https://dx.doi.org/10.4314/dujopas.v10i3b.25
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 3b September 2024*

Antibacterial and Antifungal Analysis of methanol Extract of *Calotropis Procera* Root

Abdullahi, M. Abdullahi.[1*], Odih, C.[2], Akabuogu, O. W[2], Olime M. Nkemakonam[3] and Ferdinand, C[4].

[1]Department of Chemistry,
Directorate of Science,
Remedial and General Studies,
Federal University of Health Sciences Azare,
Bauchi State,
Nigeria.

[2]Department of Chemistry,
Michael Okpara University of Agriculture,
Umudike, Umuahia,
P.M.B 7267.
Abia State,
Nigeria.

[3]Sheda Science and Technology Complex
Federal Capital Territory,
Abuja,
Nigeria.

[4]Department of Chemistry,
University of Abuja,
Abuja,
Nigeria.

Email: abdullahi.muhammadjika@fuhsa.edu.ng

Abstract

The majority of recorded deaths in tropical nations are attributed to infectious diseases, which are becoming more common as germs become more resistant to antibiotics. Therefore, the development of more practical and potent therapeutic antibacterial agents as well as other active natural compounds from medicinal plants like Calotropis procera is necessary. The aim of this study is to test for antimicrobial properties of Calotropis Procera root. Calotropis Procera root was cold macerated for 48 hours using (1.5L) of methanol. The extracts (filtrate) were concentrated using a rotary evaporator. The antimicrobial effect of methanol extract of Calotropis procera on two bacteria: E. coli, H. pyroli and two fungi: Aspergillus niger, yeast Candida albicans were determined using disk diffusion. The results revealed that methanol extract of Calotropis procera showed antimicrobial properties. The growth of four bacterial and fungal isolates was inhibited by methanol extract of Calotropis Procera root. The minimum inhibitory concentration (MIC) for the methanol extract was between 200 and 400 µg/mL, the minimum bactericidal concentration was between 300 and 400 µg/mL for fungal and bacterial isolates. This study revealed that the C. procera root demonstrated strong inhibitory effect on the test organisms. The results therefore established a good support for the use of C. procera in traditional medicine.

Author for Correspondence

INTRODUCTION

According to Kareem *et al.,* (2008), *Calotropis Procera* is native to the Arabian Peninsula (which includes Saudi Arabia, Oman, and Yemen), the Middle East, and southern Asia, *Calotropis procera* is found in African countries including Nigeria. This specie was once grown as a decorative garden plant, but because it is deadly and often considered to be a weed, it is no longer in popularity (Semimul and Hussain, 2019). *Calotropis procera* is primarily found in drier sections of tropical and subtropical regions, as well as semi-arid and dry inland areas (Kareem *et al.,* 2008). *Calotropis procera* is a weed found in disturbed regions, waste places, roadsides, inland watercourses, grasslands, open forests, and pastures. A tiny tree or shrub that is upright, typically reaching a height of 1-4 meters (Parakash *et al.,* 2011). It's a big shrub with milky sap-filled leaves and waxy stalks. Its comparatively big, stem-clasping, greyish-green leaves, measuring 5–20 cm in length and 4–10 cm in width, are borne in pairs (Prashanthi et al., 2021).

Calotropis procera, a member of the Asclepiadaceae family of wild growing plants, is widely recognized for its therapeutic qualities. There have been reports of anti-inflammatory, analgesic, and antioxidant qualities in various components of this plant (Raginee *et al.,* 2010). Both conventional and modern medicine can benefit from the use of medicinal herbs. About 80% of people worldwide, primarily in developing nations like Africa, rely on traditional herbal therapy to manage their illnesses (Akinyemi, 2010). Since ancient times, medicinal plants have been utilized as a source of cures for various illnesses. Early people healed their sicknesses based on instinct, taste, and experience (Chinwuba *et al*., 2017). Medicinal herbs that are used to treat skin conditions include; *Artemisia nilagrica, Vaccinium microcarp, Senellgalia visco*, and *Calotropis procera.* Over half of all pharmaceuticals marketed for therapeutic use are traditional medicinal plants (Sherman and Pabbithi, 2022). These plants contain bioactive compounds including hydroquinone and epicatechin, which are used to treat skin pigmentation problems and skin cancer (Sherman and Pabbithi, 2022). Numerous kinds of phytochemicals, including alkaloids, saponins, flavonoids, anthraquinones, terpenoids, coumarins, polysaccharides, polypeptides, and proteins, have been found in medicinal plant extract, which comprises thousands of secondary metabolites (Mulholland *et al*., 2010).

