Quality assessment of essential oils of *Eucalyptus globulus* and *Boswellia rivae*

Mirtachew Tihar[1*] and Ermias Dagne[2]

[1]Department of Chemistry, College of science, Bahir Dar University, P.O. Box: 79, E-mail:mfedlu94@gmail.com phone No.: +251918022046

[2]African Laboratory for Natural Products (ALANP), Department of Chemistry, Addis Ababa University, P.O. Box 30270, Addis Ababa Ethiopia. E-mail: edagne@gmail.com, Phone No.: +251911645303

ABSTRACT

In the course of this study, essential oils of *Eucalyptus globulus* and three *Boswellia rivae* species were analyzed using GC-MS. Comparison of the chemical compositions of 1,8-cineole and α-pinene in the assessment of these oils' quality will help in the production of high value essential oils that will enhance the economic condition of the nation. The *Eucalyptus* oil samples were collected from commercial distillers in Shashemene, Addis Ababa and Bonga. The *Boswellia* species studied were *B. rivae* from Ethiopia, *B. sacra* (Beeyo) and *B. frereana* (Meydi) from Somalia. The analysis of oil samples of *E. globulus* showed that the oil samples obtained from Shashemene and Addis Ababa had high content of 1,8-cineole (70%) and 17% and 12% α-pinene content , respectively. But when compared to the standard South African oil sample, they showed lower concentration of 1,8-cineole (*ca* 86% vs. 70%). However, the oil from Bonga exhibited relatively higher α-pinene content (20%). Two species of *B. sacra* (synonym *B. carteri*) and *B. frereana*, occurring in Somalia are well known worldwide as the source of high quality frankincense. In this study, Ogaden etan-2 and Ogaden etan-3 showed a close resemblance to some extent with better quality essential oils of *B. sacra and B. frereana* species of Somalia.

Key words: *Quality Assessment, GC-MS, Eucalyptus globulus, 1,8-cineole, α-pinene, Boswellia rivae*

INTRODUCTION

Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from various parts of the plant, including leaves, flowers, seeds, rhizomes, roots and barks. It is usually obtained by water and steam distillation. Today, essential oils are used as perfumes, flavors and for medicinal purposes. The trend to use essential oils or essential oil-containing plants in foods, which may act as natural antimicrobials or antioxidant preservatives, may also influence health of consumers as well as prolong the shelf-life of relevant food products (Tuba *et al.*, 2012).

*Corresponding author

The quality of essential oils can be negatively affected by the use of pesticides and other chemicals, the variability in altitude, soil conditions and rainfall and the difficulty of differentiating plant species and varieties (Jason *et al.*, 2007). Quality control deals with the identification and quantification of chemical constituents in essential oils. For the analysis of volatile compounds in herbal medicines, Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS) are widely used (Mi-Hee *et al.*, 2011).

Eucalyptus globulus belongs to Myrtaceae family which comprises about 900 species and sub-species. The genus *Eucalyptus* is native to Australia but it is presently spreading all over the world. Essential oils of *Eucalyptus* are widely used as spices, flavors, perfumes, industrial raw materials and in pharmaceutical applications (Kanko *et al.*, 2012). The essential oil of *E. globulus* is known to have 1,8-cineole (45.4%), limonene (17.8%), *p*-cymene (9.5%), γ-terpinene (8.8%), α-pinene (4.2%) and α-terpineol (3.6%) as major components (Amit and Anushree, 2011).

In order to use pure individual *E. globulus* essential oil for pharmaceutical applications, the minimum 1,8-cineole content should be 70% (Ashwin and Saiful Nizam, 2009). The essential oils of the leaves of *E. globulus* grown in Morocco contained 1,8-cineole (72.8%) as a major component (Abera and Ermias, 1997). *E. globulus* oil with cineole as a major component is added to wine to enhance its flavor (Anthony *et al.*, 2009). 1,8-Cineole which is also known as eucalyptol exhibited a strong insecticidal activity against housefly (Peeyush *et al.*, 2012). It is used as a preservative for the enhancement of shelf-life of food due to its antifungal and aflatoxin inhibition activity (Shukla *et al.*, 2012).

