Global Science Research Journals

ISSN: 0274-3248 Vol. 2 (10), pp. 259-264, October, 2016.

http://www.globalscienceresearchjournals.org/

Global Journal of Medicinal Plants Research

Full Length Research Paper

Efflux pumps inhibitory actions of *E. grandis* vital oil against respiratory tract infectious bacteria

Ahuomafo Matthew*, Olukoye Adeorimi and Adebayor Adewale

Department of Biochemistry and Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa.

Accepted 29 September, 2016

Essential oils obtained by hydrodistillation from the fresh and dry leaves of *Eucalyptus grandis* were analyzed by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The main components of the fresh leaf oil were α-pinene (29.6%), *p*-cymene (19.8%), 1,8-cineole (12.8%) and α-terpineol (6.4%). While, the dry leaf oil had 1,8-cineole (47.4%), limonene (13.3%), α-pinene (7.5%) and spathulenol (7.1%). The antimicrobial activities of the essential oils were tested against respiratory tract infectious microorganisms (*Klebsiella pneumoniae, Staphylococcus aureus* and *Moraxella catarrhalis*) using the microdilution-broth methods. The minimum inhibitory concentration and minimum bactericidal concentration values of the oils ranged between (0.31 to 1.25) mg/ml and (0.63 - >5) mg/ml respectively. The minimum bactericidal concentration values caused the release of cytosolic lactate dehydrogenase (membrane damage) which ranges from 8 to 24% in comparison with Triton X-100. The accumulation of rhodamine 6G in bacterial cells showed that the essential oils were effective as efflux pump inhibitors. The results of this study support the use of the plant in folk medicine.

Key words: *Eucalyptus grandis*, myrtaceae, essential oil, antimicrobial activity, efflux pump, R6G.

INTRODUCTION

Eucalyptus (Myrtaceae), previously native to Australia, now grows in both tropical and subtropical climates round the world. Different species of these plants are known, but *Eucalyptus globulus* is the most studied (Nagpal et al., 2012). In many countries around the world, traditional healers reportedly use the leaves (fresh and dry) of different species of the genus *Eucalyptus* for asthma, cough colds, flu, sore throats, bronchitis, pneumonia, aching, stiffness, neuralgia, and as an antibiotic (Bajpai et al., 2008; Hutchings et al., 2006; Hopkins-Broyles et al., 2004). The dry leaves are also consumed as teas or used in bathing (Chen et al., 2006). Sisay (2010), reported that the essential oils of *E. globules* and *Corymbia citriodora,* which have 70% of their constituent to be 1.8 cineole

*Corresponding author: E-mail: matthew.ahomafo@ymail.com

are used for the management of bronchitis, asthma, catarrh, sinusitis and throat infections. Among the *Eucalyptus* species, *E. grandis* is the most widely distributed in KwaZulu-Natal Province, South Africa. Traditional healers in this region uses both the fresh and dry leaves of *E. grandis* to treat many illnesses such as infections, colds, flu, sore throats, bronchitis, pneumonia, aching, stiffness, neuralgia, and as an antibiotic (Hutchings et al., 2006; Hopkins-Broyles et al., 2004). Bajpai et al. (2008), reported its use as an anti-fungal agent for some skin infections.

Respiratory tract diseases are diseases that affect the air passages (the nasal passages, bronchi and the lungs) and it includes pneumonia, bronchitis, asthma and pulmonary diseases (Nunez et al., 2000; Nester et al., 2001; van Wyk and Wink 2004; lindell et al., 2005). The respiratory pathogens which affect the respiratory tracts that have been observed to trigger asthma attacks include *Crytococcus neoformans*, a fungal infection that causes cough and chest pains (Nunez et al., 2000; lindell et al., 2005). *Klebsiella pneumonia,* a gram negative bacterium also known to cause pneumonia with chills, fever and the development of mucoid sputum, coughing and chest pain (Nester et al., 2001). *Moraxella catarrhalis* is a fastidious and nonmotile gram-negative bacteria that causes lower respiratory infection, leading to otitis coupled with sinusitis, shortness of breath, chronic bronchitis and cough (Nester et al., 2001, van Wyk and Wink, 2004).

