

*Full Length Research Paper*

# Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*

L. Kambizi<sup>1\*</sup> and A.J. Afolayan<sup>2</sup>

<sup>1</sup>Walter Sisulu University, Biological Sciences Department, P. Bag X1 Mthatha 5117, South Africa.

<sup>2</sup>Fort Hare University, Botany Department, Alice 5700, South Africa.

Accepted 14 November, 2007

Herbal medicines play a vital role in the treatment of sexually transmitted infections (STIs), especially in the remote areas of South Africa where clinics and hospitals are sparsely located. *Aloe ferox* and *Withania somnifera* are among the southern African plants commonly used for the treatment of (STIs). This paper reports on the *in vitro* antimicrobial activities of water and methanol extracts from the two plants on *Neisseria gonorrhoea* and *Candida albicans*, common causes of STIs in rural South Africa. Extracts from both species together with pure aloin from *A. ferox*, were evaluated for activity against six strains of *N. gonorrhoea* and nine strains of *C. albicans*. The extracts showed activity against *N. gonorrhoea* at concentrations of ranging from 0.5 (methanol extracts from both) to 10 (water extract of *W. somnifera* only) mg/ml while pure aloin inhibited the growth of both microorganisms. Only the methanol extract of *W. somnifera* was effective against *C. albicans* at a concentration of 20 mg/ml.

**Key words:** Antimicrobial, *Aloe ferox*, *Candida albicans*, *Neisseria gonorrhoea*, *Withania somnifera*.

## INTRODUCTION

The indigenous people of the Eastern Cape Province in South Africa have a long history of traditional plant usage for the treatment of various diseases and ailments (Van Wyk et al., 1997; Grierson and Afolayan, 1999) including sexually transmitted infections (STIs). In the remote rural communities of the province, STIs are common among young adults and herbalists take advantage of the biodiversity of plant species to treat these infections.

The commonest and most frequently reported STIs in the Eastern Cape province of South Africa, apart from the HIV and AIDS, are gonorrhoea, herpes and syphilis (Kambizi and Afolayan, 2003). These diseases are caused by bacteria and viruses. The commonest disease is gonorrhoea which is caused by *Neisseria gonorrhoea*, a Gram-negative bacterium. It was discovered in 1879 by Neisser (Kelly, 1992). The organism attacks mucous membranes, especially the columnar epithelium found in the urethra, the cervix uteri, rectum and conjunctivae. An intense inflammatory reaction follows with a massive polymorphonuclear response facilitating penetration of

the mucosal lining by *N. gonorrhoea* (Daly et al., 1994; Mariam et al., 2004). The incubation period is 2 - 5 days. In men the main symptom of the disease is a profuse purulent urethral discharge while it is asymptomatic in women. The microorganism causes some complications in men such as epididymitis and prostatitis while in women the most important complication is pelvic inflammatory disease (Fenton and Lowndes, 2004; Mariam et al., 2004).

Candidiasis is one of the diseases treated by herbalists in the Eastern Cape. It is caused by a dimorphic fungus called *Candida albicans*. This fungus exists as oval, single yeast cells, which reproduce by budding (de Repentigny, 2004). Although *C. albicans* most frequently infects the skin and mucosal surfaces, it can cause systemic infections manifesting as pneumonia, septicaemia or endocarditis in severely immunocompromised patients. According to Lamb et al., (2000), it grows vigorously in the vagina and pregnant women transmit the infection to the babies during birth.

In a previous survey of plants used for the treatment of STIs (Kambizi and Afolayan, 2003), traditional healers and other knowledgeable rural dwellers of the Eastern Cape listed *Aloe ferox* (Family Asphodelaceae) and *Withania somnifera* (Family Solanaceae) as two of the

\*Corresponding author. E-mail: [Lkambizi@wsu.ac.za](mailto:Lkambizi@wsu.ac.za).

commonest plant species used for the treatment of gonorrhoea and candidiasis. *A. ferox* is widely used in traditional medicine. According to many authors, the species is used as an anti-inflammatory and anticancer agent such as in the treatment of leukemia and against neuroectodermal tumors (Van Wyk et al., 1997; Capasso et al., 1998; Pecere, 2000). *Withania somnifera* grows widely in the Eastern Cape and is used for the treatment of arthritis, tuberculosis, cancer and STIs (Devi, 1996; Van Wyk et al., 1997; Singh and Kumar, 1998).

