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Effects of Time on Chemical Composition of the Hydrocarbon and Oxygenated Monoterpenes of *Hoslundia opposita* Leaf Essential Oils

O. E. Ogunjinmi, [1]J. A. Awotoye and [2] N. O. Olawore

[1]Chemistry Department, The Polytechnic, Ibadan. Oyo State. Nigeria
[2] Pure and Applied Chemistry Department, Ladoke Akintola University of Technology, Ogbomoso. Oyo State Nigeria
Main Author's E-mail : oluwasayoesther@yahoo.com

ABSTRACT

An essential oil is any concentrated, hydrophobic liquid containing volatile aroma compounds produced by plants. It has been established that several factors affect the component of the plants such as texture of the soil, relative humidity, wind and time of collection. This study is aimed at investigating the effect of time collection on chemical composition of monoterpenes and sesquiterpenes of this essential oil. Pulverized leaves (500 g) of Hoslundia opposita collected in the morning (7 am) and afternoon (2 pm) of the same day were separately hydrodistilled for four hours using Clevenger apparatus to obtain the essential oils from the leaves. The leaf oils collected in the morning (7 am) and afternoon (2 pm) harvests yielded 0.54 and 0.65 %w/w respectively. Analysis of the leaf oil obtained in the morning, using gas chromatography (GC) and gas chromatography combined mass spectrometry (GC-MS) revealed the most abundant components of the leaf oil collected in 7 am harvest were p-cymene (28.7 %), sabinene (7.1 %) and 1, 8-cineole (6.6 %); meanwhile the major components of leaf oil in 2 pm harvest were p-cymene (26.4 %), thymol (15.3 %), 1, 8-cineole (15.0 %) and γ- terpinene (10.4 %). Hydrocarbon monoterpenes detected in the leaf oil obtained in the morning harvest that were not found in the afternoon harvest are sabinene and trans-β-ocimene. β-Pinene, α-caryophyllene, and cis-β-ocimene were present in the oil obtained in the afternoon harvest but absent in the oil collected in the morning harvest. Oxygenated hydrocarbon (Fenchol, α-campholenal and pinocarvone) detected in the leaf oil obtained in the morning harvest but were not found in the afternoon harvest; only Linalool and Cis-β-terpineol found in the afternoon but not yet form in the morning harvestThe composition pattern of leaf oil obtained in the morning and afternoon harvests of Hoslundia opposita revealed significant differences in qualitative and quantitative.

Keywords- *Essential oils, Hoslundia opposita and oxygenated hydrocarbon.*

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1. INTRODUCTION

The name *Hoslundia* was named for *O. Hoslund-Smith*, a naturalist from Guinea. The Latin name *opposita* refers to the leaves and fruits, which are set in *opposita* pairs. People eat tasty fruits. Leaves are reported to have a strong unpleasant scent, which is alleged to repel bees (Anchebach *et al.*, 1992). This herbaceous perennial is a hardy garden plant in southern African gardens, but it might not prove hardy in colder climates. Plants require well-drained soil, and perform well in full sun. They can be used successfully to line an informal shrub border or driveways; however, enough space must be left to allow plants to spread comfortably. *Hoslundia opposita* herbaceous perennial shrub widely grown in Nigeria; where it is commonly known as Oke ota by the Igbos and Efirin odan by the Yorubas according to *Usman et al., 2010 reports*. *Hoslundia opposita* is widely used to cure abdominal pains; oral wounds sort throat, epilepsy, malaria, mental disturbance, malarial, fungal and bacterial (Anchebach *et al.*, 1992, Tonzibo *et al.*,2005 and Usman *et al.*,2010). An essential oil is any concentrated, hydrophobic liquid containing volatile aroma compounds produced by plants (Lahlou *et al.*, 2000 & Lahlou, 2003). According to Ana *et al.*, 2009, essential oils are responsible for the characteristic odour and flavour of the odourous plants and they are very volatile at room temperature unlike other oils and highly volatile substance isolated by a physical process from an odoriferous plant of a single botanical species. The constituents of the oils are mainly monoterpenoids and sesquiterpenoids which are hydrocarbons with the general formula $(C_5H_8)_n$. It has been reported that there are more than 1000 monoterpenoids and 3000 sesquiternoids structures, others compounds are phenylpropenes, compounds containing sulphur or nitrogen, etc. (Svobode et al., 1999; Bouvier, 2005). The Thermal decomposition of most terpenoids gives isoprene unit (2-methylbuta-1,3 diene.) It is believed that the skeletal structure of all naturally occurring terpenoids are built up from isoprene units. They may be defined as a group of molecules whose structure is based on a various but definite number of isoprene units that is methylbuta-1,3-diene, named hemiterpene, with 5 carbon atoms (Wallach 1887) and re-emphasized by Ruzicka 1935; Eisenreich, 2004 and Bouvier, 2005. Kobakobbi *et al.*, 2008 reported the effect of Geographical location on chemical composition of essential oils of the aerial part of *Ocimum basillicum* from five locations in Togo which shows five different chemotypes: estragole type, linalool or estragole, methyleugenol, methyleugenol, methyleugenol/ t-anathole type and t-anathole type. The effect of time of collection in Brazil with Erlanio *et al* (2010) reports the variations in chemical composition of *Lantana camara* essential oils. Seventeen (17), fourteen (14), thirteen (13), sixteen(16) and twelve (12) compounds were detected in 7:00am, 10:00am, 1:00pm, 4:00pm and 7:00pm harvests

