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Antimicrobial activity, cytotoxicity, and phytochemical assays of organic and aqueous extracts from *Sarcophyte piriei*

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ABSTRACT

Traditional medicine practice systems employ plants in the era of rising antimicrobial resistance. The current research investigates the antimicrobial activities of *Sarcophyte piriei*, whose tuber is utilized to manage diseases. There are limited studies on the efficacy, safety, and chemical composition of the *Sarcophyte piriei*. The objective was to investigate the antimicrobial activity, cytotoxicity, and phytochemical components. The powdered material was extracted by maceration using water and organic solvents progressively from petroleum ether (100%), dichloromethane (100%), dichloromethane: methanol (1:1) and methanol (100%) and water. Disk diffusion method was used to investigate antimicrobial activity at 100 mg/ml and minimum inhibitory concentration determined. Cytotoxicity of the extracts were evaluated using brine shrimp lethality assay. Phytochemical screening was done using standard procedures. The extracts revealed varied antimicrobial effects, the methanol: dichloromethane (1:1) extracts revealed the highest zone of inhibition 18.0 ± 0.0 mm against *S. aureus* while aqueous had a zone of 10.0 ± 0.0 mm against *C. albicans*. The lowest minimum inhibitory concentration of the active dichloromethane: methanol (1:1) against *Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Minimum inhibitory concentration values obtained were: 18.75 ± 00, 14.06 ± 4.7 and 28. 13 ± 9.4 mg/ml respectively. The minimum bactericidal concentrations values were: 62.5 ± 12.5, 20.31 ± 9.5 and 40.63 ± 19 mg/ml respectively. The petroleum ether and dichloromethane extracts had the highest cytotoxic effects against brine shrimp with LC50 value of 55.0 µg/ml while the aqueous extract was nontoxic. Alkaloids, terpenoids, amino acids, carbohydrates, flavonoids, saponins, tannins, cardiac glycosides, diterpenes, xanthoproteins, anthocyanins, coumarins and oxalates were detected. It was concluded that the extracts of *Sarcophyte piriei* has antimicrobial activity and the water extracts is safe. Therefore, the secondary metabolites responsible for antimicrobial activity require to be isolated.

Keywords: *Sarcophyte piriei*, Phytochemical components, Traditional medicine.

INTRODUCTION

Plants secondary metabolites are exploited in traditional medicine practices for primary health care, over 80% of human population in developing countries of the world, characterized by resource constraints have from prehistoric times to date consistently utilize plants as sources of medicines and also in livestock and crop farming [1–3]. In the present times, plants continue to provide solutions to human ailments in the middle of the challenges of antimicrobial resistance and limited accessibility to quality and effective antibiotics [4].

Antimicrobial plant extracts are characterized with alkaloids, phenolic acids, polyphenols, terpenes and essential oils which destroy microorganisms by cell wall and membrane rupture, cell leakage or disruption of DNA-related processes [5].

Nations around the world are contemplating on improving the health status of their citizens as per Global Goals number three which requires that the Nations ensure a health lives and promotes the well-being for all citizens of all ages [6,7]. Most population in the sub-Saharan Africa are characterized by hand to mouth economically. The inadequacy of health facilities and qualified medical practitioners has become an impetus for the use of plant medicines [8]. The mixing of conventional medicines and herbal concoctions require research to prove their safety and efficacy as some plants are toxic [9,10]. The goals envisaged in Vision 2030 by Kenya are aimed at improved livelihood by the overall Kenyan Population and also the Kenyan government is committed to provide Universal health care [11]. The Kenyan Government holds that it is imperative for the responsible authorities to validate plants in traditional medicine practice to allow the integration of plants into the healthcare system [12]. The plant *Sarcophyte piriei* is used in traditional medicine system to treat conditions of the skin like acne, sores, eruptions, burns, wounds, snakebites, and shingles. In addition, sore throat, diarrhoea, abdominal pains, menstrual

pain, toothache, and cancer are treated by the extracts from *S. piriei* [13–20]. Despite, the wide distribution this plant in Zambia, Malawi, Mozambique, Zimbabwe, East Africa from Somalia, and Ethiopia, to Mozambique and Zimbabwe, there is scant scientific research to validate the traditional medicinal usage. The current study on the antimicrobial activity, cytotoxic effects and phytochemical screening, provides scientific data to back up the use of *Sarcophyte piriei* in traditional medicine to treat microbial related infections.

MATERIALS AND METHODS

Sample Collection:

The *Sarcophyte piriei* stem tubers were obtained from Embu County, Mbeere South Constituency, Kiambere Location, Ntharawe sublocation, Rwagiri village with the aid of a local recognised traditional medicine practitioner. Plant voucher specimen was authenticated and deposited at University of Nairobi Herbarium. Stem tubers were pilled, chopped into small pieces and air dried for 7 days. Grinding of the stem tubers into a coarsely powder was done at the Department of Public Health, Pharmacology and Toxicology at Kabete Campus, University of Nairobi using an electric laboratory plant mill. The powder obtained was kept in well labelled manila airtight bag and stored in a dry place in the herbarium cabinet.

