Open Access

Contemporary Research Analysis Journal

e-ISSN: 3050-5909
p-ISSN: 3050-5895

Volume 03 Issue 03 March 2026

CrossRef DOI: 10.55677/CRAJ/08-2026-Vol03I03

Page no: 184-190

Characterization of the Ethanolic Extract of Tridax Procumbens Leaf Powder, and Evaluation of the Antimicrobial Properties of the Topical Ethanolic Extract Formulation

Oladapo A. Adetunji[1], Taiwo P. Fakunle[2], Oludele A. Itiola[3]

[1,2,3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of Ibadan, Ibadan, Nigeria.

ABSTRACT: The ethanolic extract of *Tridax procumbens* leaves, known traditionally for wound healing, was characterized through photomicrography, FTIR, pH measurement, phytochemical screening, and antimicrobial susceptibility testing. This extract was formulated into a topical ointment and evaluated for its antimicrobial activity in comparison to gentamicin. The results were analyzed using mean and standard deviation. *Tridax procumbens* leaves were washed, shade-dried and ground into powder, which was then extracted using 99.9% ethanol (TPEt). TPEt was characterized using photomicrography, FTIR, pH, phytochemical screening and antimicrobial susceptibility tests. Ointment containing 11.8%w/w *TPEt* was prepared using the fusion method (TrOt) and evaluated for homogeneity, colour, pH, spreadability, viscosity, antimicrobial susceptibility, minimum inhibitory and bactericidal concentrations. TPEt, exhibited dark patches composed of round (90.12±0.17µm) and irregular (180.12±1.06µm) particles. FTIR analysis revealed O-H stretching peaks around 3300-3500cm^{-1}, with aliphatic alkanes and methylene groups at 2850 cm^{-1} and 1456 cm^{-1}. TPEt had a pH of 5.87, contained alkaloids, cardenolides and flavonoids, but lacked anthraquinones, saponins and tannins. It inhibited *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, with inhibition zones ranging from 12.5 to 50.0 mg/mL. TrOt appeared as a smooth dark brown substance with a spreadability of 2.5cm in 10 seconds under 200g load, pH of 5.7, pseudoplastic flow behavior, and exhibited bacteriostatic and bactericidal effects against *Staphylococcus* species at 6.25 mg/mL and 6.72 mg/mL, respectively, comparable to gentamycin ointment. The ethanolic extract of Tridax procumbens leaves and its ointment demonstrated comparable antimicrobial activity. The ointment displayed favorable physicochemical properties, including good flow and compatibility with skin.

KEYWORDS: Antimicrobial resistance, *Tridax procumbens*, Ethanolic leaf extract, Topical antimicrobial formulation, Herbal ointment.

Cite the Article: *Adetunji, O. A., Fakunle, T.P., Itiola, O.A. (2026). Characterization of the Ethanolic Extract of Tridax Procumbens Leaf Powder, and Evaluation of the Antimicrobial Properties of the Topical Ethanolic Extract Formulation. Contemporary Research Analysis Journal, 3(3), 184–190. https://doi.org/10.55677/CRAJ/08-2026-Vol03I03*

Publication Date: March 16, 2026

**Corresponding Author: Oladapo A. Adetunji*

INTRODUCTION

Medicinal plants are important sources of therapeutic agents, which have been used for the management of various disease conditions. This is due to the various bioactive molecules present in them (Egamberdieva et al, 2021). A lot of factors contribute to their continual use; one of which is that these plants biosynthesize a lot of metabolites e.g. alkaloids, flavonoids, terpenoids, phenolics and glycosides. These metabolites have bioactivity profiles that are difficult to replicate by combinatorial chemistry alone (Arpita et al, 2022). Also, these compounds often interact with biological targets in novel ways (Najmi et al, 2022). Examples of such bioactive substances include paclitaxel, which is obtained from *Taxus brevifolia*, and is used to manage lung, ovarian, and breast cancer (Gallego-Jara et al, 2020). Silymarin, a flavonolignan complex, is extracted from the seeds of *Silybum marianum* and is used in the management of hepatic disorders (Gillessen and Schmidt, 2020). Digitoxin, extracted from the foxglove plant, is used to manage congestive heart failure. Codeine, obtained from *Opium poppy,* is used as an analgesic and as a cough-suppressing agent (Khandelwal et al, 2024).