Frankincense which belongs to the genus *Boswellia*, family Burseraceae, includes approximately 23 species of small trees that grow mainly in Arabia, on the eastern coast of Africa and in India (Sandrine *et al.*, 2005). Ethiopia is well endowed with various species of *Acacia*, *Boswellia* and *Commiphora* that are known sources for economically valuable products, principally oleo-gum resins such as gum acacia, frankincense, and myrrh (Mulugeta and Demel, 2003).The essential oil of frankincense is one of the most commonly known essential oils that are used in aromatherapy, treatment of asthma patients and against respiration disorders. The essential oil of *Boswellia rivae* contains α-thujene (2.9%), α-pinene (16.7%), *o*-cymene (3.9%), Δ-3-carene (17.3%), *p*-cymene (3.2%) and limonene (21.1%) (Melese and Ermias, 2007). The resin which has a woody, spicy smell is usually steam distilled to afford one of the most important commercial essential oils available on the international market (Vuuren *et al.*, 2010).

The purpose of this study was to assess the chemical constituents of essential oils of white eucalypt and different species of frankincense to assess their quality and to investigate whether these oils meet the quality standard.

MATERIALS AND METHODS

Plant Materials

E. globulus and *B. rivae*, *B. sacra* and *B. frereana* essential oil samples were obtained from Distillers in Ethiopia, South Africa and Somalia. Essential oil samples of *E. globulus* were obtained from Distillers in Ethiopia, particularly, Shashemene, Addis Ababa and Bonga and typical commercial essential oil of *E. globulus* from South Africa. Essential oil samples of *B. rivae* from Ethiopia *and B. sacra* and *B. frereana* from Somalia were also brought from local markets.

Chemicals and Instruments

Dichloromethane, chloroform, ethanol, sodium sulphate, clevenger apparatus, separatory funnel, rotary evaporator, refrigerator, and HP 5890 Gas Chromatograph- 307 Mass Spectrometry (GC-MS).

Extraction of Essential Oils

Powdered leaves and gum resins of *E. globulus* and *B. rivae* that give essential oils were hydrodistilled using Clevenger apparatus for 1.5 h. The oils from the distillate were extracted with dichloromethane and separated using a separatory funnel. The dichloromethane extract was dried over anhydrous sodium sulphate, filtered and concentrated to afford oils.

Chemical Composition of Essential oils

Gas Chromatographic-Mass Spectrometry (GC-MS) analysis

The essential oil samples analyses were done using 5890 Hp GC-307 MS instrument coupled with an auto sampler equipped with a fused silica capillary column (50 m x 0.25 mm, film thickness 0.25 mm) and the MS operating conditions were: ionization voltage 70 eV, ion source of 240^{o}C. The oven temperature was programmed from 50^{0}C up to 240^{o}C at 3^{0}C/min. and helium was used as carrier gas. The compounds percentage composition of compounds for the oil samples was found out by the chemstation software of GC-MS. Peak identification was carried out by comparison of the mass spectra with mass spectra available on data base of Wiley Library.

RESULTS

Five oil samples extracted from *E. globulus* leaves in Shashemene private distiller company were analyzed using GC-MS. The results obtained from the GC-MS chromatograms indicated the presence of five monoterpenes, namely, α-pinene, β-pinene, *p*-cymene, limonene, and 1,8-cineole. The α-pinene percentage composition of each of the five oil samples of *E. globulus* collected from Shashemene were in the range of 14 to 20%. It was found out that the 1,8-cineole content of the five oil samples was in the range of 68-73%. Among the six batch oil samples of Shashemene batch 6 exhibited the highest 1,8-cineole content (77%). This enhancement in 1,8-cineole content by 8% from the lowest value oil was observed after subjecting Shashemene batch 5 oil to rectification and it was labeled as Shashemene batch 6. The results obtained are summarized in Table 1.

Table 1. Chemical composition of *E. globulus* leaf oil samples collected from Shashemene.