Extraction:

Sequential extraction of *Sarcophyte piriei* powder

Approximately six hundred of the powder from *Sarcophyte piriei* tuber were extracted successfully by cold maceration using different organic solvents in increasing polarity [21–23]. The solvents used for extraction were petroleum ether, dichloromethane, mixture of 50% dichloromethane in methanol and methanol. Six hundred grams of the powder were soaked separately in 500 mL petroleum ether and were allowed to be extracted for 72 hours and the miscella was obtained by filtration, first, through muslin cloth and secondly by using Whatman number one filter paper. The residue was further re-extracted two times by using fresh petroleum ether and the filtrates were pooled together. The marc was air dried and further extracted three times using dichloromethane, the procedure was repeated using a mixture of 50% dichloromethane in methanol and finally pure methanol. The filtrates were reduced using rotary evaporator under reduced pressure and low temperature and the extracts were dried completely using oven at 40 ^{0}C. The yield of each extract was weighed and stored at 4 ^{o}C until used.

Direct extractions of *Sarcophyte piriei* powder

Two extracts, methanol and water were obtained through direct extraction. The methanol extracts were done by soaking 600 g powder of *Sarcophyte piriei* tuber directly in 500 mL of methanol with intermittent shaking for 72 hours. The extract was strained using muslin cloth followed with further refining of the filtrate by using Whatman no1 filter paper. The residue was subjected to further extraction in fresh petroleum ether solvent (two times) and the filtrates were put together and filtered similarly as the first extract and then reduced using a rotary evaporator and thereafter dried completely in the oven at temperature of 30 ^{o}C. The water extract of *Sarcophyte piriei* powder was prepared by mixing one hundred grams of the powder with 500 ml distilled water in volumetric flask. The mixtures were boiled at 100 ^{o}C. for 10 minutes and left to cool. The extracts were then separated utilizing Whatman paper no. 1, after which the filtrate was centrifuged at 3000 rpm for 10 min. The supernatant was further separated on sintered glass and lyophilization of the filtrate was carried out for 48 hours to constitute a water extract [21].

Preparation of working extract solutions

The working solution of 100 mg/ml were prepared for each of the extracts. The *Sarcophyte piriei* extracts in the study were wetted using 0.1% dimethyl sulfoxide (DMSO) to make the working concentrations. The petroleum ether, dichloromethane, dichloromethane: methanol, methanol sequential, methanol direct and water extracts were evaluated at a concentration of 100 mg/ml. Hundred milligrams of each extract were dissolved in 1000 µL of dimethyl sulfoxide (1ml of the 0.1 % DMSO) with the aid of ultrasonic machine (sonicator) to make the higher concentration (100 mg/ml). Then the extracts were transferred into Eppendorf tubes. Extracts that exhibited activity against bacteria were then serial diluted to constitute the different concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, and 1.5625 mg/ml that were used for determining the minimum inhibitory concentration (MIC) and minimum bactericidal and or minimum fungicidal concentration.

Determination of antimicrobial activity:

Test microorganisms and preparation of culture media

Five microorganisms were used in this study. Two Gram-positive bacteria which were *Staphylococcus aureus* (ATCC 12393) and *Bacillus subtilis* (ATCC 6051). *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15442) the Gram-negative and one fungal strain that was *Candida albicans* (ATCC 10231). The stock cultures of the microorganism were maintained at 4 ^{o}C on nutrient agar slants. Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) were used as culture media for bacterial and fungal strains respectively. Both culture media were prepared as per manufacturer's instructions. Nutrient agar was prepared by suspending 14.0 g of Nutrient in 500 mL distilled water while Sabouraud Dextrose Agar was prepared by suspending 13 g of Sabouraud Dextrose Agar in 200 mL of distilled water. The mixtures of the respective media were heated to boiling on a hot plate under constant stirring to dissolve the medium completely. This was followed by sterilization which was done by autoclaving at 15 lbs. pressure (121 ^{o} C) for 15 minutes and left to cool to about 45-50 ^{o} C before it was well mixed and exactly 20 mL of the agar was poured into sterile petri dishes. Each plate contained 20 ml and four plates per microorganism.

Antimicrobial assay:

Disk diffusion assay

The agar disk diffusion assay method was used to screen for antibacterial and antifungal activity of the water and organic extracts from *Sarcophyte piriei* [22]. Bacterial and fungal inoculum were prepared by direct colony suspension method. Briefly, 24-hours old morphologically similar bacteria and fungi colonies previously sub-cultured on nutrient agar and Sabouraud Dextrose Agar were fetched using a sterile wire loop and suspended in 10 ml of normal saline test tube then capped and vortexed. The turbidity of the inoculum suspension was adjusted to the 0.5 McFarland standard by either adding more colonies of the respective micro-organism or normal saline to attain inoculum of 1.5×10^8 Colony forming unit per millilitre (CFU/mL).

The sterilized and cooled nutrient agar (20 ml) was rapidly but carefully poured into Petri dishes to form uniform thickness of 3 mm. The agar was allowed cool and settle to form a solid gel that was suitable for plating out operation. Each 0.5 McFarland standardized bacteria inoculum was plated uniformly on the media plates using sterile cotton wool swabs. Sterile disks of diameter 6 mm were loaded with 10 µl of the *Sarcophyte piriei* extracts. Experiments for each sample were conducted in triplicates and respective to commercial antimicrobial were used as positive controls (Ciprofloxacin 0.5 mg/ml and Nystatin 100 µg/mL). The disk containing plates were put in the incubator that was set at a temperature of 37 ºC for 18 hours. The zones of inhibition were read using digital zone of inhibition measuring calliper.

