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Evaluation of Antigonorrhea Activity and Cytotoxicity of *Helichrysum caespititium* (DC) Harv. Whole Plant Extracts

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Abstract

Over 80% of African population depends on traditional knowledge for their well-being, and especially on plants as medicines. Although *Helichrysum caespititium* is among plants that are commonly used by traditional healers in Africa, its biological activities are still not scientifically proven and reported. The primary objective of this study is to assess the anti-gonorrhea activity and cytotoxicity of *H. caespititium* whole plant. The plant material was subjected to a serial exhaustive extraction to obtain different solvent extracts using *n*-hexane, dichloromethane, methanol, and water. The antigonorrhea activity of the four plant extracts (*n*-hexane, dichloromethane, methanol, and water extracts) against 2008 WHO *Neisseria gonorrhea* reference strains and the toxicity of the extracts against rat liver cells were investigated. All four *H. caespititium* extracts showed good activity against the four 2008 WHO *N. gonorrhea* strains (F, O, N, G strains) under study in the range of 0.037 to 0.33 mg/ml. *n*-Hexane extract was observed to be the most potent against all the four strains with a lowest Minimum Inhibitory Concentration (MIC) value of 0.037 ± 0.0 mg/ml against G strain, which was comparable to gentamicin (standard 1) and more active compared to amoxicillin (standard 2), and also the most less toxic of all with LC_{50} value of 428.77 ± 4.76 μg/ml followed by water extract (394.36 ± 5.41 μg/ml) and methanol (357 ± 2.81 μg/ml). The results justify the usage of *H. caespititium* in the traditional medicine against gonorrhea infections.

Keywords: Antigonorrhea; Cytotoxicity; *H. caespititium*; MIC

Introduction

Over 80% of African population depends on traditional knowledge for their well-being, and especially on plants as medicines. The Southern African region contains more than 350,000 species of flowering plants, in which many of them have been used by traditional healers. However, their biological activities are still not scientifically proven and reported. Among the 500 species that contain *Helichrysum* family, which are discarded worldwide, considerable species, approximately 245-246, occurs in Africa and Madagascar [1-4]. *Helichrysum caespititium* is one of those plants growing in Southern African region where it is referred to as *impepo* (Zulu), *impepho* (isiXhosa), *seledu-sa-phoko* (South Sotho), *moriri-wa-naha* (Kwena), and *sephanyane* (Kgatla).

Helichrysum species name "*caespititium*" was derived from the Latin word "*caespitose*," which means very much tufted, matted, referring to the cushion-forming growth habit. The plant has been referred to as everlasting [1]. Botanically, *H. caespititium* (DC) is presented as a prostate, perennial, mat-forming herb that is profusely compact shrublet with branched and densely tufted. Leaves are scattered with orange glands. Silvery white flowers appear in late summer with yellow centers and pale furry underneath [1].

The plant has been used since ancient time for treatment of several diseases, such as bronco-pneumonia, tuberculosis, and intestinal ulceration. Moreover, it is used in styptic wound dressing particularly during the circumcision rites [5], bruises, cuts, and sores [3]. Furthermore, the plant has been involved in the treatment of skin infection diseases, respiratory problems, gastro-intestinal tracts, and diarrhea in Sekhukhune and Waterberg municipality districts in the Limpopo province, South Africa [6]. The Basotho population inhales the smoke emerging from burning of *H. caespiticium* plant material for the treatment of headache, chest colds as well as for the treatment of internal wounds such as intestinal ulceration. Moreover, the concoction of *H. caespititium* has been drunk by Bakwena and Bakgatla populations in ancient time for the treatment of gonorrhoea infection [1].

