Full Length Research Paper

Antipseudomonal potential of *Colophospermum mopane* and *Acrotome inflata*, medicinal plants indigenous to Namibia

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Plants with diverse therapeutic properties are indigenous to Namibia. Concoctions of *Colophospermum mopane* and *Acrotome inflata* are widely used traditionally in the management of respiratory, gastrointestinal and wound infections. Limited studies have validated these traditional uses particularly against resistant bacteria strains such as *Pseudomonas* spp. This study aimed to determine the antimicrobial activity and phytochemistry of extracts of *C. mopane* and *A. inflata*, medicinal plants indigenous to Namibia. Phytochemical analysis and antimicrobial testing were done on leaves and barks of *C. mopane* and *A. inflata* whole plant. Voucher specimens were collected from Omugulugoonime village, Oshikoto region and validated at the National Botanical Research institute, Windhoek. Crude extraction of dried plants was done by maceration with ethanolic and aqueous solvents. Phytochemical screening was done using methods described by Harborne (1998) and/or Tiwari et al (2011). The antimicrobial activity against wild types of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtillis* and *Candida albicans* was done using the disc-agar-diffusion method. The mean diameters of the zones of inhibition (mm) for each extract were determined against each test organism. The antimicrobial activity (zones of inhibition) of ethanolic extracts (5 mg/ml) of *C. mopane* bark (10±0.6 mm) and leaves (10±1.2 mm) and *A. inflata* (8.7±0.6 mm) against *P. aeruginosa* is comparable to that of penicillin G (14±1.4 mm). Aqueous extracts leaves and bark of *C. mopane* showed activity against *P. aeruginosa* and *S. aureus*. The activity of the ethanolic extracts against *B. subtillis* was: *C. mopane* leaves (7.3±0.6 mm), *C. mopane* bark (8.7±0.6 mm) and *A. inflata* (11.3±3.21 mm) and Penicillin G (26±1.4 mm). Both ethanolic and aqueous extracts did not have activity on *C. albicans*. Aqueous extracts of *A. inflata* had no activity on *Pseudomonas* and *B. subtillis*. Organic extracts of *Colophospermum mopane* and *A. inflata* exhibit antimicrobial potential against *Pseudomonas* and *Bacillus* species. The alkaloids, flavonoids and tannins should be further purified and characterized for antipseudomonal activity.

**Key words:** Antipseudomonal, Namibia, *Colophospermum mopane*, *Acrotome inflata*.

INTRODUCTION

The sub-Saharan Africa is indigenous to over 50,000 species flora and ethno-medicines (Clarkson et al., 2004; Cowan, 1999; Hosttetmann et al., 1996; Iwu, 2014). Namibia is home to about 8159 plant species with
therapeutic potential (Hedimbi and Chinsembu, 2012; Ilonga, 2012; Cunningham, 1993; Grote, 2003; Cheikhhyoussef et al., 2011; Thomas, 2015). Over 80% of the world’s population, mostly in low and middle income countries (LMIC) rely on traditional medicine for primary health care of common ailments (Fabricant and Farnsworth, 2001; Ganatra et al., 2012; Thirumurugan, 2010; WHO, Traditional Medicines, 2012). Despite the wide practice of ethno-medicine the sub-Saharan Africa, the region bares highest global burden of infectious diseases such as HIV/AIDS, tuberculosis and malaria. Out of the 500 000 estimated plant species found in nature, a limited proportion undergone phytochemicals, biological and pharmacological characterization (Mahesh and Satish, 2008; Dushimemaria, 2014).

The antimicrobial potential of higher medicinal plants as sources for new drugs in Namibia is still largely unexplored. Recently, there is a rise in patients living with non-communicable diseases and the WHO has estimated that 3 out of 4 patients with hypertension reside in sub-Saharan region (WHO, 2016). This calls for action for further research for efficacious and cost-effective medicines from the indigenous plants and/or ethno-medicines. In addition, the global surge in resistance against essential antimicrobials has had its greatest impact on health care in the sub-Saharan region and is a public health concern (Wagate et al., 2009; Parekh and Chanda, 2007). As a result, antimicrobial resistance has led to use of more expensive medicines and or treatments, further constraining health care systems (Kamaraj et al., 2012).