Broth micro-dilution assay

Broth microdilution assay was used to establish the minimum inhibitory concentration (MIC) of the of the extract from the mixture of 50% dichloromethane and methanol extract of *Sarcophyte piriei*. A procedure described by Mogana et al. in 2020 [24] on the basis of Clinical and Laboratory Standards Institute (CLSI) guidelines using sterile 96 well microtiter plates with lids [25]. 100 µL of Mueller Hinton broth were placed in the wells of second to the eleventh column in row B to G of the microtiter plates. A multichannel pipette was used to introduce hundred microliters of *Sarcophyte piriei* tuber extracts (500 µg/mL) and 0.8 µg/mL of ciprofloxacin or nystatin into row B of column 2 to 9 in duplicates [26,27].

100 µL of the mixture in row B (Columns 2^{nd} – 9^{th}) were transferred to row C and were mixed gently, after which, 100 µL of the mixture were picked and transferred to row C. The procedure was repeated throughout up to row G and the resultant was a twofold serially diluted preparations in the wells from row B to G. Finally, 100 µL were removed from row G and discarded. 100 µL of 1 % DMSO in MH broth were added to the 10^{th} and 11^{th} columns. Two-fold serial dilutions were done following the procedure in the 2^{nd} to 9^{th} columns and was referred to as negative control. 5 µL of the bacteria inoculum (1×10^6 CFU/mL) was transferred into all wells excluding the wells of row G [26–28].

The set up was incubated at temperature of 37 °C for 18 hours after which, 60 µL of Alamar blue resazurin was added to all the wells and further incubated for 20 minutes. Thereafter, observation of change of colour from blue/purple to pink was indicative of the presence of microorganisms. Viable cells produce oxidoreductases which convert resazurin (blue) to resorufin (pink). The microbial growth was used to determine minimum inhibitory concentration [26–28].

Minimum bactericidal concentration test

Minimum bactericidal concentrations (MBC) was tested based on the procedure of Man et al. in 2019 with slight changes [28]. Mueller Hinton broth (3 µL) were taken from the last three wells of each row where there was no bacterial growth. The media that was collected were sub-cultured in 100 µL fresh Mueller Hinton agar (MHA) in petri dishes plates. The plates were then put in the incubator for 24 h at a temperature of 37 ºC. Minimum bactericidal concentration was assigned at the lowest concentration, where there was no visible microbial growth as observed under light microscope. This was indicative of 99.5% deaths of the original bacterial inoculum.

Cytotoxicity assay by Brine shrimp lethality test:

Preparation of brine solution and hatching the brine shrimps

Brine shrimp lethality assay was carried out by the method described by Meyer et al. was adopted with modifications [29]. Briefly, 19.25 g of brine salt were dissolved in 500 ml of distilled water and mixed in by a sonicator to make brine solution. Fifty milligrams of brine shrimp eggs were gently scattered in the dark side of hatching tray already half filled with filtered brine solution. The hatching tray was incubated for 24 hours under continuous normal bulb illumination at 25 – 29 °C temperature.

Brine shrimp lethality assay

Brine shrimp lethality assay was carried out to investigate cytotoxicity of the water and organic extracts of *Sarcophyte piriei*. After 24 hours the eggs had hatched into larvae (nauplii) which were observed in the open and light section of the hatching box. Ten larvae were picked from the open side using a Pasteur pipette with the aid of a magnifying glass and transferred into 14 mL falcon tubes that were filled with 5 mL of varying concentrations of *Sarcophyte piriei* extracts in brine salt. The concentrations of the extracts varied from 0 to 1000 µg/mL. Vincristine sulphate injection 100 µg/mL was used as positive control while 1% dimethyl sulfoxide solvent was used as a negative control and all experiments were set in triplicates. The nauplii were incubated for 24 hours and the number of survivors in each falcon tube were counted and documented. The lethality percentages were determined as a ratio of surviving nauplii in the test groups to those in the control (vehicle-treated) group.

Phytochemical screening:

The composition of phytochemical group of compounds (plant sterols, terpenoids (diterpenes), phenols, glycosides, fatty acids and tannins were evaluated using standard methods described by Harbone [30], Alfalluos et al. [31], Evans [32], and with slight modification done by Muthee et al. [33].

Test for phenols

Approximately 1 g of the four organic and one water extracts of *Sarcophyte piriei* were boiled in 10 ml of 70% ethanol in water bath using boiling tubes for 5 minutes. 2 ml of 5% ferric chloride was added to the mixture and the formation of bluish black colour indicated presence of phenols [28, 31].

Test for tannins

One gram of *Sarcophyte piriei* extracts were each measured into separate test tubes containing with 5 ml of water and boiled by heating in a water bath. The extracted mixtures were filtered using filter paper no. 1. To 2 mL portions, two drops of ferric chloride solution were added and the formation of brown green colour precipitates indicated the presence of tannins [29, 30].

Test for flavonoids

Approximately 1 g of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol sequential, methanol direct and water extracts) boiled with 10 ml of 70 % ethanol in a water bath using boiling tubes for 5 minutes. The extracts filtered while hot and cooled. Filter paper dipped in each of the alcoholic solutions of the extracts and then exposed (suspended) to ammonia

vapour in the chamber. Formation of a yellow spot on the filter paper indicated the presence of flavonoids [31].

Test for saponins (Frothing test)

Approximately 0.5 g of *Sarcophyte piriei* organic and water extracts were placed in separate test tubes containing 5 mL water. The mixtures were shaken and left to stand for 5 minutes. Persistent frothing for ten minutes was considered to indicate the presence of saponins [30, 31].

