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Research Article
Evaluation of Antimicrobial Activities of Extract from *Pyrenacantha grandiflora* Baill. (Icacinaceae)

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Abstract

Background and Objective: Microbial drug resistance is a growing health problem. This has led to search for new antimicrobial compound and plants are considered as one of the most promising sources for new antimicrobials discovery. *Pyrenacantha grandiflora* (*P. grandiflora*) Baill is used for the treatment and management of diarrhea, gastrointestinal related infections, dysentery, inflammation and tooth pain by traditional healers in the Venda region. The goal of the present study was to evaluate the antimicrobial activity of *P. grandiflora* tubers using different extraction solvents against 15 bacterial and 11 fungal strains. **Materials and Methods:** Plant extracts were obtained using 5 solvents separately, boiled water, cold water, methanol, dichloromethane, chloroform and ethyl acetate. Hole plate assay was used for initial evaluation of antimicrobial properties of plant materials. Minimum inhibitory concentrations (MIC) of the most active plant extracts were determined by the broth microdilution method. One-way ANOVA was used for data analysis. **Results:** The hole plate assay revealed that the highest antibacterial activity was against *Micrococcus kristinae* with ethyl acetate extract and no extract was active against *Candida* and *Fusarium* species using this method. The MIC of the extracts was determined and all the extracts showed antimicrobial activity against all tested strains ranging from 0.06-7.5 mg mL^{-1}. Some extract appeared to be fungicidal and hot water extract were more active against *Cryptococcus neoformans* with the MFC value of 0.06 mg mL^{-1}. Methanol extract was also active against most test strains including *Candida tropicalis* with the minimum fungicidal concentration value of 3.75 mg mL^{-1}. **Conclusion:** *Pyrenacantha grandiflora* contains substances that make it active against bacterial and fungal pathogens. This is the first time the antimicrobial and antifungal activities of *P. grandiflora* have been demonstrated scientifically. Extraction with hot water as done by the traditional healers showed activity thereby justifying the traditional use of this plant.

Key words: Antibacterial, antifungal, *Pyrenacantha*, MIC, MFC, bacterial and fungal species

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

World Health Organization (WHO) 2014 report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction for the future, it is happening right now, across the world and the ability to treat common infections in the community and hospitals is at risk. Therefore, the outlook for the use of antimicrobial drugs in the future is still uncertain[1,2]. There is an urgent need for new antimicrobials or any action to effectively fight against resistant microbes. Furthermore, the search of new antimicrobial compounds should be done to better comprehend their properties, safety and efficiency/ effectiveness[3]. Plants are considered to be amongst the safest sources because of their natural origin as compared to the synthetic compounds[4,5]. Plant secondary metabolites are known to be responsible for antimicrobial action[6]. Other advantages of using plant derived antimicrobial include the fact that they have several target sites and have a variety of mechanisms of action[5,7-9]. Several studies have highlighted the antibacterial activities of South African traditional medicinal plants[10-13]. However, *Pyrenacantha grandiflora* has not been studied.

Pyrenacantha grandiflora Baill in a medicinal plant used by elders in the Venda region in Limpopo, South Africa. It is known by the common names Gwere, Velavhahleka, Mbengelele (in Tshivenda) and Velabahleke (Siswati). *Pyrenacantha grandiflora* belongs to the family Icacinaceae. There are approximately 48 species, subspecies, varieties, forms and cultivars in the genus *Pyrenacantha*. Members of the genus mostly studied includes *Pyrenacantha staudtii* which was found to possess an inhibitory activity of the derivatives of 3-Carbomethoxypyridine on isolated rat uterus[14].

Although not many specific indications have been seen for this species, it belongs to a genus where most, if not all members of the genus produce hydrogen cyanide, a toxic substance that gives almonds their characteristic flavor and it is typically present in too little amount to do any damage[15]. In small quantities, produced hydrogen cyanide has been appeared to stimulate respiration and improve digestion, it is also asserted to be of advantage in the treatment of cancer. In abundance, in any case, it can cause respiratory failure and even death[16]. In the Venda region of South Africa, *Pyrenacantha grandiflora* is used for the treatment and management of gastroenteritis, dysentery, inflammation and tooth pain[17]. Apart from the antimicrobial activities, plant extracts have also exhibited immunomodulatory effects on various cell cultures and in experimental animals[18,19].