Plant species that are utilized worldwide for a variety of reasons, including the treatment of diseases, typically have biological characteristics that are attributed to active chemicals created during secondary metabolism (Langat *et. al.,* 2011). The isolated extract of *Calotropis procera* showed antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*) which were more susceptible than the Gram-negative (*Pseudomonas aeruginosa* and *Salmonella enteritidis*) and the yeast species were more susceptible than the filamentous fungi, and the crude flavonoid fraction was the most active (Nenaah *et al.,* 2013).

The majority of recorded deaths in tropical nations are attributed to infectious diseases, which are becoming more common as germs become more resistant to antibiotics. Therefore, the development of more practical and potent therapeutic antibacterial agents as well as other active natural compounds from medicinal plants like Calotropis procera is necessary. The aim of this study is to test for antimicrobial properties of *Calotropis Procera* root.

MATERIALS AND METHODS

Sample Collection

The (*Calotropis procera*) roots were collected from near Centre for African Medicinal Plants Research (CAMPRE) North-Eastern University, Gombe Gombe-Bauchi Express way, Lafiyawo Gombe, Nigeria. Location of about 10.2881^0 N and 11.0537^0 E and was identified by Dr Hajara Sani Labaran Department of Biological Sciences, Federal University of Kashere Gombe State, Nigeria (Abdullahi *et al.,* 2024b; Ferdinand *et al.,* 2023).

Extraction

Cold maceration for 48 hours was used to extract the plant material (1 Kg) using (1.5L) of methanol. Superfluous filtrate was collected using a Winchester bottle, a glass funnel, and fluted Whatman No. 1 filter paper. The extracts (filtrate) were concentrated using a rotary evaporator. Superfluous concentrated extracts were airdried in a fume hood, prior to the analysis (Abdullahi *et al.,* 2024a).

Purification of Microbial Reagents

Bacterial and fungal isolates collected from Federal Medical Centre, Gombe State, Nigeria were sub-cultured on Mannitol salt agar (MSA) and MacConkey agar (MAC) and incubated at 37 °C for 24 hours to obtain pure isolates of bacteria. After incubation, pure bacterial isolates were stored on NA and prepared in Bijou bottles respectively. The stock cultures were then preserved in a refrigerator at 4 °C until used for further microbiological analyses (Yaghoubi *et al.,* 2021).

Characterization and Identification of Bacterial Isolates.

Bacterial isolates were characterized and identified by observation of colonial, and morphological characteristics, Gram reaction and biochemical tests. The various biochemical test that were carried out for identification are catalase, coagulase, indole and oxidase test (Yaghoubi *et al.,* 2021).

Catalase Test

Hydrogen peroxide was poured into the bottle and the organism was emulsified in it with the sterile wire loop. It produced bubbles of oxygen, the organism was catalase positive (Yaghoubi *et al.,* 2021).

Coagulase Test

A drop of normal saline was dropped on a clean slide using inoculating wire loop, the loop was then flamed and allowed to cool before being used and was added to the normal saline which was used to make a smear that gives a creamy coloration. A drop of plasma was then added to it and mixed properly to observe agglutination. Agglutination was observed (Prashanthi *et al.,* 2021).

Oxidase Test

A few drops of oxidase reagent (1% tetra methyl p-phenylene-diamic dihydrochloride) were dropped on a filter paper, a clean slide was used to pick colonies of the organism to be tested and smear on a paper. A positive test was indicated (Prashanthi *et al.,* 2021).

