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Bioactivity of acetone extract of *Sensevieria aethiopica* (Thunb) leaf

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ABSTRACT

Otitis is highly prevalent infection caused mainly by bacteria and frequently associated with secondary infections. It may lead to other permanent dysfunction in children, and multidrug-resistance has been reported in the implicated bacterial pathogens. The aim of this study is to evaluate the phytochemicals, toxicity and antibacterial potential of acetone leaf extract of *Sansevieria aethiopica* (Thunb.) against bacterial pathogens responsible for otitis. The phytochemical analyses of the acetone extract were determined using standard assay methods and the antioxidant activity was assessed using 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic-acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) and ferric reducing power. Brine shrimp lethality test was used to determine the cytotoxicity effect of the extract. Macrobroth dilution method was used for the determination of minimal inhibitory concentration (MIC). The amount of both phenolics and flavonoid in the extract were 19.69 mg tannic acid equivalent/gram and 7.198 mg quercetin equivalent/gram respectively. Flavonols and proathocyanidins concentrations in the extract were 4.351 mg quercetin equivalent/gram and 1.405 mg catechin equivalent/gram respectively. The extract expressed antibacterial effects on both Gram negative and Gram positive bacteria however the activity was more pronounced on Gram negative organisms. The extract has good antioxidant properties. Although, slightly toxic it has both bacteriostatic and bactericidal effects on the selected bacteria associated with otitis especially Gram negative.

Key words: Otitis, *Sensevieria aethiopica*, pathogens, antioxidant, phytochemicals, ear infections, antibacterial.

INTRODUCTION

Otitis (ear infection) is a spectrum of infections of the ear usually associated with microbial infections of macerated skin and subcutaneous cellular tissue [1, 2]. It is more common in children than adults, approximately 75% of children experience at least three or more ear infections during the first three years of life [3]. Acute otitis is one of the most common childhood infections and is the leading indication for prescription of antimicrobials in children [4].

Otitis is highly prevalent worldwide [5]. Over 50 % of the cases of otitis are caused by bacteria [6]. It has been reported to be the most common infection in young children [7], with the average toddlers having two to three episodes a year [8] and with mortality rate of more than 50,000 children (under 5 years) per year [9]. Children below the age of seven years are much more susceptible to otitis media [10].

Ear infection is mainly caused by mainly by bacteria followed by fungal pathogens [1,11]. Antimicrobial resistance in these pathogens has become widespread and highly prevalent globally [12]. This has led to treatment failures which have become more frequent [13]. Otitis is frequently associated with secondary bacterial and/or fungal infections [14,15].

The clinical manifestations of otitis include pain, pruritus and erythema also as the disease progresses, edema, otorrhea and conductive hearing loss may also develop [16,17]. In childhood it leads to significant hearing loss in pediatric patients, resulting in developmental problems in speech, language, and the acquisition of social skills [16, 18]. Acute cases of bacterial otitis does not only come with earache but also severe pain, fever (39°C or more) and febrile seizures [17,19,20,21].

Indiscriminate use of commercial antimicrobial drugs has lead to the development of multiple drug resistant strains of bacteria and fungi. This on the other hand informs the search for new antimicrobials from natural sources [22]. The use of medicinal plants, especially in public health care programs in developing countries is gaining recognized globally [23].

Sansevieria is a genus of xerophytic perennial herbs that occur mostly in dry tropical and subtropical habitats. About 70 species are known with a distribution range from Africa, Asia to Burma and the islands of the Indian Ocean [24,25]. *Sansevieria aethiopica* (Thunb.) is a species of the genus *Sansevieria* in the family *Asparagaceae*. It grows as a perennial, stemless, succulent plant with about 1 centimeter thick rhizomes . The 13-30 and succulent rosettes arranged closely together standing leaves are spread out in ascending order. The concave upper surface and the base sometimes narrowed easily [26]. *S. aethiopica* is commonly found in Kenya and southern Africa. In South Africa it is found in the

provinces of Northern Cape, North West , Eastern Cape and Gauteng spread to dry, open places or in the bush on permeable soils [26,27] where it is used for the treatment of ear infections, dental caries and ulcers [28].
The aim of this study is to evaluate the antioxidant activity, toxicity and antibacterial potential of acetone leaf extract of *S. aethiopica* against bacterial pathogens responsible for otitis.