respectively. Many aromatic and/or medicinal plants species have been the target of studies that relate the time influence on essential oil chemical composition, this occurs because the time of material collection is a relevant aspect to be taken into account when dealing with essential oil production. (Erlanio *et al* 2010) Previous reports showed chemical composition variation in *Hoslundia opposita* leaves essential oils from different origins (Anchebbach, 1992; Tonzibo, 2005 Usman *et al*., 2010). Despite this, until this moment, to the best of our knowledge, there is no literature record regarding the daytime chemical variation, and these justify this work. Variations in the percentage yield of essential oils can only be established after the oils have been extracted from plant materials (Dabri and Sefidkan, 2001; Omidbaigi *et al*., 2003, Erlanio *et al.*, 2010 and Memet, 2011).

The presence of B caryohyllene in many essential oils might contribute strongly to their antiviral ability. These results indicate that phenylopanoids and sesquiterpene present in essential oils contribute to their activity against HIV (Akram *et al*., 2009). Early reports had indicated that essential oil components, especially monoterpenes, have multiple pharmacological effects on mevalonate metabolism which could account for the terpene-tumor suppressive activity (Edris, 2007). Monoterpenes have been shown to exert chemopreventive as well as chemotherapeutic activities in mammary tumor models and thus may represent a new class of therapeutic agents. The mechanism of action of monoterpenes is based on two main approaches, chemoprevention and chemotherapy.

To achieve the chemical composition of essential oils, the analysis of essential oil will be gotten by using the standard analytical techniques such as gas chromatography (GC) and gas chromatography combined mass spectrometry (GC-MS). Analysis of essential oils is to identify the quantity, quantify the constituents present, to evaluate the quality of the oil and detect factors that may affect the oil. Gas chromatography combined with mass spectrometry is used to analyze the essential oil that is the GC separates the compounds from each other, while the mass spectrometer helps to identify them based on their fragmentation pattern.

2. METHODS

The leaves of *Hoslundia opposita* were harvested at Awotan Forest in Ibadan, Oyo State. The sample materials were identified by the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan with herbarium number FH10086637-0. The leaves samples were harvested at two different times, in the morning (7am) and afternoon (2pm) to know effect of time on the microbiological activities of the plants and effect of time on the constituents of the oil (Dabiri and Sefidkan 2001, Erlanio *et al* 2010). The harvested leaves sample was immediately air dried in shade at room temperature for one day and then analyzed. 500grammes of *Hoslundia opposita* morning

(7am) harvest and 500 grammes of *Hoslundia opposita* afternoon (2pm) were pound separately with mortar and pestle. Leaf oil of *Hoslundia opposita* were obtained by hydrodistillation in a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia (2006). Then the oils were characterized using GC, GC-MS

Gas Chromatography Analysis Analysis was performed on an Orion Micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with CP-Sil 5 and CP-Sil 9 (fused silica, 25µm x 0.25mm, 0.15µm film thickness) and flame ionization detector (FID). The volume injected was 0.2ml and split ratio was 1:3. Oven temperature was programmed from 50^0C-230^0C at 5^0C/min, using hydrogen as a carrier gas. Injection and detector temperatures were maintained at 200^0C and 250^0C respectively. Qualitative data were obtained by electronic integration of the Flame Ionization Detector (FID) area percent without the use of a correction factor.

