Full Length Research Paper

The possibility of using *Sansevieria aethiopica* (Thunb) leaf extracts in combinations with gentamicin for the treatment of oral enterococcal infections

David, O. M. and Afolayan, A. J.*

Department of Botany, Phytomedicine Research Centre, University of Fort Hare, Alice, 5700, South Africa.

Accepted 5 November, 2012

Sansevieria aethiopica (Thunb) has been reported to be used for the treatment of oral infections in Eastern Cape of South Africa. Based on ethnobotanical survey, the plant was selected for the possible synergistic effects of its acetone, ethanol and methanolic extracts with gentamicin on the planktonic and sessile cells of *Enterococcus faecalis* ATCC 29212 and *Enterococcus faecalis* KZN. *In vitro* interactions between the plant extracts and gentamicin were studied using checkerboard macrodilution method and anti-biofilm activity of the iso-effective combinations was determined by semi-quantitative adherence assay. Acetone extract of *S. aethiopica* has the highest inhibitory activity. The minimum concentration of gentamicin that inhibited the two isolates was the same (0.016 mg/ml). Different iso-effective points were observed with fractional inhibitory concentration indexes ranged between 0.375 and 1.9313. Out of the iso-effective points observed only four were synergistic while one was additive. The maximum biofilm reduction was observed when the two antibacterial agents were combined. We therefore suggest that the extracts of the plant at the test concentrations can be used in combination with gentamicin for oral hygiene.

Key words: *Enterococcus faecalis*, *Sansevieria aethiopica*, biofilm, planktonic cell, dental infection, medicinal plant.

INTRODUCTION

The prevalence of dental caries in school aged children is up to 90% and the majority of adults are also affected (Petersen et al., 2005) and chronic diseases like diabetes and HIV have been reported to enhance oral infections (Petersen, 2003). Oral infection is caused by different bacteria out of which *Enterococcus faecalis* is emerging. *E. faecalis* has been mentioned with increased frequency with regard to oral with post-treatment diseases. Enterococci are Gram positive coccus bacterium and a part of the normal flora in the oral cavity and gastrointestinal tract. *E. faecalis* has been reported as the most common bacterium recovered from different oral infections with failed treatment and persistence (Rams et al., 1992; Colombo et al., 2002; Rocas et al., 2004). The pathogen survives in situations where conventional therapy has eliminated other bacteria; this attribute has

resulted in the emergence of drug resistance strain of the bacterium (Sundqvist et al., 1998).

E. faecalis accounts for around 80% of all infections caused by enterococci (Sahm, 2000). Enterococci pose increasing problems in medicine due to an increased resistance to various antibiotics. It account for 10% of the dental infections among healthy individuals (Smyth et al., 1987) and higher rate of 60% was observed among long-term patients (Murray, 1990).

The genus *Enterococcus* has been implicated in 60% of dental infections among school children and 75% of patients with endodontic infection (Gold et al., 1975). Sedgley et al. (2004) reported the presence of *E. faecalis* in 29% of oral rinse samples, 55% of tongue dorsum and 22% of gingival sulcus samples from 41 endodontic subjects. The genus is well known for biofilm formation

*Corresponding author. E-mail: aafolayan@ufh.ac.za. Fax: +27-866-282-295.

which serves as an important reservoir of cells that can repopulate colonized sites hence lead to persistency (Costerton et al., 2011; Li and Yu-mei, 2011). Biofilms are responsible for persistent pathogens on infection sites (Costerone et al., 1999; Anderson et al., 2004; Li and Yu-mei, 2011).

The use of plant for oral hygiene is age long. The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman Empires (Almas and al-Lafi, 1995). It has been documented that medicinal plants confer considerable antibacterial activity against various bacterial pathogens including those responsible for dental infections (Jonathan et al., 2000).

Sansevieria aethiopica (Thunb) is a medicinal plant belonging to the family *Asparagaceae*. It is a perennial shrub with tough, semi-succulent and erected leaves (Van Wyk et al., 2000). The hard, mottled leaves arise from thick fleshy rhizomes and like other members of the genus, it flourishes under low light conditions. *S. aethiopica* is called *isikhokotho*, *isikwendle* or *isitokotoko* in South Africa where it has been long recognized as a remedy for the treatment of dental infections, otitis, ulcers among others (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Newton, 2001; Von Koenen, 2001).