Test for alkaloids (Dragendorffs reagent)

About 1 g of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were warmed on a water bath with 10 ml of 10% Sulphuric acid for 5 minutes at 95 ºC. The solution was made alkaline with dilute ammonia solution with the aid of a red litmus paper turning blue. Extracted with 2 ml chloroform using a separating funnel. Formation of orange red colour precipitates indicated presence of alkaloids [32].

Test for alkaloids (Wegner's test

To 3 ml of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were dissolved in 5 ml dilute hydrochloric acid and filtered. Filtrates were treated with Wegner's reagent (Iodine in potassium iodide). Formation of brownish precipitates indicated the presence of alkaloids [32].

Test for coumarins

Approximately 1 g of *Sarcophyte piriei* extracts were boiled with 10 ml of 70% ethanol in a water bath using boiling tubes for 5 minutes. The extracts were filtered while hot and cooled. To 2 ml of each extract a few drops of alcoholic ferric chloride solution was added and a yellow colour indicated the presence of coumarins [34].

Test for diterpenes (Copper acetate test)

20 g of copper acetate monohydrate was dissolved in 200 ml of hot water to prepare copper acetate monohydrate solution. 1 g of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol (1:1), methanol and water extracts) were dissolved in 2 ml of water and treated with 3 drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpenes [35].

Test for anthocyanin

Two millilitres of aqueous *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were added to 2 ml of 2 N Hydrochloric acid and ammonia. The appearance of pink red colour turns to blue violet colour indicated the presence of anthocyanin [36].

Test for xanthoproteins

One millilitre of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were treated with 3 drops of concentrated nitric acid and ammonia solution. Formation of reddish-orange colour precipitates indicated the presence of xanthoproteins [37].

Test for terpenoids (Salkowski's)

About 0.5 g of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were added with 2 ml of chloroform followed by 2 ml concentrated Sulphuric acid to form a layer. Formation of reddish-brown colour at the interface indicated the presence of terpenoids [33].

Test for Carboxylic acid

Saturated solution of sodium bicarbonate was prepared by dissolving 8 g of sodium bicarbonate in 100 ml of water. The one ml of each of the *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were treated with a 2 ml saturated solution of sodium bicarbonate. The solution was shaken well. Evolution of brisk effervescence indicated the presence of carboxylic acid [32].

Test for amino acids (Ninhydrin test)

To 1 ml of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated the presence of amino acid [31].

Test for carbohydrates (Benedict's test)

To 5 ml of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts), 0.5 ml hydrochloric acid was added and heated for 30 minutes. Dissolved in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrate. The samples were treated with Benedict's reagent and heated gently. Formation of orange red precipitates indicated the presence of reducing sugars [32].

Test for oxalate

To 3 ml of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) 3 drops of glacial ethanoic acid (acetic acid) was added. Formation of a greenish colour indicated the presence of oxalates [32].

Test for cardiac glycosides/ 2-Deoxy sugar (Keller-Killian test)

About 200 µl of *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol sequential, methanol sequential and water extracts) were dissolved in 100 µl of glacial acetic acid. 3 drops of 5 % ferric chloride solution were added followed by 3 drops of concentrated sulfuric acid. Formation of a greenish blue colour confirmed the presence of glycoside (2 -deoxy sugar) [32].

Test for unsaturated lactone ring (Kedde test)

Evaporated *Sarcophyte piriei* extracts (Petroleum ether, dichloromethane, dichloromethane: methanol, methanol, and water extracts) were to dryness and added 1 drop of 90% alcohol and 2 drops of 2% 3, 5-dinitrobenzoic acid in 90% alcohol. The solution was made alkaline with 20% sodium hydroxide solution. A purple colour was produced. The colour reacted with 3,5-dinitrobenzoic acid and depended upon the presence of a beta-unsaturated – lactones ring in the aglycone [30, 36].

Data analysis:

The obtained data was transferred to excel sheet and analysed by Prism GraphPad software package 5.00 for Windows to calculate mean and standard error of the mean (SEM) values of the zones of inhibition, minimum inhibition concentrations and minimum bactericidal concentrations. Zones of inhibition of more than 13 mm was interpreted as high antimicrobial activity based on the criterion of De Almeida Alves et al. in 2000 [39]. Minimum inhibition zones (MIC) was also be interpreted according to Voukeng et al. (2016) [40], any plant extract with MIC values of less than 8 mg/ml was considered to be active. Extracts with MIC values less than 100 µg/ml will be considered as potentially active, 100 µg/ml to 625 µg/ml moderately active and more than 625 µg/ml lowly active. Cytotoxicity of the extracts against the brine shrimps will be presented by calculating the concentrations that will kill 50 % of the nauplii following a 24-hour exposure (LC_{50} values). The LC_{50} values will be presented as $\tilde{x} \pm$ SEM means with their standard errors and the phytochemical composition results will be tabulated. Data was analysed using one-way ANOVA followed by Tukey test using GraphPad Prism5 software. A significant difference will be considered at the level of $P < 0.05$.

Ethical consideration:

The approval to conduct this research was granted by the University of Nairobi Biosafety, Animal welfare and Ethics Committee with certificate number REF: FVM BAUEC/2022/347. Also, the research permit was obtained from National Commission for Science, Technology, and Innovation (NACOSTI) with permit number: NACOSTI/P/22/15856.