Despite the global efforts to increase access to essential medicines, access to essential and alternative antimicrobial remains a challenge, particularly in remote settings where indigenous systems are commonly practiced as part of the primary health care (WHO, Traditional Medicines, 2012; Carlet et al., 2012). In recent years, the clinical efficacy of essential antibiotics such as cotrimoxazole and penicillin used in the sub-Saharan region against common pathogens has reduced tremendously (Mutembei et al., 2011; Monroe and Polk, 2000). Namibia has one of the highest case notification rates for multi-drug resistant tuberculosis (MDR-TB) in the world (World Health Organization, 2013). There is a growing burden of multi-drug resistance to bacterial pathogens in the public health care in Namibia including strains of Escherichia coli, Enterococcal pneumoniae, Proteus mirabilis, Klebsiella (Khan and Musharraff, 2004). The emergence of multi-drug resistant pathogens threatens the clinical efficacy of many existing antibiotics (Mutembei et al., 2011). In particular, there are high rates of resistance to amoxicillin, cotrimoxazole and nitrofurantoin. The resistance against amoxicillin is rising among strains of E. coli (79.6%) Klebsiella (96.72) and Proteus (55.91%). Similar patterns of resistance against cotrimoxazole have been documented on isolates of E. coli (78.6%), Proteus (57.85%) and Klebsiella (56.52%). The resistance against nitrofurantoin on Proteus mirabilis isolates is reported at 77.37% (Mengistu et al., 2014). The rising burden of antimicrobial resistance is a wake up call for further research and development of cost-effective medicines, particularly from the indigenous ethno-medicines of Namibia.

In Namibia, concoctions of Colophospermum mopane and Acrotome inflata are widely used traditionally in the management of infectious diseases (Musvoto et al., 2007; Cheikhhyoussef et al., 2011; Bainbridge H, 2012). The traditional uses include cure and/or prevention of wound infections and treatment of syphilis, diarrhea, coughs, inflammatory diseases and fevers (Cheikhhyoussef et al., 2011). However, despite the wide use of C. mopane and A. inflata in Namibia, the safety and efficacy profiles in the treatment of diseases have not been validated. C. mopane and A. inflata belong to the Fabaceae and Lamiaceae families, respectively. Secondary metabolites of these families including tannins, alkaloids, and flavonoids have exhibit antimicrobial activity (Cheikhhyoussef et al., 2011; Mahesh and Satish, 2008; Lewis and Ausubel, 2006). Thus, extracts of these ethno-medicines from C. mopane and A. inflata may be a potential source of phytochemicals with antimicrobial activity conceivably with better safety profile and/or novel mechanisms against resistant bacteria (Thirumurugan, 2010; Parekh and Chanda, 2007; Tiwari et al., 2011; Cowan, 1999) Phytochemicals such as alkaloids, tannins, saponins and flavonoids have been reported to have antimicrobial activity (Heneman and Zidenberg-Cherr, 2008; Suliman, 2010). C. mopane (Figure 1) is a shrub or a small tree of the family Fabaceae (Leguminosae) and in Namibia, it is popularly known as Mopane or Omusati. C. mopane covers about 9% of Namibia’s surface area and is more common in the Northern parts of Namibia (Dushimemaria, 2014). Decoctions of Mopane leaves, bark and gums are used by the Heikum Bushmen, Wambo, Damara and Himba communities for, management of gastrointestinal complaints, diarrhoea, coughs, wounds symptoms of inflammation, oral hygiene and diarrhoea (Von-Koenen, 2009; Van den Eynden et al., 1992). The roots of C. mopane are used to treat wounds; stomach problems, inflamed eye, and syphilis, and have been reported to contain tannins and resin (Von-Koenen E, 2009, 2001). A. inflata (Figure 2) belongs to Lamiaceae (Labiatae) family, it is a herb called tumbleweed and locally known

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A. inflata is mostly distributed in northern part of Namibia including in Oshikoto, Oshana and Omusati regions (Van Rooyen, 1988; Walt and Riche, 1999; Nordenstam, 1970). The leaves of *A. inflata* inflate are used by the Damara communities as a tea to treat coughs and stomach upsets (Mukanganyama et al., 2011). The Wambo communities use the entire plant for treatment of malaria and as an indoor insecticide. In Kavango region, ashes of the dried and burnt tumbleweed flower are used for management of pain and scratches over the temples (Von-Koenen, 2009; Nafuka, 2014).

