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Assoc. Prof. Dr. H. Turan AKKOYUN

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Busari M. B. Hamzah R. U. Yusuf R. S. Yahaya, A. S. Oladejo N. A.	*Federal University of Technology Minna*	Comparative qualitative phytochemical contents and antimicrobial activities of methanol extract of Calotropis procera flower and latex
Aloui Mourad Menana Elhalaoui	*Sidi Mohamed Ben Abdellah University*	Rational design of potent and selective survivin inhibitors based on MX-106 hydroxyquinoline derivatives: A 2D-QSAR, molecular docking, dynamics, and ADMET study

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*Busari M. B.[1], Hamzah R. U. [1], Yusuf R. S.[2], Yahaya, A. S.[3], Oladejo N. A.[1]

[1]Department of Biochemistry, Federal University of Technology Minna, Nigeria
[2].Department of Biochemistry, Bauchi State University, Nigeria
[3].Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma Katsina State, Nigeria
Corresponding Author: busari.bola@futminna.edu.ng

COMPARATIVE QUALITATIVE PHYTOCHEMICAL CONTENTS AND ANTIMICROBIAL ACTIVITIES OF METHANOL EXTRACT OF CALOTROPIS PROCERA FLOWER AND LATEX

ABSTRACT

Microbial infections are common disease among many ages and sexes. The most pathetic circumstances about their management is their resistance to the available drugs; which necessitate alternative therapy. Both *C. procera* flower and latex have been widely used locally in Nigeria to tackle microbial skin infections without any antimicrobial comparisons. Therefore, this study investigated the phytochemical constituents and in vitro activities of methanol extract of *C. procera* flower (MECPF) and its latex (CPL) against *E. coli*, *Pseudomonas aeruginosa and Staphylococcus aureus* using standard methods. Maximum inhibitory concentration of both MECPF and CPL were carried out on each bacterium at 20 mg/ml, 40 mg/ml and 60 mg/ml using standard methods. The MECPF and CPL revealed the presence of phenol, flavonoids, anthraquinone, steroids and terpenoids, while glycoside and tannins are only present in CPL. None of the concentration (20 mg/ml, 40mg/ml and 60 mg/ml) of CPL is however active agains*t E. coli*, while only 40 mg/ml and 60 mg/ml of MECPF are active against the same organism. The maximum concentration (20 mg/ml) of CPL shows higher zone of inhibition when compared with 60 mg/ml of MECPF on *pseudomonas aeruginos*a. Contradictorily, maximum concentration (20 mg/ml) of CPL shows higher zone of inhibition on staphylococcus aureus compared to the maximum concentration (60 mg/ml) of MECPF. Therefore, CPL possesses higher antimicrobial activities on *pseudomonas aeruginosa and Staphylococcus aureus* but lower antimicrobial activities against E. coli when compared with MECPF.

Keywords: *Calotropis procera*, Microbial infections, *Pseudomonas aeruginos*a, Phytochemicals.

INTRODUCTION

Many modern antibiotics drugs have been used to battle infectious diseases after the discovery of the first drug ‘’penicillin’’ in the year 1928 by Sir Alexander Fleming (Bello et al, 2020; Alshiekheid, 2023). However, overuse and indiscriminate uses of antibiotics in human and veterinary medicine have led to dramatically increased resistance by the microbes (Bello et al, 2020). Bacterial infections have become issue of major concern due to the evolution of bacterial resistance over the years (Ahmed et al., 2024). There is a concerning rise in the number of deaths linked to bacterial infections as a result of bacteria developing resistant to antibiotics (Zhang et al., 2024).

The antibiotic resistance among Nigerians are more compounded probably due to inadequate medical services (Bello et al., 2020). Additionally, it has been projected that, by 2050, nearly 10 million annual death will be as a result of antibiotic resistance (Church and McKillip, 2021). In order to curtail this menace, novel and alternative treatment options need to be sought. Therefore, natural sources like medicinal plants could be explored as affordable antimicrobial agents.

Plants have been used as a medicinal source since primitive times. The traditional and folk medicines utilize plant, animals and their products for the treatment of various transmittable illnesses (Asefa et al., 2025). Furthermore, numerous studies have confirm the potential use of plant and animals materials for novel drug discovery and development (Asefa et al., 2025).

Calotropis procera is commonly called Sodom apple, dead sea apple, swallowwort, milkweed (English), kisher (Arabic), pomme de sodome (French), bomubomu (Yoruba), and tumfafiya (Hausa), is a wild-growing plant that belongs to the family *Asclepiadaceae*. It is widely distributed in the tropical and subtropical regions of Africa and Asia (Bello et al., 2020). Conventionally, *C. procera* is used to treat fever, rheumatism, pain, asthma, bronchitis, ulcer, indigestion, cold, eczema, measles, diarrhea, abscesses, and jaundice. elephantiasis, cough among others (Sigh et al., 2024).

Hence, the aim of this study was to determine the phytochemical contents and the antimicrobial effect of the methanol extract and latex of *Calotropis procera*.