2.0 RESULTS AND DISCUSSION

2.1 Phytochemical constituents of the extract

Plants play a major roles development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. As shown in Fig 1, the amount of both phenolics and flavonoid concentrations in the extract were 19.69 mg tannic acid equivalent/g and 7.198 mg quercetin equivalent/g respectively. Polyphenols have been reported to be the major plant compounds with antioxidant activity due to their redox properties [29]. They adsorbed, neutralize free radicals and quenching singlet and triplet oxygen [30]. Flavonoids have been reported to possess strong antioxidant properties [31,32]. For flavonols and proathocyanidins the amounts present in the extract were 4.351 mg quercetin equivalent/g and 1.405 mg catechin equivalent/g.

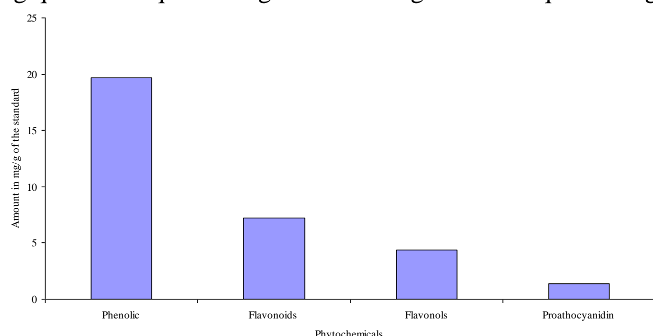

Fig 1. Phytochemical analysis of acetone leaf extracts of *S. aethiopica*

2.2 ABTS radical scavenging activity

In the recent years, attempts have been made to combat the detrimental effects of oxidative damages using biological antioxidants nutrients [33]. Enthnobotanical studies have confirmed that some medicinal plants are very rich in antioxidant in addition to their antimicrobial activities. In this study antioxidant activity of the acetone extract of the *S. aethiopica* was determined using different assay methods. ABTS is one of the free radicals that have been used for assessing antioxidant activity [34]. It is frequently used to screen the medicinal plant and food materials for their antioxidant properties. It generates free radicals by loss of electrons and produced a colored nitrogen centered cation by reacting with potassium persulfate for 12–14 h Prior et al. [35]. The acetone extract of *S. aethiopica* was able to scavenge generated by ABTS and the activity was comparable with the with the phenolic-derivative standards: BHT and rutin. The ABTS radical scavenging activity of the extract at 0.4 mg/mL (the highest concentration of the extract tested) was 82.02 %. This value was very close to those of the standard drugs: BTH and rutin which were 90.1 and 83.73% respectively as shown in Fig 2.

2.3 Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant effect of the acetone extracts of *S. aethiopica* was analyzed for the scavenging activity on free radical DPPH in a concentration-dependent manner. The maximum scavenging effect exerted by the extract to that exerted by the standards (BTH and Rutin) controls. The activity of the extract is dose-dependent. The extract of *S. aethiopica* had a good DPPH scavenging property (Fig 3). Therefore, the data obtained from this study justified the ethnomedicinal use of this plant in the treatment of ear infections initiated by oxidative stress [36]. The antioxidant ability of this extract to donate hydrogen to DPPH radical, thus converting it into stable molecules may be due to the presence of the Phytochemicals like phenolic compounds, such as flavonoid, polyphenol, tannins and terpenes [37,38]. The redox properties by the Phytochemicals play an important role in adsorbing and neutralizing free radicals [39]. Antioxidant potentials of the extract determined based on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers [40].

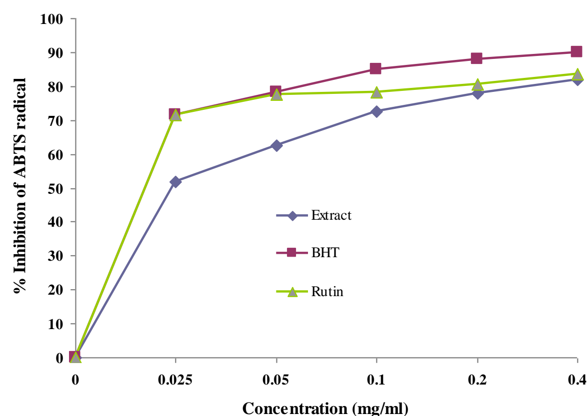

Fig. 2: ABTS radical scavenging activity of acetone extract of *S. aethiopica* leaf