Previously, Ramalivhana and colleagues[17] conducted a study to determine the antimicrobial activities of *Pyrenacantha grandiflora* Baill and *Ficus sycomorus* used by traditional healers in Limpopo province against *Aeromonas* spp. However, there is a dearth of information on the antimicrobial activity of extract from the genus *Pyrenacantha*. The objective of the current research was to evaluate the antimicrobial activities of extracts from *Pyrenacantha grandiflora* prepared using different solvents.

MATERIALS AND METHODS

Microorganism and growth conditions: Following microorganisms were used as test strains, *Streptococcus faecalis* (ATCC 29212), *Staphylococcus epidermidis* (clinical isolate), *Staphylococcus aureus* (clinical isolate), *Pseudomonas aeruginosa* (ATCC19582), *Serratia marsecens* (ATCC 9986), *Klebsiella pneumonia* (clinical isolate), *Acinetobacter calcoaceticus*, *Escherichia coli* (clinical isolate), *Micrococcus kristinae* (clinical isolate), *Escherichia coli* (ATTC 8739), *Proteus vulgaris* (ATCC 6830), *Pseudomonas aeruginosa* (ATCC 7700), *Salmonella* spp. (clinical isolate), *Salmonella typhi* (clinical isolate), *Candida krusei*, *Candida tropicalis*, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Cryptococcus neoformans*, *Fusarium oxysporum*, *Fusarium graminearum*, *Fusarium nygamai*, *Fusarium verticillioides*, *Fusarium proliferatum*. Mueller-Hinton broth medium (MHB) (Rochelle chemical, SA) and Mueller-Hinton agar medium (MHA) (Rochelle chemicals, SA) were used to cultivate bacteria and Mueller-Hinton broth medium and sabouraud dextrose agar were used for yeast cultivation.

Collection, identification and drying of plant materials: Plants material collection was done in October, 2014 a village called Masisi situated at about 30 km outside of Thohoyandou. The tubers were harvested and were washed with tap water to remove any contaminants and subsequently placed in a drying room for 2 weeks. Dried plant materials were finely grounded into powder and stored in a sealed glass jars at room temperature.

Preparation of plant crude extract: Plant materials were extracted using different solvent which included boiled and cold water, chloroform, methanol, ethyl acetate and dichloromethane extract (Rochelle chemical, SA). For boiled water extract, the stock solution was prepared by adding 50 g of crude dried tuber in 500 mL of distilled water then boiled for 15 min followed by cooling and kept at 4°C. For cold water

extraction, chloroform, methanol, ethyl acetate and dichloromethane extract, 50 g of dried tuber was added to 500 mL of those solvent and allowed to homogenize for 3 days at room temperature. The mixture was filtered through 22 µm filter and the extracts were further dried using a rotary evaporator.

Hole plate assay: Antimicrobial activity of *P. grandiflora* was initially evaluated using hole plate assay[20]. Growth medium was poured into petri dishes at 50-70°C and left to solidify under ultraviolet (UV) light (265 nm wavelength) for about an hour. A sterile cotton swab was dipped into overnight culture of each bacterial strain (adjusted to turbidity of 0.5 McFarland standard). An agar plate was inoculated by evenly streaking cotton swab over the agar medium surface. Small holes with a diameter of 8 mm were punched in the medium with a sterile cock borer and 30 µL of the extracts (of 100 mg mL^{-1}) were dispensed into the holes and the plates were incubated at 37°C for 24 h. The diameter of growth inhibition zone around the holes were measured.