Mycobacterium tuberculosis is a bacterium that causes tuberculosis, chronic cough, fever and bloody sputum (Nester et al., 2001) and *Staphylococcus aureus* a bacterium responsible for lower respiratory tract infections like *K. pneumonia* (Nester et al., 2001). Despite the availability of variety of antibiotics, the pathogens are becoming more resistant to currently used drugs. There is a need to develop new antibacterials, either by improving the molecular design of old antibiotics or by developing efflux pump inhibitors (EPIs) (Nikaido, 1996; Zechini and Versace, 2009). EPIs can become active against multidrug resistance (MDR) pumps by binding directly to the pump and blocking it, in a competitive or non-competitive manner (Mahamoud et al., 2007; Lomovskaya and Bostian, 2006). As a result, there is need to find a plant based therapy to manage these pathogens. The aim of this study was to investigate the antimicrobial and efflux pumps inhibitory activities of *E. grandis* essential oil against respiratory tract infectious bacteria.

MATERIALS AND METHODS

Plant material

E. grandis Hill ex Maiden was collected from the Mbakanathubana area of Eshowe, KwaZulu-Natal Province, South Africa. The plant sample was identified at the Department of Botany, University of Zululand, KwaDlangezwa and voucher specimens (OS.01UZ) was deposited at the University Herbarium.

Extraction of essential oils

Fresh and air-dry leaves of *E. grandis* (300 g) were separately subjected to more than three hours of hydrodistillation, using a Clevenger-type apparatus, according to the standard method recommended by the British Pharmacopoeia (1988). The essential oil obtained was dried over anhydrous sodium sulfate, dissolved in methanol and then stored at - 4°C until required.

Gas chromatography (GC)

GC essential oils were carried out using an Agilent Gas Chromatography (7890A) equipped with an Agilent 190915 (30 m × 250 µm × 0.25 µm calibrated). The oven temperature was programmed from 50°C (after 2 min) to 240°C at 5°C/ min and the final temperature was held for 10 min. Injection and detector temperatures were 200°C and 240°C respectively. Hydrogen was the carrier gas at flow rate of 1 ml/min. 0.5 µl of the diluted oil was injected into the GC. Peaks were measured by electronic integration. *n*-Alkanes were run at the same condition for retention indices determination.

Gas chromatography/mass spectrometer (GC/MS)

GC/MS essential oils were carried out using an Agilent Gas Chromatography (7890A) equipped with an Agilent 190915 (30 m × 250 µm × 0.25 µm calibrated) attached with an Agilent mass spectrometer system (5975C VL MSD with Triple Axis Detector). The oven temperature was programmed from 45°C to 310°C. Helium was used as the carrier gas at a flow rate of 5 ml/min with a split ratio of 1:200. The essential oil (1 µl) was diluted in hexane and 0.5 µl of the solution was manually injected into the GC/MS. The chemical compositions of the essential oil of the leaves of *E. grandis* were determined according to their retention time, and spectrometric electronic libraries (WILEY, NIST).

Bacteria strains

Bacteria strains used in the study were collected from the Water Department, Umhlathuze municipality and Nkonjeni Hospital, Nongoma, both in KwaZulu-Natal Province, South Africa. These microbes were *K. pneumonia* (ATCC 31488), *S. aureus* (ATCC 25925) and *M. catarrhalis*. The stock cultures were maintained at 4°C in Müeller-Hinton agar (Oxoid, Germany).

Antimicrobial assay

The minimum inhibitory concentration (MIC) of the essential oils was determined by the method described by Eloff (1998). Nutrient broth (50 µl) was added to all wells of the microtitre plate; 50 µl of the essential oils (10 mg/ml) in 1% DMSO was added to the well in row A and then serially diluted down the rows from row A. The remaining 50 µl was discarded. Bacteria culture (50 µl) of McFarland standard was then added to all the wells and then incubated at 37°C for 24 h. P-iodonitrotetrazolium violet (INT) solution (20 µl of 0.2 mg/ml) was then added to each well and incubated at 37°C for 30 min. The MIC is the lowest concentration at which no visible microbial growth is observed. The minimum bactericidal concentration (MBC) is the lowest concentration of the sample at which inoculated bacterial strains are completely killed. This was confirmed by re-inoculating 10 µl of each culture medium

from the microtiter plates on nutrient agar plates and incubating at 37°C for 24 h. Bacteria treated with ampicillin and neomycin were used as positive controls.

