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Full Length Research Article

Phytochemical and Antibacterial Properties of Root and Leaf Extracts of *Calotropis procera*

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ABSTRACT: Phytochemical and antibacterial properties of water, methanol and ethanol extracts obtained from root and leaf of *Calotropis procera* were investigated. Antibacterial growth inhibition was determined using Ditch method against *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Streptococcus pyrogenes*. The phytochemical screening reveals the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, balsams and volatile oil and steroids with higher amount in water extracts. The concentration of the phytochemical constituents were in the order of water > methanol > ethanol. Water extracts showed broad spectrum activity against the tested organisms at concentrations of 30, 60, 90 and 120 mg/ml. Methanol and ethanol extracts did not show a significant effect against the tested organisms at 120 mg/ml as compared with those of tetracycline. The result of this study validates the use of water extract of this species in ethnomedicine and could provide a lead in the isolation of antibacterial agents from water extracts of *Calotropis procera*.
Keywords: *Calotropis procera*, antibacterial, phytochemical, ethnomedicine

INTRODUCTION

Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in trade and medicinal practices in recent years (Ojo *et al.,* 2005). Many researchers have examined the effects of plants used traditionally by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated. In many developed countries 70- 80% of the population have used some forms of alternative or complementary medicine e.g. acupuncture, while in most African countries 80% of the population depends on traditional medicine for primary health care (WHO 2005).

Calotropis procera (Ait) R. Br. (Sodom Apple) belongs to the family Asdepiadaceae. It occurs in most parts of the tropical world, in dry sandy and alkaline soils, in waste land and grows abundantly as a weed. It is a shrub which grows up to 5.5 m high, occasionally branchless to a height of 2.5 m. All parts of the plant exude white latex when cut or broken, leaf is large ovate, opposite, sessile, up to 30 cm long and 16 cm wide (Aliyu, 2006). Medicinally, the pungent sap latex is used to treat boils, infected wounds and other skin problems in people and to treat parasitic skin infestation in animals. It also yields ash for making gun powder; the latex is processed and used in treating vertigo, baldness, hair fall, tooth aches, intermittent fevers, rheumatoid /joints swellings and paralysis (Vohra, 2004). The whole plant when dried and consumed is a good tonic, antihelmintic and as an expectorant (Agharkar, 1991; Warrier *et al.,* 1996). The dried root is used to cure bronchitis, asthma, leprosy, eczema and elephantiasis, hepatic and splenic enlargement and as eye tonic (Vohra, 2004). The milky juice and caustic flowers were considered to improve digestion, catarrh and increases appetite (Oudhia, 2001). The juice was also found to induce abortion in women and tanners use the milky juice to remove hair from the hides (Singh *et al.,* 1996). The presence of alkaloids calotropin, calotaxein and uskerin has been reported and are stimulant to heart (Ashwari, 2009). It is also used by traditional medicine practitioners in Gwari communities for the treatment of ring worms (Kuta, 2008).

In North Western Nigeria, *C. procera* is used by local communities for the treatment of infectious diseases. There is the need to carry out scientific investigations to ascertain the authenticity of the claims on the medicinal properties of this species. The aim of this study is to evaluate the phytochemical and antibacterial properties of root and leaf extracts of *C. procera*.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh leaves and root of *C. procera* were collected from Tudun Wada ward of Gusau, Zamfara state. The species was identified and authenticated at the Herbarium Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, where the

voucher specimen was prepared and deposited. The plant parts were sun dried and pulverized into coarse powder using mortar and pestle. The powdered samples were sieved and stored in polythene bags until when required for use, in accordance with method of Onomire and Olorunfemi (1998).

Extraction and Preparation of Material for Phytochemical Screening

The powdered plant parts were separately extracted with 95% ethanol, methanol and distilled water. The extracts were filtered using Whitman No. 1 filter paper and the solutions were concentrated using water bath. The extracts were stored in different containers labelled and kept in polythene bags before analysis. The methods of Trease and Evans (1989) and Sofowora (1982) were used for the phytochemical screening of the extracts.