The aim of this study was to investigate the *in vitro* interactions between extracts of *S. aethiopica* and gentamicin and the activities of the resultant iso-effective combinations against biofilm of *E. faecalis* ATCC 29212 and *E. faecalis* KZN.

MATERIALS AND METHODS

Plant material

Plant material was collected in March 2012 from a single population of *S. aethiopica* growing around Alice Township in Nkokobe Municipality, Eastern Cape Province (32°47' S, 26°50' E and altitude 589 m). The species was authenticated by Prof. Donald S. Grierson, University of Fort Hare, Alice, South Africa and a voucher specimen (DavMed, 2012/2) was prepared and deposited in the Giffen Herbarium of the University of Fort Hare, Alice, South Africa for future reference. Plant sample was dried in the oven at the temperature (40°C).

The dried leaves of the plant sample were pulverized. Powdered plant material (40 g each) was separately extracted in acetone, ethanol and methanol for 48 h on an orbital shaker (Stuart Scientific, Manchester, UK). The extracts were filtered through Whatman No. 1 filter paper. The extracts were evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000-efficient, Heidolph, Germany). Individual crude extracts was diluted using 50% dimethylsulphoxide to give 50 mg/mL stock solution (Taylor et al., 1995). This was then diluted to the required concentrations for the bioassay.

Test organisms

Two strains of *E. faecalis* (*E. faecalis* KZN and *E. faecalis* ATCC 29212) used in this study were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. Each

strain was maintained on Nutrient Agar (Biolab, South Africa) plates. The grown cultures were used for preparation of bacterial suspensions in sterile distilled water with densities adjusted to 0.5 McFarland standard.

Antimicrobial susceptibility tests (AST)

The checkerboard method was used to examine the effects of combinations of *S. aethiopica* extract and gentamicin against strains of *E. faecalis*. Twofold serial dilutions of crude extract of *S. aethiopica* and gentamicin were added to make up to final concentrations of 0.0488 to 50.0mg/ml and 1.0 to 64.0 µl/l, respectively in Mueller Hinton broth. The tubes were incubated for 24 h at 37°C. The tube with least concentration and with no sign of growth was taken for MIC of each of the antibacterials and the iso-effective combinations. Fractional inhibitory concentration (FIC) index, which represents the sum of the FICs of each drug tested, where the FIC is determined for each drug by dividing the MIC of each drug when used in combination by the MIC of each drug when used alone.

$$FICI = FIC_{Ext} + FIC_{Gen} = (C_{Ext} \text{ comb}/MIC_{Ext} \text{ alone}) + (C_{Gen} \text{ comb}/MIC_{Gen} \text{ alone})$$

Where, $MIC_{Ext} \text{ alone}$ and $MIC_{Gen} \text{ alone}$ were respectively the MICs of extract and gentamicin when acting alone, and $C_{Ext} \text{ comb}$ and $C_{Gen} \text{ comb}$ are concentrations of extract and gentamicin at the iso-effective combinations respectively. The interpretation of FICI was made as follows: synergistic (<0.5), additive (0.5 to 1.0), indifferent (>1), or antagonistic (>4.0) according to White et al. (1996).

Biofilm eradication test

Mueller Hinton broth (5 ml) was prepared in the test tubes and inoculated with then test organism. The tubes were incubated at 37°C for 6 h after which the MICs of the *S. aethiopica* extracts and the iso-effective combinations were added to separate tubes except the control. The tubes were further incubated aerobically for 24 h at 35°C. After the incubation, the contents of tubes were discarded and the tubes washed three times with sterile distilled water. The remaining attached bacteria were fixed with methanol after 15 min of which the tubes were emptied, air dried and stained with 10 ml of 1.0% crystal violet for 5 min. Excess stain was rinsed off with water. After the plates were air dried, the dye bound to the adherent cells was extracted with 10 ml of 33% (v/v) glacial acetic acid (Merck, Darmstadt, Germany) per tube. The optical density of each tube was measured at 570 nm using UV-3000 PC spectrophotometer (Optima Scientific). The percentage reduction in biofilm was calculated as

$$\% \text{Biofilm reduction} = (A_C - A_T)/A_C \times 100$$

Where, A_C = absorbance of the control, A_T = absorbance of the test

RESULTS AND DISCUSSION

In this study, *S. aethiopica* leaf extracts in combination with gentamicin were screened against the *E. faecalis* strains planktonic and sessile cells. *S. aethiopica* alone and in combinations with gentamicin produced good inhibitory activity on the isolates. The result of MIC study is represented in Table 1. Against *E. faecalis* KZN, methanolic and ethanolic extracts had lower inhibitory activity, compared to acetone extract. The MICs of ethanolic extract of *S. aethiopica* were 0.3906 and 0.1953 mg/ml against *E. faecalis* KZN and *E. faecalis* ATCC 29212 respectively. The minimum concentration of genta-