Gonorrhea is a common sexually transmitted disease that affects thousands of men and women annually, particularly in the United States [7]. Although gonorrhea is easily treated, it can cause serious and sometimes enduring complications such as pelvic inflammatory disease in women and epididymitis and barrenness in men [7-9]. Regimens for the treatment of gonorrhea are increasingly being based on oral and/or injectable expanded-spectrum third generation cephalosporins such as cefixime and ceftriaxone, but worries have recently been uttered about their continuing efficacy [10-15]. This condition, as well as the emergence of reduced susceptibility and resistance to azithromycin, has called for improved efforts for the control of gonococcal disease [12,14,15].

In our endeavor to find cure for infectious disease particularly gonorrhea, we decided to investigate the claimed antigonorrhea activity of *H. caespititium* by traditional healers and the cytotoxicity of *H. caespititium* plant extracts. Although the antibacterial activity of the plant has been proven by Mathekga *et al.* [5], a search in the literature reveals that *H. caespititium* plant's antigonorrhea activities and cytotoxicity have not yet been studied and proven scientifically. As there has been a concern about the efficacy of some current antigonorrhea drugs toward gonorrhea infections [10-15], it is of considerable urgency to find other drugs that can surmount the difficulty experienced at present.

In this study, to the best of our knowledge, we are the first to report the antigonorrhea activities of the plant against 2008 WHO *Neisseria gonorrhea* reference strains and the cytotoxicity of the plant extracts.

Materials and Methods

Material

The solvents that were obtained from Sigma (South Africa) for extraction were *n*-hexane, dichloromethane, and methanol (reagent grade). The water was purified from water distillation plants in our laboratory. All other chemicals were of analytical grade or GC grade.

Collection and identification of the plant

The whole plant material of *H. caespititium* was collected from Masealama village, which is situated at 29.88° East longitude and -23.83° South latitude in Polokwane Municipality, Capricorn District Municipality in the Limpopo Province, South Africa. The plant was then taken to the South African National Biodiversity (SANBI) in Pretoria for identification, and the identification code is DTH 9006000.

Processing of plant material

The collected plant material was dried at room temperature before being grinded into powder using Mellerware Coffee Bean Grinder machine (Aromatic, 29105A, South Africa). The resulted powder was kept in dark at 4°C for further usage.

Extraction

A mass of 160 g of powdered plant material was subjected to a serial exhaustive extraction using the maceration method in 3000 ml of *n*-hexane, dichloromethane, methanol, and water (starting with less polar to more polar solvent). The mixtures were shaken for 24 h at 120 rpm using Labtech shaker. The filtration was performed using the Whitman filter paper No. 1. The extracts were concentrated using rotary evaporator with reduced pressure at temperature up to 40°C. Each solvent used in the extraction was repeated three times, and the extracts were combined. Moreover, the extracts were used in this study.

Microorganisms

The MIC test involved four *N. gonorrhoea* strains that are used by WHO as reference strain for global quality assurance and quality control of gonococcal antimicrobial resistance testing, and the strains are identified as F, N, O, and G [16]. The selection of the 2008 WHO *N. gonorrhea* strains in this study was based on their type. 2008 WHO *N. gonorrhea* strains have eight strains among which two are African type (M and O strains), one is an Asian type (N strain), one is Dutch type (G strain), three are wild-type (F, L, and P strains), and one is an unknown type (K strain). It was decided to use one strain in each type namely F (wild-type), G (Dutch type), N (Asian type), and O (African type). The adopted experimental procedure was that proposed by Eloff [17] and that from the Clinical and Laboratory Standards Institute (CLSI) [18], but with slight modification. Hundred and fifty microliter (150 µl) of Mueller Hinton broth was pipetted into each well of the 96-wells plate. Thereafter, 75 µl of the extract solution (*n*-hexane, dichloromethane, methanol, or water) (1 mg/ml) was prepared in water and 0.5 ml of acetone to allow the extracts to dissolve, because it does not completely dissolve in water alone. This was added to first wells followed by a threefold serial dilution. Thirty five microliters (35 µl) of an overnight bacterial suspension grown on New York City plate (GC-agar) was added to each well. The microtiter plate was sealed and then anaerobically incubated for 24 h at 37°C in the presence of 5-10% of CO_2 for the survival of the bacteria under study. After incubation, 40 µl of *p*-iodonitrotetrazolium chloride (INTC) (0.1 mg/ml) was added to each well, and then the plate was further incubated for approximately 40 min to 1 h at the similar condition. Gentamicin and amoxicillin solutions were used as positive control. The wells that showed pink color explained the growth of bacteria. However, the yellow colored wells or colorless wells explained no bacteria growth (formation of formazan). The MIC was recorded as the lowest concentration that could not produce the visible bacteria growth.

