OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.

Research Article
Evaluation of the Antimicrobial Properties of *Vachellia karroo* Hayne Banfi and Galasso Pods Used Traditionally for the Treatment of Venereal Diseases

S. Maposa, A.J. Afolayan and G.A. Otunola

Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, 5700 Alice, South Africa

Abstract

Background and Objective: *Vachellia karroo* is a forage plant for goats belonging to Fabaceae family, that is also used for the treatment of sexually transmitted infections in the Eastern Cape Province of South Africa. However, the pods from the tree are often discarded as waste causing environmental pollution. This study, therefore, aimed at evaluating the antimicrobial potentials of the pods towards minimizing their loss and negative impact on the environment. **Materials and Methods:** Antimicrobial evaluation of acetone, aqueous, hexane and methanol extracts of *V. karroo* pods using agar dilution assay was done. Eight bacterial strains and 6 fungal isolates were screened. **Results:** The results revealed that methanol extract had a broad-spectrum activity, with greater sensitivity against Gram-positive than Gram-negative strains. The extracts also showed great inhibition against *Candida albicans* the fungal isolate responsible for causing candidiasis in women. **Conclusion:** Since the pods showed a promising antimicrobial activity, they could serve as a cheap and ready source of antibiotic for the treatment and management of sexually transmitted infections, caused by these organisms.

Key words: *Vachellia karroo*, broad spectrum, antimicrobial, Gram-positive and Gram-negative strains sexually transmitted infections

Received: November 14, 2018 Accepted: April 27, 2019 Published:

Citation: S. Maposa, A.J. Afolayan and G.A. Otunola, 2019. Evaluation of the antimicrobial properties of *Vachellia karroo* hayne banfi and galasso pods used traditionally for the treatment of venereal diseases. Int. J. Pharmacol., CC: CC-CC.

Corresponding Author: Gloria Aderonke Otunola, Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, 5700 Alice, South Africa Tel: +27 745276130

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

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

INTRODUCTION

Micro-organisms that can survive in warm, dark places including the anus, the genital areas of both males and females, as well as the mouth cause sexually transmitted diseases. According to the World Health Organization, more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. The report further stated that an estimated 357 million new infections with 1 out of 4 STIs caused by chlamydia, gonorrhoea, syphilis or trichomoniasis occur annually. The STIs such as; gonorrhoea and chlamydia are the major causes of pelvic inflammatory disease (PID) and infertility in women[1]. In South Africa, about 11 million cases are reported annually and young women are particularly vulnerable to STIs, which is also one of the major contributors to the human immunodeficiency virus (HIV) epidemic[2].

Herbal medicines are gaining popularity because of several advantages such as; fewer side effects, better patient tolerance and acceptance due to long history of use[3]. The emergence of drug resistant strains have complicated the treatment of these infectious diseases and has led the search for new antimicrobial substances from various sources. Plant extracts possess active compounds that act positively against sexually transmitted pathogens and are thus the first resource for new active agents[2]. The prevention of the spread of STDs, alongside with early detection and appropriate therapy, have the potential of reducing infections that might damage the reproductive tract.

The alarming trend of drug resistance to synthetic antioxidants as well as antimicrobial agents have resulted in the search for medicinal plants with potent antimicrobial and antioxidant capacity[4]. Medicinal plants are sought after because they possess certain bioactive compounds such as; flavonoids, alkaloids, tannins and phenolics with reported antimicrobial as well as other biological activities[2]. Herbal medicines are also considered to be relatively inexpensive and safer than synthetic drugs. This has prompted the increased use of medicinal plants in the treatment and management of myriad of ailments. In recent times, the amount of polyphenols in plants and antioxidant activities depends on biological factors such as; genotypes or plant part and environmental conditions, such as; water stress, temperature and light intensity[5,2].

Vachellia karroo (Hayne) Banfi and Galasso, (sweet thorn), commonly known as *Acacia karroo*, belongs to the legume family Fabaceae. This plant has a wide distribution in South Africa and is abundantly spread through the dry region. The common name for the plant by the Xhosa speaking people is Umnga. It is common to the southern Africa region and is widely distributed throughout South Africa, Zambia, Zimbabwe and Angola[6]. The *V. karroo* is a multipurpose tree with great potential for increasing productivity in agroforestry and silvopastoral systems over a wide range of sites in the dry zones of the tropics and subtropics. It is also categorized as a species with potential commercial value in Botswana, South Africa and Zimbabwe[7].

The *V. Karroo* pods are brown, linear, falcate, usually constricted between the seeds, glabrous or with apices of lobes pubescent and size[8] (4)5-10.5(21)×0.5-0.7(1.1) cm. The pods which are protein rich are dropped in winter and they can be used as a goat supplement during dry season. There is no information in the scientific literature on the antimicrobial activity of the pods extract of this plant. Therefore, the present study was aimed at determining the antibacterial and antifungal activities of the pods of *Vachelia karroo* using selected pathogens.

