Phytochemical Screening and *In vitro* Antimicrobial Activity of *Cyperus rotundus* Leaf Extracts

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doi: https//doi.org/10.46912/napas.236

Abstract
Cyperus rotundus, a member of family *Cyperaceae* is a perennial, stoloniferous herb. The study investigated the secondary metabolites present in the leaf of *Cyperus rotundus* and antimicrobial activities of the hexane, ethyl acetate and methanol extracts. Phytochemical analysis revealed the presence of saponins, alkaloids, phenolic compounds, tannins, steroids, flavonoids, cardiac glycosides and terpenoids. The qualitative test of the extracts show the presence of steroid only in the hexane extract; flavonoid, cardiac glycoside in both the ethyl acetate and methanol extracts; saponin and tannin and phenolics in just the methanol extract. While alkaloid was present in all the extracts, terpenoid was absent in all. Antimicrobial activity of plant extracts were monitored using the agar disk-diffusion method against strains gram-positive bacterial: *Staphylococcus aureus, Pseudomonas aeruginosa;* and gram-negative bacteria: *Escherichia coli, Salmonela typhi,* and strains of fungi: *Candida albicans* and *Aspergillus niger*. The antimicrobial activity of the extracts was monitored at different concentrations of 50, 25, 12.5 and 6.25 mg/mL prepared from the stock concentration (100mg/mL) by serial dilution; with standard drugs: ciprofloxacin and fluconazole. The inhibition of the test organisms were concentration dependent. The methanol extract showed significantly higher Inhibition Zone (IZ) and Activity Index (AI) against the microbes at all concentrations compared to the ethyl acetate and hexane extracts due to the much phytochemicals (saponins, tanins, phenols, flavonoids, cardiac glycocides and alkaloids) found present in its extract. The overall study results signify the potential of *Cyperus rotundus* as a source of therapeutic agents. The methanol extract showed the best activity against both bacterial and fungal pathogens at the highest concentration of 100 mg/mL, especially the most susceptible bacteria: *Staphylococcus aureus* (IZ of 25.0±1.5; AI of 0.96) and *Candida albicans* (IZ of 25.5±0.5; AI of 0.91). At MIC, MBC and MFC of 6.25 mg/mL and 12.5 mg/mL, the plant extracts showed effective bactericidal and fungicidal effect against the test microbes.

Keywords: *Cyperus rotundus*, Phytochemical, antimicrobial activity, agar disk-diffusion, activity index

Introduction

Mankind has been depending on plants and its products from ancient times, for health care needs in particular, and various other reasons. Until the introduction of chemotherapeutic agents, this dependence on herbal medicines has diminished (Gera *et al.*, 2018).

Medicinal plants are a blessing of nature to human and have been used for ages to cure a good number of diseases. In many parts of the world, medicinal plants are used against bacterial, viral and fungal infections. Recently, the evaluation of plants which possess efficiency in curing various diseases is increasing (Premlata *et al.*, 2012). Infectious diseases have increased to a great extent during the recent years (Mothana and Lindequist 2005), and they are the second leading cause of death across the world and the third leading cause of death of economically developed countries (Luqman *et al.*, 2005).

Due to unselective use of commercial antimicrobials, the increase in resistance of multiple drug has slowed down the development of new synthetic antimicrobial drugs, and has imposed the search for new antimicrobials from other alternative sources (Kunle *et al.*, 2012; Neelam and Padma, 2008). Thus, there is progressively justified notion which claims that medicines derived from plants are cheaper and more active than contemporary medicine. The studies of medicinal plants used as folklore medications have consequently attracted enormous attention of researchers in the scientific world in an effort to find possible solutions to the problems of multiple resistances to the existing synthetic and conventional antimicrobials (Abbas *et al.*, 2017; VanWhyk 2002; Teklehaymano *et al.*, 2007).