No	Sample code	% α-pinene	% β-pinene	% p-cymene	% limonene	% 1,8-cineole
1	Shashemene-1	16	0.3	-	2	70
2	Shashemene-2	14	0.2	-	2	73
3	Shashemene-3	20	0.3	0.1	-	68
4	Shashemene-4	18	0.2	0.1	-	70
5	Shashemene-5	19	0.3	-	-	69
6	Shashemene-6	11	0.2	-	-	77

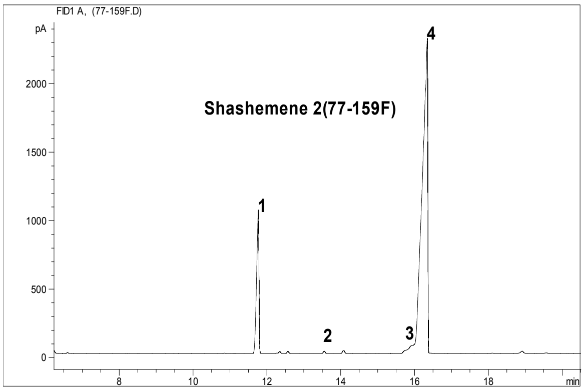

Figure 1 GC-MS chromatogram of oil sample of *E. globulus* collected from Shashemene

The Eucalyptus oil obtained from shashemene distiller showed high content of 1,8-cineole and it was shown in Figure 1.

Two essential oil samples of *Eucalyptus* that were collected at different times from distillers in Addis Ababa were analyzed and compared. The α-pinene content of Addis-1 and 2 oil samples of *Eucalyptus* were 5% and 22%, respectively. In the same fashion, the composition of 1,8-cineole of the above two oil samples were found to be 80% and 72%, respectively. The summary of the results obtained is indicated in Table 2.

Table 2. Chemical composition of oil samples of *E. globulus* from Addis Ababa

Sample Name	% α-pinene	% β-pinene	% *p*-cymene	% limonene	% 1,8-cineole
Addis-1	5	-	1	-	80
Addis-2	22	0.4	-	-	72

The GC-MS chromatograms of Addis-1 and Addis-2 exhibited high composition of 1,8-cineole and it was shown in Figure 2.

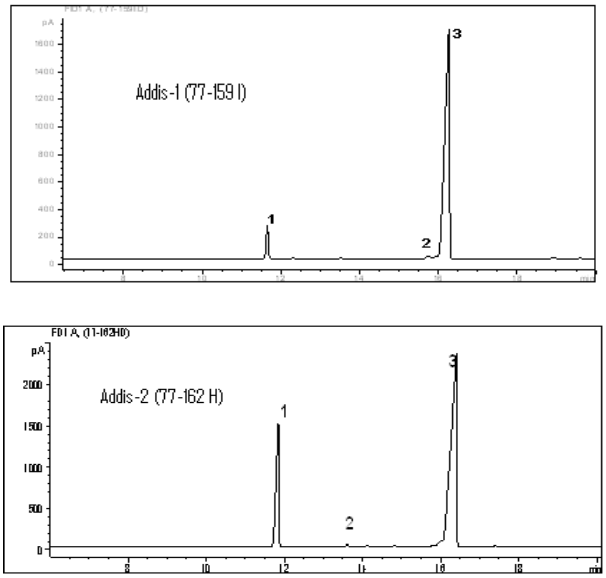

Figure 2 GC-MS Chromatograms of *E. globulus* oil samples from Addis Ababa

The chemical compositions of eleven oil samples of *E. globulus* collected from Bonga at different times of the year were analyzed using GC-MS. The 1,8-cineole content of 11 oil samples of *E. globulus* collected from Bonga was found in the range of 61 to 73%. The summary of the chemical constituents of these oil samples is shown in Table 3.

In this work, six essential oil samples of *Boswellia* species which were obtained from distillers in Ethiopia and Somalia were studied. These were *B. rivae* (Ogaden etan) from Ethiopia, *B. sacra* (Beeyo) and *B. frereana* (Meydi) from Somalia.

When the Ogaden frankincense (etan-Amharic) oil which was labeled as Ogaden etan-1 was compared to the oil samples analyzed, it contained the lowest α-pinene content (39%). However, it exhibited the highest percent composition of Δ-3-carene (14%). Comparison of the chemical constituents of oil samples of Ogaden etan-2 and Ogaden etan-3 oil samples showed much closer α-pinene and limonene percent composition with the better known *B. sacra* and *B. frereana* oils from Somalia which were labeled as Somalia etan-2 (Beeyo) and Somalia etan-3 (Meydi), respectively. The chemical analysis of the above oil samples are summarized in Table 4.