The antimicrobial properties of these two plant species have been widely reported in literatures (Afolayan et al., 2002; Arora et al., 2004). However, the reports have focused mainly on their activities against commensal microbial flora while information on their activity against human pathogens is scanty. In this study, we examined the antimicrobial activities of the aqueous extracts from *A. ferox* and *W. somnifera* on *N. gonorrhoea* and *C. albicans*.

## MATERIALS AND METHOD

### Plant collection

The leaves of *A. ferox* and the roots of *W. somnifera* were collected from villages around Alice in the Eastern Cape. According to the traditional healers in the study area, only the roots of *W. somnifera* are used for the treatment of STIs. Voucher specimens (Kambizi Med.2003/1 and Kambizi Med.2003/2) were prepared, properly identified and deposited at the University of Fort Hare Botany Department (Kambizi and Afolayan 2003).

### Preparation of the extracts

Air-dried plant materials (leaves of *A. ferox* and the roots of *W. somnifera*) (50 g each) were pulverised and shaken for 30 min in 300 ml of water and 300ml of methanol respectively (Afolayan and Meyer 1997). The extracts were filtered through Whatman No.1 filter paper and concentrated to dryness under reduced pressure. Each dry extract was later redissolved in its respective solvent, either water or methanol to final concentrations of 0.1, 5.0, 10 and 20 mg/ml (Taylor et al., 1996). Stock solutions (0.1 and 5 mg/ml) of pure aloin, which was previously isolated from *A. ferox* (Kambizi et al., 2004), were similarly prepared.

### Microorganisms

Six clinical isolates of *N. gonorrhoea* were procured from the STI unit of the National Health Laboratory Service in Johannesburg. They were maintained on New York City agar in the laboratory. Nine laboratory strains of *C. albicans* were cultured and maintained on Sabouraud Dextrose agar.

### Sensitivity testing

Sensitivity testing of the two microorganisms to the water and methanol extracts of the plants was carried out on Potato Dextrose agar. 5 ml of the agar was sterilised at 121°C for 20 min in the autoclave and then mixed with the different extracts to make final concentrations of 0.5, 1.0, 5.0, 10 and 20 mg of extract ml<sup>-1</sup> of agar. The agar-extract mixture was then poured into 65 mm Petri dishes

and was allowed to cool and set. All extracts were also plated out on normal Blood agar plates to ensure sterility. The minimum inhibitory concentration (MIC) value was considered as the lowest extract concentration with no visible growth. Blank plates containing only PDA or 2% methanol served as controls (Afolayan and Meyer, 1997).

### Antibacterial testing

*N. gonorrhoea* was cultured anaerobically in Mueller-Hinton broth (Merck, Darmstadt, Germany) at 37°C for 48 h based on the National Committee for Clinical Laboratory Standards (NCCLS) (1997) protocols. The strains were multipoint-inoculated using a MacFarland standard into 5 ml of Mueller-Hinton broth at 37°C. The extract plates were inoculated with 6 strains of *N. gonorrhoea* and were incubated in anaerobic conditions at 37°C. All extracts were plated out on normal Blood Agar plates to ensure sterility. Minimum inhibitory concentrations (MIC) of the compounds were determined after 48 h of incubation. The MIC value was considered as the lowest extract concentration with no visible growth.

### Antifungal testing

Strains of *C. albicans* were cultured and maintained on Sabouraud Dextrose Agar. Pure colonies of *C. albicans* were inoculated onto the prepared extract plates using McFarland standard and incubated for 72 h at 37°C.