RESULTS

Antimicrobial activity of *Sarcophyte piriei* extracts

The *Sarcophyte piriei* tuber organic and aqueous extracts showed antimicrobial activities of varied ranges against the bacterial and fungal strains in the current study (Table 1). According to De Almeida Alves et al. [39], the dichloromethane: methanol (1:1) extract revealed high antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and very low activity against *Escherichia coli.* All the other extracts have activity shown by zone of inhibition of less than 13 mm and therefore interpreted as low. The study, pursued to find the minimum inhibitory concentration and minimum bactericidal concentration of the active dichloromethane: methanol (1:1) extract against *S. aureus, B. subtilis* and *P. aeruginosa*. There were no observed differences in the activity of the *Sarcophyte piriei* extracts at 100 mg/ml. The zones of inhibition of the water, petroleum ether and dichloromethane extract against S. aureus was 6 ± 0.0 and there was no significant difference between the activities between these extracts. However, there were significant differences in activities between the dichloromethane: methanol (1;1) extracts and all the other extracts against *S. aureus*. In *B. subtilis*, the highest zone of inhibition as recorded at 15.7± 0.3 mm. Figure 1 indicates the statistical differences of extract activities at of the $P < 0.05$. there were There was statistical difference in the zones of inhibition between the other extracts in this study, except between the extracts that were obtained by methanol sequential and methanol direct extraction methods. All extracts were found to have low activity against *E. coli* and there were no statistical differences between their activities.

Table 1: Mean Diameter zone of inhibition in millimeters of *Sarcophyte piriei* tuber crude extracts at 100 mg/ml.

Plant extracts	Mean diameter (mm) Zone of inhibition				
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***
Petroleum ether	6 ± 0.0	7.7± 0.3	6 ± 0.0	**10.0 ± 0.0**	6 ± 0.00
Dichloromethane	6 ± 0.0	6 ± 0.0	6 ± 0.0	8.0 ± 0.0	6 ± 0.00
DCM: MeOH (1:1)	18 ± 0.0	15.7± 0.3	6 ± 0.0	15.0 ± 0.0	6 ± 0.00
$MeOH^s$	9.7 ± 0.3	12.3± 0.3	6 ± 0.0	9.7 ±0.3	8 ± 0.00
$MeOH^d$	13.3 ± 0.3	12.7± 0.3	7± 0.0	11.7 ± 0.3	8 ± 0.00
Aqueous	6 ± 0.0	9.7 ± 0.3	6 ± 0.00	6 ± 0.00	10 ± 0.00
Ciprofloxacin	21.3 ± 0.3	21.7± 0.3	32 ± 0.6	27.7 ± 0.3	NT
Nystatin	NT	NT	NT	NT	17.7 ± 0.33

Key: DCM: MeOH; Mixture of dichloromethane and methanol (1:1), $MeOH^d$; methanol extract from direct extraction, $MeOH^s$; methanol extract from sequential extraction, Pet ether; extract obtained from petroleum ether solvent, NT; Not tested.

Minimum inhibitory concentration studies of the extracts that revealed higher zone of inhibition that is; dichloromethane/methanol (1:1) revealed MIC values of 18.75 $\pm$ 00, 14.06 + 4.7, and 28.13 $\pm$ 9.4 mg/ml against *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively (Table 2).