Cytotoxicity assay

The extracts of *H. caespititium* were screened for cytotoxic activity in H411E rat hepatoma (liver) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [19,20]. Cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin solution. Previously established 80% confluent monolayer was trypsinized and resuspended in fresh MEM for seeding at a density of 1.0×10^5 cells/ml into each well (100 µl) of 96-well plate for the toxicity assays. Following overnight incubation at 37°C in a 5% CO_2, 100 µl of fresh media containing plant extracts was added to the cells in the plates for cytotoxicity assay. Prior to addition of fresh medium with plant extracts, stock solutions (100 mg/ml) were prepared and further serial dilutions with growth media to six concentrations were made and used for the assays. DMSO was used as solvent (negative) control, while doxorubicin was used as the positive control. The cells were then incubated for 24 h. After the incubation, the medium was removed by aspiration and fresh media together with 20 µl of MTT (5 mg/ml in phosphate buffered saline, PBS) were added into each well. After a further incubation period (4 h), the medium was carefully aspirated without disturbing the MTT crystals at the bottom of the wells and replaced with 40 µl of undiluted DMSO. The concentration of MTT reduced was measured at 570 nm after gentle shaking. The wells containing only medium and MTT was used to blank the microplate reader (Epoch BioTek). The LC_{50} calculated from a linear curve of log of concentrations versus average absorbances represents the concentration of extract that resulted in a 50% reduction of absorbance in comparison to the untreated cells.

Statistical analysis

The results of all the experiments involved in this study were performed in triplicate. One-way analysis of variance (ANOVA) followed by the t-test were used in the data analysis. Therefore, all results are presented as mean values ± standard deviation (SD). All P values lower than 0.05 were considered as significant ($p < 0.05$).

Results and Discussion

All four plant extracts tested were active against the four WHO *N. gonorrhea* strains with MIC values ranging from 0.037 to 0.3 mg/ml (Table 1). It is stipulated that the antimicrobial activity of a crude extract is considered significant when the MIC is below 100 µg/ml (0.1 mg/ml), moderate when between 100 and 625 µg/ml (0.1 and 0.625 mg/ml), and low when more than 625 µg/ml (0.625 mg/ml) [21,22]. For pure compounds, the activity is considered significant when the MIC is below 10 µg/ml (0.01 mg/ml), moderate when between 10 and 100 µg/ml ($0.01 < \text{MIC} < 0.1$ mg/ml), or low when greater than 100 µg/ml (0.1 mg/ml) [21,22]. Moreover, according to Gibbons *et al.* [23], the values of MIC below 1 mg/ml for extracts and 64 µg/ml (0.064 mg/ml) for single chemical entities are considered significant. Therefore, these results (Table 1) are worth considering.