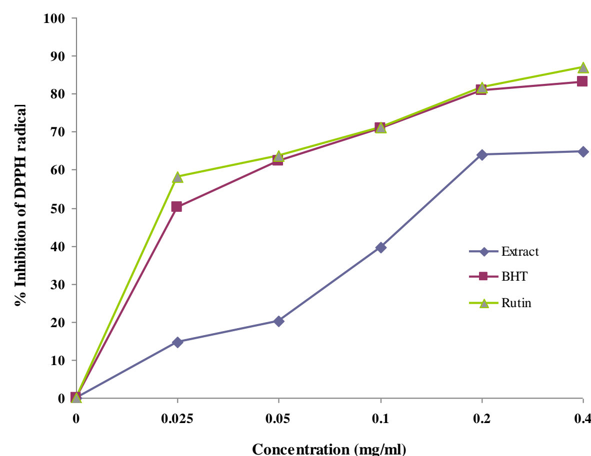

Fig. 3: DPPH radical scavenging activity of acetone extract of *S. aethiopica* leaf

2.4 Hydrogen peroxide inhibition activity

Partial oxygen metabolism generates hydrogen peroxide (H_20_2) this reactive oxygen species is versatile and diffusible. Hydrogen peroxides generally lead to the damage of cells [42]. However, in the presence of reduced transition metals, such iron, H_2O_2 can produce the highly reactive OH•, which can cause extensive damage to DNA, proteins and lipids [43]. The acetone extract of S. aethiopica showed high level of scavenging ability against hydrogen peroxide at 0.4 mg/ml were 66.78, 62.81 and 82.05% for acetone extract, BTH and rutin respectively (Fig 4). At all the concentrations tested the hydrogen peroxide scavenging activity of the extract was higher than BTH but lower than rutin. Hydrogen peroxide is a highly important reactive oxygen species it has ability to penetrate biological membranes. It may also have a detrimental effect on the cell if it is converted to hydroxyl radical which is toxic to the cell [44]. The acetone extract of *S. aethiopica* was capable of scavenging hydrogen peroxide in a concentration dependent manner.

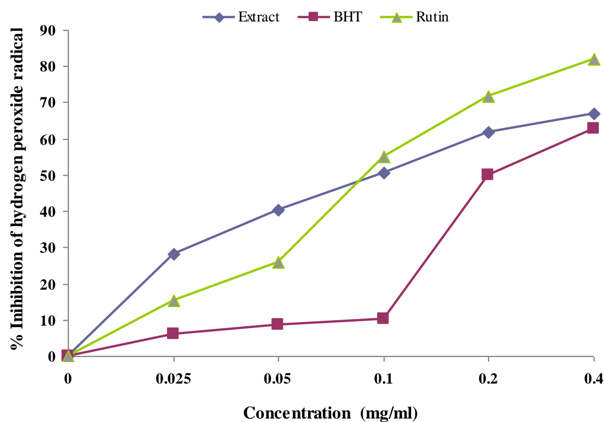

Fig. 4: Hydrogen peroxide radical scavenging activity of acetone extract of *S. aethiopica* leaf

2.5 Ferrous reducing antioxidant power (FRAP) assay

This is non-inhibition and direct test of total antioxidant power method of determining antioxidant potency of biological substances [45]. The ferric reducing/antioxidant power (FRAP) assay is simple, speedy and robust assay [35]. It is regarded as a direct test of total antioxidant power. It treats both chemically defined reductant and biologically defined antioxidants as basically equal. The FRAP assay involves neither a pro-oxidant nor an oxidizable substrate. It depends upon the reduction of a ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH [45,46]. In the present study, the highest antioxidant potential was observed in Vitamin C followed by the two the other standard chemicals: Vitamin E and BHT (in decreasing order) (Fig. 5). The reducing power of the extract showed to be concentration dependent. Though lower than the than the standard drugs the extract reducing potential can be compared favorably with them. The activity of the extract could be linked with the presence of the Phytochemicals present in it.