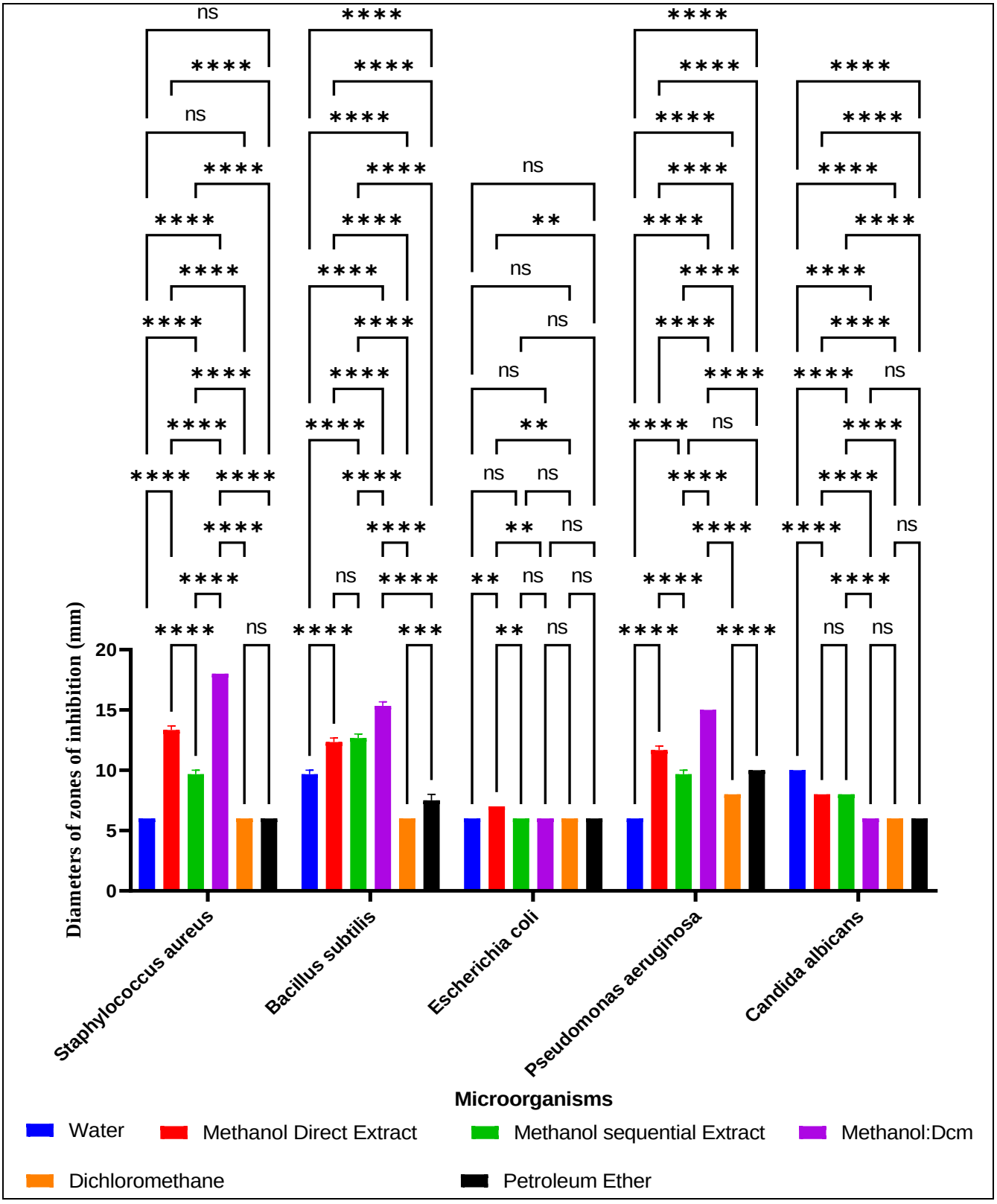

Figure 1: Graphical presentation of a comparison of antimicrobial activities of the different concentrations of extracts against different microorganisms

Table 2: MIC and MBC of Dichloromethane: Methanol (1:1)

Microorganisms	MIC mg/mL	MBC mg/mL	MBC/MIC Ratio
Bacillus subtilis	18.75 ± 00	62.5 ± 12.5	3.33
Pseudomonas aeruginosa	14.06 ± 4.7	20..31 ± 9.5	1.44
Staphylococcus aureus	28.13 ± 9.4	40.63 ± 19	1.44

Key: MIC; MBC; Minimum bactericidal concentration, Minimum inhibitory concentration,

Cytotoxicity of the *Sarcophyte piriei* extracts

The results from the present study indicate that over 83% of the tested extracts were toxic against brine shrimp nauplii with LC_{50} values of < 1000 µg/mL (Table 3). Only the aqueous extract had the LC_{50} of > 1000 µg/mL and therefore was regarded nontoxic [41]. The petroleum

ether and dichloromethane extract of *Sarcophyte piriei* found to have to have LC_{50} values of 55 µg/mL (Less than 100) therefore were highly toxic against brine shrimp nauplii. On the other hand, the extracts obtained from the mixture of 50% dichloromethane in methanol, sequential methanol and direct methanol extraction procedures revealed LC_{50} values of 338.33, 398.33 and 228.33 µg/mL that were within the range of 100 to 500 µg/mL and therefore were considered of medium toxicity against brine shrimp nauplii. The observed mortalities were directly proportional to the concentration of the extracts. There were no death of the brine shrimp nauplii in the control set ups and at the concentration of 1 µg/mL of all the extracts that were tested in this study. The increase in concentration of the extracts from 10 to 1000 µg/mL was accompanied with increase in the percentages of brine shrimp nauplii deaths. Hundred percent nauplii deaths were observed at 100 µg/mL, which was the positive control and recorded LC_{50} values of 25 µg/mL.

Table 3: % Mortality of shrimp nauplii after treating with aqueous and organic extracts of *Sarcophyte piriei* tuber

Plant extract	**Concentration µg/mL)**	**Number of deaths Nauplii (after 24 h)**					**Total Number of Nauplii death**	**Percentage Mortality**	**LC_{50} (µg/mL)**	**Inference**
		T1	T2	T3	T4	T5				
Control (Distilled water)	1	0	0	0	0	0	0	0%		
	100	0	0	0	0	0	0	0%		
Standard (Vincristine Sulfate USP)	1	0	0	0	0	0	0	0%	25.0	High toxicity
	10	4	5	6	3	3	21	42%		
	100	10	10	10	10	10	50	100%		
Petroleum ether extract	1	0	0	0	0	0	0	0%	55.0	High toxicity
	10	1	0	0	0	0	1	2%		
	100	10	10	9	9	8	45	90%		
	1000	10	10	8	9	10	47	98%		
Dichloromethane extract	1	0	0	0	0	0	0	0%	55.0	High toxicity
	10	2	0	0	0	0	2	4%		
	100	10	10	8	10	10	48	96%		
	1000	10	10	10	10	10	50	100%		
Dichloromethane: methanol (1:1) extract	1	0	0	0	0	0	0	0%	338.33	Medium toxicity
	10	0	2	0	0	0	2	4%		
	100	3	3	4	1	2	13	26%		
	1000	10	10	10	10	10	50	100%		
Methanol extract obtained through sequential method.	1	0	0	0	0	0	0	0%	398.33	Medium toxicity
	10	0	0	0	3	0	3	6%		
	100	5	3	3	2	3	16	32%		
	1000	10	10	10	10	10	50	100%		
Methanol obtained through direct extraction method	1	0	0	0	0	0	0	0%	228.33	Medium toxicity
	10	2	3	3	1	1	10	20%		
	100	2	4	5	2	3	16	32%		
	1000	10	10	10	10	10	50	100%		
Water	1	0	0	0	0	0	0	0%	>1000	Non-toxic
	10	0	0	0	0	0	0	0%		
	100	0	0	0	0	0	0	0%		
	1000	3	5	4	7	5	24	48%		

Fig. 2. indicates the statistical differences in mortality effects of different concentration of extracts of *Sarcophyte piriei* tuber. There was statistical difference of mortalities between concentrations 10 to 1000 µg/mL for methanol direct, methanol sequential and mixture of 50% dichloromethane in methanol extracts. The dichloromethane, petroleum ether extracts and the positive control (vincristine sulphate) had statistical differences in mortality effects between concentrations of 10 to 100 µg/mL. However, there were no statistical difference between the concentration of 100 to 1000 of the extracts.