Recently, studies have exposed the presence of powerful healing agents: the phytochemicals in diversity of plants. Traditionally used medicinal and aromatic plants contribute a broad spectrum of biologically active compounds of known therapeutic activities (Neelam and Padma, 2008). Thus, this study is aimed at investigating and estimating the antibacterial and antifungal activity of *Cyperus rotundus.* This plant is one of the oldest known medicinal plant. It is a weed belonging to the family Cyperaceae, the largest family in the monocotyledons. *C. rotundus* is indigenous to India and one of the most invasive weeds known, spreading out to a world-wide distribution in tropical and temperate regions. It is also found in warm temperate regions often as a rice-field weed (Thanabhorn 2015; Al Esmail 2016). It is commonly known as nut grass and referred to as 'giragiri' in Hausa, 'Danda' in Yoruba, 'Iklegbe' in Idoma and 'Kpan-ishoho' in Tiv (Edward 2007).

Cyperus rotundus is a versatile plant, widely used in traditional medicine around the world to treat stomach ailments, wounds, boils and blisters. A number of pharmacological and biological activities including anti-*Candida*, anti-inflammatory, antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic and apoptotic, anti-pyretic and analgesic activities have been reported for this plant (Oladipupo and Adebola, 2009) The tuber has been used for the treatment of dysmenorrheal and menstrual abnormalities. The root has been reported suitable in the treatment of gastrointestinal spasms, stomach and bowel conditions, food poisoning, gastritis, fever and inflammatory diseases. Infusion of the leaves has been used in fever, pain, diarrhea, dysentery and other abdominal problems. It is also used in the treatment of wounds boils, blisters and stomach disorders. The rhizomes have been found to possess antibacterial activity and is used as an analgesic, diuretic, stimulant and as a tranquillizer (Puratuchikody *et al.*,2008; Pal *et al.*, 2009; Sri and Prince 2012; Sri 2013).

Materials and methods

Plant collection and identification

The leaves *Cyperus rotundus* were collected from Benue State University Makurdi environs and was authenticated by a senior botanist in the department of Biology, Benue State University Makurdi.

Cyperus rotundus plant

Plant preparation

The leaves were rinsed with water, air-dried for two weeks. The dried leaves were pulverized into powder using mortar and pestle, then stored in a dry and airtight bottles.

Plant extraction

250g of the powdered plant leaves were macerated with 1500mL, 750mL and 400mL of hexane, ethyl acetate and methanol in a clean flat bottom flask successively for 72 h each with occasional swirling. It was then filtered using Whatman filter paper No. 2, and the filtrate concentrated under pressure using the rotary evaporator at 60^{o}C. The concentrated filtrate was then air-dried at room temperature in a fume hood to obtain the hexane, ethyl acetate and methanol crude extracts.

Phytochemical Screening

The qualitative test for secondary metabolites like saponins, terpenoids, steroids, tannins and phenols, flavonoids, cardiac glycosides, and alkaloids in the hexane, ethyl acetate and methanol extracts were carried out according to the method described by Harborne (2016). The result is as tabulated in table 1.

Test for Saponins (Froth's Test)

0.1 g each of the extracts was mixed with 5 mL of distilled water in a test tube and shaken vigorously. The formation of stable foam (froth) which persisted was an indication for the presence of saponins.

Test for Terpenoids (Liebermann-Burchard Test)

0.1 g each of the extracts was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2ml of concentrated sulphuric was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

Test for Steroids (Salkowski's Test)

0.1 g each of the extracts was dissolved with 2 mL of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

Test for Tannins and Phenols (Ferric Chloride Test)

0.1 g each of the extracts was dissolved with 2 mL of 2 % solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoids (Alkaline Reagent Test)

0.1 g each of the extracts was dissolved with 2 mL NaOH. An intense yellow colour formed which turned colorless on addition of few drops of hydrochloric acid indicated the presence of flavonoids.

Test for Cardiac Glycosides (Keller-killiani Test)

0.1 g each of the extracts was dissolved with 2 mL chloroform and 1 mL of glacial acetic acid containing a trace of ferric chloride solution. The mixture was carefully poured on the surface of sulphuric acid already contained in a test tube. A separate layer was formed. A reddish-brown colour at the interface of the layer indicates the presence of glycosides.