Gas Chromatography - Mass Spectrometry Analysis A Hewlett – Packard (HP) 5890A Gas Chromatograph, interfaced with a VG analytical 70-50s double focusing mass spectrometer was used. Helium was used as the carrier gas at 1.2ml/min. The MS operating conditions were; ionization voltage 70ev, ion source 230^0C. The GC was fitted with a 25m x 0.25mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15µm; the Gas chromatograph component operating conditions were identified with those of gas chromatograph analysis. The mass spectrometry data were acquired and processed by on-line desktop with a computer equipped with disk memory. The percentage composition of the oils was computed in each case from gas chromatogram peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature (Adams and Babcock, 1994; Adams, 1995; Juolain and Koenig, 1998)

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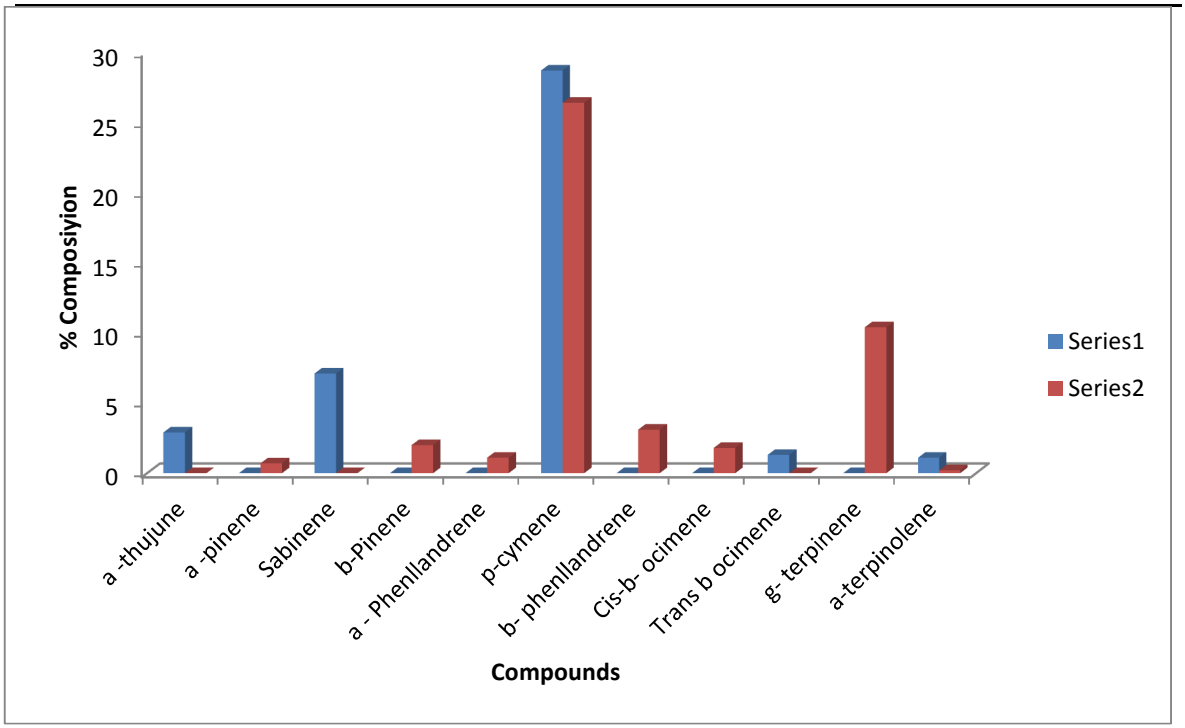
3. RESULTS AND DISCUSSION

Table1: Percentage composition of Hydrocarbon monoterpenes of *Hoslundia opposita* leaf oil collected in the morning (7am) and afternoon (2pm) harvests.