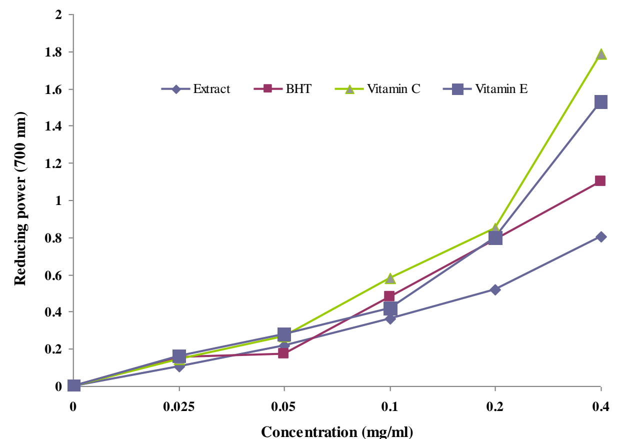

Fig. 5: Reducing power activities of acetone extract of *S. aethiopica* leaf

2.6 Brine shrimp lethality test

Brine shrimp is a low cost bench bioassay indicative of toxicity to different biological and chemical agents.The plant extract had lethal effects on the brine shrimp nauplii with LD_{50} of 3.17 ppm. The extract was classified as toxic to *Artemia salina* as the observed LC_{50} was higher than standard limit (1000 μg/mL) [47]. The extract could be considered toxic (Fig 6).

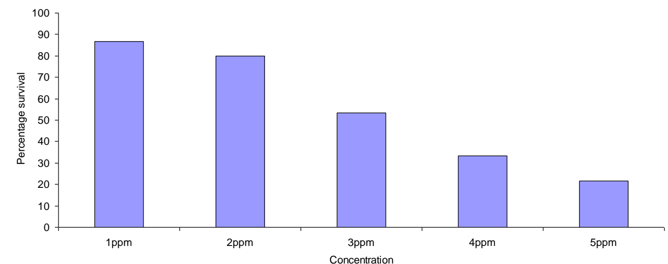

Fig. 6: Brine shrimp cytotoxicity of acetone extract of *S. aethiopica* leaf

2.7 Antibacterial properties of the extract

The antibacterial activity of acetone extract of *S. aethiopica on the test organisms* varied among the tested bacteria. The extract was more effective against gram negative organisms than Gram positive organisms. This report contradicts the report of Sharma and colleagues [48] that reported Gram negative bacteria pathogens associated with ear infections to be more resistant to plant extracts than Gram positives. Phytochemicals have different mechanisms of actions against pathogens [49]. Antimicrobial action of the extract may not be targeted against the cell wall that makes a gram negative pathogen to be more resistant to most antimicrobials. Medicinal plants have been reported to be DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition [50,51]. Among the Gram negative bacterial screened *P. aeruginosa* ATCC 19582 had the highest MIC of 3.125 mg/ml. The MIC of the pathogens ranges between 0.3906 and 3.125 mg/ml for the Gram negative while for Gram positive the value ranges between 1.5625 and 3.125 mg/ml respectively. As shown in Table 1, the MBC values ranged between 0.7812 mg/mL and 6.25 mg/mL for the different bacterial ear pathogens. Except for *Ent. cloaca* ATCC 13047 and *S. aureus* ATCC 6538 the MBC values were higher than the MIC. The MIC index of was highest for *K.*

pneumoniae ATCC 10031 (8.0) followed *E. coli* ATCC 25922 (4.0) which indicate microbiostatic effect on the pathogens.

3.0 Experimentals

3.1 Plant material

Fresh leaves of *S. aethiopica* were collected in February, 2012, at the Alice Township in Nkokobe Municipality, Eastern Cape Province (32°78'98"S, 26°84'28"E and Altitude 589 m). The plant was identified in the Department of Botany, University of Fort Hare, Alice, Eastern Cape, South Africa. A voucher specimen (DavMed, 2012/2) was prepared and deposited in the Giffen Herbarium of the University. Plant sample was dried in the oven at the temperature (40°C).

The dried plant sample was pulverized and 40 g was separately extracted in acetone for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, Greater Manchester UK). The extracts were filtered through Whatman No. 1 filter paper. The extracts were evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). The extract of the plant was kept in the refrigerator until used.

Table 1: Antibacterial activity of acetone extract of *S. aethiopica* leaf

Pathogens	**Gram Reaction**	**MIC**	**MBC**	**MBC/MIC**
P. aeruginosa ATCC 19582	-ve	3.125	6.25	2.0
K. pneumoniae ATCC 10031	-ve	0.3906	3.125	8.0
Ent. cloaca ATCC 13047	-ve	0.7812	0.7812	1.0
E. coli ATCC 25922	-ve	0.7812	3.125	4.0
S. sonnei ATCC 29930	-ve	0.7812	3.125	4.0
S. Typhi ATCC 1331	-ve	0.7812	1.5625	2.0
B. cereus ATCC 10702	+ve	1.5625	3.125	2.0
S. aureus ATCC 6538	+ve	3.125	3.125	10
S. aureus OK1	+ve	1.5625	3.125	2.0
B. pumilus ATCC 14884	+ve	3.125	6.25	2