Limited studies provide scientific evidence of the antibacterial potential of these plants against commonly resistant bacteria such as *Pseudomonas*. Consequently, the aim of this study was to screen for phytochemicals and validate the use of *C. mopane* and *A. inflata*, in folk medicine in Namibia.

**METHODOLOGY**

Laboratory analysis of the phytochemistry and antimicrobial activity of crude extracts of *C. mopane* and *A. inflata* was done. The experiments were conducted at the laboratory of School of Pharmacy, University of Namibia.

**Plant material preparation**

Plant specimens of *A. inflata* and *C. mopane* were collected from the Omugulugoonime village, Omuntele constituency in the
Oshikoto region with the help of a local traditional healer. These specimens were subsequently validated at the National Botanical Research Institute, Windhoek (voucher numbers: C. mophane------, A. inflata -----). The plant samples, that is, the bark and leaves of C. mophane and the whole plant of A. inflata were rinsed with tap water, chopped into small pieces and air-dried at room temperature (23 to 26°C). The dry plant samples were then pulverized into fine powder using a laboratory mechanical mill. Crude extraction of the powders of the whole plant of Ac. inflata as well as the leaves and bark of C. mophane was done by maceration using ethanolic and aqueous solvents as described by Mutembei et al., (2011). This extraction method mimics the traditional processes for preparing concoctions of C. mophane and A. inflata. Aqueous extraction was done by soaking 10 g of the crude plant powder in 200 mL of distilled water in a conical flask. The aqueous mixture was subsequently heated at 70°C for 2 h using a hot plate. The aqueous extraction was done in triplicate so as to obtain the mean percentage yield. The resulting mixture was cooled at room temperature and was subsequently filtered with Whatman Filter Paper No. 1 to remove the solvent and was subsequently concentrated by heating at 70°C. Organic extraction was done by macerating 10 g of the crude plant powders of C. mophane and A. inflata in 200 mL of ethanol at room temperature for 4 days. The organic extraction was also done in triplicate. The resulting mixture were filtered using Whatman Filter Paper No. 1 to remove the solvent and the filtrate was concentrate on a Rota evaporator and were subsequently filtered using Whatman Filter Paper No. 1. The filtrate was concentrated using a Rota evaporator and were subsequently refrigerated until phytochemical and antimicrobial activity tests.

**Test microorganisms**

**Phytochemical analysis**

Phytochemical analysis of the aqueous and ethanolic extracts of C. mophane and A. inflata were performed according to standard methods described by Harbone (1989) and Tiwari et al., (2011). A mixture of aqueous (2 ml) extract and Ferling’s solution (3 ml) was heated to near boiling, a color change indicated the presence of reducing sugars. To 2 ml of the aqueous extract were diluted with distilled water and 1-2 drops of 0.1% ferric chloride solution were added- a dark-green, blue-green or blue-black color indicated the presence of tannins.

**Saponin test**

3 ml of the aqueous extract was shaken vigorously in a stoppered test tube- the persistence of froth for at least 5 min indicated presence of saponins. To 3 ml of the aqueous extract, dilute ammonia solution (3 ml) and sulphuric acid (1 ml) were added- a yellow color that disappeared on standing indicated the presence of flavonoids.

**Test for cardiac glycosides**

To a test tube containing 5 ml of the aqueous extract, 2 ml of glacial acetic acid added followed by a drop wise addition of 1 ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxy-sugars.

**Anthraquinones test**

To a test tube containing 2 ml of the ethanolic extract, a drop wise addition of 1 ml of dilute ammonia was done. A reddish color in the upper layer indicated the presence of anthraquinones.

**Alkaloids test**

A thin layer chromatogram (TLC) separation was done on the ethanolic extract. The dried TLC spots were subsequently sprayed with Dragendorff reagent. A pink color indicated the presence of alkaloids.

**Salkowski test for sterols and steroid**

To the 2 ml of the ethanolic extract, a drop wise addition of 3 ml of concentrated sulphuric acid was done. A reddish brown color at the interface indicated the presence of phytosterols.

