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PHYTOCHEMICAL ANALYSIS OF FRUIT AND BARK OF *CALOTROPIS PROCERA* (AIT.) R.BR. MEDICINAL PLANT COLLECTED FROM *BOKARO* DISTRICT, JHARKHAND

Shailendra Kumar Roy*[1], Bharat Bhushan[2] and Devendra Kumar[3]

[1,3]Department of Botany, Radha Govind University, Jharkhand.

[2]Department of Botany, Central University of Jammu, J&K.

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***Corresponding Author**
Shailendra Kumar Roy
Department of Botany,
Radha Govind University,
Jharkhand.

ABSTRACT

An erect, perennial shrub in the Asclepiadaceae family, *Calotropis procera* which flourishes in dry soil and is widely cultivated throughout most of India and other tropical nations. Long before prehistoric times, it was employed for medical purposes. Several manuscripts discussed the application of *C. procera*, also known as Apple of Sodom. According to several Hindu religious scriptures, the shrub *C. procera* is employed in traditional medical and folklore systems to treat a variety of ailments. Along with the Arabic, Unani, and Sudanese systems, it is commonly used in Indian traditional medicine system. The plant is frequently used to treat a variety of illnesses, including skin conditions and in particular elephantiasis. Some components can be extracted for use in treating colic as well as skin conditions, ulcers, spleen enlargement, and illnesses of the liver. The milky exudate is beneficial for skin conditions, tumours, and ascites. It is an excellent purgative. Our phytochemical studies on the plant parts of *C. procera* such as stems, leaves, fruits, latex, etc. revealed the presence of numerous phytochemicals such as flavonoids, glycosides, terpenoids, tannins, alkaloids, coumarins, phenolics, saponins, proteins, and steroids. The presence of these phytochemicals showed that this plant is effective against a number of microorganisms and have anti-cancer, anti-malarial, anti-oxidant and immunomodulatory properties.

KEYWORDS: *Calotropis*, pharmacological, phytochemical, flavonoids.

INTRODUCTION

Calotropis procera is a member of the Asclepiadaceae family and is one of the six species of *Calotropis* weed that possess numerous therapeutic characteristics. The natural habitats of this species are wastelands in Asia and Africa; it is found in South China, Pakistan, Malaysia, Indonesia, Vietnam, Cambodia, Bangladesh, Sri Lanka, India, and Thailand. There are several names for this plant, including "giant milkweed," "shallow wort," and so on. Their distinctive thick, broad leaves and unscented, purplish-colored blossoms facilitate identification. The 4–8 inch long, decussate, obovate, or briefly acute leaves of the perennial *Calotropis* plant, which grows to a height of 4–6 meters, are cordate or frequently amplexicaul at the base. The plant produces a white, milky latex that contains cardiac glycosides such as gigantin, uscharidin, calotoxin, uscharin, and calactin. These glycosides have been shown to have significant wound healing potential. In contrast, the leaves are said to have antibacterial and anti-inflammatory qualities.

Many illnesses, including as syphilis, boils, inflammation, epilepsy, hysteria, fever, muscle spasms, warts, leprosy, gout, snakebites, and cancer, are said to be treated by the entire tree, which is believed to have medicinal properties. *C. procera* is generally used in Ayurvedic, Chinese, and homeopathic medicine to treat illnesses such as rheumatism, leprosy, leukoderma, diarrhea, fever, coughs, and asthma. It is actually used, according to the homeopathic *Materia Medica*, to treat toothaches, vomiting, and elephantiasis. It has been found that *C. procera's* alcoholic extract may increase skin permeability. Therefore, the pharmacological qualities of *Calotropis procera*, such as its anti-diabetic, anti-toxin, anti-hepatotoxin, antioxidant, and wound-healing effect, attract researchers from all over the world. Therefore, the goal of the current study is to gather information on the phytochemical activities of the extracts in order to determine the chemical composition of the active ingredients.

Components of chemicals Studies on *Calotropis* phytochemical makeup have produced a variety of chemicals, including flavonoids, tannins, sterol, saponins, cardiac glycosides, alkaloids, resins, anthocyanins, and proteolytic enzymes in latex (Patil and Saini, 2012; Kirtikar and Basu, 1999). However, the *Calotropis gigantea* leaves contain five main chemicals: Pleurone, Calotropagenin, Calotoxin, Methyl β-carboline-1-carboxylate, and (+)-dehydrovomifoliol (Bhandari et al., 2019; Wadhwani et al., 2021).