Table 1. Combined activity of extracts of *S. aethiopica* and gentamicin on planktonic cells of *E. faecalis* strains.

Extract	<i>E. faecalis</i> KZN				<i>E. faecalis</i> ATCC 29212			
	MIC A	MIC B	FICI		MIC A	MIC B	FICI	
			MIN	MAX			MIN	MAX
Ethanollic	0.3906	16	0.625	0.1249	0.1953	16	0.75	1.2499
Methanollic	0.3906	16	0.625	1.1249	3.1250	16	0.375	1.9313
Acetone	6.250	16	0.375	1.0625	0.7812	16	0.625	1.0625

MICs values were expressed in mg/ml.

Table 2. Numbers of the iso-effective combinations and interactions of extracts of *S. aethiopica* and gentamicin on planktonic cells of *E. faecalis* strains.

Test organism	Extract	Iso-effective combinations (n)	Synergy	Addictive	Indifference	Antagonism
<i>E. faecalis</i> KZN	Ethanollic	5	0	0	5	0
	Methanollic	5	0	0	5	0
	Acetone	8	2	0	6	0
<i>E. faecalis</i> ATCC 29212	Ethanollic	5	0	1	4	0
	Methanollic	8	2	0	6	0
	Acetone	6	0	0	6	0

micin that inhibited the two isolates was the same (0.016 mg/ml). Our report is similar to that of Van Wyk and Gericke (2002) and Aliero et al. (2008) that reported that *Sansevieria* spp. are very rich in antimicrobial compounds. The plant has also reported to inhibits bacteria associated with otitis (Hutchings et al., 1996). Philip et al. (2011) reported antibacterial activity of closer member of Genus; *Sansevieria roxburghiana* on common pathogens. They also reported, in agreement with our results, that the methanollic is more active on *E. faecalis* than acetone extract of the plant. The *S. aethiopica* extracts could be good alternatives against enterococcal infections. Enterococcal oral infections are developing resistance against antibiotics and different researchers have suggested alternates to antibiotic treatment of the infections (Rocas et al., 2004; Stuart et al., 2006; Zehnder and Guggenheim, 2009).

The FIC index is the most frequently used method to determine the interaction between antifungal drugs. The interpretation is based on the value got as synergistic interaction of anti-bacterial which has FIC that is less than one. This means that the action is better when in combination than they are separately (Isenberg, 1992). FIC results were provided to allow re-evaluation of the results and also indicate whether the degree of interaction (Eliopoulos and Eliopoulos, 1988). Though bulk of the thirty six iso-effective points observed were indifferent, only four showed synergistic interactions and one was addictive, none was antagonistic as shown in

Table 2. The FIC indexes calculated for different iso-effectives concentrations ranged between 0.375 and 1.9313. The interaction between the combinations of *S. aethiopica* extracts and gentamicin against two strains of *E. faecalis* were predominantly indifference. FICI values greater than 1 were considered as indifference and as the value decreases the interaction tends towards synergism. The FIC index is based on the assumption that neither the *S. aethiopica* extract nor gentamicin would interact with itself, therefore the effect of a self-drug combination will always be additive, with an FIC index of 1. An FIC index lower or higher than 1 indicates synergy or antagonism, respectively, because less or more of either of the extracts of gentamicin would be required in order to produce the same effect as either alone.