Test for Alkaloids (Wagner's Reagent Test)

1.0 mL each of the extracts was shaken with 5.0 mL of 2 % HCl on a steam bath and filtered. To 1 mL of the filtrate, Wagners reagent (solution of iodine in potassium iodide) was added. The formation of a brown coloured precipitate confirmed the presence of alkaloids.

Test microorganisms

Strains of bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi,* and fungi: *Candida albicans* and *Aspergillus niger* were obtained from the microbiology department, College of Health Science, Makurdi, Benue State

University. They were subjected to Gram staining and biochemical tests for proper identification. Bacterial strains were grown and maintained on nutrient agar medium and the fungal strains were grown and maintained on Sabouraud Dextrose Agar (SDA) medium.

Determination of antimicrobial activity

The antimicrobial activity of the extracts was determined in accordance with the agar disk-diffusion method described by Madland (2013). The bacteria isolates were first sub-cultured into McConkey agar and incubated at 37ºC for 18 h, while fungi isolates were sub-cultured on SDA. The isolates were then standardized to correspond to 0.5 Macfarland turbidity standard.

Nutrient agar and SDA were used as growth media for bacteria and fungi species respectively. The media were autoclaved and sterilized at 45ºC and poured into sterile petri dishes approximately 4mm depth, then allowed to solidify at room temperature.

The solidified media were seeded with 0.2 mL of the standardized inoculum of the test microbes using a sterile swab stick. Test solutions of each extract was prepared by dissolving 0.1 g of extract in 1 mL of Dimethylsulphoxide (DMSO) in a conical flask to make up stock concentration of 100 mg/mL. Then concentrations of 50, 25, 12.5 and 6.25 mg/mL were prepared from the stock concentration by serial dilution.

Disks of filter paper (5 mm in diameter) were drenched with the different concentration solutions of the extract, allowed to dry to remove residual solvent, and then introduced on the upper layer of the seeded agar plates along with disks impregnated with standard drugs (ciprofloxacin and fluconazole used as positive controls for bacteria and fungi respectively, while DMSO served as negative control). These plates were kept for 30 min for the diffusion of extracts into the media and thereafter were incubated at 37ºC for 24 h for bacteria and 72 h for fungi. Zone of inhibition (IZ) produced by the extracts around the disks were measured in mm and the 'Activity Index' (AI) were calculated by the established formula. The experiment was performed three times to minimize the error and the mean values were recorded (tables 2-4).

Activity Index (AI) = Inhibition zone produced by extract/Inhibition zone produced by standard

Determination of minimum inhibitory concentration (MIC)

The nutrient agar was prepared and sterilized, then poured into sterile petri dishes and allowed to solidify. The surface of the medium was inoculated with the test isolates. The discs soaked in different concentrations of each extract (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) were placed on the surface of the seeded nutrient agar. The plates were incubated at 37ºC for 24 h, after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms (Oludare, 2018).

Determination of Minimum bactericidal concentrations (MBC) and Minimum fungicidal concentrations (MBF)

A loopful of the content of each plate in the MIC determination, which did not show any visible growth after the period of incubation was streaked unto freshly prepared nutrient agar, to determine their MBC and then incubated at 37ºC for 24 h after which it was observed for visible growth. The lowest concentration of the subculture with no growth was considered as the minimum bactericidal concentration. This was carried out in triplicates and the mean result taken (Oludare, 2018).

Results

Qualitative test

Table 1: Phytochemicals of the leaf extracts of *C. rotundus*

	Extracts		
Phytochemicals	**Hexane**	**Ethyl acetate**	**Methanol**
Saponins	-	-	+
Terpenoids	-	-	-
Steriods	+	-	-
Tannins and phenols	-	-	+
Flavonoids	-	+	+
Cardiac glycosides	-	+	+
Alkaloids	+	+	+