Plant extracts/ standards	MIC[a] (mg/ml)				Cytotoxicity (μg/ml)
	F	G	N	O	
H.E	0.33 ± 0.00	0.037 ± 0.00	0.33 ± 0.00	0.106 ± 0.067	428.77 ± 4.76
D.E	>0.33	>0.33	>0.33	>0.33	82.86 ± 3.36
M.E	>0.33	>0.33	>0.33	>0.33	357.39 ± 2.81
W.E	>0.33	>0.33	>0.33	>0.33	394.36 ± 5.41
Amoxicillin[b]	>0.33	>0.33	>0.33	>0.33	–
Gentamicin[b]	0.22 ± 0.11	>0.33	>0.33	0.22 ± 0.11	–
Doxorubicin[c]	–	–	–	–	10.80 ± 1.63

[a]Minimum inhibitory concentration (MIC, value expressed as mean ± standard deviation with $n = 2$), H.E (hexane extract), D.E (dichloromethane extract), M.E (methanol extract), W.E (water extract).
[b]Standards for antigonorrhea assay.
[c]Standard for cytotoxicity (LC_{50} values expressed as mean ± standard deviation with $n = 3$). This process was repeated twice.

Table 1: Antigonorrhea activities (MIC in mg/ml) and cytotoxicity values (LC_{50} in μg/ml) of *H. caespititium* extracts

n-Hexane extract was found to be the most active against all four *N. gonorrhea* strains under study with MIC value ranging from 0.037 ± 0.00 mg/ml to 0.33 ± 0.00 mg/ml. This extract was found more active against G strain compared to other strains. The plant extract being active against African type gonorrhea strain (O strain) was also observed by Olivier *et al.* [24] where it was discovered that the *n*-hexane extract of *Asparagus suaveolens* whole plant was active against 2008 WHO *N. gonorrhea* O strain, which was the most active strain compared with all other 2008 WHO *N. gonorrhea* strains. In this study, Dutch type strain (G strain) was found to be the most active compared to other strains under study. These results suggest that although these plants, *Asparagus suaveolens* [24] and *H. caespititium*, are from the same area (Limpopo Province, South Africa), these plants cannot be used interchangeably against *N. gonorrhea* infections in the area because the phytochemical responsible for their activities against the infection could be different.

The cytotoxicity of the four plant extracts was also evaluated, and Doxorubicin was the drug of reference. All four plant extracts were found to be far less toxic against H-4-11-E rat hepatoma (liver) cell with LC_{50} values ranging from 82.86 ± 3.36 μg/ml to 428.77 ± 4.76 μg/ml compared to the reference drug (Doxorubicin, 10.80 ± 1.63). *n*-Hexane extract was found to be the most less toxic among all the plant extract with LC_{50} value of 428.77 ± 4.76 μg/ml. These results suggest that the plant extract can safely be used without any worries of being toxic to the cells. Moreover, the most interesting result is of water extract, which was also far less toxic than the reference. It can also be concluded that even if traditional healers do not have all these organic solvents to get the extracts, the water that is always available to them can also provide a nontoxic extract of the plant. Therefore, the traditional healer's water extract is also safe to use.

This plant may, thus, be a source of drugs that can improve the treatment of infection caused by these microorganisms. This finding provides a clear understanding of the utilization of the whole plant by indigenous people to treat *N. gonorrhea* infections without anticipated toxicity.

Conclusion

In conclusion, this study managed to reveal some truth about the plant antigonorrhea activities and toxicity character toward cells. The plant in general is a strong antigonorrhea agent with *n*-hexane extract being the most compared to other extracts (dichloromethane, methanol, and water extracts). In addition, the plant can be used to address the problem of gonorrhea infections like other plant extracts that have been published elsewhere [24]. Looking at the cytotoxicity character of the plant extracts, this plant is proven to be nontoxic to cells.

This study is a preliminary study toward the isolation of the phytochemical compounds responsible for the claimed bioactivity of *H. caespititium* plant. An in-depth study of the extract in terms of structure elucidation of the bioactive compounds is under way to provide a good base for all the phytochemical functions mentioned above and their bioactivity studies.

