4 to form a layer. Observe the

formation of reddish-brown coloration at the junction of two layers shows positive results for the presence of triterpenoids and steroids [12].

Test for Saponin: Boil 2 g of the powdered sample in 20 ml of distilled water in a water bath and then filter. Take 10 ml of the filtrate and mix with 5 ml of distilled water and shake vigorously for a stable, persistent froth. Then add 3 drops of olive oil into a froth and shake vigorously. After vigorous shaking observes for the formation of an emulsion. Formation of the emulsion is considered as a positive test of saponin [12].

RESULTS AND DISCUSSION: The various extracts of *Calotropis procera* leaves *viz.*, methanolic, ethanolic and chloroform shows the following phytochemical as,

TABLE 1: PHYTOCHEMICAL SCREENING OF *CALOTROPIS PROCERA* LEAVES EXTRACT

S. no.	Phytochemical Tests	Methanolic extract	Ethanolic extract	Chloroform extract
1	Alkaloids	+	+	+
2	Glycosides	+	-	+
3	Tannins and phenolics	-	+	-
4	Saponin	+	+	+
5	Steroids and triterpenoids	-	-	-
6	Proteins and amino acid	+	+	+
7	Flavonoids	+	+	+

(+) indicates present; (-) indicates absent.

TABLE 2: PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *CALOTROPIS PROCERA* LEAVES

S. no.	Phytochemical Tests	Ethyl acetate fraction	Acetone fraction
1	Alkaloids	+	+
2	Glycosides	++	-
3	Tannins and phenolics	-	+
4	Saponin	+	+
5	Steroids and triterpenoids	-	-
6	Proteins and amino acid	+	+
7	Flavonoids	++	+

(++) indicates present in higher amount; (-) indicates absent.

The result of phytochemical screening of methanolic, ethanolic, and chloroform leaf extract of *Calotropis procera* revealed the presence of alkaloids, flavonoids, proteins, and saponins. The methanolic and chloroform extracts show the presence of alkaloids, glycosides, saponins, proteins, and flavonoids. While the ethanolic extract shows the absence of glycosides and steroids.

There while the fractions of methanolic extract show an elevated amount of glycosides and flavonoid content in ethyl acetate fraction in comparison to the acetone fraction. The presence of these components in this species is an indication that it may have some medicinal potential. The parts of the plant used in Ayurvedic medicine are leaves, root, root bark, and flowers. The powdered leaves are used in healing wounds, as a purgative and to treat indigestion. The leaves used to treat joint pain and reduce swelling. It also used in homeopathic medicine [2].

CONCLUSION: *Calotropis procera* leaf extract made in chloroform, methanol, and ethanol contains different phytochemical constituents with biological activities. **Table 1** shows the preliminary phytochemical screening of *Calotropis procera* leaf extract and elevated concentration of that flavonoids and glycosides in ethyl acetate fraction a depicted in **Table 2**. It is interesting to note that the action of the extract of *C. procera* is non-toxic. The obtained results provide support for the use of this plant in traditional medicine. The presence of flavonoids and glycosides which revealing some medicinal uses of these fractions.

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CONFLICT OF INTEREST: Nil

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