MATERIALS AND METHODS

Collection of plant: The pods of *Vachellia karroo* were collected from Alice in the Central Eastern Cape province of South Africa, situated at 32.79 01°S, 26.83 30°E. The plant was identified and validated by Professor Cupido at the Giffen Herbarium of the Botany Department, University of Fort Hare and voucher specimens was deposited in the herbarium.

Preparation of extracts: Pods of *V. karroo* were dried at room temperature to constant weight, then ground to fine powder. About 60 g each of the powder was extracted separately in distilled water, methanol, acetone and hexane solvents for 48 h on an orbital shaker. Extracts of *V. karroo* pods were filtered and concentrated to dryness using a rotary evaporator for the acetone, hexane and methanol extracts and freeze dryer for the aqueous extract.

Antimicrobial assay

Selection of micro-organisms: The bacteria and fungi used for this study were selected based on their roles as opportunistic pathogens to humans and primarily to validate the ethnopharmacological claims of *V. karroo* pods as a remedy for venereal infections. The strains were obtained from the microbiology unit of the Medicinal Plants and Economic Development (MPED) Research Centre, University of Fort Hare, South Africa. Four Gram-positive strains, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (OK), *Bacillus subtilis* KZN, *Bacillus cereus* and *Streptococcus pyogenes* and 4 Gram-negative strains, *Vibrio cholera*, *Klebsiella pneumonia*

(ATCC 4352), *Pseudomonas aeruginosa* (ATCC 19582), *Salmonella typhi* (OK) were used for the antibacterial activity. The fungi isolates used were *Trichophyton mucoides* ATCC 201382, *Candida albicans* (ATCC 10231), *Candida glabatra*, *Penicillium chrysogenum*, *Aspergillus fumigatus*, *Penicillum aurantiogriseum*.

Preparation of bacterial and fungal inoculum: Direct colony suspension method as described by Unuofin *et al.*[9] using Mueller Hinton and Sabouraud Dextrose agars was used to prepare the bacterial and fungal inoculum, respectively. The suspensions were adjusted to give turbidites equivalent to 0.5 McFarland standard and fungal colony suspensions of 1×10^4 CFU/spot.

Agar dilution assay: A stock solution of the various extracts of *V. karroo* pods was prepared in Dimethyl sulfoxide (DMSO). Two fold serial dilutions of the stock to give a range of concentrations from 7.8125-250 mg mL^{-1} were pipeted into 96 well plates[10]. Erythromycin and Nystatin were used as standard antibiotics for the bacterial and antifungal assays, respectively.

RESULTS

Table 1 showed that methanol extract of *V. karroo* pods inhibited most of the tested bacterial strains compared to the other extracts; while the aqueous extract had the least activity but all the organisms were susceptible to erythromycin which was used as the standard. Gram-positive bacteria strains were more susceptible than the Gram-negative bacteria. *Staphylococcus aureus* which is a Gram-positive bacteria showed highest susceptibility amongst others, while *Vibro cholerea* which is a Gram-negative bacteria showed resistance to the extracts at the concentrations evaluated.

Table 2 showed that the aqueous and methanol extracts of *V. karroo* pods exhibited the best inhibition against the selected fungal strains compared to acetone and hexane extracts. All the extracts of *V. karroo* pods showed good inhibition against *Candida albicans* fungal isolate and were able to inhibit the growth of this isolate, while *Penicillum chrysogenum* was resistant.

DISCUSSION

Sexually transmitted infections are highly prevalent in many rural areas of developing countries due to poor sanitary conditions and lack of proper hygiene. Most people in these communities depend on traditional healers and medicinal plants to treat sexually transmitted infections, because they are too shy to talk to unknown western doctors or they do not have access to modern medical facilities[2].

The results obtained showed that, methanol, acetone, hexane and aqueous extracts of *V. karroo* pods exhibited appreciable antimicrobial activities against the selected pathogens. The Gram-positive bacteria were more susceptible to the extracts compared to Gram-negative bacteria. The observed resistant susceptibility of the Gram negative bacteria was not surprising as they have been reported to be more resistant than Gram-positive to antibiotics[11]. The

Table 1: Minimum Inhibitory Concentrations (MIC) of extracts of *V. karroo* pods on selected Gram-negative and Gram-positive bacteria (mg mL^{-1})