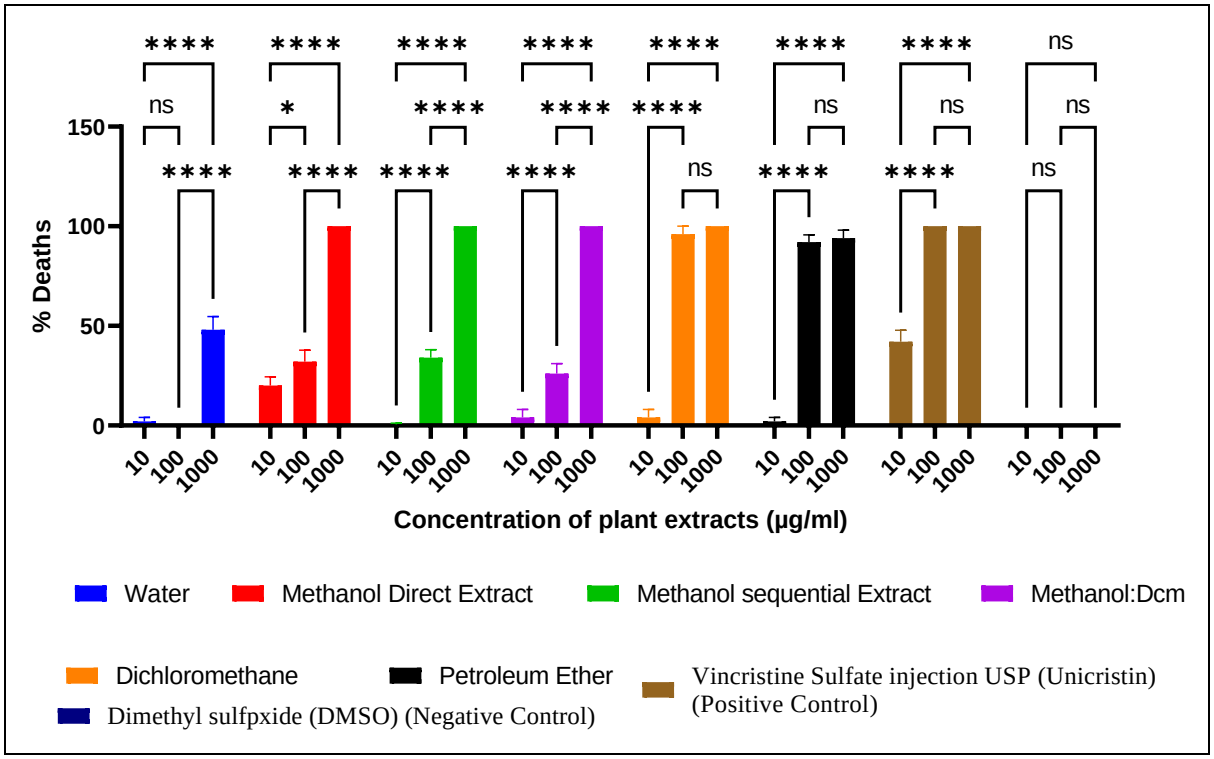

Figure 2: Graphical comparison representation of average % mortality against various extract concentrations

Phytochemical screening

The phytochemical screening *Sarcophyte piriei* tuber extracts revealed a wide range of secondary metabolites. All organic extracts and aqueous extracts tested positive for alkaloids, cardiac glycosides, saponins, phenolics, amino acids and terpenoids. Carboxylic acid was not detected in any of the extracts. Flavonoid, tannins, xanthoproteins, composition of phytochemical group of compounds: plants phenols, tannins, flavonoids, saponins, alkaloids, coumarins, diterpenes, anthocyanin, xanthoproteins, terpenoids, carboxylic acid, amino acids, carbohydrates, oxalate and cardiac glycosides were evaluated using standard methods as described by Evans [32]. The results were observed and tabulated as shown in Table 4.

The stem tuber showed the presence of several secondary metabolites. The phenols, saponins. Alkaloids, terpenoids, amino acids and cardiac glycosides were present in all the petroleum ether extracts, dichloromethane extracts, dichloromethane: methanol (1:1) extract, methanol extract and the water extracts. Diterpenes and carbohydrates were present in dichloromethane extracts, dichloromethane: methanol (1:1) extract, methanol extract and the water extracts. Tannins, flavonoids, coumarins, anthocyanins and xanthoproteins were shown to be present in dichloromethane: methanol (1:1) extract, methanol extract and water extracts. Oxalates were only present in the water extract. Carboxylic acid was absent in all the extracts of the petroleum ether extracts, dichloromethane extracts, dichloromethane: methanol (1:1) extract, methanol extract and the water extracts.