Tridax procumbens is a specie of flowering plants that belongs to the *Asteraceae* family. It is one of the most potent species among the 30 species of the genus Tridax. It originated from Mexico, Central America and South America, and is a widespread weed in

Africa and other topical regions (Emmanuel et al, 2024). The plant is an ayurvedic herb of Asia with a history of traditional use. The wound healing activities of *T. Procumbens* was reported by Thalkari et al. in 2020. Prior to that time the plant was prepared as a drink to treat bronchial catarrh, diarrhea as well as dysentery (Andriana et al, 2019). The degree of antimicrobial activities elicited varies depending on the plant part used. Ambulkar *et al* discovered that the ability of the fresh leaf juice to inhibit microbial growth was greater than that of the fresh flower juice (Ambulkar et al, 2020).

Antimicrobial resistance is an increasingly urgent global problem. The increasing number of resistant microbial strains skin or wound pathogens (eg *Staphylococcus aureus*, *Pseudomonas aeruginosa*) drives the research to focus on alternatives like plant-based remedies with potential antimicrobial properties as source of lead compounds for the development of new topical drugs (Seyed-Alinaghi et al, 2025). In Nigeria, data to support the use of leaves obtained from *Tridax procumbens* indigenous to our environment as a topical formulation is limited in literature. This study was therefore aimed at harvesting leaves from locally available *Tridax procumbens*, extracting the leaves using ethanol and evaluating the antimicrobial properties of both the ethanolic extract of the leaves and its topical ointment formulation.

MATERIALS AND METHODS

Materials

Freshly collected *Tridax procumbens* plants from within the premises of the University of Ibadan, Ibadan, Nigeria were authenticated at the University of Ibadan herbarium, University of Ibadan with assigned number: UIH-23743. Cetostearyl alcohol, white soft paraffin, wool fat and stearic acid (Iangsu Juming Chemical Technology Co., Ltd, Yangzhou, China) were obtained as gifts from Bond Chemical Industries limited Aawe, Nigeria. 99.9% ethanol was obtained from Unichem Nigeria limited, Ikeja, Lagos, Nigeria, Gentamicin ointment (manufactured by DrugField Pharmaceuticals Limited, SangoOtta, Nigeria) was purchased from a registered pharmacy. All the other reagents and equipment used were of analytical grade, and were made available at the Research Laboratories, Faculty of Pharmaceutical Sciences, University of Ibadan, Ibadan, Nigeria.

Methods

1. Sample collection, identification and drying

Samples of *Tridax procumbens* plants were collected within the University of Ibadan campus and were taken to the University Herbarium, where they were identified and authenticated with voucher number UIH-23743. The leaves of the authenticated plant samples were detached, washed with distilled water, shade-dried for 14 days and grinded into powder. The powdered sample was weighed and stored in airtight containers.

2. Ethanolic extraction of powdered *Tridax procumbens* leaves

Exactly 83 gm of powdered *Tridax procumbens* leaves was weighed into a covered glass jar and macerated with 99.9% of ethanol in ratio 1:8. The mixture was shaken for ten minutes. This process was repeated every 4 hours for 72 hours and filtered using Whatman filter paper number one. While the supernatant was stored, 250 mL of ethanol was added to the filtrate and the process was repeated for another 48 h. The supernatant was then concentrated to give a paste, shade-dried to make the paste thicker and stored in an airtight container.