Plate 1 : *Calotropis procera*

MATERIALS AND METHODS

MATERIALS

Collection and Identification of the Plant Material

The *Calotropis procera* flower and latex was collected within the school environment in Federal University of Technology, Minna Gidan kwano Campus, Bosso Local Government, Niger State, Nigeria on 4th of April, 2024. The plant was identified at the Department of Plant Biology, Federal University of Technology, Minna by Dr. Daudu O. A. Y. Voucher number FUT/PLB/ASC/004 was allotted to the plant and the sample was deposited at the herbarium of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

Experimental Organisms

The *E. coli, staphylococcus aureus and Pseudomonas aeruginosa* used were obtained from Microbiology section of Centre of Genetic Engineering Federal University of Technology, Minna. The bacteria were cultured in agar plates in a temperature of about 37°c (body temperature) under aerobic condition in a sterile environment to prevent contamination.

Methods

Samples preparation

The flowers were removed from the stalk and washed thoroughly to remove unwanted materials. Thereafter, the flowers were spread under the shade at 30℃. The dried samples were later pulverized using electric blender and later transferred into air tight plastic container before being refrigerated at 2℃ for further use.

Furthermore, the latex sample was collected from the whole plant in a clean conical flask afterwhich the sample was lyophilized using freeze dryer. The dried sample was later transferred into a air tight glass container before being refrigerated at 2℃ for further use.

Extraction of *C. procera* Flower

Extraction of *C. procera* performed by soaking 250 g powder of *C. procera* flower in 500 mL of absolute methanol in 1 Liter volumetric flask. The mixture was left for 72 hours with constant vortexing. Subsequently, the liquid extract was separated with Whatman filter paper (No 1). Finally, the methanol fraction of the extract was obtained by evaporating methanol solvent using rotary evaporator, then with water bath at 40℃. Thereafter, the resulting molten solution was lyophilized using freeze dryer

Phytochemical Screening

Qualitative and quantitative tests were carried out on the powdered sample of the dried flower and latex sample using standard procedures described by (Sofowara, 1993),

Qualitative Phytochemical Screening of *C. procera*

Test for Flavonoids

A portion of powdered plant in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed thus indicating the presence of flavonoids.

Test for Tannins

0.5g of the dried powdered sample was boiled in 20ml distilled water in a test tube and filtered. 0.1% ferric chloride ($FeCl_3$) solution was added to the filtered. The appearance of brownish green or a blue-black colouration indicate the presence of tannins in the test sample

Test for Saponins

2.0g of the powdered sample was boiled in 20ml of distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins

Test for Alkaloid

0.5 of extract was stirred with $5cm^3$ of 1% aqueous HCI on a steam bath, few drops of picric acid solution was added to $2cm^3$ of the extract. The formation of a reddish-brown precipitate was taken as a preliminary evidence for the presence of alkaloids.

Test for steroids

0.5g of ethanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Cardiac glycosides (Keller-Killani test)

5ml of each plant extract was mixed with 2ml of glacial acetic acid containing one drop of ferric chloride (Fecl3) solution, followed by the addition of 1ml concentrated sulphuric acid. Brown ring formed at the interface indicates deoxy sugar characteristics of cardenloides. A violet ring may appear beneath the brown ring, while in the acetic acid, layer; a greenish ring may also form just gradually throughout the thin layer.

Test for Anthraquinones (Borntrager's test)

0.5g of plant extract was shaken with 5ml chloroform, the chloroform layer was filtered and 0.5cm3 of 10% ammonia was added to the filtrate. The mixture was shaken thoroughly, the formation of a pink/violet or red, yellow colour in the ammonical phase indicates the presence of anthraquinones.

Test for Reducing Sugar (Benedict test)

0.5g of the plant extract was mixed thoroughly with 3cm3 distil water and filtered, 3 drops of the filtrate were added to 3cm3 Benedict reagents and placed in a boiling water bath for 5mins. The formation of a brick red precipitate indicates reducing sugar.

Collection of standard cultures

The standard cultures of bacteria under study were collected from different samples. After obtaining the culture, the test organisms were streaked on nutrient agar plates and incubated at 37^0C for 24 hours before gram staining was performed on the isolated colony.

The organism thus obtained were tested for their purity and confirmed by their morphological, cultural and biochemical characteristics.

Preparation of Media and **Standard Culture Inoculum**

The media used in the study were prepared according to the manufacturer's recommendation. Thereafter, two to three similar appearance colonies from nutrient agar plate were touched with the inoculating loop aseptically. It was then transferred to a tube containing 2 ml of sterile nutrient broth.

Screening and Evaluation of Antimicrobial (antibacterial) Activities

Agar well diffusion method was used in this study for screening and evaluation of antibacterial activity of *C. Procera* flower and latex extract. The antimicrobial activity test has carried out as described by Rajamohan et al. (2014). Briefly, agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45˚C. The cooled media have poured on to a sterile petri plates and allowed for solidification. The plates with the media have seeded with the respective microbial suspension using sterile swab. The plant extracts have prepared at different dose individually placed in each petri plates, discs and placed control and standard (Gentamycin 10 µg/ml) discs. The plates have incubated at 37˚C for 24 hrs. After incubation period, the zone of inhibition surrounding the discs has measured using a transparent ruler and the diameter recorded in mm.