Microdilution assay: The microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the plant extracts using 96 well microtitration plates[21]. Briefly, 185 µL of the broth was added into each well in the first row of the microtitration plate and 100 µL to the rest of the wells from the second row downwards. Plant crude extracts (15 µL) was then added into each well on the first row (row A), starting with the positive control penicillin (Sigma, Germany), followed by the negative control (20% DMSO (Sigma-Aldrich, Germany) used to dissolve the plant extracts) and the plant extracts in the rest of the wells in that row. A two-fold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µL to the next well of the same column and the same procedure was done up to the last well of the same column and the last 100 µL from the last well was discarded. Then 100 µL of different microorganism suspensions was added and the plate was incubated overnight at 37°C. The next day, 40 µL of INT (Iodo-nitro tetrazolium) was added in each well and the plate was incubated for 10 min. After 10 min incubation, the results were read using a spectrophotometer (SpectraMax M2, Sunnyvale, USA), observing the color change and determining the MIC. All the extract that showed activity (no color change) were inoculated again on the agar plate and incubated overnight to determine the MBC and MFC.

Determination of MBC/MFC values: Minimum bactericidal concentration (MBC) and minimum fungicidal concentrations (MFC) of the plant crude extracts were determined by sub-culturing the samples (5 µL) taken from the wells without growth during MIC determination on the agar medium. The lowest concentration of crude extracts with the absence of growth after 24 h incubation at 37°C was considered as MBC/MFC.

Growth inhibition assay: Growth inhibition assay for 3 extracts including methanol, cold and hot water was conducted to evaluate the antimicrobial activity of *P. grandiflora* against *Fusarium species*. Different plant extracts were inoculated in the growth medium of *Fusarium species* and the diameter of inhibition of fungal growth were recorded daily for 7 days.

Statistical analysis: All the data were loaded onto access and one way ANOVA was used for statistical analysis. The difference was considered statistically significant if the $p<0.05$.

RESULTS

The activity of the extracts from *P. grandiflora* prepared with solvents such as boiling and cold water, chloroform, methanol, ethyl acetate and dichloromethane were evaluated against different microorganism including bacteria and fungi (*Candida* spp., *Cryptococcus neoformans* and *Fusarium* spp.).

Hole plate assay: The antimicrobial assay of different plant extracts was initially evaluated using hole plate assay. The hole plate assay revealed that the ethyl acetate extract had the highest antibacterial activity showing a diameter of inhibition of 18 mm against *Micrococcus kristinae*. Dichloromethane and chloroform extracts showed no activity against all bacteria. Methanol extract appeared to inhibit the growth of most bacteria with the highest diameter of 15 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table 1). There was no extract active against *Candida* and *Fusarium* species using the hole plate assay.

Microdilution assay: The minimum inhibition concentration was determined by broth microdilution method. The most active plant extract using this method was chloroform extract with the concentration of 0.12 mg mL^{-1} against *Escherichia coli*. Ethyl acetate extracts had the lowest overall MIC value compared to all the other extracts. Dichloromethane showed the highest MIC value against all different bacteria (Table 2).

Table 1: Antibacterial activity of *P. grandiflora* using the hole plate method

Organisms	Methanol extract	Hot water extract	Cold water extract	Dichloromethane extract	Chloroform extract	Ethyl acetate extract
Streptococcus faecalis (ATCC 29212)	8	12	0	0	0	0
Staphylococcus epidermidis (clinical isolate)	12	10	10	0	0	0
Staphylococcus aureus (Clinical isolate)	15	0	0	0	0	0
Pseudomonas aeruginosa (ATCC 19582)	15	0	15	0	0	10
Serratia marsecens (ATCC 9986)	8	0	8	0	0	10
Klebsiella pneumonia (clinical isolate)	12	0	0	0	0	10
Acinetobacter calcoaceticus	13	0	0	0	0	5
Escherichia coli (Clinical isolate)	10	15	15	0	0	0
Micrococcus kristinae (Clinical isolate)	0	10	0	0	0	18
Escherichia coli (ATCC 8739)	0	0	0	0	0	7
Proteus vulgaris (ATCC6830)	7	10	8	0	0	0
Shigella flexneri (Clinical isolate)	0	5	0	0	0	0
Pseudomonas aeruginosa (ATCC 7700)	0	8	0	0	0	14
Salmonella spp. (Clinical isolate)	0	10	0	0	0	0
Salmonella typhi (Clinical isolate)	0	0	5	0	0	0

The amount of extracts was 30 µL. The results are presented as diameters of inhibition zones in mm

Table 2: Minimum inhibition concentration (MIC) of the extracts against different bacteria using the microdilution method