Large number of researchers worked on Calotropis so far. Few of them are Lewis and Lewis (1995); Kirtikar and Basu (1999); Cowman (1999); Onomir and Olorinfemi (1998); Oudhia (2001); Vohra (2004); Arya and Kumar (2005); Quaquebeke et al., (2005); Hassan et al. (2006); Kuta (2008); Yesmin et al. (2008); Bhagar et al., (2009); Mainsara et al., (2011); Patil and Sainia (2012); Shobowale et al., (2013); Kori and Alawa (2014); Aliyu et al., (2015); Uthirasamy and Chitra (2018); Bhandari et al., (2019); Ahmed (2020) and Wadhwani et al., (2021).

MATERIALS AND METHODS

Plant Collection

In August 2022, fresh leaves, roots, bark, and fruits of *Calotropis procera* were gathered from the Bokaro district in Jharkhand. The voucher specimen was created and placed at the Herbarium, Department of Botany, Radha Govind University, Ramgarh, Jharkhand, where the species was recognized and authenticated. Using a mortar and pestle, the plant components were ground into a coarse powder after being sun dried. The powdered samples were sieved using Sieve No. 80 and kept in polythene bags until needed for usage, following Onomire and Olorunfemi's (1998) procedure.

Preparation of extract for phytochemical screening

The plant parts that were powdered were extracted separately using water, methanol, and 95% ethanol. The solutions were concentrated in a water bath after the extracts were filtered through Whatman No. 1 filter paper. The extracts were placed in polythene bags and maintained in various containers that were labeled before examination.

Phytochemical Screening

Following Sofowara's (1982) procedures, the water, methanol, and ethanol extracts of the fruit and bark of C. procera were examined to determine whether any of the following substances were present: proteins, amino acids, phenols, glycosides, tannins, alkaloids, and tannin-producing compounds.1 gm of the extract was dissolved in 25 ml of water to create the test solution.

A. Test for Carbohydrates

Following tests were carried out for carbohydrates.

a) Molisch's test: Two drops of freshly made 20% napthol and a mixed alcoholic solution of concentrated sulphuric acid were added to the test tube holding the drug extract, and the test

tube was then sealed. If there are any carbohydrates present, the intersection of two liquids will turn purple or reddish violet in color.

b) Benedict's test: Benedict's solution should be added to a test tube containing drug extract. The combination should be properly mixed, heated for two minutes, and then cooled. Red precipitate formation brought on by the presence of carbohydrates.

c) Barfoed's test: After heating the 0.5 ml solution under inspection to a boil, the barfoed's solution was added. Carbohydrate presence was revealed by the formation of a red copper oxide precipitate.

d) Anthrone test: Add the drug extract to the two milliliters of anthrone test solution. The presence of carbohydrates was indicated by a green or blue color.

B. Test for Alkaloids

a) Dragendorff's Test: A small amount of the drug extract, dissolved in 5 milliliters of water, was mixed with 2 milliliters of hydrochloric acid to cause an acid reaction. After adding 1 milliliter of Dragendorff's reagent (potassium bismuth iodide solution), an orange-red precipitate was added, signifying the presence of alkaloids.

b) Wagner's test: Use 1.5% v/v hydrochloric acid to acidify the drug extract, then add a few drops of Wagner's reagent (iodine potassium iodide solution). Precipitate with a reddish brown coloration suggested the presence of alkaloids.

c) Mayer's Test: The presence of alkaloid was shown by the production of a dull white precipitate in two millilitres of extract solution when two to three drops of Mayer's reagent (potassium mercuric iodide solution) were added.

d) Hager's Test: When three millilitres of Hager's reagent—a saturated solution of picric acid—were added to the drug solution extract, the presence of alkaloids was verified by the production of a yellow precipitate.

C. Test for Glycosides

a) Legal's test: After the extract solution was dissolved in pyridine, an alkaline solution of sodium nitroprusside was added. The presence of glycosides was indicated by the colour pink red.

b) Baljet's test: When sodium picrate solution was added to the drug extract, the presence of glycosides was detected by a yellow to orange hue.

c) Borntrager's test: A few millilitres of a diluted solution of sulfuric acid were added to the extract test solution. After filtering, ether or chloroform was boiled with the filtrate. After the

organic layer was separated and ammonia was added, the orange layer's production of pink, red, or violet colour indicated the existence of glycosides.

d) Keller Kiliani test: One millilitre of very concentrated sulfuric acid was cautiously added at the test tube's edge after the methanolic extract had been dissolved in glacial acetic acid with a hint of ferric chloride. Glycosides were indicated by a red hue at the interface of the two liquids and a blue colour in the acetic acid layer.