Indole Test

Peptone water was pipetted into the epidoff tube with the help of a micropipette, the organism to be tested was emulsified in the medium, and incubated overnight. After incubation a drop

of Kovacs reagent was added. If there is a pinkish ring in the solution was observed (Yaghoubi *et al.,* 2021)..

Agar Plate and Inoculum Preparation

All aspects of the Kirby–Bauer procedure were standardized to ensure consistent and accurate results. The media used in Kirby–Bauer testing was Mueller–Hinton Agar (MHA) at only 4 mm deep, poured into 100 mm Petri dishes. Bacterial inocula were prepared by diluting a broth culture to match a 0.5 McFarland turbidity standard (Balouiri *et al.,* 2016).

Inoculation and Incubation

Using the aseptic technique, broth culture of all organisms was collected with a sterile swab. Excess liquid was removed from the swab by gently pressing it against the inside of the tube. The swab was then streaked across a Mueller–Hinton agar plate to form a bacterial lawn. The agar plate was streaked with the swab in one direction, rotated 120° and streaked again, rotated another 120° and streaked again to obtain uniform growth. Using an antibiotic disk dispenser, filter paper disks containing specific antibiotics were then applied to the plate within 15 minutes of inoculation. Flame-sterilized forceps were used to gently press each disk onto the agar and ensure it was attached. Plates were then incubated overnight at a temperature of 35 °C. Plates were incubated within 15 minutes of applying antibiotic disks (Balouiri *et al.,* 2016).

Agar Disk-diffusion Method

In this well-known procedure, agar plates were inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6mm in diameter), containing the crude extract and isolated compounds at different concentrations were placed on the agar surface. The petri dishes were incubated at 35 °C. Antimicrobial agents was diffuse into the agar and inhibits growth of the organisms [*E. coli, H. pyroli,* Yeast *(Candida albicans), A. niger*)] the diameters of inhibition were measured in (mm) (Balouiri *et al.,* 2016).

RESULTS AND DISCUSSION

Table 1. Antimicrobial Sensitivity Test of Methanol Extract of *Calotropis Procera* Root

S/N	Organism	Concentrations(µg/ml) /zone of inhibition(mm)				Positive Control (µg/ml)zone of inhibition(mm)
		400	300	200	100	Aug /Keto
1	*E. coli Sp*	19	13	11	8	Augmentin 30/26
2	*H. pylori Sp*	17	12	9	7	Augmentin 30 /22
3	*Yeast Sp*	15	11	8	7	Ketoconazole100 /31
4	*A. niger*	16	8	7	6	Ketoconazole100 /22

Key: mm = millimeter, Keto = Ketoconazole 100µg/ml, Aug = Augmentin, 30µg/ml = microgram (µg).

As presented in Table. 1, the results indicate the sensitivity of the *E.coli Sp, H. pylori Sp, Yeast Sp, A. niger* to different concentrations of antimicrobial agents. The diameter zone of inhibition (measured in millimeters, mm) around the antimicrobial disc indicates the effectiveness of the tested concentration against the organisms. A larger zone of inhibition suggests higher sensitivity or effectiveness of the antimicrobial agent. The control results provide a reference point for comparison, indicating the expected sensitivity of the organisms to the control agents used. For *E. coli sp* the concentration of 100 µg/mL had a zone of inhibition of 8 mm, 200 µg/mL had 11 mm, 300 µg/mL had 13 mm and 400 µg/mL had 19 mm. *H. pyroli sp.* showed highest zone of inhibition at a concentration of 400 µg/mL and lowest zone at 100 µg/mL. *Yeast sp.* showed highest inhibition zone at 400 µg/mL and lowest at 100 µg/mL. Finally *A. niger* also showed highest inhibition zone of 16mm at 400 µg/mL