As shown in Tables 3 and 4, at the MIC of ethanollic extract, there was reduction in biofilm formed by the two test organisms. The maximum biofilm reduction was observed when two antibacterial agents were combined as shown in Table 5. The inhibitory effect of the extracts (alone) on the biofilm of the two enterococci was noticed to be more pronounced than the gentamicin except for the methanollic extract on the biofilm of *E. faecalis* ATCC 29212. The combinations of extracts and gentamicin achieved inhibition of cells in suspension and eradication of the biofilm of the test bacteria. The recognition that most oral infections developed from a biofilm explains why some dental infections like periodontitis have been

Table 3. Effect of ethanolic extracts of *S. aethiopica* in combination with gentamicin on biofilm of *E. faecalis* strains.

Isolate	Ethanolic extract-gentamicin combination		
	Extract (mg/ml)	Gentamicin (mg/ml)	Biofilm eradication (%)
<i>E. faecalis</i> KZN	0.3906	0	29.31
	0.3906	0.001	12.07
	0.1953	0.002	24.14
	0.1953	0.004	32.76
	0.0977	0.008	15.52
	0.0488	0.016	31.03
	0	16 (MIC)	17.24
	0.1953 MIC	0	32.11
<i>E. faecalis</i> ATCC 29212	0.1953	0.001	35.78
	0.1953	0.002	38.53
	0.0977	0.004	11.01
	0.0977	0.008	21.10
	0.0488	0.016	24.77
	0	0.016 (MIC)	15.60

Table 4. Effect of methanolic extracts of *S. aethiopica* in combination with gentamicin on biofilm of *E. faecalis* strains.

Isolate	Methanolic extract combinations		
	Extract (mg/ml)	Gentamicin (mg/ml)	Biofilm eradication (%)
<i>E. faecalis</i> KZN	0.3906 (MIC)	0	44.68
	0.1953	0.001	46.81
	0.1953	0.002	41.13
	0.0977	0.004	47.52
	0.0488	0.008	46.10
	0.0488	0.008	15.60
	0	(MIC)	15.92
	3.1250 (MIC)	0	33.33
<i>E. faecalis</i> ATCC 29212	1.5625	0.001	38.10
	1.5625	0.002	24.76
	1.5625	0.004	26.67
	0.7813	0.004	21.90
	0.3906	0.004	29.52
	0.1953	0.008	19.05
	0.0977	0.016	8.58
	0.0488	0.016	15.23
	0	16 (MIC)	20.95

reported to be very difficult to prevent and treat (Kishen et al., 2004; Rocas et al., 2004; Arias-Moliz et al., 2009). All the *S. aethiopica* extracts were effective at reducing the *E. faecalis* biofilms at concentrations that will inhibit the

planktonic cells of the pathogens. Gentamicin, like other aminoglycoside antibiotics, affects Gram positive bacteria by production of transferase enzyme and impairment of permeability of cell membrane (Simjee et al., 1999;

Table 5. Effect of acetone extracts of *S. aethiopica* in combination with gentamicin on biofilm of *E. faecalis* strains

Isolate	Acetone extract combination		
	Extract (mg/ml)	Gentamicin (mg/ml)	Biofilm eradication (%)
<i>E. faecalis</i> KZN	6.250 (MIC)	0	34.23
	6.250	0.001	41.18
	3.1250	0.002	32.29
	1.5625	0.004	40.34
	0.7812	0.004	31.09
	0.3906	0.008	32.77
	0.1953	0.016	8.40
	0.0977	0.016	20.17
	0.0488	0.016	35.29
	0	0.016 (MIC)	37.82
<i>E. faecalis</i> ATCC 29212	0.7812 (MIC)	0	26.45
	0.7812	0.001	25.00
	0.7812	0.002	38.02
	0.3906	0.004	42.98
	0.1953	0.008	42.15
	0.0977	0.008	31.40
	0.0488	0.016	33.88
	0	0.016 (MIC)	17.36

Katzung, 2006). It also impaired 30S ribosomal subunit and inhibits protein synthesis by causing misreading of mRNA and complete inhibition of protein synthesis at low and high levels, respectively (Kaye, 2004; Poole, 2005). Medicinal plants have different sites of actions due to the different bioactive compounds (Cushman et al., 1991; Qin et al., 2010). The actions of these antimicrobial must have complemented each other to achieve this activity. The variation in the activities of the combinations may be due to different bioactive compounds present in the *S. aethiopica* extracts as suggested by Gatsing et al. (2010).