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References

1. Mathekga ADM (2001) New Phloroglucinol from Helichrysum Caespititium. Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophiae plant physiology in the faculty of biological and agricultural sciences, University of Pretoria, South Africa.
2. Lourens ACU, Reddy D, Baser KHC, Viljoen AM, Van Vuuren SF (2004) In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J Ethnopharmacol 95: 253-258.
3. Swartz VG (2006) Phytochemical Studies of *Helichrysum patulum* [Dissertation]. Retrieved from University of the Western Cape, South Africa.
4. Süzgeç-Selçuk S, Birteksöz AS (2011) Flavonoids of *Helichrysum chasmolycicum* and its antioxidant and antimicrobial activities. S Afr J Bot 77: 170-174.
5. Mathekga ADM, Meyer JJM, Horn MM, Drewes SE (2000) An acylated phloroglucinol with antimicrobial properties from *Helichrysum caespititium*. Phytochemistry 53: 93-96.
6. Semenya SS, Maroyi A (2012) Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. J Ethnopharmacol 144: 395-401.
7. Satterwhite CL, Torrone E, Meites E, Dunne EF, Mahajan R, *et al.* (2013) Sexually transmitted infections among US women and men: prevalence and incidence estimates, 2008. Sex Transm Dis 40: 187-193.
8. Handsfield HH, Lipman TO, Harnisch JP, Tronca E, Holmes KK (1974) Asymptomatic gonorrhea in men. N Engl J Med 290: 117-123.

9. Centers for Disease Control and Prevention (CDCP) (2015) Sexually Transmitted Disease Surveillance 2014, U.S. Department of Health and Human Services, Atlanta.

10. Lo JYC, Ho KM, Leung AOC, Tiu FS, Tsang GK, *et al.* (2008) Ceftibuten resistance and treatment failure in gonococcal infection. Antimicrob Agents Chemother 52: 3564-3567.

11. Lindberg R, Fredlund H, Nicholas R, Unemo M (2007) Neisseria gonorrhoeae isolates with reduced susceptibility to cefixime and ceftriaxone: association with genetic polymorphisms in penA, mtrR, porB1b, and ponA. Antimicrob Agents Chemother 51: 2117-2122.

12. Tapsall JW, Dillon J-A, Ison CA, Ng LK, Knapp JS (2008) 16th International Pathogenic Neisseria Conference, 7-12 September. Rotterdam, The Netherlands. Abstract O18, 34. Congress Care, IPNC.

13. Yokoi S, Deguchi T, Ozawa T, Yasuda M, Ito S, *et al.* (2007) Threat to cefixime treatment for gonorrhea. Emerg Infect Dis 13: 1275-1277.

14. Workowski KA, Berman SM, Douglas JM Jr (2008) Emerging antibacterial resistance in *Neisseria gonorrheae*: urgent need to strengthen prevention strategies. Ann Intern Med 148: 606-613.

15. Deguchi T, Yasuda M (2008) Lack of nationwide surveillance of antimicrobial resistance in *Neisseria gonorrhea*. Ann Intern Med 149: 363-364.

16. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J (2009) Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. J Antimicrob Chemother 63: 1142-1151.

17. Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64: 711-713.

18. Clinical and Laboratory Standards Institute (CLSI) (2006) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Seventh Edition: Approved Standard M7-A7. Wayne, PA: CLSI.

19. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

20. Olaokun OO, McGaw LJ, Awouafack MD, Eloff JN, Naidoo V (2014) The potential role of GLUT4 transporters and insulin receptors in the hypoglycaemic activity of *Ficus lutea* acetone leaf extract. BMC Complement Altern Med 14: 269.

21. Kuete V, Efferth T (2010) Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol 1: 123.

22. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Alibert-Franco S, *et al.* (2010) Efflux pumps are involved in the defense of gram-negative bacteria against the natural products isobavachalcone and diospyrone. Antimicrob Agents Chemother 54: 1749-1752.

23. Gibbons S (2004) Anti-staphylococcal plant natural products. Nat Prod Rep 21: 263-277.

24. Olivier MT, Muganza FM, Shai LJ, Gololo SS, Nemutavhanani LD (2017) Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of *Asparagus suaveolens* aerial parts. S Afr J Bot 108: 41-46.