Antibacterial Screening

The microorganisms used for antibacterial screening, were *Staphylococcus aureus*. *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Streptococcus pyrogenes*, obtained from Microbiology Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto. The well plate diffusion method was used to determine the growth inhibition of bacteria by plant extracts as described by Mohammad and Dabai (2008). The nutrient agar (Muller Hinton) plates were prepared and seeded with the test organisms. Four holes of 6.0 mm diameter each were made on to the plates with a sterile cork borer and filled with 30, 60, 90 and 120 mg/ml extracts. The inoculated plates were allowed to congeal for 30 min to allow pre diffusion time and then incubated at 37^0C for 24 hrs. The plates were examined for zones of inhibition (Cheesbrough, 2001). The diameter of such zone of inhibition was measured using a transparent meter ruler and the value was recorded and expressed to the nearest millimeter.

RESULTS AND DISCUSSION

The results of phytochemical screening of water, methanol and ethanol root and leaf extracts of *C. procera* revealed the presence of alkaloids, flavonoids, saponins, tannin and glycosides (Table 1). The concentrations of the various classes of secondary metabolite vary amongst the extracts evaluated. The concentrations of the constituents are in order of water > methanol > ethanol. The presence of these components in this species is an indication that it may have some medicinal potential. This is due to the fact that each of the components identified has one therapeutic usage or another. For instance, plants rich in saponins have immune boosting and anti inflammatory properties (Kenner and Requena, 1996). Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors (Hassan *et al.,* 2005; Aliero *et al.,* 2008; Yesmin *et al.,* 2008).

The antibacterial activity of *C. procera* water, methanol and ethanol extracts obtained from the root and leaves on the test organisms exhibited antibacterial activity (Figures 1-6). Generally, *Salmonella typhi* and *Streptococcus pyrogenes* were observed to be the most susceptible organisms, this result is similar to that of Yesmin *et al.* (2008). Similarly, *Staphylococcus aureus* is least susceptible in this study and same observation was reported by Aliero *et al.* (2008). The methanol extracts of *C. procera* root did not show appreciable activity against, *S. pyrogenes* and *S. aureus* (Figure 4).

Table 1: Phytochemical constituents of root and leaf extracts of *C. procera*

	Extracts					
	Water		Methanol		Ethanol	
Constituents	Root	Leaf	Root	Leaf	Root	Leaf
Tannins	-	+	+	-	-	+
Flavonoids	-	+++	-	+	-	+
Saponins	++	+	-	-	-	-
Alkaloids	++	+++	+++	+++	++	++
Saponins Glycosides	-	++	-	+	-	+
Cardiac Glycosides	++	+++	+	+	+	+
Steroids	+++	++	++	-	++	-
Balsams	-	+			-	-
Volatile Oils	++	+++	+	++	+	+
Glycosides	+	+	-	+	-	-

Key: +++ = present in high amount, ++ = moderately present, + = Trace amounts, - = absent

The ethanol extracts *C. procera* root showed no activity on *S. aureus, Salmonella typhi* and *E. coli* (Figure 6). A similar observation was also reported by Yesmin *et al.* (2008) on *C. procera* growing in India. Likewise, water extracts obtained from the root did not show activity on all the test organisms except against *Pseudomonas aeruginosa* (Figure 2). This lack of activity was also observed by Usman *et al.* (2005) on leaf extracts of *Celtis integrifolia*. Similarly, methanol, ethanol and water extracts of *C. procera* showed significant antibacterial activity against both the Gram positive and Gram negative bacterial strains (Yesmin *et al.,* 2008). Zone of inhibition of tetracycline on the five test organism is presented in Table 2, *Salmonella typhi* showed the highest inhibition with 22 mm followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 20 mm each. The result of this study justifies the use of water extracts of *C. procera* in ethnomedicine for the treatment of infectious diseases caused by susceptible bacterial species.

Table 2: Zone of inhibition (mm) by synthesized standard antibiotic drug (Tetracycline) on the five Test organisms

Organisms	Zone of inhibition (mm)
Streptococcus pyrogenes	18
Staphylococcus aureus	20
Salmonella typhi	22
Escherichia coli	17
Pseudomonas aeruginosa	20

Note; Diameter of zone of inhibition is 6 mm; any zone of inhibition greater than 6 mm implies activity