The *S. aethiopica* extracts reduced the pre-formed biofilm at varying degrees, though there was no total eradication at the MICs (of both the extracts and gentamicin) and also at the iso-effective concentrations. This is in agreement with the work of George et al. (2005), Arias-Moliz et al., (2009, 2010) and Nett et al., (2008) that reported that higher concentrations than MIC are needed to clear already established biofilm due to the protection offered by the glycoprotein. Pathogens within a biofilm environment behave very differently from free-floating bacteria (Lima et al., 2001; Kishen et al., 2008; Arias-Moliz et al., 2010; Balaei-Gajan et al., 2010). Protective extra-cellular slime matrix makes bacteria extremely resistant to antibiotics, antimicrobial agents and host defense mechanisms (Colombo et al., 2002; Rocas et al., 2004; Rolland et al., 2006; Daneshmehr et al., 2008).

The combinations of the agents were able to break the barrier created by the biofilm. This is necessary before dental pathogen embedded in the biofilm could be eradicated or reduced. The efficacies of the extracts of *S. aethiopica* (singly and in combination with gentamicin) justifies its usage in oral hygiene and also suggests it as an important candidate for the formulation of paste or tincture for oral hygiene and treatment of enterococcal infections. However, isolation and characterization of the active ingredients in this plant together with their mechanisms of actions on pathogens are still open for further investigations.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial supports of the National Research Foundation, South Africa and the University of Fort Hare, Alice 5700, South Africa.

REFERENCES

- Aliero AA, Jimoh FO, Afolayan AJ (2008). Antioxidant and antibacterial properties of *Sansevieria hyacinthoides*. Inter. J. Pure Appl. Sci. 3:103-110.
- Almas K, al-Lafi TR (1995). The natural toothbrush. World Health Forum. 16:206-210.
- Anderson GG, Dodson KW, Hooton TM, Hultgren SJ (2004). Intracellular bacterial communities of uropathogenic *Escherichia coli*