	Bacterial strains							
Samples	*P. aeruginosa* (-)	*S. typhi* (-)	*V. cholerea* (-)	*K. pneumoniae* (-)	*S. pyogene* (+)	*B. subtilis* (+)	*B. cereus* (+)	*S. aureus* (+)
Methanol	1.25	1.25	50.0	0.625	<0.3125	1.25	<0.312	0.625
Acetone	2.50	5.00	2.5	0.625	2.50	2.50	1.25	<0.3125
Hexane	1.25	5.00	5.0	2.500	5.00	5.00	5.00	<0.3125
Aqueous	5.00	5.00	>5.0	5.000	5.00	5.00	5.00	1.25
Erythromycin (µg mL^{-1})	8.00	8.00	8.0	8.000	<1.00	8.00	1.00	2.00

>: Value greater than the highest concentration tested and ≤: Value lesser than or equal to the lowest concentration tested

Table 2: Minimum Inhibitory Concentrations (MICs) of extracts of *V. karroo* pods on selected fungi strains (mg mL^{-1})

	Fungal isolates					
Samples	*Trichosporon mucoides*	*Candida albicans*	*Candida glabrata*	*Penicillium chrysogenum*	*Aspergillus fumigatus*	*Penicillum aurantiogriseum*
Methanol	<0.3125	<0.3125	<0.3125	1.25	1.25	2.50
Acetone	1.250	<0.3125	1.250	2.50	2.50	1.25
Hexane	1.250	0.6250	0.625	2.50	1.25	1.25
Aqueous	0.625	<0.3125	0.625	1.25	<0.3125	<0.3125
Nystatin (µg mL^{-1})	2.000	1.0000	1.000	4 .00	1.00	1.00

>: Value greater than the highest concentration tested and ≤: Value lesser than or equal to the lowest concentration tested

Gram-negative bacteria pose a great medical challenge as they have an outer membrane that acts as a barrier to many environmental substances and protects them from many antibiotics (including penicillin) and detergents that would normally damage the peptidoglycans of their (inner) cell membrane.

This resistance of Gram-negative to plant extracts has been documented by Koohsari *et al.*[12], who reported that the extracts were more effective in Gram-positive bacteria compared to Gram-negative bacteria. The authors justified that this may be because of inherent tolerance of Gram-negatives and the nature and composition of herbs and also the cell walls of Gram-positive bacteria compared with Gram-negative bacteria are more sensitive to many of antibiotics, antimicrobial chemical compounds and even many herbal drugs. Lipopolysaccharides layer and periplasmic space of Gram-negative bacteria are the reasons of relative resistance of Gram-negative bacteria[13].

Methanol extract showed the greatest antibacterial activity against *Staphylococcus aureus* these results were similar to the results reported by Madureira *et al.*[14], who reported that the methanol extract of *V. karroo* leaves gave the best inhibitory activity against selected bacterial strains.

Aqueous extract had the lowest antibacterial activity. The weak activity of the aqueous extract in this study could be due in part to the inability of water to extract as much antibacterial bioactive compounds as other solvent from the pods of the plant. The results in this study are in agreement with previous reports by Palombo and Semple[11]. However, on the contrary, Mulaudzi *et al.*[15] reported that the aqueous extract of *V. karroo* bark had the best inhibitory activity against selected organisms compared to other solvent extracts.

The MIC values of V*. karroo* pod extracts against bacteria were higher than those for fungi and is an indication that fungi were more susceptible to the crude pods extracts of *V. karroo* pods than bacteria. Methanol, acetone and aqueous extracts showed good inhibition on *Candida albicans.* This fungal isolate causes candidiasis that is responsible for vaginal yeast infections in women and a recent report has shown that *Candida albicans* had been found in the sperm of men whose partners suffered from yeast infections[16]. Recently Mamba *et al.*[17] evaluated antifungal activities of ethanol extracts of *V. karroo* leaf against *C. albicans* and reported that the extract demonstrated good activity. In a separate study[18], it has previously been established that the ethanol extracts of *V. karroo* roots possess antifungal activities against *C. albicans*.

The antifungal activity of methanol and aqueous leaf extracts of *A. karroo* against *Aspergillus niger*, *C. albicans* and *Rhizopus stolonifer* has also been previously established. Antifungal activities were observed in methanol and aqueous leaf extracts against *A. niger* with minimal inhibition concentration (MIC) values of 486 and 325 mg mL^{-1}, respectively. Observations in this study showed that methanol and aqueous extracts of *V. karroo* pods showed high antifungal activities against *C. albicans.* These observations indicated that the pods of *V. karroo* could be a good source of antifungal medication for the treatment and management of *Candida albicans.* However, the resistance of *Penicillium chrysogenum* against all the extracts used could be because of the inability of the extracts to penetrate through the cell walls of this fungal isolate.