3.2 Phytochemical screening of the plant

3.2.1. Determination of total phenolics content

The total phenolics content of the extract were determined by Folin-Ciocalteu method described by Wolfe *et al*. [52] with little modification. To 5.0 ml of plant extract with concentration of 0.1 mg/mL, 5.0 mL of 10% Folin-Ciocalteu reagent and 4.0 mL of sodium carbonate (75% w/v) was added. The mixture was vortexed for 15 s and incubated at 40 ^{o}C for 30 min for colour appearance. The absorbance was measured at 765 nm using spectrophotometer. Samples of the extract were evaluated at the final concentration of 0.1 mg/mL. The amount of total phenolic content was expressed as mg/g tannic acid equivalent using the expression obtained from the calibration curve: $Y = 0.1231x$, $R^2 = 0.9742$, where x is the absorbance and Y is the tannic acid equivalent in mg/g.

3.2.2 Determination of total flavonoids content

The total flavonoids were determined using the method of Ordonez *et al*. [53]. A volume of 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of extract solution. The mixture was incubated for 1 h at room temperature for yellow color appearance; the absorbance was measured at 420 nm. Plant extracts were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation obtained from the curve: $Y = 0.0263x$, $R^2 = 0.9701$, where x is the absorbance and Y is the quercetin equivalent.

3.2.3 Determination of total flavonols content

The total flavonols content were determined using the method of Kumaran and Karunakaran [54]. Two milliliter (2.0 mL) of the sample was mixed with 2.0 mL of $AlCl_3$ prepared in ethanol and 3.0 mL of 50 g/L sodium acetate solution were added. The mixture was incubated at 20 °C for 2.5 h after which the absorption was read at 440 nm using spectrophotometer. Plant extracts were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve $Y = 0.0263x$, $R^2 = 0.9824$, where x is the absorbance and Y is the quercetin equivalent.

3.2.4 Determination of proanthocyanidins content

The total proanthocyanidin were determined using the procedure reported by Sun *et al*. [55]. A volume of 0.5 mL of 0.1 mg/mL of extract solution was mixed with 3.0 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid, the mixture was allowed to stand for 15 min at room temperature, the absorbance was measured at 500 nm. Total proanthocyanidin contents were expressed as catechin (mg/g) using the following equation of the curve: $Y= 0.5902x$, $R^2 = 0.9714$, where x is the absorbance and Y is the catechin equivalent.

3.3 Antioxidant screening of the extract

3.3.1 ABTS radical scavenging activity

The method of Re et al. [56] was adopted for the determination of ABTS activity of the plant extract. The working solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowed to react for 12 h at room temperature in the dark. The resulting solution was further diluted by mixing 1 ml of freshly prepared ABTS solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm after 7 min using spectrophotometer. The percentage inhibition of $ABTS^{\cdot+}$ by the extract was calculated and compared with that of BHT and rutin using the following equation:

$ABTS^{\cdot+}$ scavenging activity $= [(A_0 – A_1)/ (A_0)] \times 100$

Where; A_0 is the absorbance of $ABTS^{\cdot+}$ + methanol; A_1 is the absorbance of $ABTS^{\cdot+}$ + sample extract or standard.

3.3.2 Determination of diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method of Liyana-Pathiranan and Shahidi [57] was adopted for the determination of scavenging activity of DPPH free radical in the solution of the extract. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was added into 1.0 ml of the extract prepared in methanol with concentrations ranging between 0.025 and 0.400 mg/ml and standard drugs BHT and Rutin. The reaction mixture was vortexed thoroughly and was left in the dark at room temperature for 30 min. The absorbance of the mixture was measured in the spectrophotometer at 517 nm. The ability of the plant extract to scavenge DPPH radical was calculated by the equation: DPPH radical scavenging activity = $[(A_0 - A_1)/(A_0)] \times 100$; where A_0 is the control and A_1 is tge absorbance of the extract.