Table 3. Chemical composition of oil samples of *E. globulus* obtained from Bonga

No	Sample Code	% α-pinene	% β-pinene	% *p*-cymene	% limonene	% 1,8-cineole
1	Bonga-1	31	0.8	0.2	-	62
2	Bonga-2	31	0.8	-	-	62
3	Bonga-3	29	0.7	-	-	64
4	Bonga-4	31	0.5	-	-	62
5	Bonga-5	29	-	0.1	0.3	61
6	Bonga-6	4	-	-	-	66
7	Bonga-7	6	0.1	0.1	1.8	70
8	Bonga-8	3	-	-	1.5	71
9	Bonga-9	0.2	-	-	1.5	73
10	Bonga-10	27	0.8	-	-	67
11	Bonga-11	30	0.8	-	-	63

DISCUSSION

A comparison was made regarding the content of monoterpenes in oil samples of *Eucalyptus* that were collected from Shashemene, Addis Ababa and Bonga. Those essential oils collected from the three different places in Ethiopia possess a minor composition of *β*-pinene, *p*-cymene and limonene. The oil samples of *E. globulus* obtained from Shashemene contained 1,8-cineole as major component and had an average content of 70%. Similarly, the average

Table 4. Chemical constituents of oil samples of *Boswellia* species from Ethiopia and Somalia

No	Sample Code	% α-thujene	% α-pinene	% β-pinene	% Δ-3-carene	% *p*-cymene	% limonene
1	Ogaden etan-1	3	39	6	14	7	12
2	Ogaden etan-2	1	54	10	9	4	7
3	Ogaden etan-3	2	56	11	8	3	5
4	Somalia etan1(Meydi)	1.0	52.0	10.0	-	-	6.0
5	Somalia etan 2 (Beeyo)	7.0	54.0	2.0	-	-	11
6	Somalia etan 3 (Meydi)	18.0	61.0	3.2	-	-	15

*Ogaden etan 1, 2 and 3 describes the *B. rivae* commercial frankincense essential oil samples while Somalia etan 1, 2 and 3 refer to known somalian *B. freareana* and *B. sacra* essential oil samples.

1,8-cineole contents of oil samples of *Eucalyptus* obtained from Addis Ababa were found to be 70% . This result showed that the oil samples from Addis Ababa had remarkable similarities in both α-pinene and 1,8-cineole content values with the oil samples collected from Shashemene. However, the eucalyptus oil of Bonga exhibited a lower average content of 1,8-cineole (66%).

In this preliminary assessment of the quality of *E. globulus* oil samples, the distillers were reluctant to give genuine information about the nature of these samples such as the way the samples are harvested, stored prior to distillation, the length of time that passes between when the samples were harvested and when they are distilled into an essential oils, the exact part of the plants used in the distillation of the oil, etc.

In general, oil samples of *Eucalyptus* collected from Shashemene and Addis Ababa showed similar 1,8-cineole content. *E. globulus* oils that have a higher value of 1,8-cineole enjoys a huge demand in the international market for pharmaceutical applications (Bachheti *et al*., 2011).

As many scholars indicated, the minimum percentage of 1,8-cineole required for high pharmaceutical grade is 70% (Ashwin and Saiful Nizam, 2009). The overall result obtained indicated that the *E. globulus* oil samples collected from Shashemene and Addis Ababa likely satisfy the minimum requirement to be used as a high grade raw material for different pharmaceutical applications. But, they exhibited lower resemblance in the 1,8-cineole composition compared to the typical high grade standard oil of South Africa(*ca* 86 vs. 70%). However, it is difficult to conclude that the essential oil of *E. globulus* distilled in Ethiopia is competent in the international market for pharmaceutical applications. This is due to the inconsistency in chemical constituents of the oil samples analyzed in the three areas of Ethiopia. The oil samples collected from Bonga had exhibited relatively lower 1,8-cineole content . Rectification could be used in order to enhance the 1,8-cineole content so that some unnecessary impurities will be removed.

The chemical constituents of oil sample of *B. frereana* which was labeled as Somalia etan-1 (Meydi) and Ogaden etan-2 were also compared in terms of their percent monoterpene contents. They exhibited the highest β-pinene content (10%). They were also found to have very similar content in other monoterpenes which were analyzed except in Δ-3-carene and *p*-cymene.

Two species of *B. sacra* (synonym *B. carteri*) and *B. frereana*, occurring in Somalia are well known worldwide as the source of high quality frankincense (Melese and Dagne, 2007). These species of Somalian frankincense trees are locally known as Beeyo and Meydi, respectively.