Compounds	KI	% composition		Mass Spectra Data's
		Morning collected	Afternoon collected	
Sabinene	976	7.1	-	136, 121, 93, 57, 27
β-Pinene	980	-	2.0	136, 121, 93, 41, 27
α - Phenllandrene	1005	-	1.1	136, 121, 93, 77, 27
p-cymene	1026	28.7	26.4	134, 119, 91, 41, 27
β- phenllandrene	1031	-	3.1	136, 121, 93, 77, 27
Cis-β- ocimene	1040	-	1.8	136, 105, 93, 41, 27
Trans β ocimene	1050	1.3	-	136, 121, 93, 79, 27
γ- terpinene	1062	-	10.4	136, 121, 93, 79, 27
α-terpinolene	1088	1.1	0.2	136, 121, 93, 71, 43
Total		**38.2**	**45.0**	



Key: Series1- Morning (7am) collection

Series2- Afternoon (2pm) collection

Fig 1: Plots of Hydrocarbon monoterpenes of Hoslundia opposita leaf oils collected in the morning (7am) and afternoon (2pm) harvests

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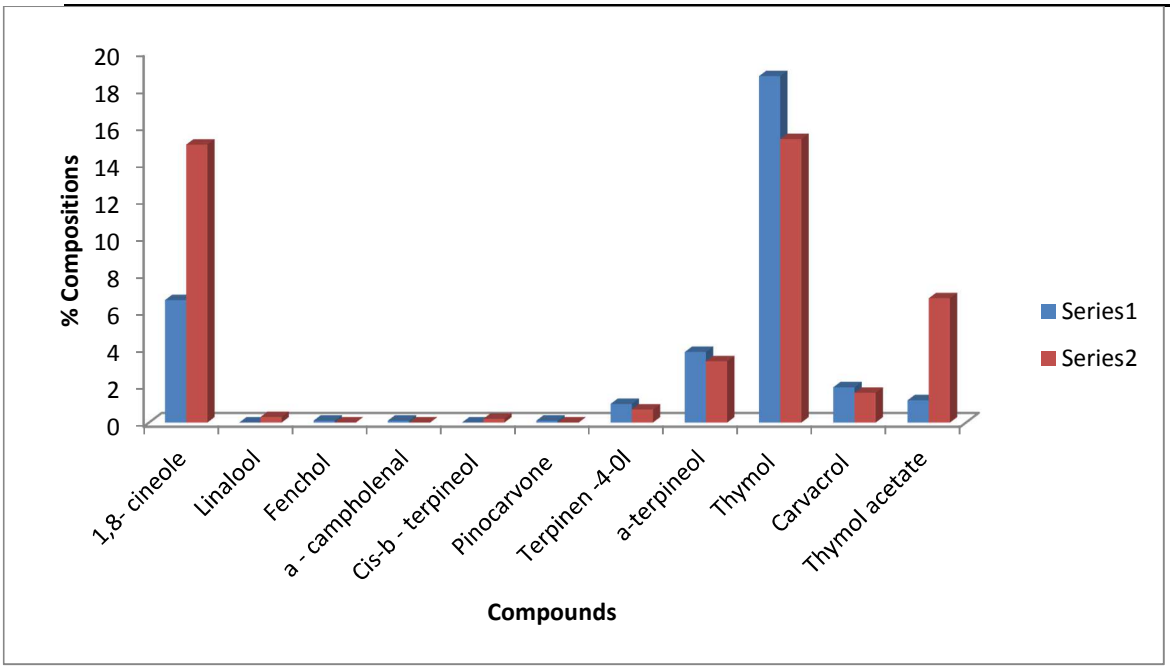
Table2: Percentage composition of Oxygenated monoterpenes of *Hoslundia opposita* leaf oil collected in the morning (7am) and afternoon (2pm) harvests

Compounds	KI	% composition		Mass Spectra Data's
		Morning collected	Afternoon collected	
1,8- cineole	1033	6.6	15.0	154, 108, 81, 43, 27
Linalool	1098	-	0.3	154, 121, 93, 71, 43
Fenchol	1111	0.1	-	154, 134, 81, 67, 28
α - campholenal	1125	0.1	-	134, 108, 93, 53, 28
Cis-β - terpineol	1144	-	0.2	154, 121, 93, 71, 43
Pinocarvone	1162	0.1	-	150, 108, 93, 53, 28
Terpinen -4-0l	1177	1.0	0.7	154, 111, 93, 71, 43
α-terpineol	1189	3.8	3.3	136, 121, 93, 59, 31
Thymol	1290	18.7	15.3	150, 135, 91, 77, 39
Carvacrol	1298	1.9	1.6	150, 135, 91, 51, 39
Thymol acetate	1355	1.2	6.7	192, 135, 91, 77, 43
Total		**33.5**	**43.1**	