Lactate dehydrogenase (LDH) release assay (Membrane Damage)

The cytosolic lactate dehydrogenase release assay was carried out according to the method described by Korzeniewski and Callewaert, (1983) and modified by Badovinac et al. (2000) and Tadić et al. (2012). The susceptible organisms were grown and incubated with the MBC concentrations of the essential oils overnight. The microbial cultures were then centrifuged (5000 g; 5 min). The supernatant (100 µl) was then mixed with 100 µl of lactic acid dehydrogenase substrate mixture of 54 mM lactic acid, 0.28 mM of phenazinemethosulfate, 0.66 mM p-iodonitrotetrazolium violet and 1.3 mM NAD. The pyruvate-mediated conversion of 2,4-dinitrophenyl-hydrazine into visible hydrazone precipitate was measured on an auto microplate reader (BiotekELx 808) at 492 nm. The total loss of membrane integrity resulting in complete loss of cell viability was determined by lysing the cells of untreated organisms with 3% Triton X-100 and using this sample as a positive control. The cytotoxicity in the LDH release test was calculated using the formula:

$$(E-C) / (T-C) \times 100$$

Where E is the experimental absorbance of the cell cultures, C is the control absorbance of the cell medium, and T is the absorbance corresponding to the maximal (100%) LDH release of Triton X-100 lysed cells (positive control).

Rhodamine 6G uptake

The activities of the essential oils were tested for their MDR inhibition of Rhodamine 6G (R6G) accumulation using the method of Maesaki et al. (1999) with some modifications. Bacteria were cultured overnight at 37°C with shaking (110 rpm). After 24 h, cells were centrifuged at 4000xg for 5 min and washed twice with phosphate buffer saline (PBS, pH 7.2). Cells were centrifuged again and re-suspended at 40 mg/ml in PBS containing 10 mM sodium azide (NaN_3). R6G was added to a final concentration of 10 µM, and cells placed in an incubator for 1 h. Cells were then divided into two aliquots, tube 1 and tube 2. Cells were centrifuged for 5 min at 4000 rpm. Cells in tube 1 were re-suspended in PBS containing 1 M glucose while the cells in tube 2 were re-suspended in PBS alone. Essential oils were then added to the cells containing glucose to a final concentration of 100 µM. Both tubes were then placed in an incubator with agitation for 30 min at 37°C. Cells were centrifuged and the supernatant discarded. The remaining pellet was re-suspended in 0.1M glycine HCl, pH 3 and placed in the shaking incubator overnight. After 24 h, cells were centrifuged for 10 min at 4000xg and the supernatant collected, and absorbance read at 527 nm. The accumulation of the R6G was expressed as percentage accumulation in the cells. The percentage accumulation of R6G inside cells after exposure to glucose, essential oil and standards was calculated using this formula:

$$(1-A_t / A_o) \times 100$$

Where A_t is the absorbance of the test compound, and A_o is the absorbance of the control in the presence of glucose only.

Statistical analysis

The mean and standard error of four experiments for accumulation of R6G inside cells and controls were determined. Statistical analyses of the differences between mean values obtained were calculated using Graphpad Prism 6. Data were subjected to one way analysis of variance (ANOVA). *P* values ≤ 0.05 were regarded as significant and *P* values ≤ 0.01 as very significant.

RESULTS

The chemical composition Table 1 of the essential oil from fresh leaves revealed 31 compounds which were about 99.3% of the essential oil. The most abundant compounds found in the oil were α- pinene (29.6%), p-cymene (19.8%) and 1, 8-cineole (12.8%). In the essential oil from the dry leaves, 13 compounds were identified which together constituted 89.2% of the total oil. The major compounds were 1, 8-cineole (47.4%), limonene (13.3%), α - pinene (7.5%) and spathulenol (7.1%). The minimum inhibitory and minimum bactericidal concentrations of the essential oils from the fresh and dry leaves of *E. grandis* against the microorganisms tested are shown in Table 2. The results obtained form the MIC and MBC for the fresh oil revealed *K. pneumonia* to be the most sensitive microorganism with the lowest MIC and MBC values of (0.31 and 0.63) mg/ml, respectively. While, *S. aureus* (1.25 and 2.5) mg/ml and *M. catarrhalis* (1.25) mg/ml, respectively exhibit high MIC and MBC values for the fresh leaf oil. On the contrary, the dry leaf oil displays highest MIC and MBC values, expect for the MIC of *S. aureus* (1.25) mg/ml. % LDH release in comparison to Triton X-100 presented in Table 2 shows low level release of cytosolic LDH from bacteria cells (8 to 24%). The percentage accumulation of R6G inside cells after exposure to glucose, essential oils and the standard inhibitor (beberine) were summarized in Table 2. While, the accumulation over time were displayed in Table 3. The result shows that R6G was bacteria strain specific as observed in Table 2.