## RESULTS AND DISCUSSION

Water extracts from *A. ferox* did not show any activity against *N. gonorrhoea*. The organism is a Gram-negative bacterium which belongs to a group of microbes that have been reported to be more resistant to plant extracts than the Gram-positive organisms (Geyid, 2002). Similar observations were noted by Afolayan et al. (2002) who reported that a pure compound (3,5,7-trihydroxyflavone) isolated from the shoots of *Helichrysum aureonitens* was not active on the following Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Serratia marcescens*. Kambizi and Afolayan (2003) also observed that water; methanol and acetone extracts did not show activity on *S. marcescens*, a gram-negative bacterium. Water extracts from *W. somnifera*, however, exhibited complete inhibition of *N. gonorrhoea* at 10 mg/ml. The ability of water extract from this plant to inhibit the growth of *N. gonorrhoea* may be the reason for its use by the herbalists for the treatment of gonorrhoea. Methanol extracts from *A. ferox* and *W. somnifera* exhibited activity against all the strains of *N. gonorrhoea* at a concentration of 0.5 mg/ml. However, aloin (a pure compound isolated from *A. ferox*) inhibited the bacteria at a much lower concentration of 0.1 mg/ml (Table 1). However, cytotoxicity studies (Kambizi et al., 2007) on aloin have shown that at a concentration of 0.1 mg/ml, the compound does not exhibit any toxic effects in cell culture. Previous work (Kambizi et al., 2004) have shown that other compounds such as aloe emodin and chrysophanol present in the extracts of *A. ferox* are also

**Table 1.** Inhibitory effects of aqueous extracts from *Aloe ferox* and *W. somnifera* on *N. gonorrhoea*.

<i>N. gonorrhoea</i> strains	Minimum inhibitory concentration (mg/ml)				
	<i>A. ferox</i>			<i>W. somnifera</i>	
	Water	Methanol	Aloin	Water	Methanol
1 N	na	0.5	0.1	10.0	0.5
2 N	na	0.5	0.1	10.0	0.5
3 N	na	0.5	0.1	10.0	0.5
4 N	na	0.5	0.1	10.0	0.5
5 N	na	0.5	0.1	10.0	0.5
6 N	na	0.5	0.1	10.0	0.5

na: Not active.

**Table 2.** Inhibitory effects of aqueous extracts from *A. ferox* and *W. somnifera* on *C. albicans*.

<i>C. albicans</i> strains	Minimum inhibitory concentration (mg/ml)				
	<i>A. ferox</i>			<i>W. somnifera</i>	
	Water	Methanol	Aloin	Water	Methanol
1 C	na	na	5.0	na	20.0
2 C	na	na	5.0	na	20.0
3 C	na	na	5.0	na	20.0
4 C	na	na	5.0	na	20.0
5 C	na	na	5.0	na	20.0
6 C	na	na	5.0	na	20.0
7 C	na	na	5.0	na	20.0
8 C	na	na	5.0	na	20.0
9 C	na	na	5.0	na	20.0

na: Not active.

responsible for the antimicrobial activity exhibited.

The antifungal screening showed that aloin was active against all strains of *C. albicans* at a concentration of 5 mg/ml. Water extracts from both plants did not show any activity against *C. albicans*. Methanol extract from *A. ferox* exhibited low activity at a concentration of 20 mg/ml (Table 2). Some researchers (Vahidi et al., 2002; Bonjar 2004, Iwalokun et al., 2004) have reported high resistance of *C. albicans* to plant extracts and hence recommended antifungal testing at concentrations as high as 100 mg/ml. The herbalists in the study area prescribe decoctions (water extracts) to their patients with no upper limit to their concentrations. This may suggest that the high concentration of the extracts administered to patients may be able to cure the disease.

It was important to screen extracts from *A. ferox* and *W. somnifera* against *C. albicans* because many fungal infections have been reported to be the primary cause of mortality in patients with severely impaired host defence mechanisms (Kulberg, 1997). The increase of AIDS-related fungal opportunistic pathogens and the emergence of resistant strains in recent years have lent additional urgency to studies on potential antifungals. In the present study it is of interest to note the correlation

between the claims of herbalists and the demonstrated antimicrobial activity of the extracts from *W. somnifera* on *N. gonorrhoea*. However, further studies are needed to determine whether the plants are effective against candidiasis at higher concentrations.

## ACKNOWLEDGEMENT

This research was supported by the National Research Foundation of South Africa.

## REFERENCES

- Afolayan AJ, Meyer JJM (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. *J. Ethnopharmacol.* 57: 177-181.
- Afolayan AJ, Grierson DS, Kambizi L, Madamombe I, Masika PJ (2002). *In vitro* antifungal activity of some South African medicinal plants. *S. Afri J. Bot.* 68: 72-76.
- Arora S, Dhillon S, Rani G, Nagpal A (2004). The *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. *Fitoterapia* 75: 385-388.
- Bonjar GH (2004). Inhibition of Clotrimazole-resistant *Candida albicans* by plants used in Iranian folkloric medicine. *Fitoterapia* 75: 74-76.
- Capasso F, Borrelli F, Capasso R (1998). Aloe and its therapeutic use. *Phytother. Res.* 12: 124-127.