**Test for coumarins**

1 ml of the ethanolic extract was spotted on the TLC plate for separation. Also, a mixture of 0.5 ml of diluted ammonia solution and the ethanolic extract was spotted on the other end of the TLC plate. The two spots were observed under UV light. Intense fluorescence indicated the presence of coumarins.

**Antimicrobial activity analysis**

The antimicrobial activities of the extracts were tested on four human pathogenic organisms: P. aeruginosa, B. subtilis, S. aureus and C. albicans (yeast). The test organisms were obtained from an accredited national laboratory, the Namibia Institute of Pathology (NIP) laboratory. The bacterial strains- P. aeruginosa, B. subtilis and S. aureus were cultured in a nutrient broth at 37°C for 24 h. The fungal strain- C. albicans was cultured on the potato dextrose agar at 25°C for 24 h.

The antimicrobial activity of the extracts were determined with the mean zones of inhibition using Filter-paper disc agar diffusion procedure as described by Kirby-Bauer (Bauer et al., 1966). In the Kirby-Bauer method, Whatman’s filter papers were punched into discs of diameters of 6 mm and 10 μL of varying concentrations (20, 10 and 5 mg/ml) of aqueous and ethanolic extracts incorporated using a micropipette. The concentrations were prepared by dissolving the respective quantities of dried ethanolic extract of C. mophane and A. inflata in the dimethyl sulfoxide (DMSO). The discs were allowed to dry and were subsequently stored at room temperature. A volume of 25 ml of sterilized nutrient agar was added on sterile Petri-plates and allowed to solidify. A volume of 100 μl of fresh culture of human pathogens- P. aeruginosa, B. subtilis and S. aureus were separately applied on the nutrient Agar using a sterile spreader. Whatman’s paper discs with varying concentration of the extracts (20, 10 and 5 mg/ml) were placed on separate petri-plates containing the cultured microorganisms using sterile forceps. The plates were then incubated at 37°C for 24 h, except the C. albicans which was incubated at 25°C for 24 h.

The zones of inhibition of each extract on each test organism at the three different concentrations were measured for three replicates. Penicillin G and streptomycin were used as positive controls for the antimicrobial susceptibility tests for bacterial activity and antifungal activity, respectively.

DMSO was used as the negative control for all experiments. The mean zone of inhibition was determined as a mean ± standard deviation.

**Data analysis**

The qualitative and quantitative methods were used to analyze the
Figure 3. Percentage yield of aqueous and ethanolic extracts of *C. mopane* and *A. inflata*.

Ethical considerations

The study was approved by the Faculty of Health Sciences, University of Namibia ethics review board, UNAM laboratory management and local leadership of Omuntele constituency council.

RESULTS

Percentage yield of crude extracts

Aqueous extracts gave higher percentage yields (23.79 to 31.62%) as compared to ethanolic extracts (8.16 to 18.11%). Leaves of *C. mopane* gave the highest yield for both the aqueous and ethanolic extracts, as compared to the bark of *C. mopane* and whole plant of *A. inflata* (Figure 3).

Phytochemical profile

Table 1 shows the phytochemicals identified in extracts of *C. mopane* and *A. inflata*. Aqueous extracts of *inflata* leaves and bark *C. mopane* gave positive tests for tannins, saponins, flavonoids and cardiac glycosides. The test was more reactive with the bark as compared to the leaf extracts. Aqueous extract of *A. inflata* tested positive for tannins and cardiac glycosides. The test for reducing sugars in the *C. mopane* and *A. inflata* were negative. Only the ethanolic extracts of the bark of *C. mopane* were positive for alkaloids, sterols and steroids and coumarins. The ethanolic extracts of leaves of *C. mopane* and the whole plant of *A. inflata* were also positive for coumarins (Table 1).