D. Test of Saponins

a) After diluting 1 ml of alcoholic extract with 20 ml of distilled water, the mixture was agitated in a graduated cylinder for fifteen minutes. A single centimetre of foam suggested the existence of saponins.

E. Test for Flavonoids

a) Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

F. Test for Protein and Amino acid

a) Biuret's test: When two to three millilitres of the drug extract are combined with one millilitre of 40% sodium hydroxide solutions and two drops of 1% copper sulphate solution, a purplish-violet or pinkish-violet hue is formed, signifying the presence of proteins.

b) Ninhydrin's test: The extract was mixed with two drops of freshly made 0.2% ninhydrin reagent, brought to a boil for one to two minutes, and then allowed to cool. Proteins, peptides, or amino acids are indicated by the development of a blue hue.

c) Xanthoprotein test: Conc. nitric acid should be added to the extract in a test tube. After heating, the white precipitate turns yellow, so gently cool the solution. An excess of 20% sodium hydroxide solution revealed the existence of aromatic amino acids, as shown by the orange hue.

d) Millon's test: The drug extract was diluted in distilled water and a little amount of Millon's reagent (five to six drops) was added. When heated, a white precipitate appeared, indicating the presence of proteins.

e) Lead Acetate test: After taking the extract, two millilitres of a 40% sodium hydroxide solution were added, boiled, glacial acetic acid was added, cooled, and then one millilitre of a lead acetate solution was added. A grey-black precipitate that included sulfur-containing amino acid was created.

G. Detection of phenols Ferric Chloride Test: Three to four drops of a ferric chloride solution were applied to the extracts. Presence of phenols is indicated by the formation of bluish black hue. Three to four drops of a ferric chloride solution were applied to the extracts. Presence of phenols is indicated by the formation of bluish black hue.

RESULTS AND DISCUSSION

The phytochemical components of *Calotropis procera* fruit and bark that were determined through phytochemical testing are listed in table 1.1. Tannins, flavonoids, saponins, alkaloids, glycosides, cardiac glycosides, steroids, and volatile oil were the phytochemical components that were found.

Table 1.1: Phytochemical constituents of *C. procera* fruit and bark.

Constituents	Extracts					
	Water		Methanol		Ethanol	
	Fruit	Bark	Fruit	Bark	Fruit	Bark
Flavonoids	++	++	+		++	-
Tannins	+	+	+	+	-	-
Saponins						
Frothing	++	-	-	-	-	-
Emulsification	++	++	-	-	-	-
Alkaloids						
Wagner’s Reagent	+	++	-	-	+	++
Mayer’s reagent	+++	++	-	-	-	-
Glycosides	+	-	+	+	-	-
Steroids	++	+	+	-	+	-
Cardiac glycosides	++	+	-	-	+	+
Volatile Oil	++	+	+	+	+	-

Key: +++ = present in high concentration, ++ = moderately present, + = Trace, - = Absent

Fruit and bark extracts in aqueous and methanol extracts were found to contain tannins, whereas no tannins were detected in the ethanol extract. Flavonoids were found in fruit and bark aqueous extracts. Flavonoids were detected in fruit methanol extract but not in bark extract. Flavonoids were detected in fruit ethanol extracts but not in bark extracts. Tests such as emulsification and foaming were used to monitor saponins. By using an emulsification test, fruit and bark aqueous extracts both demonstrated the presence of saponins, however methanol and ethanol extracts did not exhibit any saponins. By using a foaming test, saponins were found in the fruit extract only and not in the bark extract in the aqueous extract of the two. The methanol and ethanol extracts did not contain any saponins. Wagner's and Mayer's

reagent tests were used to observe alkaloids. In fruit and bark water extracts, Mayer's reagent produced favourable results; however, in methanol and ethanol extracts, it produced negative results. Wagner's reagent test produced favourable results for fruit and bark aqueous and ethanol extracts, but negative results for methanol extract.

Glycosides were detected in fruit aqueous extract, however they had an adverse effect on *Calotropis procera* bark aqueous extract. When it came to the fruit and bark extracts, methanol extract produced positive results, but the ethanol extract produced negative ones. Cardiac glycosides were detected in fruit and bark aqueous and ethanol extracts, but they had adverse effects in fruit and bark methanol extracts. In aqueous extracts of fruits and bark, as well as in methanolic and ethanolic fruit extracts, steroids produced favourable outcomes; however, they produced unfavourable results in methanolic and ethanolic bark extracts. All fruit and bark extracts, with the exception of the ethanol extract of the bark, were found to contain volatile oil.

CONCLUSION

Because the plant extract contains a variety of phytochemicals that either actively prevent the growth of microorganisms or have the potential to do so, it may be helpful as an antibacterial agent.

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