Evaluation of Antigonorhea 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 antigonorhea 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 antigonorhea 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 LC₅₀ 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:** Antigonorhea; 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 insepe (Zulu), insepeho (isiXhosa), seledu-sa-phoko (South Sotho), moriri-wa-na-ha (Kwena), and sepahanye (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 infections 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. caespititium* 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 antigonorhea 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 Matheka et al. [5], a search in the literature reveals that *H. caespititium* plant’s antigonorhea activities and cytotoxicity have not yet been studied and proven scientifically. As there has been a concern about the efficacy of some current antigonorhea 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 gonorrhoea 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.

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. gonorrhoea 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 < 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.
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 LC50 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 LC50 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 antagononorrhea activities and toxicity character toward cells. The plant in general is a strong antagononorrhea 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**