- Daly C, Maggwa N, Mati JK (1994). Risk factors for gonorrhoea, syphilis, and trichomonas infections among women attending FP clinics in Nairobi, Kenya. *Genitourin. Med.* 70: 155-161.
- de Repentigny L (2004). Animal models in the analysis of *Candida* host-pathogen interactions. *Curr. Op. Microbiol.* 7: 324-329.
- Devi PU (1996). *Withania somnifera* Dunal (Ashwagandha): Potential plant source of a promising drug for cancer chemotherapy and radiosensitization. *Indian J. Exp. Biol.* 34: 927-932.
- Fenton KA, Lowndes CM (2004). European Surveillance of Sexually Transmitted Infections (ESSTI) Network. Recent trends in the epidemiology of sexually transmitted infections in the European Union. *S.T.I.* 80: 255-263.
- Geyid A (2002). A study of surface proteins, other adhesins and iron acquiring mechanisms in the pathogenesis of *Neisseria gonorrhoea* strains isolated from STD patients in Ethiopia. *J. Ethiop. Med. Pract* 4: 14-20.
- Grierson DS, Afolayan AJ (1999). Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. *J. Ethnopharmacol.* 66: 103-106.
- Iwalokun BA, Ogunledun A, Ogbolu O, Bamiro BS, Omojola J (2004). *In Vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and *Candida* species from Nigeria. *J. Med. Food* 7: 327-333.
- Kelly GF (1992). *Sexuality Today: Human Perspective*, The Dushkin Publishing Group, USA, pp. 375-386.
- Kambizi L, Afolayan AJ (2003). Phytomedicinal studies of four selected medicinal plants used for the treatment of STIs in the Eastern Cape. *Fort Hare Papers* 12: 10-24.
- Kambizi L, Sultana N, Afolayan AJ (2004). Bioactive compounds isolated from *Aloe ferox*: A plant traditionally used for the treatment of sexually transmitted infections in the Eastern Cape, South Africa. *J. Pharm. Biol.* 42 (8): 636-639.
- Kambizi L, Goosen BM, Taylor MB, Afolayan AJ (2007). Anti-viral effects of *Aloe ferox* and *Withania somnifera* on herpes simplex virus type 1 in cell culture. *S. Afr. J. Sci.* (In Press).
- Kulberg BJ (1997). Trends in immunotherapy of fungal infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 16: 51-55.
- Lamb DC, Kelly DE, Baldwin BC, Kelly SL (2000). Differential inhibition of Human CYP3A4 and *Candida albicans* CYP51 with azole antifungal agents. *Chemico- Biol. Interact.* 25: 165-175.
- Mariam R, Chacko MD, Kirk Von Sternberg MSW, Velasquez PD (2004). Gonorrhea and chlamydia screening in sexually active young women: the processes of change. *J. Adolesc. Health* 34: 424-427.
- National Committee for Clinical Laboratory Standards (NCCLS) (1997). Performance standards for antimicrobial disk susceptibility tests-Sixth Edition: approved standard M2-A6. NCCLS, Wayne, PA J. Pharmaceut. Res. 1: 33-35.
- Pecere T, Gazzola MV, Mucignat C, Parolin C, Vecchia FD, Cavaggion A, Baso G, Diaspro A, Salvato B, Carli M, Palù G (2000). Aloe emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Res.* 60: 2800-2804.
- Singh S, Kumar S (1998). *Withania somnifera*: The Indian ginseng ashwagandha. Central Institute of Medicinal and Aromatic Plants, p. 293.
- Taylor RSL, Edel F, Manandhar NP, Towers GHN (1996). Antimicrobial activity of Southern Nepalese medicinal plants. *J. Ethnopharmacol* 50: 97-102.
- Vahidi H, Kamalinejad M, Sedaghati M (2002). Antimicrobial properties of *Crocus sativus* L. *Iranian J. Pharm. Res.* 1: 33-35.
- Van Wyk BE, Van Oudtshoorn B, Gercke N (1997). *Medicinal Plants of South Africa*. Briza, Pretoria, pp. 1-304.