Organisms	Methanol extract	Hot water extract	Cold water extract	Dichloromethane extract	Chloroform extract	Ethyl acetate extract
	mg mL^{-1}					
Streptococcus faecalis (ATCC 29212)	0.95	0.95	0.95	>7.5	>7.5	1.90
Staphylococcus epidermidis (Clinical isolate)	>7.50	7.5	3.75	>7.5	>7.5	3.75
Staphylococcus aureus (Clinical isolate)	0.06	1.9	0.95	>7.5	>7.5	3.75
Pseudomonas aeruginosa (ATCC 19582)	3.75	7.5	>7.50	>7.5	>7.5	3.75
Serratia marsecens (ATCC 9986)	1.90	7.5	>7.50	>7.5	>7.5	>7.50
Klebsiella pneumonia (Clinical isolate)	0.48	1.9	3.75	>7.5	>7.5	3.75
Acinetobacter calcoaceticus (Clinical isolate)	>7.50	>7.5	7.50	>7.5	>7.5	7.50
Escherichia coli (Clinical isolate)	>7.50	7.5	7.50	>7.5	0.12	3.75
Micrococcus kristinae (Clinical isolate)	1.90	0.12	7.50	>7.5	>7.5	3.75
Escherichia coli (ATCC 8739)	>7.50	7.5	7.50	>7.5	>7.5	3.75
Proteus vulgaris (ATCC 6830)	>7.50	7.5	7.50	>7.5	>7.5	3.75
Shigella flexneri (Clinical isolate)	>7.50	7.5	>7.50	>7.5	>7.5	3.75
Pseudomonas aeruginosa (ATCC 7700)	>7.50	7.5	3.75	>7.5	7.5	3.75
Salmonella spp. (Clinical isolate)	>7.50	>7.5	>7.50	>7.5	>7.5	3.75
Salmonella typhi (Clinical isolate)	>7.50	>7.5	>7.50	>7.5	>7.5	7.50

Methanol extract was the most active with the MIC value of 0.12 mg mL^{-1} against *Candida parapsilosis*. Ethyl acetate extract showed the lowest MIC overall value against all *Candida* spp. and *Cryptococcus neoformans*. Dichloromethane and chloroform extract were least active with MIC values 7.5 mg mL^{-1} against all *Candida* spp. and *Cryptococcus neoformans* (Table 3).

Fusarium species was also used in evaluation of *P. grandiflora* activity using broth microdilution method. Methanol extract was the most active with the MIC value of 0.06 mg mL^{-1} against *Fusarium nygamai*. Cold water extract showed the lowest MIC overall value in all *Fusarium* spp. Dichloromethane and chloroform extract were less active with MIC value of 7.5 mg mL^{-1} against all *Fusarium* spp. (Table 4).

Determination of MBC/MFC values: All the wells containing extracts that showed activity (no color change) during microdilution assay were inoculated again in the agar to check if they can kill the microorganisms. Most extracts were active against the organisms tested and hot water extract appeared to be more active against *C. neoformans* with the MFC value of 0.06 mg mL^{-1}. Methanol extract also showed to be active against *C. tropicalis* with the MFC value of 3.75 mg mL^{-1} (Table 5). Cold water, dichloromethane, chloroform and ethyl acetate extracts were not fungicidal to any of the fungal organism tested. None of the extracts was bactericidal and none of the extracts was fungicidal against the fusarium species.

Growth inhibition assay: Growth inhibition assay of three extracts including methanol, cold and hot water was conducted to evaluate the cytotoxicity of *P. grandiflora* against *Fusarium species*. Methanol extract was observed to be most active against *Fusarium verticillioides* (Fig. 1a), *Fusarium oxysporum* (Fig. 1b) and *Fusarium proliferatum*

Table 3: Minimum inhibition concentration (MIC) of *P. grandiflora* against yeast using microdilution method

Organisms	Methanol extract	Hot water extract	Cold water extract	Dichloromethane extract	Chloroform extract	Ethyl acetate extract
	----------mg mL^{-1}----------					
Candida krusei	3.75	>7.50	>7.5	>7.5	>7.5	3.75
Candida tropicalis	3.75	>7.50	7.5	>7.5	>7.5	3.75
Candida albicans	>7.50	>7.50	>7.5	>7.5	>7.5	3.75
Candida parapsilosis	0.12	0.95	7.5	>7.5	>7.5	3.75
Candida glabrata	3.75	3.75	7.5	>7.5	>7.5	3.75
Cryptococcus neoformans	>7.50	0.06	>7.5	>7.5	>7.5	3.75