DISCUSSION

Secondary metabolites from plants that are historically used to treat infectious disease in human beings and animals are consistently validated by scientific evidence based on laboratory studies. In addition to previous research on African plants with antibacterial and antifungal activities in the African Herbal Pharmacopoeia [42,43]. To the best of our knowledge, the antimicrobial activities of the sequentially prepared extracts from the tuber of *Sarcophyte piriei* tuber are recorded for the first time against *Bacillus subtilis* in the current study. The results are consistent with the findings of Mbakazi et al. [44] and Mahammed et al. [45] where antibacterial activity of the methanol, dichloromethane and ethanol extracts have been recorded as active. Also, the toxicity studies against brine shrimp nauplii were reported in the current study for the first time and the extracts from the polar solvents, dichloromethane: methanol (1:1), methanol sequential and methanol direct were found to have IC_{50} values ranging from 100 to 500 µg/mL and therefore were concluded to have medium toxicity while for the water extract was more than 1000 µg/mL therefore non-toxic. However, the extracts from non-polar solvents, that is petroleum ether and dichloromethane had high toxicity against brine shrimp nauplii (IC_{50} = 55 µG/mL).

The varying antimicrobial activity and brine shrimp toxicity of different extracts of *Sarcophyte piriei* is indicative that the extracts contain different compounds. Water is a universal solvent and extracts high percentagee of inert chemicals including carbohydrates. Despite the use of water to prepare traditional medicines by herbalists. Usually, the water-soluble flavonoids have no antimicrobial significance. In the current study, water extract had appreciable anticandidal activity which can be attributed to the presence of saponins. The saponins form complexes with membrane sterols on the fungi causing loss of membrane integrity [46]. The antimicrobial activity of the methanol extracts is attributed to the presence of alkaloids and polyphenolic compounds. Terpenoids are selectively selected by dichloromethane. The mixture of methanol and dichloromethane (1:1) has ability of extracting the alkaloids, polyphenols and terpenoids which both have antimicrobial activities by different mode of actions. Terpenoids generally act by membrane disruption. In addition, polyphenols bind to adhesins. inhibit enzymes, substrate deprivation, complex with cell wall, metal ion complexation. The high antimicrobial activity of the dichloromethane: methanol (1:1) mixture in this study can be owed to the presence of the

polyphenols and terpenoids in the extract [47]. The petroleum and dichloromethane solvents extracted compounds which had poor antimicrobial activity.

The presence of alkaloids could be attributed to the high toxic effects against brine shrimp of the petroleum and dichloromethane extracts. Similar findings have been reported by Waghulde et al. [48]. However, compound that are highly toxic against brine shrimp are not limited to the petroleum and dichloromethane extracts but can also occur in water, methanol, ethanol, or other polar extracts. Also, in other plants the petroleum and dichloromethane or non-polar solvent extracts may lack toxic effects against brine shrimp nauplii [43, 47, 48].

Table 4: Phytochemistry of *Sarcophyte piriei* stem tuber crude extracts

Phytochemical	Test/reagent	Extracts					
		Pet ether	DCM	DCM: MeOH (1:1)	$MeOH^s$	$MeOH^d$	Water
Alkaloids	Wagner	+	+	+	+	+	+
	Dragendorff	+	+	+	+	+	+
Carboxylic acid	Sodium bicarbonate	-	-	-	-	-	-
Saponins	Foam Test	+	+	+	+	+	+
Phenols	Ferric Chloride	+	+	+	+	+	+
Tannins	Gelatin	-	-	+	+	+	+
Flavonoids	Ammonia	-	-	+	+	+	+
Carbohydrates	Benedict	-	+	+	+	+	+
Amino acids	Nihydrin	+	+	+	+	+	+
Xanthoproteins	Ammonia	-	-	+	+	+	+
Terpenoids	Salkowski	+	+	+	+	+	+
Diterpenes	Copper acetate	-	+	+	+	+	+
Oxalates	Glacial acetic acid	-	-	-	-	-	+
Glycosides	Kedde	+	+	+	+	+	+
	Keller killian	+	+	+	+	+	+
Anthocyanins	Ammonia	-	-	+	+	+	+
Coumarins	Nitric acid	-	-	+	+	+	+

KEY: +; presence, –; absence, DCM; extract obtained from dichloromethane, DCM:MeOH; extract obtained from the mixture of dichloromethane and methanol (1:1), $MeOH^d$; methanol extract from direct extraction, $MeOH^s$; methanol extract from sequential extraction, Pet ether; extract obtained from petroleum ether solvent.

CONCLUSION

Based on the results of this study, it was concluded that the extract of *Sarcophyte piriei* tuber from the dichloromethane: methanol (1:1) solvent mixture has compounds with bactericidal activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtills.* Also, there are components in the water extracts that have anticandidal activity and therefore these finding validates the claims of traditional medicine practitioners to treat infectious diseases that cause some skin, urinogenital and gastrointestinal disorders. It can be further presumed that the water extract is non-toxic, whereas all the organic solvent extracts in the current study contain toxic compounds.

Future perspectives

The future research is to be able to isolate and identify the compounds with antibacterial and antifungal activities and establish their mode of action. Since literature greatly indicate the use *of Sarcophyte piriei* for treating viral conditions, it will be important to research on the antiviral activities too. Furthermore, it shall be necessary to explore the activity of the extracts against resistant microbial strains in the effort towards discovery of alternative forms of drugs that bring out solutions to the current problem of antimicrobial drug resistance. Future researchers are obliged to establish the toxic effects of the active extracts on suitable animal model and answer the question about the safety profile of the extracts from *Sarcophyte piriei.*

Data availability

The data that has been used to support the findings of this study have been incorporated in this article.

Conflict of Interest

The author declares that there are no conflicts of interest.

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