and lowest zone of inhibition of 6 mm at 100 µg/mL. The zone of inhibition of hexane extract of *Calotropis Procera* leaf extract against *E. coli* was 24 mm in diameter (Amna *et al.*, 2022). The results are in agreement with the present study with slight difference which might result from solvent used. The two fractions showed antimicrobial activity against tested organisms, diameter of inhibition zones ranged between 15.5 and 28.5mm against the tested bacterial strains (Gomah, 2013). According to Salem *et al.*, (2014) the zone of inhibition of aqueous extract of *Calotropis procera* leaves and latex against *E. coli* was 7 and 11mm in diameter which is in agreement with the result obtained in the study. The plant extract of *C. procera* was considered significantly active against *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Escherichia coli*, with inhibition zones of 18.66 mm, 21.26 mm, and 21.93 mm, respectively (Amna *et al.*, 2022).

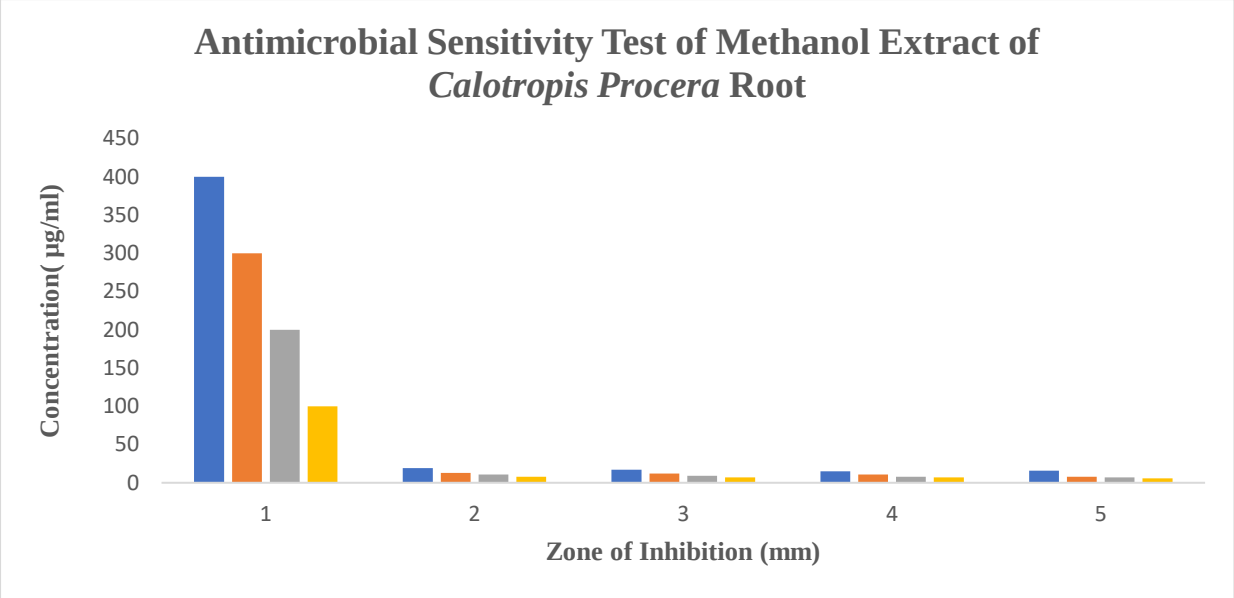

Figure 1. Antimicrobial Sensitivity test of methanol extract of *Calotropis Procera* root

Table 2. Minimum Inhibitory Concentration (MIC) of Methanol Extract of *Calotropis Procera*

S/N	Organism	Concentrations(µg/ml)/ Inhibition			
		400	300	200	100
1	*E. coli sp*	-	-	-	+
2	*H. pylori sp*	-	-	-	+
3	*Yeast sp*	-	-	-	+
4	*A. niger*	-	-	+	+