DISCUSSION

Although, drying of plant material has been reported to increase essential oil yields and accelerate distillation by improving the heat transfer (Whish and Willam, 1998). Other advantages of drying include the reduction of microbial growth and the inhibition of some biochemical reactions in the dried materials (Baritaux et al., 1992; Combrinck et al., 2006). Concentrations of various volatile substances, when leaves are dried, have been observed to increase in numerous species of plants and have been attributed to the breakdown of glycosylated forms, dehydration reactions, and oxidation reactions (Moyler, 1994; Bartley and Jacob, 2000) or due to ruptures in plant cells where the volatile compounds are stored. Some compounds also arise from dehydration of oxygenated compounds which could have occurred during the process of drying (Combrinck et al., 2006). It is

Table 1. Chemical composition of essential oils of *E. grandis.*

Compound	KI[a]	KI[b]	Percentage composition	
			Fresh	Dry
α- Pinene	8.10	936	29.6	7.5
Camphene	8.50	950	1.5	-
β- Pinene	9.31	964	-	0.2
β-Myrcene	9.69	993	-	0.3
p-Cymene	10.72	1025	19.8	5.4
Limonene	10.84	1029	3.1	13.3
1,8-cineole	10.92	1031	12.8	47.4
-Terpinene	11.71	1060	2.1	1.5
Terpinolene	12.60	1089	0.3	1.6
Carene	12.90	1148	0.2	-
β-Fenchol	13.35	1122	1.2	-
trans-Pinocarveol	13.73	1139	0.6	-
Camphor	14.11	1146	2.5	-
Sabinyl acetate	14.82	1166	0.5	-
Borneol	14.90	1169	3.4	-
Terpinen-4-ol	15.22	1177	0.8	-
α-Terpineol	15.62	1189	6.4	1.2
cis-Carveol	16.38	1231	0.4	-
Thymol	18.19	1290	0.2	-
Carvacrol	18.63	1299	0.3	-
Terpinyl acetate	19.97	1349	1.6	-
Caryophyllene	21.90	1419	1.8	-
Alloaromadendrene	26.12	1441	0.2	-
γ-Gurjunene	25.53	1447	0.3	-
Viridiflorene	22.83	1497	1.5	-
α-Calacorene	24.87	1546	0.4	-
Spathulenol	25.76	1578	1.4	7.1
Caryophyllene oxide	25.92	1583	1.5	2.7
α-Eudesmol	26.35	1632	0.7	0.3
cis-Cadin-4-en-7-ol	26.65	1637	1.7	-
Epoxy-allo- alloaromadendrene	26.82	1641	1.0	-
Cadine-1,4-diene	26.82	1646	0.4	0.5
Amiteol	27.13	1660	0.1	-
Monoterpene hydrocarbons	-	-	56.6	29.9
Oxygenated monoterpenes	-	-	31.0	48.7
Sesquiterpene hydrocarbons	-		4.9	0.5
Oxygenated sesquiterpenes	-	-	6.8	10.1
Total identified	-	-	99.3	89.2

*Kovats index on a DB-5 column in reference to *n*-alkanes (Adams 1995, 2001). MS, NIST and Wiley libraries spectra and the literature; KI, Kovats index; RT, Retention time.

obvious that the drying process does not only affect the composition of the oils, but the concentration of the components as well. For example, while the concentrations of α-pinene and p-cymene decreased in dry leaf, the concentration of 1,8- cineole and limonene increased in the dry leaf.

However, this study shows that the fresh leaf oil possessed significant antibacterial properties than the dry leaf oil. These compounds have been reported to possess antimicrobial properties (Raju and Maridas, 2011). In addition, readily react with air and heat sources thereby reducing their antimicrobial activity of the dry leaf

Table 2. Antibacterial, cytosolic lactate dehydrogenase assay and R6G inside the cell after exposure to *E. grandis* essential oils and Berberine.