Key: Series1- Morning (7am) collection

Series2- Afternoon (2pm) collection

Fig.2: Plots of Oxygenated monoterpenes of Hoslundia opposita leaf oil collected in the morning (7am) and afternoon (2pm) harvests

Discussion

The essential oil was collected in *n*-hexane and stored at 4°C in the dark. The leaf oils collected in the morning (7 am) and afternoon (2 pm) harvests yielded 0.54 and 0.65 % w/w respectively. Essential oils of *Hoslundia opposita* are characterized by monoterpenoid and oxygenated monoterpenoid. Percentage composition of hydrocarbon monoterpenes in the leaf oil collected from the morning and afternoon harvests are represented in Table1. In the table, a total of four (4) and seven (7) compounds were identified in the leaf oil collected from the morning (7am) and afternoon (2pm) harvests, the number represent 38.2% and 45.0% of the oil respectively. Predominant Hydrocarbon monoterpene in the oil obtained from morning harvest were, P-Cymene (28.7%) and Sabinene (7.1%) (Figure17). Trans β-ocimene (1.3%) and α-Terpinolene (1.1%) were occurred in moderate quantity. In the oil obtained in the afternoon harvest, predominant hydrocarbon monoterpenes detected were P-cymene (26.4%), γ-terpinene (10.4%), β-phenllandrene (3.1%) and β-pinene (2.0%). (Figure 17) The moderate quantities are cis-β-ocimene (1.8%) and α- phenllandrene (1.1%) while α-Terpinolene (0.2%) occurred in minor quantities. Percentage composition of hydrocarbon oxygenated monoterpenes in the leaf oil collected from morning and afternoon harvests were represented in Table2. In the table, a total of nine (9) and eight (8) compounds were detected in the oil gotten from oils collected from the morning (7am) and afternoon (2pm) harvests with the total number of 33.5 and 43.1% respectively. In the leaf oil collected from the morning, the major compounds were Thymol (18.7%), 1,8-cineole (6.6%). α-Terpinolene (3.8%) and carvacrol (1.9%). (figure18) Thymol acetate (1.2%) and terpinen-4-ol (1.0%) has significant proportions whereas Fenchol (0.1%), α-campholenal (0.1%), pinocarvone (0.1%) exists as minor constituents. The amajor constituents detected in the leaf oil obtained in the afternoon harvest were 1,8-cineole (15.0%), thymol (15.3%), thymol acetate (6.7%) and α-Terpineol (3.3%) (figure18). carvacrol (1.6%) and terpinen-4-ol (0.7%) has moderate proportions but Linalool (0.3%) and cis-β-terpineol (0.2%) has minor significance. The chemical components detected in the leaf oil collected from the morning harvest p-cymene (3.8%), thymol (18.7%), α-terpineol (3.8%), β caryophyllene (2.3%), carvacrol (1.9%), α-terpinolene (1.1%), and terpinen-4-ol (1.0%) have higher quantities compared with the chemical components in the leaf oil collected in the afternoon(26.4, 15.3, 3.3, 1.4, 1.6, 0.2 and 0.7% respectively); this may be as a result of sunlight meanwhile 1,8 cineole(6.6%), thymol acetate (1.2%) detected from oil gotten from the morning (7am) harvest has lower proportions compared to the constituents detected in the afternoon leaf oil (15.0, 6.7 %), which means that the sunlight has increased the yields of the

leaf oil collected from afternoon harvest. (Erlanio *et al* 2010, Dabiri and Sefidkan 2001) Sabinene (7.1%), Trans β ocimene (1.3%),Fenchol (0.1%), α-campholenal (0.1%) pinocarvone (0.1%), were absent in the oil collected from the morning (7am) whereas present in the afternoon harvest. Meanwhile, β-pinene (2.0%), α-Phenllandrene (1.1%), γ-terpinene (10.4%), Linalool (0.3%), cis - β terpineol (0.2%) were present in the oil collected from the afternoon (2pm) but absent in the oil collected from morning harvest. The two essential oils were very rich in hydrocarbon monoterpenes compared with oxygenated monoterpenes. Hydrocarbon monoterpenes detected in the leaf oil collected in the morning harvest that were not found in the afternoon harvest are sabinene and Trans β-ocimene. In the same vein, β-pinene, α-caryophyllene, β-caryophyllene and cis β-ocimene were present in the oil collected in the afternoon harvest.