Antimicrobial potential of *C. mopane* and *A. inflata*

Table 2 and Figure 4 show the mean zone of inhibition (±SD) of the aqueous and ethanolic extracts of *C. mopane* and *A. inflata*. Generally, the ethanolic extracts showed higher antimicrobial activity than the aqueous extracts on all the bacterial test organisms. Antimicrobial activity against *S. aureus* was higher with the aqueous (10.7±1.3 mm) than the ethanolic leaf (9.7±2.1 mm) and bark (9.0±1.0 mm) extracts of *C. mopane*. This was however lower than the positive controls. The aqueous and ethanolic extracts of *A. inflata* had no activity against *S. aureus*. There was antimicrobial activity against strains of *P. aeruginosa* with the aqueous extract of the bark of *C. mopane* (10.7±3.1 mm), ethanolic extract of the leaves (12.7±0.6 mm) and bark (11.3±0.6 mm) of *C. mopane*, as well as the ethanolic extract of *A. inflata* (11.7±2.1 mm).
This was however lower than the activity of the positive controls against *Pseudomonas*. None of the aqueous extracts of the leaves and bark of *C. mopane* as well as *A. inflata* had activity against *B. subtilis*. All the ethanolic extracts of *C. mopane* leaves (9.0±1.0 mm) and bark (10.7±1.2 mm) as well as *A. inflata* (11.3±3.2 mm) had activity against strains of *B. subtilis*. *C. albicans* was not susceptible to any of the aqueous and ethanolic extracts.

Ethanolic extracts of *C. mopane* leaves and bark had significant antimicrobial activity against *P. aeruginosa*, *S. aureus* and *B. subtilis* (Table 2).

The antimicrobial activities of ethanolic extracts against *Pseudomonas* were comparable to the positive control penicillin G. The zone of inhibition for Penicillin G was highest with *B. subtilis* (26 mm) and lowest with *S. aureus*. Econazole had minimum zone of inhibition against *C. albicans.*
Chemical analysis revealed the presence of antimicrobial activity in the study extracts. This suggests the potential of certain plants and their parts to manage inflammatory conditions as well as acute infections of the gastrointestinal, respiratory systems and wounds. However, the presence of coumarins may have safety implications as they have been documented to have anticoagulation effects that may aggravate bleeding. This calls for further investigation of the safety profile of preparations of C. mopane used in folk medicine in Namibia.

The extracts of C. mopane had antibacterial activity against S. aureus, Pseudomonas and B. subtilis. The plant extracts exhibited more antimicrobial activity against P. aeruginosa than B. subtilis and S. aureus. The antimicrobial activity of the ethanolic extracts of C. mopane on Pseudomonas strains was comparable to penicillin G and streptomycin, the positive control. This result suggests that phytochemicals in C. mopane have activity against both aerobic Gram positive and facultative Gram negative bacteria. This may explain why preparations of C. mopane are widely used in the management of wounds, diarrhea, coughs and inflammatory diseases. Thus, further research is required to purify, characterize and test for the presence of flavonoids, tannins and alkaloids in C. mopane that have been associated with antimicrobial activity (Table 1).

### DISCUSSION

C. mopane and A. inflata gave significant yields of crude extracts. C. mopane and A. inflata are both higher plant and cover a wide area of Namibia and thus cultivation of these plants on large scale is commercially viable (Dushimemaria, 2014).

The phytochemical analysis revealed the presence of tannins, saponins, flavonoids and cardiac glycosides in the leaf and bark aqueous extracts of C. mopane. The aqueous extracts of A. inflata were positive for tannins and cardiac glycosides (Table 1). The findings in this study are similar to a study on Sphenostylis stenocarpa, both belonging to the Fabaceae family, also tested positive for presence of flavonoids, tannins and alkaloids (Nyananyo and Nyingifa, 2011). Similar studies have associated antimicrobial and/or activity of certain plants to the presence of tannins (Athanasiadou, 2001) and saponins (Avato et al., 2006) have been reported to have antimicrobial properties. The organic extract of the bark of C. mopane was positive for alkaloids, steroids and sterols and coumarins. Alkaloids have been documented to have several pharmacological properties including antimicrobial activity, antioxidant activity and analgesic properties. A study by Tiwari et al. (2011) has associated flavonoids, coumarins and tannins with antimicrobial activities in medicinal plants (Tiwari et al., 2011). This may explain why the bark of C. mopane is widely used as a folk medicine to manage inflammatory conditions as well as acute infections of the gastrointestinal, respiratory systems and wounds. However, the presence of coumarins may have safety implications as they have been documented to have anticoagulation effects that may aggravate bleeding. This calls for further investigation of the safety profile of preparations of C. mopane used in folk medicine in Namibia.