3.3.3 Determination of hydrogen peroxide inhibition activity

The H_2O_2 inhibition effect of the extract was determined by the method of Gulcin [58]. A 1.0 ml of the sample was added to a 0.6 ml of 40 mM hydrogen peroxide solution prepared in phosphate buffer (pH 7.4). The absorbance of the hydrogen peroxide at 230 nm was determined after 10 min at room temperature against a blank solution containing phosphate buffer solution alone. BHT and ascorbic acid were used as positive controls. The percentage scavenging of hydrogen peroxide of the samples was calculated as follow

H_2O_2 inhibition capacity (%) = [1 - (H_2O_2 concentration of sample /H_2O_2 concentration of control)] × 100.

3.3.4 The ferrous reducing antioxidant power (FRAP) Assay

Total Antioxidant Activity (FRAP Assay)

The FRAP assay was carried out according to Benzie and Strain [46] with a slight modification. FRAP reagent solution consisted of 300 mmol/l acetate buffer (pH 3.6), 10 mmol/l of 2,4,4-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mmol/l hydrochloric acid, and 20 mmol/l iron (III)-chloride hexahydrate. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37 °C before using. Plant extracts (150 µL) were allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO4. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid and catechin.

3.4 Brine shrimp lethality test

Toxicity was studied using the larvae of brine shrimp nauplii, *Artemia salina* L [56]. Shrimp eggs were allowed to hatch and mature as nauplii in two days in a hatching tank filled with seawater. The free-swimming nauplii were attracted by a light to a compartment from which they could be collected for the assay proper. Vials containing 4 to 20 µg ml-1 samples were prepared by dissolving the extracts in distilled water and transferring the solution to each vial. The crude extract was initially dissolved in dimethyl sulfoxide (DMSO) to make the extracts hydrophilic. Then distilled water was added to get final concentrations. In the control tube the same volume of DMSO and sea water were taken. After 24 h of exposure the mortality was determined and corrected according to Abbott [60].

$C_m = [(O_m-C_t)/(100-C_t)] \times 100$

Where C_m = Corrected mortality, O_m = Observed mortality, C_t = Control mortality.

The observed data was the subject to Probit analysis was calculated according to Finney [61] to get the dose that will kill 50% of the brine shrimps (LD_{50}).

3.5 Antimicrobial properties of the extract

3.5.1 Source and standardization of test bacteria

Eight bacterial isolates were used in this study are *Pseudomonas aeruginosa* ATCC 19582, *Klebsiella pneumoniae* ATCC 10031, *Enterobacter cloaca* ATCC 13047, *Esherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10702, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* OK1 and *B. pumilus* ATCC 14884. The isolates were collected from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice. Each of the bacteria was maintained on nutrient agar (Biolab No. 2, Wadeville, Gauteng, South Africa) plates. The grown cultures were used for preparation of bacterial suspensions in sterile distilled water with densities adjusted to 0.5 McFarland standard. A 0.25ml of the standardized suspension was added to the 25ml Mueller Hinton Broth and used for the inoculation of the tubes.

3.5.2 Determination of minimum inhibitory concentration (MIC)

Macrobroth dilution method was used for the determination of minimal inhibitory concentration (MIC) of the extract as described by CLSI [62]. Nutrient broth medium was used to prepare different concentrations ranging from 0.0977 to 25 mg/ml by serial dilutions. Each prepared concentration in tubes was inoculated with 100 µl of each of the standardized culture of the test bacteria. Tube containing nutrient agar without extract was used as negative control. The tubes were incubated aerobically at 37°C for 18 h. The first tube in the series with no sign of visible growth was taken as the MIC.

3.5.3 Determination of minimum bactericidal concentration (MBC)

MBC was determined by taken one standard loopful of culture from each of the first three broth tubes that showed no growth in the MIC tubes and inoculated on fresh nutrient agar plates. After incubation for 24 h, the least concentration of the extracts that showed no colony formation on the agar was taken as the MBC. The ratio of MBC to MIC was determined to predict effect of the extract on the test bacteria. MBC/MIC ratios greater than 1 was considered bacteriostatic while other MBC/MIC ratios were considered bactericidal [63].

4.0 CONCLUSION

Acetone leaf extract of *S. aethiopica* (Thunb.) compare with the standard chemicals is very rich in antioxidant and phytochemicals. It also showed good antibacterial activity on both Gram negative and Gram positive bacteria although, slightly toxic. This work serves as basic scientific validation of the folkloric uses of the plant for the treatment of ear infection in South Africa. The extract will certainly contain bioactive compounds that could be good candidates for the treatment of drug-resistant ear bacterial pathogens.

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