Key: (-) = Negative not detected, (+) = Positive Detected, S/N = Serial Number

According to Table 2. The lowest concentration of the crude extract that inhibited the growth for *E. coli, H. pyroli, Yeast,* was 200 µg/mL and that of *A. niger* was 300 µg/mL. The concentration of 100 µg/ml does not affect the growth of all the tested organisms. At 200 µg/ml concentration, all the organism's growth were inhibited except for A. niger. At concentrations of 300 µg/ml, 400 µg/ml all the organism's growth were inhibited. *Calotropis procera* latex extract had more antimicrobial activity than flowers, the MIC of *Calotropis Procera* was 6.3mg/mL (Elshaima *et al.*, 2021). The plant extract of *Calotropis Procera* was considered to be a moderate inhibitor against *Bacillus subtilis*, with MIC ranging from 0.60–1.50 mg/mL. On the other hand, the isolated actinobacteria were considered to be a moderate inhibitor against *S. aureus* (MIC of 86 µg/mL), and a potent inhibitor against *Candida albicans* (MIC of

35 µg/mL) (Amna *et al.,* 2022). The minimum inhibitory concentration (MIC) of aqueous and organic extract varies from 5-20mg/ml (Varsha *et al.,* 2015).

Table 3. Minimum Bacteriocidal Concentration (MBC) of Methanol Extract of *Calotropis Procera*

S/N	Organism	Concentrations(µg/ml)/Inhibition			
		400	300	200	100
1	*E. coli sp*	-	-	-	+
2	*H. Pylori sp*	-	-	+	+
3	*Yeast sp*	-	-	+	+
4	*A. niger*	-	-	+	+

Key: (-) = Negative not detected, (+) = Positive Detected, S/N = Serial Number

At concentration of 100 µg/ml the extract does not affect the growth of all the organisms. At concentration of 200 µg/ml the extract inhibited the growth of only E. coli while others were not affected. At concentration of 300 µg/ml, 400 µg/ml, all organism's growth were inhibited. The minimum bacteriocidal concentration (MBC) that can kill *H. pyroli, Yeast, A. niger was* 300 µg/ml and that of E. coli was 200 µg/ml. according to Table 3. The minimum bactericidal concentration of the latex ethanol extract of *Calotropis Procera* was 200 µg/ml (Kawo *et al.,* 2009). This is in agreement with the results obtained in the study.

CONCLUSION

The methanol extract of *Calotropis procera root,* showed strong antibacterial and antifungal activity against *E. coli, H. pyroli, Yeast and A. niger*. Therefore, there is need for more research on antimicrobial activities of *Calotropis Procera* root, such that it can be used directly or indirectly in pharmaceutical production of antibiotics. This research could serve as an alternative to modern expensive drugs in developing country like Nigeria, where the majority of population are low income earners.

REFERENCES

Abdullahi, M. A., John, V. A., Esther, M. T., and Isyaka, M. S. (2024a). Isolation and Characterization of Betulinic Acid, Stigmasterol and β-Sitosterol from *Calotropis Procera. Nigerian Research Journal of Chemical Sciences.* 12(1):387-401.

Abdullahi, M. A., Olime, M. N., Usman, G., Muhammad, A. M., Muhammad, I. N., and Muhammad, B. U. *et al.,* (2024b). Isolation of squalene from dichloromethane extract of *Croton nigritanus* leaf. *FUDMA Journal of Sciences (FJS)*. 8(2): 212-216.

Akinyemi, K. O, Mendie, U. E., Smith, S. T., Oyefolu, A.O., and Coker A.O. (2010). C. Screening of some medicinal plants for anti-salmonella activity. 36:45-60.

Amna, A. S., Hend, M. T., Nada, M. D., Asmaa, S. S., and Nashwa, H., (2022). Antimicrobial, Antigenotoxicity, and Characterization of *Calotropis procera* and Its Rhizosphere-Inhabiting Actinobacteria: In Vitro and In Vivo Studies. *Journal of Pharmacognosy and Phytochemistry.* 27(10): 3123.

Balouiri, M., Moulay, S. and Saad K. I. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analyis.* 6(2): 71-79.

Chinwuba, D., Murugananthan, G., and Pabbithi, S. C. (2017). Medicinal plants with potent Antioxidant constituents. *International Journal of Pharmaceutical Science and Research,* 1(17): 14-18.