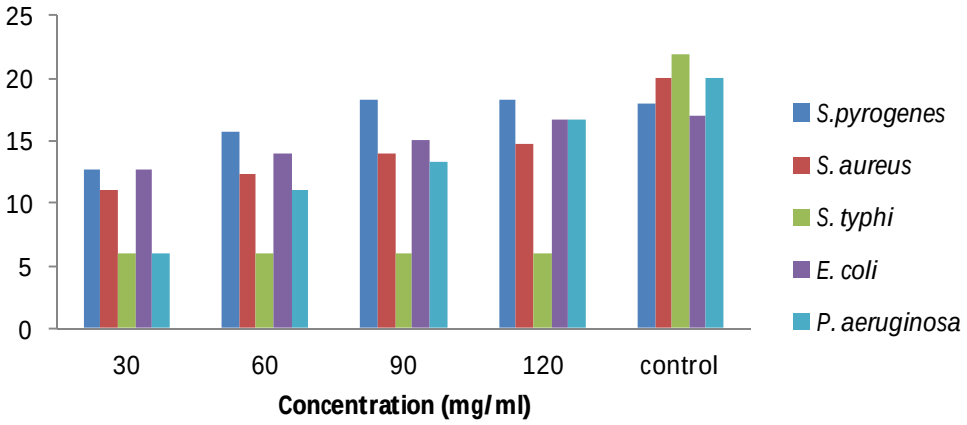

Figure 1: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of water extracts of *C. procera* leaf.

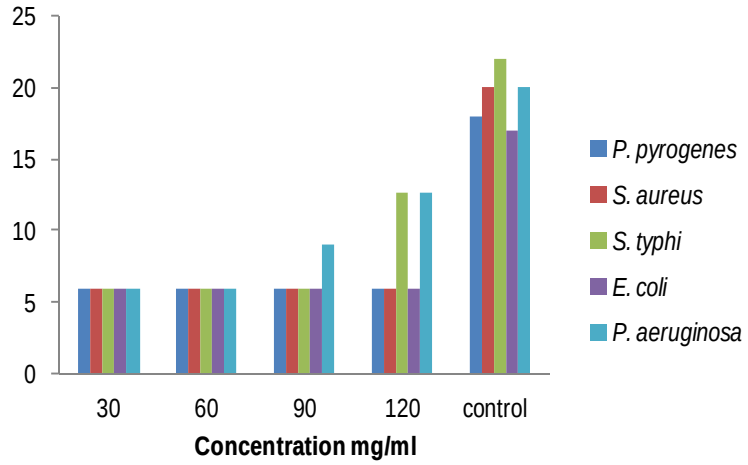

Figure 2: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of water extract of *C. procera* root.

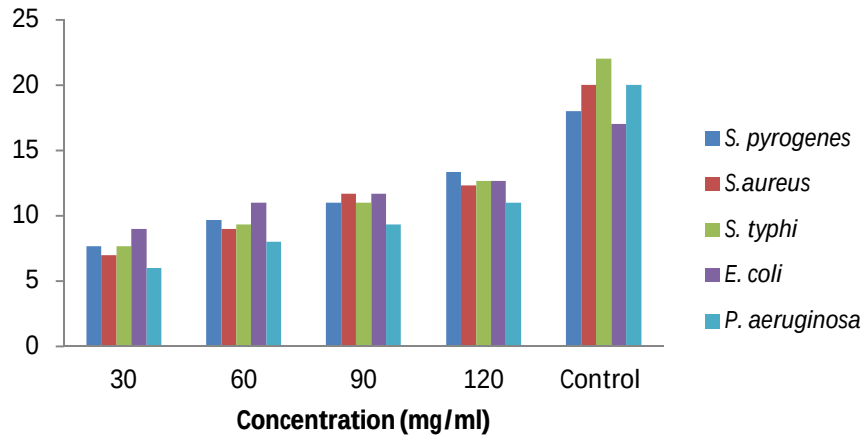

Figure 3: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of methanol extract of *C. procera* leaf.