Microorganism	MIC[a]		MBC[b]		Percentage accumulation and release				
					LDH[c]		R6G[d]		Beberine
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	
S. aureus[e]	1.25	1.25	2.5	> 5	11	8	53	11	98
K. pneumonia[e]	0.31	2.5	0.63	> 5	13	24	9	3	74
M. catarrhalis[f]	1.25	2.5	1.25	> 5	11	20	25	4	67

[a]MIC and [b]MBC values are given as mg/ml for essential oils; [c]LDH release (membrane damage) activity % LDH Release in comparison to Triton X-100; [d]Values of drug accumulation in the presence of glucose were taken as the control; [e] - American Type Culture Collection (ATCC), USA; [f] - Clinical isolates.

Table 3. Accumulation of R6G against respiratory tract infectious bacteri[a].

Microorganism	FLEO	DLEO	Plus Glucose	No Glucose	Beberine
S. aureus	2.01 ± 0.50**	2.43 ± 0.75**	0.75 ± 0.20****	4.30 ± 1.00	2.50 ± 0.80**
K. pneumonia	3.24 ± 0.50*	2.30 ± 0.5***	0.89 ± 1.00****	4.75 ± 0.90	2.37 ± 0.60***
M. catarrhalis	2.84 ± 1.00	2.22 ± 1.00*	1.38 ± 0.50**	4.00 ± 0.50	2.75 ± 1.00

[a](n = 3, mean ± S.D); FLEO – fresh leaf essential oil; DLEO – dry leaf essential oil.

(Abdelmajeed et al., 2012).

The low levels of cytosolic LDH released (8 to 24%, Table 2) does suggest that, microbial cell membrane damage contributes very little to microbial death. Living cells (including bacteria) have mechanisms that expel toxic substances. These systems (such as resistant-nodulation-division pump) are mostly found in bacteria in which a pump structure (efflux pump) is anchored to the inner membrane to release noxious substances, including antibiotics which are aimed to kill the bacteria (Wexler, 2012). The efflux pump confers bacterial resistance (Amusan et al., 2007). It is therefore important that new antibiotics, specifically efflux pump inhibitors (EPIs) are developed to reduce the emergence of multidrug resistance (MDR). In this study, the essential oils of *E. grandis* were able to increase the accumulation of rhodamine 6G inside bacterial cells, which revealed that the essential oils can apparently be used as efflux pump inhibitors.

The R6G absorption was greater for *K. pneumoniae* and *M. catarrhalis* than *S. aureus* in the presence of the essential oil for the fresh leaves. On the other hand, *S. aureus* had a high accumulation with the dry leaf oil. The uptake of R6G by oil of the fresh leaf was even higher than that of the standard used, while for *Staphylococcus aureus,* the essential oils from the dry leaves were more effective, which showed that plants extracts increase R6G concentration. It is also noted that *S. aureus* had the highest percentage accumulation than the other organisms (Table 3), because gram positive organism have a single layer of cell wall (which make them to be more susceptible to antibiotics) than gram negative which have a double membrane, making them less susceptible to antimicrobial agents (Kaur and Arora, 2009).

Conclusion

The essential oils of both the fresh and dry leaves of *E. grandis* showed considerable activities against respiratory tract bacteria. Various respiratory pathogens affect the respiratory tracts which lead to oxidative stress which in turn triggers asthmatic attack (Soyingbe et al. 2013) by destroying the membrane integrity of the bacteria and also blocking the efflux pump mechanism of the bacteria. It is concluded that the essential oils could be used as part of the existing anti-asthma therapy, and also justify its rationale use by traditional healer in the treatment of asthma.

ACKNOWLEDGEMENTS

The authors are grateful to the University of Zululand Research Committee for their financial support.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

Abdelmajeed NA, Danial EN, Ayad HS (2013). The effect of environmental stress on qualitative and quantitative essential oil of