Key: + indicate present and - indicate absence

Table 2: Antimicrobial activity of hexane extract of *C. rotundus* leaf

	Concentration (mg/L)										
	6. 25		12.5		25		50		100		*Standards
Microbes	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ
S. aureus	7.3±1.5	0.28	10.5±0.5	0.40	12.2±0.7	0.47	14.2±0.5	0.55	17.2±1.5	0.66	26
P. aeruginosa	5.0±0.0	0.21	9.0±0.7	0.38	11.3±1.5	0.47	14.0±1.0	0.58	15.7±0.7	0.65	24
E. coli	5.0±0.0	0.21	7.0±0.5	0.29	8.3±1.2	0.35	10.0±1.0	0.42	11.7±1.5	0.49	24
S. typhi	5.0±0.0	0.20	8.0±1.5	0.32	10.3±1.0	0.41	14.0±1.0	0.56	15.3±1.5	0.61	25
C. albicans	8.0±1.0	0.29	10.5±1.0	0.38	13.5±0.5	0.48	14.5±0.5	0.52	17.0±1.0	0.61	28
A. niger	5.0±0.0	0.19	8.5±1.3	0.31	10.5±1.0	0.39	12.5±0.5	0.46	16.0±1.0	0.59	27

IZ values are mean±SD; n=3; IZ = Inhibition zone in mm, AI = Activity index (IZ developed by extract/ IZ developed by standard); *Standards: for bacteria (Ciprofloxacin, 5 mg/mL); for fungi (Fluconazole, 5 mg/mL)

Table 3: Antimicrobial activity of ethyl acetate extract of *C. rotundus* leaf

`	Concentration (mg/L)										
	6. 25		12.5		25		50		100		*Standards
Microbes	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ
S. aureus	12.7±1.5	0.49	15.0±1.2	0.58	20.0±1.0	0.77	21.0±1.0	0.81	24.0±1.5	0.92	26
P. aeruginosa	11.3±0.5	0.45	14.3±1.0	0.57	18.0±1.0	0.72	20.7±0.5	0.83	23.3±1.0	0.93	25
E. coli	8.0±0.6	0.33	9.3±1.5	0.39	11.7±1.5	0.49	13.7±1.5	0.57	17.0±1.0	0.71	24
S. typhi	9.0±1.5	0.38	10.5±1.5	0.44	12.7±1.5	0.53	14.5±1.5	0.60	19.0±1.0	0.79	24
C. albicans	10.5±0.5	0.35	12.5±0.5	0.42	16.5±0.5	0.55	19.5±1.0	0.65	21.5±1.0	0.72	30
A. niger	8.5±1.0	0.30	12.0±1.0	0.43	14.5±0.5	0.52	17.0±0.5	0.61	19.5±1.0	0.70	28

IZ values are mean±SD; n=3; IZ = Inhibition zone in mm, AI = Activity index (IZ developed by extract/ IZ developed by standard); *Standards: for bacteria (Ciprofloxacin, 5 mg/mL); for fungi (Fluconazole, 5 mg/mL)

Table 4: Antimicrobial activity of methanol extract of *C. rotundus* leaf

`	Concentration (mg/L)										
	6. 25		12.5		25		50		100		*Standards
Microbes	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ
S. aureus	12.7±1.0	0.49	16.7±1.5	0.64	21.0±1.0	0.81	23.0±1.0	0.88	25.0±1.5	0.96	26
P. aeruginosa	12.2±1.5	0.49	14.7±0.5	0.59	17.0±1.0	0.68	17.3±1.0	0.69	19.7±1.2	0.79	25
E. coli	11.3±1.0	0.47	13.7±1.5	0.57	15.2±1.0	0.63	15.5±1.5	0.65	18.7±1.0	0.78	24
S. typhi	12.0±1.2	0.52	14.3±1.0	0.62	16.0±1.0	0.70	16.3±1.5	0.71	18.3±1.0	0.80	23
C. albicans	15.5±0.5	0.55	17.0±1.0	0.61	20.5±0.5	0.73	22.5±0.5	0.80	25.5±0.5	0.91	28
A. niger	13.5±0.5	0.50	16.0±1.0	0.59	18.5±0.5	0.69	19.5±0.5	0.72	21.5±0.5	0.80	27