Table 4: Minimum inhibition concentration of *P. grandiflora* against *Fusarium* species using microdilution method

Organisms	Methanol extract	Hot water extract	Cold water extract	Dichloromethane extract	Chloroform extract	Ethyl acetate extract
	----------mg mL^{-1}----------					
Fusarium oxysporum	1.9	7.5	1.90	>7.5	>7.5	3.75
Fusarium graminearum	7.5	7.5	7.50	>7.5	>7.5	3.75
Fusarium nygamai	0.06	3.75	0.95	>7.5	>7.5	3.75
Fusarium verticillioides	1.9	7.5	1.90	>7.5	>7.5	3.75
Fusarium proliferatum	7.5	7.5	3.75	>7.5	>7.5	3.75

Table 5: Minimum fungicidal concentration (MFC) using microdilution method

Organisms	Methanol extract	Hot water extract	Cold water extract	Dichloromethane extract	Chloroform extract	Ethyl acetate extract
	----------mg mL^{-1}----------					
Candida krusei	>7.5	>7.5	>7.5	>7.5	>7.5	>7.5
Candida tropicalis	3.75	>7.5	>7.5	>7.5	>7.5	>7.5
Candida albicans	>7.5	>7.5	>7.5	>7.5	>7.5	>7.5
Candida parapsilosis	>7.5	>7.5	>7.5	>7.5	>7.5	>7.5
Candida glabrata	>7.5	>7.5	>7.5	>7.5	>7.5	>7.5
Cryptococcus neoformans	>7.5	0.06	>7.5	>7.5	>7.5	>7.5

(Fig. 1c) with the lowest diameter (<25 mm) of fungal growth compared to other extract during seven days of evaluation (Fig. 1). Cold water and Methanol extract were both active against *Fusarium graminearum* (Fig. 1d) with the diameter of 25 mm of fungal growth on the last 3 days. All extracts were active against *Fusarium nygamai* (Fig. 1e).

DISCUSSION

It is found that *P. grandiflora* extract has antimicrobial activity against numerous bacteria and fungi as this was confirmed using hole plate assay as well as microdilution assay. Furthermore, *P. grandiflora* extract was able to inhibit the growth of bacteria and fungi. However, they were not able to kill the bacteria whereas fungicidal activity was observed. Since different solvent were used during extraction of plant material, methanol extract was the most active amongst them all.

The present study is the first attempt to determine the antimicrobial activities of *P. grandiflora* against organisms other than *Aeromonas* spp[17]. The study showed that water extracts as well as methanol extracts were the most active against some of the microorganisms tested particularly when the microdilution method was used. Their activities against bacteria and fungi that affect humans and plants shows that this plant could be very useful in the identification of compounds that could serve as lead in the development of drugs for the treatment of both human and animals and plant diseases. The study of other species of the genus *Pyrenacantha* showed that the leaves of *Pyrenacantha staudtii* is used as antimalarial remedies in the Democratic Republic of Congo[22].

After plant collection, the tubers were dried since Mendes *et al*.[23] suggested that drying is very important because it causes loss of volatile antimicrobials (peroxide, terpenoid and bromo-ether compounds and volatile fatty-acid) present in the fresh plant[23]. Subsequently, washing the tuber to remove soil and other contamination then ground into powder for easier further analysis is done. Different solvents were used during extraction in this study, this includes boiled water since drying followed by boiling increased the phytochemicals content and enhanced the