aromatic and medicinal plants. Arch. Sci. 66:100-120.
Amusan OOG, Sukati NA, Dlamini PS, Sibandze FG (2007). Some Swazi Phytomedicine and their constituents. Afr. J. Biotechnol. 6:267-272.
Badovinac V, Trajkovic V, Mostarica-Stojkovic M (2000). Nitric Oxide Promotes Growth and Major Histocompati- bility Complex-Unrestricted Cytotoxicity of Interleukin- 2-activated Rat lymphocytes. Scand. J. Immunol. 52:62-70.
Bajpai VK, Shukla S, Kang SC (2008). Chemical composition and antifungal activity of essential oil and various extract of *Silenearmeria* L. Bioresour. Technol. 99:8903-8908.
Baritaux O, Richard H, Touche J, Derbesy M (1992). Effects of drying and storage of herbs and species on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour Frag. J. 7:267-271.
Bartley JP, Jacob AL (2000). Effect of drying on flavour compounds in Australian grown ginger *Zingiber officinale.* J. Sci. Food Agric. 30:209-215.
British Pharmacopoeia (1988). Part 2: HMSO, London. pp. 109-110.
Chen ZZ, Ho CK, Ahn IS, Chiang VL (2006). *Eucalyptus*. Methods Mol. Biol. 344:125-34.
Combrinck S, Bosman AA, Botha BM, Plooy WD (2006). Effect of post-harvest drying on the essential oil and glandular trichomes of *Lippia scaberrima Sond.* J. Essent. Oil Res. 18:80-84.
Eloff JN (1998). A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64:711-713.
Hopkins-Broyles D, Rieger Y, Grim A, Nihill D, Jones M, Damiano R, Warren DK, Fraser VJ (2004). Risk Factors for *Staphylococcus aureus* Colonization in a Cardiac Surgery Population. Am. J. Infect. Control 32:119.
Hutchings A, Scott AH, Lewis G, Cunningham A (1996). Zulu medicinal plants: An Inventory. University of Natal Press, Scottsville, South Africa.
Kaur GJ, Arora DS (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgure* and *Trachysprmum ammi*. BMC Complement. Altern. Med. 9:30.
Korzeniewski C, Callewaert DM (1983). An enzyme- release assay for natural cytotoxicity. J. Immunol. Method 64:313-320.
Lomovskaya O, Bostian KA, (2006). Practical application and feasibility of efflux pump inhibitors in the clinic-A vision for applied use. Biochem. Pharmacol. 71:910-918.
Maesaki S, Marichal P, Bossche HV, Sangland D, Kohno S (1999). Rhodamine 6G efflux for the detection of CDR1-overrxpressing azole-resistant *Candida albicans* strains. J. Antimicrob. Chemother. 44:27-31.
Mahamoud A, Chevalier J, Alibert-Franco S, Kern WV, Pagès JM (2007). Antibiotic efflux pump in gram negative bacteria: the inhibitory response strategy. J. Antimicrob. Chemother. 59:1223-1229.
Moyler DA (1994). Spices-recent advances in Charalambous. Spices herbs and edible fungi. Amsterdam: Elseviers. pp. 1-70.
Nagpal N, Shah G, Arora MN, Shri R, Arya Y (2010). Phytochemical and pharmacological aspect of *Eucalyptus* genus. Int. J. Pharm. Sci. Res.1:28-36.
Nester EW, Anderson DG, Robert CE, Pearsall NN, Nester MT (2001). Microbiology: A Human perspective, 3rd ed. McGraw, New York, U.S.A.
Nikaido H (1996). Multidrug efflux pumps of gram-negative bacteria. J. Bacteriol. 178:5853-5859.
Raju G, Maridas M (2011). Composition antifungal and cytotoxic activities of essential oils of *Piper barberi* fruits. Int. J. Biol. Technol. 2:100-110.
Sisay F, Gil LT, Tolosana WE, López R (2010). *Eucalyptus* Species Management, History, Status and Trends in Ethiopia. Proceedings from the Congress, Addis Ababa. pp. 62-68.
Soyingbe OS, Oyedeji A, Basson AK, Opoku AR (2013). The essential Oil of *Eucalyptus grandis* W. Hill ex Maiden inhibits microbial growth by inducing membrane damage. Chin. Med. 4:7-14.
Tadić VM, Jeremic I, Dobric S, Isakovic A, Markovic I, Trajkovic V, Bojovic D, Arsic I (2012) . Anti-Inflammatory, gastroprotective and cytotoxic effect of *Sideritis scardica* extracts. Planta Med. 78:415-427.
Van Wyk B, Wink M (2004). Medicinal plants of the world. Briza Publications, South Africa.
Wexler HM (2012). Pump it up: Occurrence and regulation of multi-drug efflux pumps in *Bacteriodes fragilis*. J. Mole. Boil. Gen. Biotechnol. 18:200-208.
Whish JPM, Willam RR (1998). Effects of post -harvest drying on the yield of the tea tree oil *Melaleuca alterriba*. J. Essent. Oil Res. 8:47-51.
Zechini B, Versace I (2009). Inhibitors of Multidrug Resistant Efflux Systems in Bacteria. Recent Pat. Antiinfect. Drug Discov. 4(1):37-50.