Statistical Analysis

The results of this study were presented as mean ± standard deviation of three replicates using excel package. The experimental data were tested for homogeneity of variance and then subjected to one-way analysis of variance (ANOVA) and the difference between the samples mean were tested by Tukey post-hoc test using SPSS software version 23. A $p \leq 0.05$ was considered statistically significant.

RESULTS

Qualitative Phytochemical Analysis of methanol extract of *C. Procera* flower and latex.

Table 1 show the phytochemical constituents of methanol extract of *C. procera* flower and its latex. Methanol extract of *C. procera* flower indicates the presence of tannins, phenol, flavonoids, terpenoids, glycosides and steroids and the absence of saponins and alkaloids. The latex shows the presence of phenols, flavonoids, terpenoids, alkaloids, steroids, phlobatanins and anthraquinone and the absence of tannins and glycosides

Table 1: Qualitative Phytochemical Analysis of methanol extract of *C. Procera* flower and latex.

Phytochemicals	MECPF	CPL
Tannins	++	-
Phenols	+++	++
Flavonoids	+++	++
Terpenoids	+	+
Glycosides	+	-
Saponins	-	+++
Alkaloids	-	+++
Steroids	+	+++
Phlobatannins	+	+
Anthraquinones	+	+++

+ = Present, ++ = Moderately Present. +++ = Highly present

MECPF: Methanol extract of *C. procera* flower, CPL: *C. procera* latex

Antimicrobial Activities of Methanol Extract of *C. procera* Flower and Latex

Table 2 show antimicrobial activities of methanol extract of C. *procera* flower and latex. None of the concentration (20 mg/ml, 40mg/ml and 60 mg/ml) of CPL is however active agains*t E. coli*, while only 40 mg/ml and 60 mg/ml of MECPF are active against the same organism. The maximum concentration (20 mg/ml) of CPL shows higher zone of inhibition when compared with 60 mg/ml of MECPF on *pseudomonas aeruginos*a. Contradictorily, maximum concentration (20 mg/ml) of CPL shows higher zone of inhibition on staphylococcus aureus compared to the maximum concentration (60 mg/ml) of MECPF

Table 2: Antimicrobial Activities of Methanol Extract of *C. procera* flower and latex

GROUP	*E. Coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
CFE 20mg/ml	0.00 [a]	17.00 ± 1.00 [b]	11.33 ± 0.589 [a]
CFE 40mg/ml	13.00 ± 1.00 [c]	21.33 ± 2.08 [c]	14.00 ± 1.00 [a]
CFE 60mg/ml	21.00 ± 1.00 [d]	34.67 ± 1.15 [d]	18.00 ± 1.00 [b]
CPL 20mg/ml	0.00 ± 1.00 [d]	33.00 ± 1.00 [a]	33.00 ± 1.00 [c]
CPL 40mg/ml	0.00 ± 0.00 [a]	33.00 ± 1.00 [d]	22.67 ± 1.70 [b]
CPL 60mg/ml	0.00 ± 0.00 [a]	34.33 ± 1.15 [d]	33.00 ± 1.00 [a]
Standard	28.00 ± 26.4 [b]	26.33 ± 4.04 [a]	26.33 ± 3.51 [a]

MECPF: Methanol extract of *C. procera* flower, CPL: *C. procera* latex

DISCUSSION

E. coli is a prominent cause of enteritis and urinary tract infection and only MECPF produced reasonable antibiotic activities on this organism. This might be due to the presence of glycoside and high quantities of tannins, flavonoids and phenols when compared to CPL. Tannins, flavonoids, glycoside and phenols are good sources of antimicrobial agents (Kováč, et al., 2022). The contrast results as observed in the activity of CPL against *Pseudomonas aeruginosa* and *Staphylococcus aureus* especially at high concentration specifically for P. aeruginosa and generally for *Staphylococcus aureus* can be inferred from the presence of strong secondary metabolites presence in the CPL. Metabolites like alkaloids, saponins, steroids are strong antimicrobial agents aside from flavonoids, terpenoids and phenols that also contained in CPL (Kováč, et al., 2022). Similar activities of several parts of *C. procera* have also been reported to produced antimicrobial activities on several microbes and fungi due the presence of these aforementioned parameters (Sigh et al., 2024)

However, various mechanisms through which MECPF and CPL exhibit their antimicrobial activities are not known, but various report show that the medicinal plant could interact with microbes membrane and causes its disruption, damaging of microbes DNA, deactivate their metabolic enzymes among others.

From this study we can conclude that CPL possesses higher antimicrobial activities on *pseudomonas aeruginosa and Staphylococcus aureus* but lower antimicrobial activities against E. coli when compared with MECPF due to its minimum concentration that exhibit maximum effects.

Hence, CPL could be exploited further by isolating the specific active compound(s) the is responsible for the antimicrobial activities.

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