3. Characterization of ethanolic extract of powdered *Tridax procumbens* leaves

a. Determination of Hydrogen Potency (pH)

A 50 mg/mL of ethanolic extract of powdered *Tridax procumbens* leaves (TPEt) was prepared and poured into the pH meter (Model 720 A, Thermo Electron Corporation, MA, USA). The pH was then determined using 10 mL of the sample prepared (Zvidzayi et asl, 2021).

b. Fourier Transform Infra-Red Spectroscopy (FTIR)

The TPEt was transferred to an IR spectrophotometer (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA) using potassium bromide (KBr) disk. Transmission spectra were recorded using resolution in the spectral range of 400 – 4000cm^{-1}. Plot of percentage transmittance (%T) versus wavenumber (cm^{-1}) was created on the computer monitor, printed out and analyzed for the characteristic functional groups present in the sample.

c. Photomicrography

Photomicrograph of TPEt was taken using a digital light microscope set, VJ-2005 DN model biomicroscope® at x40, x100 and x400 magnifications. The morphometrical analyses particle diameter was done using TS View CX Image® Software, File version 6.2.4.3 and Motic Image 2000 (China).

Statistical analysis was done with Graph Pad Prism 8.

d. Antimicrobial susceptibility test

Antibacterial activity of TPEt was evaluated using well diffusion method. Two gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epididymis*) and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) were used. The organisms were streaked on Muller-Hinton agar plates and incubated at 37.0±0.1°C for 24 h. After 24 h, two distinct colonies of each organism were mixed with 10 mL of sterile distilled water. The mixture was spread on 4 Mueller Hinton Agar

plates, using sterile cotton swabs. Five 9mm diameter wells were then punched into each agar plate. The first three wells of each plate were filled with 40 microliters of a 50 mg/mL, 25 mg/mL and 12.5 mg/mL concentration of TPEt. The fourth well of each plate was filled with 40 microliters of ciprofloxacin, which served as positive control, while the fifth well of the plates were filled with 40 microliters of distilled water, which served as negative control. The zone of inhibition around each sample was measured after 24 h and recorded appropriately (Nagalakshmi et al, 2019).

4. Phytochemical Screening

a. Alkaloids

Exactly 0.1 g of TPEt was diluted to 10 mL with acid alcohol and filtered. Exactly 5 mL of the filtrate was added to 2 mL of dilute ammonia. 5 mL of chloroform was added to the resulting mixture and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids (Sofowora, 1993).

b. Cardenolides

Exactly 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added to 0.1 g/5mL of TPEt. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides (Sofowora, 1993).

c. Anthraquinones

Exactly 1.1 g of TPEt was boiled with 10 mL of sulphuric acid and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipetted into another test tube and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes (Sofowora, 1993).

d. Saponins

Exactly 0.1 g of TPEt was added to 10 mL of distilled water and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion which indicates the presence of saponins (Sofowora, 1993).

e. Tannins

Exactly 0.1g of TPEt was added to 10 mL of distilled water in a test tube and filtered. To the filtered samples, 0.1% $FeCl_3$ was added and observed for brownish green or a blue-black colouration which shows the presence of tannins (Sofowora, 1993).

f. Flavonoids

A few drops of 1% ammonia solution was added to TPEt in a test tube. The mixture was then observed for a yellow coloration which disappears with the addition of concentrated sulphuric acid (Sofowora, 1993).

5. Preparation of *Tridax procumbens* ethanolic extract ointment

The water bath was heated and when the water started to boil, 1 gram of stearic acid was weighed into the petri dish and allowed to melt, while stirring continuously. One gram of cetostearyl alcohol was then added to the melted stearic acid. Exactly 4.5 g of white soft paraffin was added next and after it had melted, 1 gram of wool fat was added. After proper melting had been achieved, the petri dish was removed from the water bath and allowed to cool while continuously stirring. After cooling, 1 g of TPEt was added and mixed appropriately. The formulated ointment was labeled as 11.8 % w/w *Tridax procumbens* ointment (TrOt) and stored appropriately (Gupta, 2015)

6. Characterization of TrOt

a. Hydrogen Potency (pH)

The pH of TrOt was determined using the pH meter (Model 720 A, Thermo Electron Corporation, MA, USA). This was done by immersing the probe stick inside the ointment while molten (Gupta, 2015).

b. Homogeneity

The TrOt was examined for uneven texture, colour variations, or visible particulates that could indicate uneven distribution of TPEt or additives (Chakole, 2009).