- in urinary tract pathogenesis. *Trends Microbiol.* 12:424-430.
- Arias-Moliz MT, Ferrer-Luque CM, González-Rodríguez MP, Valderrama MJ, Baca P (2010). Eradication of *Enterococcus faecalis* biofilms by cetrimide and chlorhexidine. *J. Endod.* 36:87-90.
- Arias-Moliz, MT, Ferrer-Luque CM, Espigares-García M, Baca P (2009). *Enterococcus faecalis* biofilms eradication by root canal irrigants. *J. Endod.* 35:711-714.
- Balaei-Gajan E, Shirmohammadi A, Abashov R, Agazadeh M, Faramarzi M (2010). Detection of *Enterococcus faecalis* in subgingival biofilm of patients with chronic refractory periodontitis. *Med. Oral Patol. Oral Cir. Bucal.* 15:667-670.
- Colombo AP, Teles RP, Torres MC, Souto R, Rosalém WJ, Mendes MC (2002). Subgingival microbiota of Brazilian subjects with untreated chronic periodontitis. *J. Periodontol.* 73:360-369.
- Costerone JW, Stewart PS, Greenberg EP (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, 284:1318-1322.
- Costerton JW, Veeh R, Shirtliff M (2011). The application of biofilm science to the study and control of chronic bacterial Biofilm is a specific type of persistent infection. *Inter. J. Oral Sci.* 3(2):72.
- Daneshmehr L, Matin K, Nikaido T, Tagami J (2008). Effects of root dentin surface coating with all-in-one adhesive materials on biofilm adherence. *J. Dent.* 36:33-41.
- Gatsing D, Nkeugoapi CFN, Nkahi BFN, Kuate JR, Tchouanguep FM (2010). Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of *Alchornea cordifolia* (Euphorbiaceae). *Int. J. Pharmacol.* 6:173-182.
- George S, Kishen A, Song KP (2005). The role of environmental changes on monospecies biofilm formation on root canal wall by *Enterococcus faecalis*. *J. Endod.*, 31:867-872.
- Gold OG, Jordan HV, van Houte, J (1975). The prevalence of enterococci in the human mouth and their pathogenicity in animal models. *Arch Oral Biol.* 20:473-7.
- Hutchings A, Scott AH, Lewis G, Cunningham AB (1996). Zulu Medicinal Plants-An Inventory. University of Natal Press, Pietermaritzburg, South Africa.
- Jonathan EK, Anna KJ, Johannes VS (2000). Zulu medicinal plants with antibacterial activity. *J. Ethnopharmacol.*, 69:241-6.
- Kishen A, Chen NN, Tan L, Asundi A (2004). Chairside sensor for rapid monitoring of *Enterococcus faecalis* activity. *J. Endod.* 30:872-875.
- Kishen A, Sum CP, Mathew S, Lim CT (2008). Influence of irrigation regimens on the adherence of *Enterococcus faecalis* to root canal dentin. *J. Endod.* 34:850-854.
- Li C, Yu-mei W (2011). The role of bacterial biofilm in persistent infections and control strategies. *Int. J. Oral. Sci.* 3:66-73.
- Lima KC, Fava LR, Siqueira JF Jr (2001). Susceptibilities of *Enterococcus faecalis* biofilms to some antimicrobial medications. *J. Endod.* 27:616-619.
- Murray B (1990). The life and times of the *Enterococcus*. *Clin. Microbiol. Rev.* 3:46-65.
- Nett JE, Guite KM, Ringeisen A, Holoyda KA, Andes DR (2008). Reduced Biocide Susceptibility in *Candida albicans* Biofilms. *Antimicrob. Agents Chemother.* 52(9):3411-3413.
- Newton LE (2001). *Sansevieria*. In: UrsEggli (ed.): *Succulents Encyclopedia*. Volume 1 monocots plants (monocots), Eugen Ulmer, Stuttgart, p. 272.
- Petersen PE (2003). The World Oral Health Report 2003: continuous improvement of oral health in the 21st century-the approach of the WHO Global Oral Health Programme. *Comm. Dent. Oral Epidemiol.* 31:3-24.
- Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C (2005). The global burden of oral diseases and risks to oral health, *Bull. World Health Org.*, 83(9):661-669.
- Philip D, Kaleena PK, Valivittan K, Kumar CPG (2011). Phytochemical Screening and Antimicrobial Activity of *Sansevieria roxburghiana* Schult. and Schult. F. *Middle-East J. Scientific Res.* 10(4):512-518.
- Rams TE, Feik D, Young V, Hammond BF, Slots J (1992). Enterococci in human periodontitis. *Oral Microbiol. Immunol.* 7:249-52.
- Rocas IN, Siqueira JF Jr, Santos KR (2004). Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J. Endod.* 30:315-320.
- Rolland SL, McCabe JF, Robinson C, Walls AW (2006). *In vitro* biofilm formation on the surface of resin-based dentine adhesives. *Eur. J. Oral Sci.* 114:243-249.
- Sahm DF (2000). Antimicrobial resistance among enterococci: a view from U.S. clinical laboratories. First International ASM Conference on Enterococci, Banff, Canada. pp. 26-31.
- Sedgley CM, Lennan SL, Clewell DB (2004). Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiol. Immunol.* 19: 95-101.
- Smyth CJ, Docent MA, Halpenny MK, Ballach SJ (1987). Carriage rates of enterococci in the dental plaque of hemodialysis patients in Dublin. *British J. Oral Maxillofac. Surg.* 25:21-33.
- Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006). *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J. Endod.* 32:93-98.
- Sundqvist G, Figdor D, Persson S, Sjögren U (1998). Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 85:86-93.
- Taylor RSL, Manandhar NP, Towers GHN (1995). Screening of selected medicinal plants of Nepal for antimicrobial activities. *J. Ethnopharmacol.* 46:153-159.
- Van Wyk BV, Gericke N (2002). *People's plants. A guide to useful plants of Southern Africa*. Briza Publications, Pretoria, South Africa, pp. 58-61.
- Van Wyk BV, Gericke N, Van Oudtshoorn B (2000). *Medicinal Plants of South Africa*. Briza Publications. Pretoria, South Africa. p. 274.
- Von Koenen E (2001). *Medicinal, poisonous and edible plants in Namibia*. Klaus Hess Publishers, Windhoek and Göttingen. p. 168
- Watt JM, Breyer-Brandwijk MG (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. Second Edition. E & S Livingstone Ltd. Edinburgh and London. p. 34
- White RL, Burgess DS, Manduru M, Bosso JA (1996). Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. *Antimicrob. Agents Chemother.* 40:1914-1918.
- Zehnder M, Guggenheim B (2009). The mysterious appearance of enterococci in filled root canals. *Int. Endod. J.* 42:277-87.