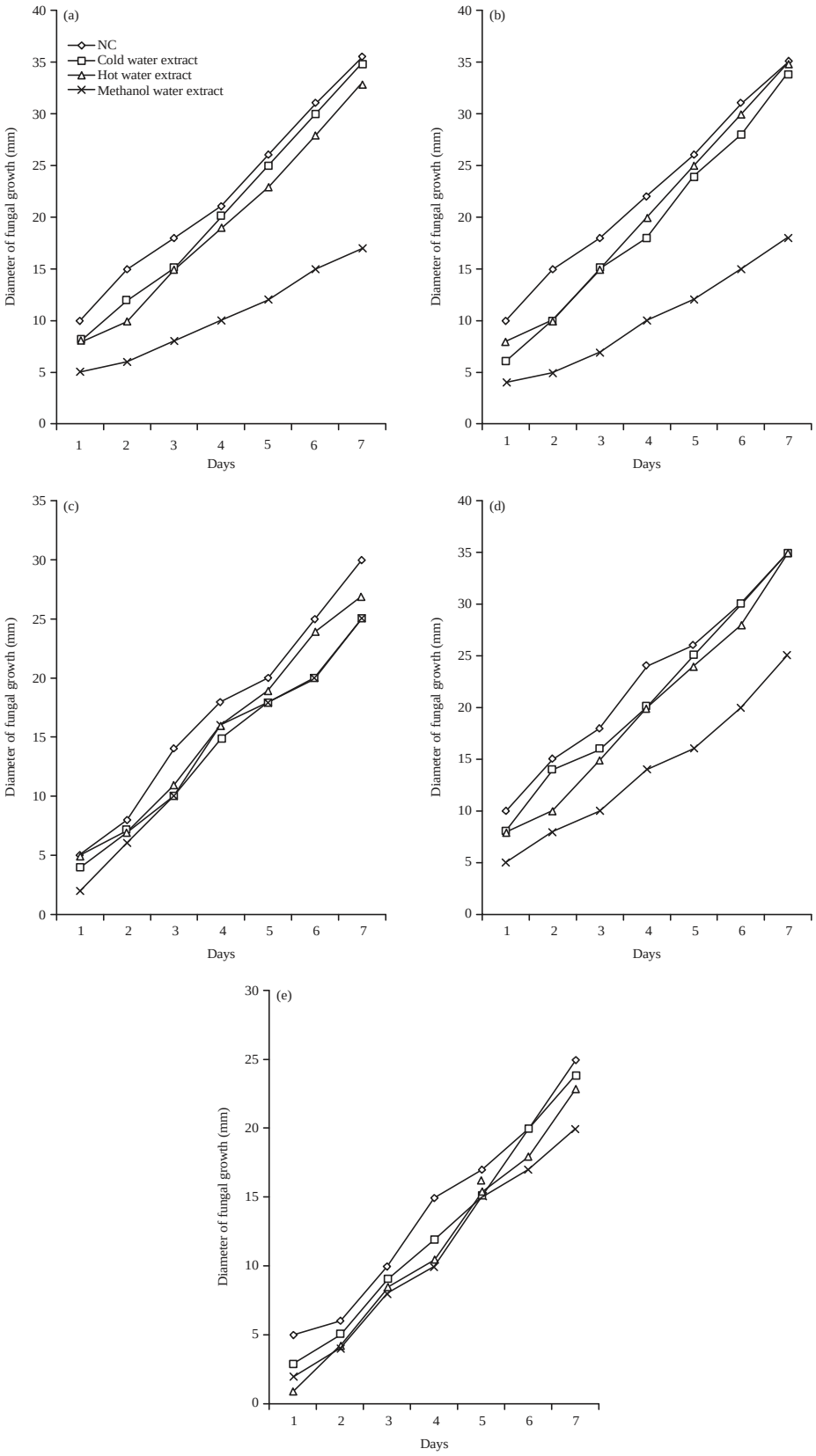

Fig. 1(a-e): Growth inhibition assay of 3 extracts against (a) *Fusarium verticillioides*, (b) *Fusarium oxysporum*, (c) *Fusarium proliferatum*, (d) *Fusarium graminearum* and (e) *Fusarium nygamai'*

inhibitory potential[24]. Probably, inhibition mechanisms are due in part to the hydrophobic nature of some components, such as fatty products[25,26]. Other studies confirmed that polar extracts have higher antibacterial activity[27-29]. Systematical evaluation and optimization of the solvent is necessary for accurate and reproducible preparation of extracts. Several studies aimed at selecting the best solvent, which was usually one of the following, water, methanol, ethanol, acetone, ethyl acetate, dichloromethane, chloroform, diethyl ether and hexane[30,31].