c. Spreadability

The parallel plate technique was used to determine spreadability of TrOt. About 1 gm of TrOt was weighed and placed on a glass slide. Another glass slide was then used to cover it and a 200 g weight was placed on the upper slide for 10 seconds. The weight was then removed, and the diameter of the formed circle was measured from three different angles to determine the average diameter (Magréault et al, 2022)

d. Viscosity

The viscosity of TrOt was measured at 5, 10, 20, 50 and 100 rpm using a Brook Field viscometer (DVIII + model, Brookfield Engineering, USA) with spindle no. 7. (Chakole, 2009).

e. Evaluation of antimicrobial susceptibility

Antibacterial activity of TrOt was evaluated using the well diffusion method. Two gram-positive bacterial organisms (*Staphylococcus aureus, Staphylococcus epididymis*) and two gram-negative bacterial organisms (*Escherichia coli, Pseudomonas aeruginosa*) were used. The organisms were inoculated on Muller-Hinton agar plates and incubated at 37°C for 24 h. After 24 h, two distinct colonies of each organism were mixed with 10 mL of sterile distilled water. Each organism mixture was spread on a Mueller Hinton Agar plate, making a total of 4 plates. Five 9 mm diameter wells were then punched into each agar plate. The first three wells of each plate were filled with 40 µL of a 50 mg/mL, 25 mg/mL and 12.5 mg/mL concentration of TrOt The fourth well of each plate was filled with 40 µL of 50 mg/mL of gentamicin ointment, which served as positive control. The fifth well of these plates were filled with 40 µL of distilled water, which served as negative control. The zone of inhibition around each sample was measured after 24 hours and recorded appropriately (Chakole, 2009).

7. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of TrOt was determined using microtiter plate-based micro broth dilution method to test their efficacy against selected organisms (Gonelimali et al, 2018). 100 µL of sterile Tryptic soy broth was dispersed into all the wells in the microtiter plate after which 100 µl of TrOt was added to the wells to obtain an initial concentration of 100mg/ml and serially diluted to other wells in the microtiter plate to obtain concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL respectively. After this, 10 µL of selected organisms was added to the wells. Wells containing only broth and formulated ointment, broth and organism and broth only used as control. This method was repeated using a separate microtiter plate. Wells containing gentamycin ointment (drug control) were also prepared. The MIC was done in triplicates using the same procedure to ensure accuracy. The microtiter plates were then incubated at 37 ºC for 24 h, after which iodonitrotetrazonium dye was added. The lowest concentration that showed no colour change was recorded as the minimum inhibitory concentration. The minimum bactericidal concentration of the ointment was determined using the method of Debalke et al, 2018). A loopful of the mixture from each well of the minimum inhibitory concentration with no visible bacteria growth after 24 h was inoculated into solidified sterile nutrient agar plates. The plates were then incubated at 37ºC for 24 h. The lowest concentration in which there was no visible growth of bacteria colony from the duplicate MIC experiment was recorded as the minimum bactericidal concentration (Nagalakshmi et al, 2019)

RESULTS AND DISCUSSION

The hydrogen ion concentration of a sample can be used to describe the acidity or alkalinity of pharmaceutical substances, therefore giving an insight to the stability of the product. For topical products, the product's pH needs to be close to that of the skin to minimize skin irritation or incompatibility. The pH values of the ethanolic extract of *Tridax procumbens* leaf and the ointment formulation were found to be 5.87 ± 0.04 and 5.70 ± 0.0 respectively, therefore, it is expected that there will be minimal skin irritation (if any) on administration of the topical ointment (Lukić et al. 2021)