CONCLUSIONS

The oils that were collected from Shashemene and Addis Ababa showed higher content of 1,8-cineole (70%) as a principal constituent. Thus, the *eucalyptus* oil samples from Addis Ababa and Shashemene could likely meet the commercially acceptable quality standard. Essential oil sample of Ogaden etan-1 exhibited the lowest content of α-pinene (39%) when compared to both the oils of *B. rivae* of Ethiopia and *Boswellia* species of Somalia while it showed the highest percent content of Δ-3-carene (14%). Thus, Ogaden etan-2 and Ogaden etan-3 oil samples likely have comparable quality to some extent with the known Somalian oils analyzed.

ACKNOWLEDGEMENTS

The authors would like to thank International Science Program (ISP), Sweden, for financial assistance used to purchase chemicals, reagents and instruments used in this work. Mr. Mirtachew Tihar is indepted to Ministry of Education for its financial support.

REFERENCES

Abera, A and Dagne, E. (1997). Essential oils of three *Eucalyptus* species acclimatized in Ethiopia. *Bulletin of Chemical Society of Ethiopia* **11** (1): 47-50.

Ahmed, A and Salim, A. (2008). Analysis of the essential oil from botanically certified oleo gum resin of *Boswellia sacra* (Omani Luban). *Molecules* **13:** 2181-2189.

Akolade, J.O., Olajide, O.O., Afolayan, Akande, M.O., Idowu, S.A., D.I and Orishadipe, A.T. (2012). Chemical composition, antioxidant and cytotoxic effects of Eucalyptus globulus grown in north-central Nigeria. *Journal of Natural Product Plant Resource* **2** (1): 1-8.

Alfreda, W and Takayuki, S. (2010). Medicinal activities of essential oils: Role in disease prevention, Bioactive foods in promoting health. F*ruits and Vegetables* **6**: 60-71.

Amit, K.T. and Anushree, M. (2011). Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapor phase against food spoilage microorganisms. *Food Chemistry* **126**: 228–235.

Anthony, J.S., Jennifer, B and Hardie, W,J. (2009). Consumer rejection threshold for 1,8-cineole (eucalyptol) in Australian red wine. *Food Quality and Preference*, **20**: 500–504.

Ashwin, C.B and Saiful, N. (2009). Extraction of the essential oil of *Aquilaria malaccensis* using hydro distillation and solvent extraction methods, MSc Thesis, University of Malaysia Pahang, Department of chemical and natural resources engineering, 1-24.

Bachheti, R.K., Joshi, A and Singh, A. (2011). Oil Content variation and Antimicrobial activity of *Eucalyptus* leaves oils of three different Species of Dehradun, Uttarakhand, India. *International Journal of Chemical Technology Research* **3** (2): 625-628.

Baser, K.H., Demirci, B., Dekebo, A and Dagne, E. (2003). Essential oils of some *Boswellia* spp., myrrh and opopanax. *Journal of Flavour Fragrance* **18**: 153–156.

Garikapati, D., Kiran, B and Bikram, S. (2009). Simulation of *Eucalyptus cinerea* oil distillation: a study on optimization of 1,8-cineole production. *Biochemical Engineering Journal* **44:** 226–231.

Jason, Q.D., Christine, A.C and Ian, E.W. (2007). Examination of the consistency of plant traits driving oil yield and quality in short-rotation coppice cultivation of *Eucalyptus polybractea.Forest Ecology and Management* **250**: 196–205.

Jossana, P.S., Geíza, A.A., Rayanne, A.T., Margarida, A.S., Maria, L.C. and Evandro, L.S. (2012). Synergies of carvacrol and 1,8-cineole to inhibit bacteria associated with minimally processed vegetables *International Journal of Food Microbiology* **154:** 145–151.

Kanko, C., Koné, S., Ramiarantsoa, H., Tue, B., Chalchat, J.C., Chalard, P., Figueredo, G and Ahibo-Coffy, A. (2012). Monoterpene hydrocarbons, major components of the dried leaves essential oils of five species of the genus *Eucalyptus* from Côte d'Ivoire *Natural Science.* **4:** 106-111.

Kumar, A. (1998). Antibacterial properties of some *Eucalyptus* oils. *Fitoterapia* **59**: 141-144.