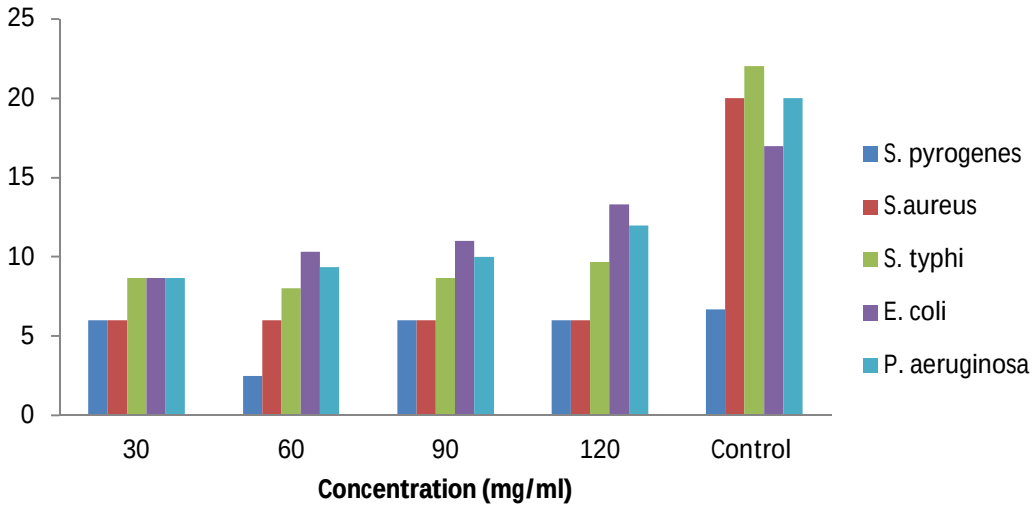

Figure 4: Diameter size of inhibition zone on the growth of different bacterial species due to application of different concentration of methanol extract of *C. procera* root.

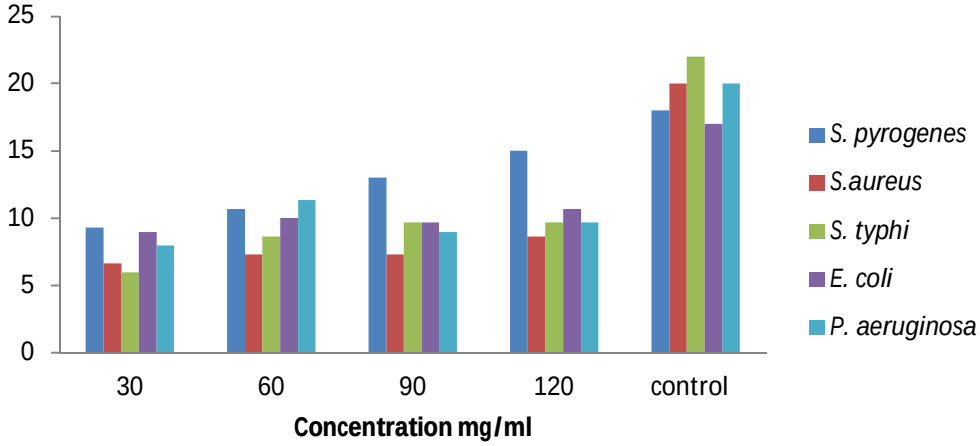

Figure 5: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of ethanol extract of *C. procera* leaf.

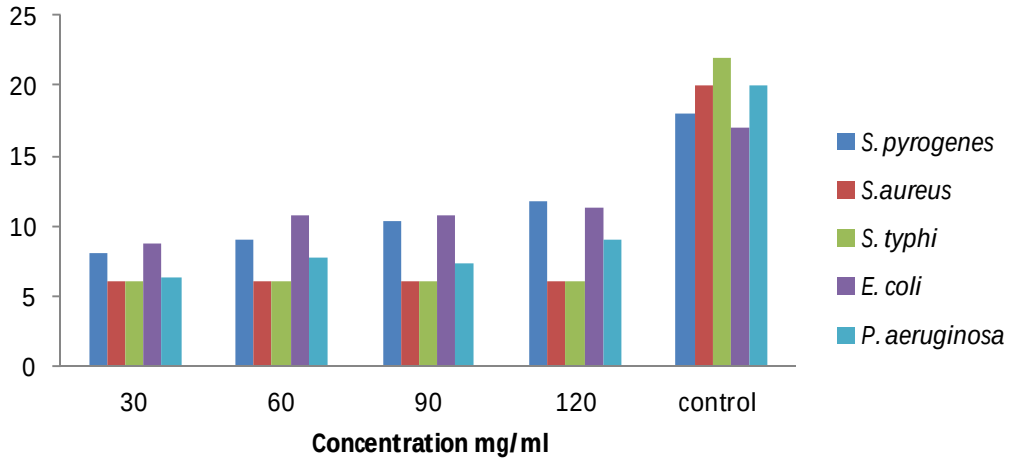

Figure 6: Diameter of inhibition zone on the growth of different bacterial species due to application of different concentration of ethanol extract of *C. procera* root.

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