The antimicrobial assay of different extracts was initially evaluated using hole plate assay and all extracts were active against bacteria only. This is an indication that the compounds active against these bacterial organisms are probably polar. Other authors have found that the polarity of the compounds was important in determining the biological activity and many non-polar compounds were active against a number of bacterial organisms[32]. Fungi (*Candida* and *Fusarium* species) are characterized by having the cell wall which might not be easily accessed by polar compounds. This could explain why none of extract were active against them when the hole plate assay was used. Methanol extract generally inhibit most microorganism[24,25]. In this study hole plate assay was performed and methanol extract inhibited the growth of most bacteria with the diameter of 15 mm against *S. aureus* and *P. aeruginosa* whereas highest antibacterial activity diameter of 18 mm zone of growth inhibition was observed with ethyl acetate extract against *Micrococcus kristinae*. In another study, it was found that methanol extracts had good activities against *P. aeruginosa* when the microdilution method was used[33]. Dichloromethane and chloroform extract showed no activity against all bacteria this may due to their low level of polarity as compared to other solvents used in this study or any other physical or chemical characteristic.

The MIC values obtained from our results ranged from 0.06-7.5 mg mL^{-1}. The MIC values were lower than those obtained with organic extracts of the *Kirkia wilmsii* leaves which ranged from 0.17-2.11 mg mL^{-1} [34]. However, 7.5 mg mL^{-1} was much higher than the reported maximum of 2.11 mg mL^{-1} in the previous study. This comparison shows that the tuber of *P. grandiflora* might be more potent. Our findings showed that the tuber extracts exhibited strong antimicrobial activity with the smallest MIC of 0.06 mg mL^{-1} which was smaller than the concentration of 1 mg mL^{-1} previously reported[35], a concentration at which the plant extracts are considered to have good potency of antimicrobial level when using the micro-plate dilution method[20]. Therefore, these extracts should be pursued as they could be a good source of bioactive compound. *Pyrenacantha grandiflora* did not show any bactericidal activity. However, Ramalivhana[17] and others obtained low bactericidal activity with *P. grandiflora* compared to *F. sycomorus*. For future study, other solvent such as ethanol and diethyl ether may also be used during extraction.

Growth inhibition assay of three extracts was conducted against *Fusarium* species. In most cases, the extracts prepared using organic solvents appeared more efficient and this is similar to previous studies[36,37]. Abundant studies have confirmed that alcoholic solutions and/or hydrophilic solvent mixtures provided better activity, i.e., methanol and acetone extracts were more active than those in lipophilic solvents such as chloroform/methanol[23,38]. In this study, methanol extract was observed to be most active against *Fusarium verticillioides* while *Fusarium oxysporum* and *Fusarium proliferatum* showed the lowest diameter of fungal growth compared to other extract on day seven of evaluation. Rajauria *et al*.[37] found considerable variations in the extraction yield and antimicrobial activity among different concentrations of methanol extract. Cold water and methanol extracts were both active against *Fusarium graminearum* with the diameter of 25 mm of fungal growth on the last days. However, the optimal solvent depends on many factors, particularly on the target solutes and microorganisms[39]. All extracts were active against *Fusarium nygamai* (E).

CONCLUSION

The present study validated the efficacy of *P. grandiflora* which is used in traditional medicine. The results revealed that water extracts which is generally used by the traditional healers is active against most microorganisms tested as well as methanol extract. Further study should be conducted to purify the plants' active compounds responsible for their antimicrobial action and examine their efficiency on other bacteria, fungi and also helminths. Synergistic effect of these plant extracts with commonly used antibiotics will also be interesting. However, apart from antimicrobial activities, these plant extracts could also be exploited to cure several disorders. The results of present investigation clearly indicate that antimicrobial activity of *Pyrenacantha grandiflora* Baill tuber vary with test strain and the type of solvent used during extraction, this clearly gives hope for future development of drug leads.

SIGNIFICANCE STATEMENT

This study discovers the antimicrobial activity of *P. grandiflora* tubers using different extraction solvents. The present study is the very first report of studied activities of the traditionally used medicinal plant against a number of bacterial and fungal species.

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