IZ values are mean±SD; n=3; IZ = Inhibition zone in mm, AI = Activity index (IZ developed by extract/ IZ developed by standard); *Standards: for bacteria (Ciprofloxacin, 5 mg/mL); for fungi (Fluconazole, 5 mg/mL)

Table 5: Minimum inhibitory concentrations of *C. rotundus* leaf extracts

	MIC (mg/mL)		
Test Organisms	Hexane extract	Ethyl acetate extract	Methanol extract
S. aureus	6.25	6.25	6.25
P. aeruginosa	12.5	6.25	6.25
E. coli	12.5	6.25	6.25
S. typhi	12.5	6.25	6.25
C. albicans	6.25	6.25	6.25
A. niger	12.5	6.25	6.25

Table 6: MBC and MFC of *Cyperus rotundus* leaf extracts

	MBC (mg/mL)			MFC (mg/mL)		
Test organisms	HE	EAE	ME	HE	EAE	ME
S. aureus	6.25	6.25	6.25	-	-	-
P. aeruginosa	6.25	12.5	12.5	-	-	-
E. coli	6.25	6.25	12.5	-	-	-
S. typhi	6.25	6.25	12.5	-	-	-
C. albicans	-	-	-	6.25	6.25	12.5
A. niger	-	-	-	6.25	6.25	12.5

KEY: HE = Hexane extract, EAE = Ethyl acetate extract, ME = Methanol extract

Discussion

The qualitative test of the extracts showed the presence of steroid only in the hexane extract; flavonoid, cardiac glycoside in both the ethyl acetate and methanol extracts; saponin and tannin in just the methanol extract. While alkaloid was present in all the extracts, terpenoid was absent in all. These phytochemicals are known to be biologically active and thus may contribute to the observed antibacterial activities in the plant. Phytochemicals exert antimicrobial activity in different ways. Flavonoids possess a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties. The antibacterial activity of flavonoids had been shown to be a result of their ability to form complexes with bacterial cell walls, extracellular and soluble proteins. Tannins act by iron removal, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides. Herbs containing tannins are astringent and are used for treating intestinal ailments such as diarrhea and dysentery, hence exhibiting antimicrobial activity. Saponins are known to produce inhibitory effects on inflammatory processes and possess antimicrobial property. One common biological properties of alkaloids is its toxicity against cells of foreign organisms. They have a wide range of pharmacological activities such as antioxidant, anticancer, antimalarial and antimicrobial activities. Cardiac glycosides are found useful in the treatment of congestive heart failure (Abdulhamid *et al.* 2018; Jebasingh *et al.*, 2012; Madland, 2013). Terpenoids reveal various important pharmacological activities such as anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Heras *et al.* 2013). Steroids produce effects regarding sex hormones and have found therapeutic applications as antimicrobials, anti-oxidants or cardiac drugs (Shailendra and Patel, 2008).

The hexane extract yielded 35.2 g (14.08 %), ethyl acetate extract yielded 15.2 g (6.08 %) and the methanol extract, 9.2 g (3.68%).

Generally, the antimicrobial activity observed for all the extracts of *Cyperus rotundus* leaf showed significant increase in growth inhibition of the test organisms as concentrations of extracts increases. All plant extracts showed varying antimicrobial potential against different test microbes. The activity were recorded in terms of inhibition zone (IZ) and activity index (AI). Maximum antimicrobial activity was recorded for the methanol extract against all test microbes compared to the ethyl acetate and hexane extracts (table 2-4). This may be attributed to the presence of a good number of phytochemicals in the extracts in the order: methanol > ethyl acetate > hexane; which is an indication of the presence of bioactive compounds exhibiting the observed antimicrobial activity.

The antibacterial activity observed for methanol extract ranged from IZ of 11.3±1.0; AI of 0.47 at 6.25 mg/mL against *E. coli* to IZ of 25.0±1.5; AI of 0.96 at 100 mg/mL against *S. aureus.* It also showed a range of antifungal activity against *A. niger* and *C. albicans* from IZ of 13.5±0.5; AI of 0.50 at 6.25 mg/mL to IZ of 25.5±0.5; AI of 0.91 at 100 mg/mL respectively. *S. aureus* and *C. albicans* were the most susceptible microbes to methanol extract as their growth were both inhibited to a greater extent than other test microbes.