The most abundant compounds in the leaf oil of *Hoslundia opposita* from both morning and afternoon harvests is P-cymene compared with 1,8-cineole that was principal constituent in Usman *et al;* 2010 reports but they did not state the time of harvest. 1,8 cineole was one of the principal constituent in the oil collected from the morning and afternoon harvests of *Hoslundia opposita* with 06 and 15% respectively, which means 1,8 cineole has not fully formed as at the time of morning harvest.

4. RECOMMENDATION

Further investigation, with a view to isolating pure components from the leaf oil is recommended; this will lead to the identification of new components.

5. REFERENCES

Ana, C. M., Jehids, M., Bibana, Z., Camilo, D., Liliana, B. and Elena, S. (2009). Citral and Carvone chemotypes from the essential oils of Colombian *Lippia alba* (Mill) N.E. Brown: composition, cytotoxity and antifungi activity. *Mem. Insti. Oswaldo crzrio de janeiro*, vol. 104(6):878-884.

Anchebach, H., Waibel, R., Nkunya, H. H. M. and Weenen, H. (1992). Antimalarial compounds from *Hoslundia opposita* Phytochemical; *Astophys Jounal* 31(11); 3781-3784

Bouvier, F ., Bohlmann, J. and Gershenzon, J. (2005). Isoprene unit and its conversion P*rog Lipid Res* 44 357-367.

Dabiri, M. and Sefidkan, F. (2001). Analysis of the essential oil from aerial parts of Perorskia antriplicito (Benth) at different concentration stages of plant growth. Flavour Frag. J., 16: 435-438.

Edris, A. E. (2007) Pharmaceutical and Therapeutic Potentials of Essential Oils and Their Individual Volatile Constituents *Phytother. Res. 1-16*

Eisenreich, W, Bacher, A., Arigoni, D. and Rohdich F. (2004). Biosynthesis of isoprenoids via the non-mevalonate pathway. *Cell Mol Life Sci.*;61:1401-1426.

Erlânio O. S, Aracelio V. C, Fabiola F. G. Adriana R. C, Sidney G. L and José Galberto M. C (2010) Effect of Collection Time on Essential Oil Composition of *Lantana camara* Linn (Verbenaceae) Growing in Brazil Northeastern *Rec. Nat. Prod.* 4:1 31-37

Kobakobbi, P. W., Poutowi, C. R., Jean-piemchanmount and Komna, S. (2008). Chemical composition and antimicrobial property of different basil essential oils chemotype from Togo. Bangladesh J. Pharmacol.,4: 1-8.

Lahlou, M. (2003). Composition and molluscicidal properties of essential oils of five Moroccan Pinaceae. *Pharm Biol* 41, 207–210.

Lahlou S, Leal-Cardoso J, H., and Magalhàes P. J. C. (2000). Essential oil of Croton nepetaefolius decreases blood pressure through an action upon vascular smooth muscle: Studies in DOCASalt hypertensive rats. *Planta Med* 66: 138–143.

Memet, I., Muzaffer, K., Kaya, D. A. and Saliha, K. (2011). Effect of harvest time on the essential oil composition of Thymbra spicata L. growing in Flora of Adiyaman Advances in Environmental Biology 5(2): 356-358

Omidbaigi R., Hadjiakhoondi, A. and Saharkhiz, M. (2003). Changes in content of ppimpinella anisun oil at various harvest time. JEOBP, 6(1) :46-50.

Ruzicka, L., Eshemmoser, A. and Heuser J.(1953). The Isoprene rule and biogenesis of terpenic compounds Experimentia. 357(7):357-367

Svoboda K. P. and Deans, S. G. (1999). Biological activity of essential oils from selected aromatic plants Actattortic. 390: 203-209.

Tonzibo, Z. F., Ahibo-coffy, J. C. and Nguessan Y. T. (2005). Chemical composition of essential of essential oils of Hoslundia opposita Vahl. fromIvory coast. *Flav and Frag* 21(5):789-791.

Usma, L., Zubair, M., Adebayo, S., Oladosu, I. N. O., Muhammad, N. and Akolade, J. (2010). Chemical Composition of Leaf and Fruit Essential Oils of *Hoslundia opposita* Vahl Grown in Nigeria *Eurasian J. Agric. & Environ. Sci.*, 8 (1): 40-43. ISSN 1818-6769).

Wallach, O. (1887). Zur kenntnis der Terpene and udatherrischen ole viette Abhandlung Bigs *Ann. Chem.*. 238: 78-88