Elshaima, F., Mosa, M., Noura, E. A., (2021). Antimicrobial activity of crude *Calotropis Procera* extract with special references to sheep salmonellosis. *Benha Veterinary Medical Journal*. 40(1):125-128.

Ferdinand, C., Isyaka, M. S., Tiwa A. A., Ibekwe, N. N., John, I., and Abdullahi, M. A. (2023). Isolation of sitosterol and stigmasterol from the roots of *Croton penduliflorus. FUDMA Journal of Sciences (FJS).* 7(6): 52-56.

Gomah, N. (2013). Antimicrobial activity of *Calotropis Procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World J Microbial Biotechnology.* 29(7): 1255-1262.

Kareem, S. O., Akpan, I., and Ojo O.P. (2008). Antimicrobial activities of *Calotropis Procera* on selected pathogenic microorganisms. *African Journal of Biomedical Research.* 11**:** 105-110.

Kawo, A. H., Mustapha, A., Abdullahi, B. A., Rogo, L. D., Gaiya, Z. A., and Kumurya, A. S. (2009). Phytochemical properties and antibacterial activities of the leaf and latex extract of *Calotropis Procera. Bayero Journal of Pure and Applied Sciences.* 2(1):34-40.

Langat, M. K., Crouch, N. R., Smith, P. J., and Mulholland, D. A. (2011). Cembranolides from the leaves of *Croton gratissimus*. *J. Nat. Prod.* 74, 2349–2355.

Mulholland, D. A., Langat, M. K., Crouch, N. R., Coley, H. M., Mutambi, E. M., and Nuzillard, J. M. (2010). Cembranolides from the stem bark of the southern African medicinal plant, *Croton gratissimus* (Euphorbiaceae). Phytochemistry 71, 1381–1386.

Nenaah, G. (2013). Antimicrobial activity of *Calotropis procera* Ait.(Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World Journal of Microbiology and Biotechnology*. 29(7): 1255-1262.

Parakash, P. K., Prasad, M.N., Kumar, M.V. and Rao, K.S. (2011). Evaluation of heptaprotective effect of *Calotropis procera* (Ait).R. Br. Root extract against paracetamol heptooxidative stress in albino rats. *Int. Res. J. Pharm*. 2(1):285-291.

Prashanthi, R., Shreevatsa, G. K., Krupalini, S., and Manoj, L. (2021). Isolation, characterization, and molecular identification of soil bacteria showing antibacterial activity against human pathogenic bacteria. *Journal of Genetic Engineering and Biotechnology.* 19**.** 120.

Raginee V., Satsangi G.P., and Shrivastava J.N. (2010). Ethnomedicinal profile of different plant parts of *Calotropis procera. Ethnobotanical Leaflets* 14: 721-42.

Salem, W. M., Sayed, W. F., Haridy, M., and Hassan, N. H., (2014). Antibacterial activity of *Calotropis procera* and Ficus Sycomorus extracts on some pathogenic microorganisms. *African Journal of Biotechnology.* 13(32):3271-3280.

Semimul, A. and Hussain ,S. (2019). An overview: Identifications and Activities of *Calotropis Procera* Leaves. *World Journal of Pharmaceutical Research.* 9(1):764-774.

Sherman, G. and Pabbithi, S.C. (2022). Medicinal Plants with Potent Antioxidant Constituents. *International Journal of Pharmaceutical Sciences and Research,* 1(17):1-4.

Varsha, G. S., Meenakshi, G. P., and Ambika, S. D. (2015). Evaluation of phytochemical and antibacterial properties of *Calotropis Procera* (AIT) R. BR. Leaves. *International Journal of Pharmaceutical Sciences.* 7(4):316-319.

Youghoubi, K. M., Strafella, S., Allegretta, I. *et al.*, (2021). Isolation of Bacteria with potential plant-promoting traits and optimization of their growth conditions. *Current Microbiology.* 78**.** 464-478.