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Extracts of A. inflata showed antimicrobial activity against P. aeruginosa and B. subtilis but not S. aureus (Figure 4). This finding suggests that A. inflata has major activity on facultative Gram negative bacteria and anaerobic Gram positive bacteria limited activity on Gram positive bacteria. This explains the use of preparations of A. inflata in management of stomach upsets that are mainly attributed to Gram negative bacteria. The

### Table 2. Antimicrobial activity of the plant extracts (zone of inhibition (mean± SD)).

<table>
<thead>
<tr>
<th>Plant/test organism</th>
<th>Aqueous extracts</th>
<th>Ethanol extracts</th>
<th>Controls</th>
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<td></td>
<td>5 mg/ml</td>
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<tr>
<td>Mopane leaves</td>
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<tr>
<td>S. aureus</td>
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<tr>
<td>P. aeruginosa</td>
<td>9.0±1.0</td>
<td>10.7±3.1</td>
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<tr>
<td>B. subtilis</td>
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<tr>
<td>C. albicans</td>
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<td>Mopane bark</td>
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<td>S. aureus</td>
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<td>B. subtilis</td>
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<td>C. albicans</td>
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<td>A. Inflata</td>
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antimicrobial activity of extracts from C. mopane and A. inflata on all the test organisms was lower than the penicillin G, the positive controls (Figure 4) (this is attributed to the insufficient quantities of phytochemicals responsible for antimicrobial activity in the crude extracts, particularly, if the activity is dose dependent.

The organic extracts had more activity against the test organisms than the aqueous extracts (Table 2). Higher activity of organic extracts has been attributed to the ability of organic solvent systems to isolate a variety of bioactive compounds and activity (Fouche et al., 2008). However, the percentage yield was higher than the aqueous extraction than the ethanolic extract (Figure 3). The water preparations of C. mopane and A. inflata are widely used traditionally in Namibia. However, in traditional practice, C. mopane leaves are prepared by boiling and simmered or applied on the wound and not boiled. Traditional therapies of A. inflata are prepared by boiling in water for few minutes or the fresh plant material is soaked in hot water to make tea.

Thus, the methods used aqueous extraction using boiling may have had an effect on the antimicrobial activity of C. mopane (Pradeepa et al., 2016; Clarkson et al., 2004). Further research is required to isolate most of the active phytochemicals using more efficient solvent systems with varying polarities and less thermolabile techniques.

The study concludes that aqueous and organic extracts of C. mopane and A. inflata contain phytochemicals with antimicrobial activity (Jäger et al., 2005). The extracts have antibacterial activity on P. aeruginosa, B. subtilis and S. aureus but lack antifungal. The lack of antifungal activity of A. inflata is contrary to the findings in Botswana that found antifungal activity (Mukanganyama et al., 2011). This is mainly because the study in Botswana used extracts from the leaf unlike our study that assessed the plant as a whole activity. Organic extracts have better antimicrobial activity as compared to the aqueous extracts for C. mopane and A. inflata and have a wide activity against aerobic Gram positive bacteria (S. aureus), Gram positive anaerobes (B. subtilis) and facultative Gram negative bacteria (Pseudomonas). The organic extracts of C. mopane have superior antibacterial activity against pseudomonas than other test organisms.

Consequently, this study provides evidence for further research on the antimicrobial potential against resistant bacterial strains including P. aeruginosa and B. subtilis of ethanolic extracts of C. mopane leaves and bark as well as A. inflata. Future studies should also focus on optimizing the extraction of phytochemicals using different solvent systems in order to achieve a greater yield of bioactive compounds. The phytochemicals with antimicrobial activity should be purified and characterized for antipseudomonal activity of these plant extracts. There is need to perform acute and chronic toxicity studies on the extracts of C. mopane and A. inflata so as to outline the safety of these plants.

Limitations

In this study, only aqueous and ethanol solvents were used to isolate phytoconstituents. In future, organic solvent systems such as methanol and chloroform should be used. The use of aqueous and ethanolic extracts was used in order to mimic the solvents and processes used to prepare the medicines in practice. Secondly, antimicrobial activity was not tested on E. coli, a common organism involved in gastrointestinal and urogenital infections. Specimens of E. coli were not accessible from the Namibia Institute of Pathology (NIP) at the time of the study. However, the use of strains of Pseudomonas represented the facultative Gram negative bacteria to which E. coli belongs.

Conflict of Interests

The authors have not declared any conflict of interests.

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