FTIR spectroscopy gives insight to the functional groups present in a material. It can also be used as a quality control tool for evaluating compatibility between the API and excipients (Adriana, 2019). The FTIR spectra of the ethanolic extract of *Tridax procumbens* leaf is presented in Fig.1. The spectra shows a broad peak around 3300-3500 cm^{-1}, indicating O-H stretching vibrations in alcohols, phenols, and carboxylic acids. Peaks around 2930 and 2850 cm^{-1} correspond to C-H stretching vibrations of alkanes, indicating aliphatic hydrocarbons. The peak around 1628 cm^{-1} is attributed to the C=C stretching vibration of alkenes, while peaks around 1737 cm-1 and 1712 cm-1 can be attributed to the C=O stretching of ketones, aldehydes, esters or carboxylic acid. These functional groups are also confirmed by the phytochemical screening that was carried out, which indicates the presence of alkaloids, flavonoids and cardenolides, but absence of tannins and saponins. Tannins have been documented to present several formulation problems due to their complex nature as polyphenols (Desmedt et al, 2016). The absence of hydrolysable tannins (which cause tissue irritation) coupled with the instability that saponins usually cause when incorporated in topical formulations are advantages for the ointment formed using the ethanolic extract of *Tridax procumbens* leaf (Desmedt et al, 2016). The photomicrograph of the ethanolic extract of *Tridax procumbens* leaf (Fig.2) shows particles that are oval to spherical in shape with average particle size of 11.26±4.45µm, supporting particle size where grittiness would most likely be avoided when incorporated as a topical formulation. (Wen-Kai, 2024)

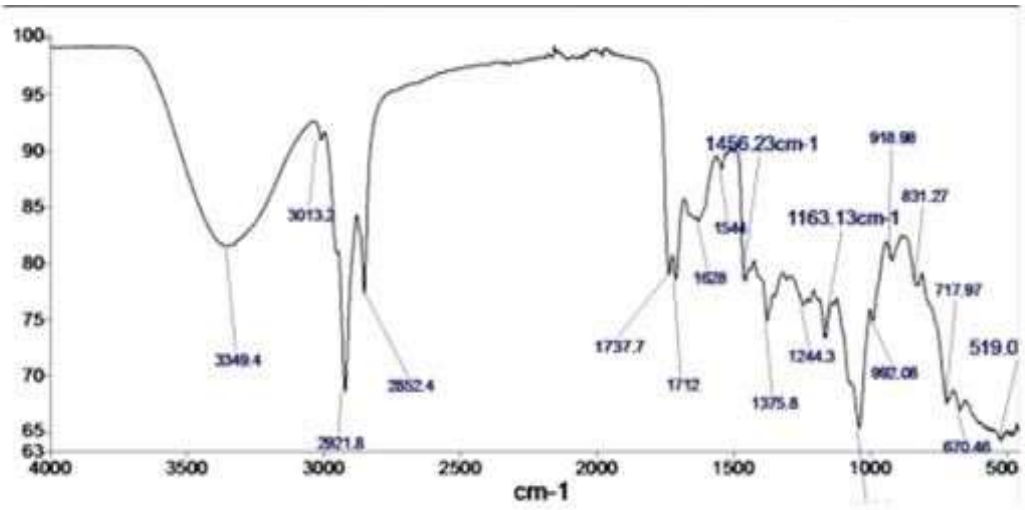

Fig.1: FTIR Spectra of ethanolic extract of *Tridax procunbens* leaf

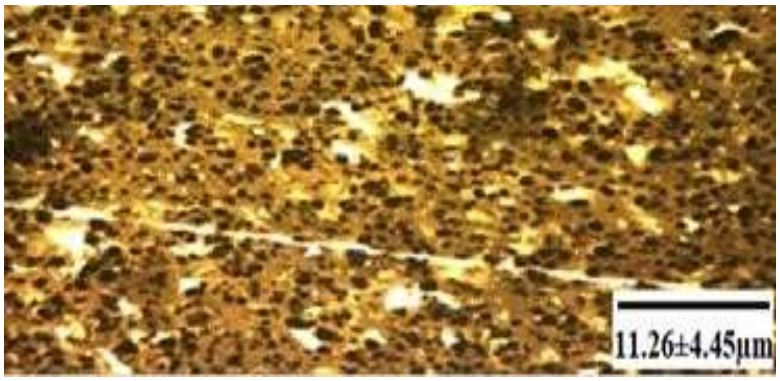

Fig.2: Photomicrograph (Mag: x 100) of ethanolic extract

The antimicrobial susceptibility evaluation (Table 1) showed that the ethanolic extract of *Tridax procumbens* leaf was able to inhibit the growth of microorganisms. The highest zone of microbial growth inhibition was recorded at 50 mg/mL, while the lowest was at 12.5 mg/mL. This shows that the effect elicited by the extract is directly proportional to the concentration. The ointment also showed a similar relationship, though the ointment's degree of microbial growth inhibition zone was slightly lower than the extract. This could be as a result of the need for the extract to diffuse out before acting on the organism, and if the diffusion is incomplete, there will be reduction in antimicrobial activity (Wen-Kai, 2024).