The ethyl acetate extract showed antibacterial activity which ranged from IZ of 8.0±0.6; AI of 0.33 at 6.25 mg/mL against *E. coli* to IZ of 24.0±1.5; AI of 0.92 at 100 mg/mL against *S. aureus.* It also showed a range of antifungal activity against *A. niger* and *C. albicans* from IZ of 8.5±1.0; AI of 0.30 at 6.25 mg/mL to IZ of 21.5±1.0; AI of 0.72 at 100 mg/mL respectively. *S. aureus* and *C. albicans* became also the most susceptible microbes to ethyl acetae extract as their growth were both inhibited to a greater extent than other test microbes.

The antibacterial activity observed for hexane extract ranged from IZ of 5.0±0.0; AI of 0.20 at 6.25 mg/mL against *S. typhi* to IZ of 17.2±1.5; AI of 0.66 at 100 mg/mL against *S. aureus.* It also showed a range of antifungal activity against *A. niger* and *C. albicans* from IZ of 5.0±0.0; AI of 0.19 at 6.25 mg/mL to IZ of 17.0±1.0; AI of 0.61 at 100 mg/mL respectively. *S. typhi* and *C. albicans* were the most susceptible microbes to hexane extract as their growth were both inhibited to a greater extent than other test microbes.

These results, is in unison with an earlier report indicating that plant extracts are more

active against gram-positive bacteria than gram-negative bacteria; and the fungi, *C. albicans* more sensitive to the extracts than *A. niger* (Prasad 2014).

The MIC as presented in table 5, showed that all tested microbes were susceptible at a concentration of 6.25 mg/mL for all extracts, except for *P. aeruginosa, E. coli, S. typhi* and *A. niger* which showed susceptibility to the hexane extract at a higher MIC of 12.5 mg/mL. This suggest that the leaf extracts at a concentration as low as 6.25mg/mL can significantly inhibit against both bacterial and fungal pathogens. This then confirms and supports the traditional use of the leaf extract in fever, diarrhea, dysentery and other intestinal problems and the research findings carried out by Pal and Dutta (2009) and Vijisaral and Subramanian (2013).

MBC and MFC which is the least concentration of the extract where no visible growth is observed for both the bacteria and fungi respectively is as shown in table 6. From the results, concentrations ranging from 6.25-12.5 mg/mL was observed for all the bacteria and fungi in the hexane, ethyl acetate and methanol extracts. At MBC as low as 6.25 mg/mL, all tested bacteria showed no visible growth to the hexane and ethyl acetate extract except for *P. aeruginosa* which showed no visible growth at a higher MBC of 12.5 mg/mL for ethyl acetate. *P. aeruginosa, E. coli* and *S. typhi* showed MBC at 12.5 mg/mL, but 6.25 mg/mL for *S. aureus* of the methanol extract. The two tested fungi, *C. albicans* and *A. niger* exhibited MFC at 6.25 mg/mL for both hexane and ethyl acetate extract but at a higher concentration for 12.5 mg/mL for the methanol extract. This result suggest that at MBC range of 6.25 mg/mL and 12.5 mg/mL, the plant extracts showed effective bactericidal effect against the test bacteria; and effective fungicidal effect against the test fungi. This is a reflection of the presence of phytochemicals and supports the research results on *C. rotundus* leaf earlier reported by Vijisaral and Subramanian (2013).

Conclusion

The study results signify the potential of *Cyperus rotundus* as a source of therapeutic agents. The methanol extract showed the best activity against the test bacterial and fungal pathogens as it showed most significant inhibition at all concentrations. The leaf extracts at a concentration as low as 6.25mg/mL can significantly inhibit against both bacterial and fungal pathogens. At MBC and MFC of 6.25 mg/mL and 12.5 mg/mL, the plant extracts showed effective bactericidal and fungicidal effect against the test microbes. However, crude extract need to be further purified through antibacterial activity guided fractionation to isolate and identify the compounds responsible for antimicrobial activity.

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