Luisa, S., Paola, D., Ivana, B. and Luigi, M. (2012). Authenticity control on lemon essential oils employing Gas Chromatography–Combustion-Isotope Ratio Mass Spectrometry (GC–C-IRMS). *Food Chemistry* **131:** 1523–1530.

Maciel, M.V., Morais, S.M., Bevilaqua, C.M., Silva, R.A., Barros, R.S., Sousa, R.N., Sousa, L.C., Brito, E.S and Souza-Neto, M. (2010). Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. *Veterinary Parasitology* **167**: 1–7.

Mahmoud, M.S., Weijuan, W., Amy, C., Fadee, G.M., Kar-Ming, F., Pin-Tsen, S., Yu-Ting, F., Cole, W., Gary, Y and Hsueh-Kung, L. (2011). *Boswellia sacra* essential oil induces tumor cell specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. *Complementary and Alternative Medicine* **11:** 121-129.

Malgorzata, B., Hartwig, S., Angela, W., Petra, R., Rolf, Q., Gerd, L and Jurgen, P. (2006). Investigation of *eucalyptus* essential oil by using vibrational spectroscopy methods. *Vibrational Spectroscopy* **42**: 341–345.

Melese, A and Dagne, E. (2007). Phytochemical investigation of *Boswellia* species collected from Kebtele area in Agew-Awi (Gojjam), MSc Thesis, Addis Ababa University, Department of chemistry, 1-53.

Mi-Hee, C., Eun, K.K., Se, M.Y., Myoung, S.Oand Ki-Young, M. (2011). GC/MS Combined with chemometrics methods for quality control of Schizonepeta tenuifolia Briq: Determination of essential oils. *Microchemical Journal* **97:** 274-281.

Mohamed, R.S and Mansoori, G.A. (2002). The uses of supercritical fluid extraction technology in food processing. *Food Technology Magazine* **14**: 1-15.

Mulugeta, L and Demel, T. (2003). Frankincense and myrrh resources of Ethiopia: medicinal and industrial uses. *SINET: Ethiopian Journal of Science* **26** (2): 161–172.

Mulyaningsiha, S., Sporera, F., Stefan, Z., Jürgen, S., Reichlinga, J and Winka, M. (2010). Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine***17**: 1061–1066.

Peeyush, K., Sapna, M., Anushree, M and Santosh, S. (2012). Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (*Musca domestica*). *Acta Tropica* **122**: 212– 218.

Prapassorn, R., Uraiwan, D., Udomlak, S., Srunya, V., Vichai, H., Potechman, P. and Putthita, P. (2009). Extraction methods for tuberose oil and their chemical componenet. *Journal of*

Natural Science **43**: 204-211.

Rapper, S., Van Vuuren, K.S., Viljoen, A.M and Dagne, E. (2012). The additive and synergistic antimicrobial effects of select frankincense and myrrh oils – a combination from the pharaonic pharmacopoeia. *Letters in Applied Microbiology* **54**: 352–358.

Salim, A., Rameshkumarb, K.B., Abdulkhader, H., Nallusamy, S and Salma, A. (2012). Composition and antibacterial activity of the essential oils of four commercial grades of Omani Luban, the Oleo-gum resin of *Boswellia sacra* Flueck. *Chemistry and Biodiversity* **9**: 615-624.

Sandrine, H., Jean, B., Jacques, C. and Alain, T. (2005). A chemical investigation by headspace SPME and GC–MS of volatile and semi-volatile terpenes in various olibanum samples. *Phytochemistry* **66**: 1499–1514.

Shukla, R., Singh, P., Prakash, B and Dubey, N.K. (2012). Antifungal, aflatoxin inhibition and antioxidant activity of *Callistemon lanceolatus* (Sm.) Sweet essential oil and its major component 1,8-cineole against fungal isolates from chickpea seeds. *Food Control* **25:** 27-33.

Tg Siti, A.T., Othman, A., Muhammad, T and Deny, S. (2012). Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. *Asian Pacific Journal of Tropical Medicine* **24**: 202-209.

Tuba, S., Nurten, Ö., Betul, D., Ayse, C., Sukran, K and Baser, K. (2012). Chemical composition of the essential oil and antioxidant activity of methanolic extracts from fruits and flowers of *Hypericum lydium*. *Industrial Crops and Products* **36:** 599–606.

Vuuren, S.F., Kamatou, G.P and Viljoen, A.M. (2010). Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples. *South African Journal of Botany* **76:** 686–691.