Ointment spreadability is the ability of an ointment to spread out and cover a surface, ensuring that a consistent amount of the active ingredient is delivered to the site of application (Al-Barghouthy et al, 2025). The ointment showed a mean spreadability of 2.5 cm under a load of 200 g within 10 secs. This shows that minimal effort would be required to apply the ointment on the skin.

The resistance to motion when an external force is applied to an ointment describes the viscosity of the ointment. There was an inverse relationship between the viscosity of the ointment and the rate of shear, thus indicating that the ointment exhibited pseudoplastic flow (Ivko et al, 2021). Shear-thinning (pseudoplastic flow) is highly desirable in pharmaceutical ointments due to the advantage of offering a balance between the stability in the container and ease of application. Shear-thinning also enhances drug release and absorption at the site of application (Qian et al, 2024).

Table 1: Antimicrobial susceptibility evaluation

Organism	**ZOI (mm) (50.0 mg/mL)**		**ZOI (mm) (25 mg/mL)**		**ZOI (mm) (12.5 mg/mL)**		**ZOI (mm) (Gent)**	**ZOI (mm) (Cipro)**	**ZOI (mm) (DW)**
	TPEt	**TrOt**	**TPEt**	**TrOt**	**TPEt**	**TrOt**			
Escherichia Coli	10.0	10.0	8.0	8.0	0.0	0.0	18.0	23.0	0.0
Staphylococcus aureus	15.0	14.0	10.0	12.0	8.0	10.0	19.0	23.0	0.0
Staphylococcus epididymis	18.0	14.0	16.0	13.0	15.0	10.0	19.0	19.0	0.0
Pseudomonas aeruginosa	10.0	10.0	10.0	10.0	8.0	8.0	12.0	12.0	0.0

TPEt = Ethanolic extract of *Tridax procumbens* leaf
TrOt = Ointment containing ethanolic extract of Tridax *procumbens* leaf
Gent = Gentamicin ointment

Cipro = Ciprofloxacin ointment
DW = Distilled water

The Minimum Inhibitory Concentration refers to the least concentration of a drug required to inhibit microbial growth, without necessarily killing them, while Minimum Bactericidal Concentration (MBC) is the concentration required to kill the microorganisms (Parvekar et al, 2020). The MIC and MBC results show the appreciable antimicrobial activity of *Tridax procumbens* against both gram-positive and gram-negative microorganisms. It elicited bacteriostatic activities at lower concentrations and bactericidal activities at higher concentrations. These values are however higher when compared to gentamicin ointment. The fact that gentamicin ointment is a pure compound while the extract contains many constituents could be responsible for the lower potency of the ointment formulated using the ethanolic extract of *Tridax procumbens* leaf. The lower the concentration required to inhibit microbial growth and/or kill the microorganisms, the higher the potency.

CONCLUSION

The ethanolic extract of *Tridax procumbens* leaf and the ointment formulated using the extract demonstrated appreciable antimicrobial activity when compared with gentamicin and ciprofloxacin ointments. The ointment containing ethanolic extract of *Tridax procumbens* leaf also displayed favorable physicochemical properties, including good flow and compatibility with skin, which justifies its application as a potential formulation for treating topical wounds. Further work may be necessary to further establish the basic constituent(s) necessary for the antimicrobial function, while the use of other solvents in extracting the leaf may also be exploited.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENT

The authors acknowledge Bond Chemical Industries, Aawe, and Bentos Pharmaceutical Products, PodoIbadan, both in Oyo State, Nigeria and Centre for Drug Discovery, Development and Production, University of Ibadan, Nigeria for the gifts of materials as described in the manuscript. Associate Professor O. Aina of the Faculty of Veterinary Medicine is acknowledged for providing technical support.

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