CONCLUSION

This study reports for the first time the antimicrobial properties of *V. karroo* pods. The findings revealed that the methanol extract had a better inhibitory potential against the bacterial strains, while aqueous extract had a better inhibition against fungi screened in this study. Since *V. karroo* pods showed promising antimicrobial activity, they could serve as a cheap source of therapeutic agents for the treatment and management of sexually transmitted diseases.

REFERENCES

1. WHO., 2015. WHO Global prevalence and incidence of selected curable sexually transmitted infections. Fact sheet No-110, World Health Organization, Geneva.
2. Tshikalange, T.E., P. Mamba and S.A. Adebayo, 2016. Antimicrobial, antioxidant and cytotoxicity studies of medicinal plants used in the treatment of sexually transmitted diseases. Int. J. Pharmacogn. Phytochem. Res., 8: 1891-1895.
3. Unuofin, J.O., G.A. Otunola and A.J. Afolayan, 2018. Polyphenolic content, antioxidant and antimicrobial activities of *Vernonia mespilifolia* Less. Used in folk medicine in the Eastern Cape province, South Africa. J. Evidence-Based Integr. Med., Vol. 23. 10.1177/2515690X18773990
4. Mohamed, A.A., S.I. Ali and F.K. El-Baz, 2013. Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* Leaves. PLos One, Vol. 8, No. 4. 10.1371/journal.pone.0060269.
5. Bano, M.J.D., J. Castillo, O. Benavente-García, J. Lorente, R. Martín-Gil, C. Acevedo and M. Alcaraz, 2006. Radioprotective-antimutagenic effects of rosemary phenolics against chromosomal damage induced in human lymphocytes by γ-rays. J. Agric. Food Chem., 54: 2064-2068.

6. Barnes, M.E., 2001. Effects of large herbivores and fire on the regeneration of Acacia erioloba woodlands in Chobe National Park, Botswana. Afr. J. Ecol., 39: 340-350.
7. Van Wyk, B.E., 2011. The potential of South African plants in the development of new medicinal products. S. Afr. J. Bot., 77: 812-829.
8. Muvengwi, J., 2017. Relationships between termite (Macrotermes) mound distribution, plant diversity and large mammalian herbivory patterns in Gonarezhou National Paark, Zimbabwe. Ph.D. Thesis, Faculty of Science, University of the Witwatersrand, Johannesburg, South Africa.
9. Unuofin, J.O., G.A. Otunola and A.J. Afolayan, 2017. Phytochemical screening and *in vitro* evaluation of antioxidant and antimicrobial activities of *Kedrostis africana* (L.) Cogn. Asian Pac. J. Trop. Biomed., 7: 901-908.
10. Ohikhena, F.U., O.A. Wintola and A.J. Afolayan, 2017. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. Scient. World J., Vol. 2017. 10.1155/2017/9658598
11. Palombo, E.A. and S.J. Semple, 2001. Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharmacol., 77: 151-157.
12. Koohsari, H., E.A. Ghaemi, M.S. Sheshpoli, M. Jahedi and M. Zahiri, 2015. The investigation of antibacterial activity of selected native plants from North of Iran. J. Med. Life, 8: 38-42.
13. Sharifa, A.A., Y.L. Neoh, M.I. Iswadi, O. Khairul and M.A. Halim *et al*., 2008. Effects of methanol, ethanol and aqueous extract of Plantago major on Gram-positive bacteria, Gram-negative bacteria and yeast. Ann. Microsci., 8: 42-44.
14. Madureira, A.M., C. Ramalhete, S. Mulhovo, A. Duarte and M.J.U. Ferreira, 2012. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. Pharm. Bio., 50: 481-489.
15. Mulaudzi, R.B., A.R. Ndhlala, M.G. Kulkarni, J.F. Finnie and J. van Staden, 2011. Antimicrobial properties and phenolic contents of medicinal plants used by the Venda people for conditions related to venereal diseases. J. Ethnopharmacol., 135: 330-337.
16. MediResource Inc., 2019. Candidiasis (Yeast infections, thrush). https://medbroadcast.com/condition/getcondition/candidiasis
17. Mamba, P., S.A. Adebayo and T.E. Tshikalange, 2016. Anti-microbial, anti-inflammatory and HIV-1 reverse transcriptase activity of selected South African plants used to treat sexually transmitted diseases. J. Pharmacol. Phyto. Res., 8: 1870-1876.
18. Tshikalange, T.E., 2002. The traditional use of medicinal plants to treat sexually transmitted diseases. Ph.D. Thesis, University of Pretoria, South Africa.
19. Cock, I.E. and S.F. van Vuuren, 2015. South African food and medicinal plant extracts as potential antimicrobial food agents. J. Food Sci. Technol., 52: 6879-6899.