J. Pl. Res. Vol. 17, No. 1, pp 86-93, 2019

Variation in Chemical Composition of Essential Oil Extracted From the Fruits and Leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) of Nepal

Tara Datt Bhatt*, Amit Dhungana and Jyoti Joshi
Department of Plant Resources, Thapathali, Kathmandu, Nepal
**Email: tdbhatt@gmail.com*

Abstract

The purpose of this study was to find out the variation of major chemical constituents present in the essential oil of leaves and fruits of *Cinnamomum tenuipile* Kosterm analyzed by GCMS instrument. Leaves and Fruits of *Cinnamomum tenuipile* Kosterm were collected from Brindaban Botanical Garden of Makawanpur district of Nepal. The extraction of essential oil was performed by hydro-distillation using Clevenger apparatus and then their chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS). The results of chromatographic analysis have shown somehow similar compounds except camphor which was found in fruits whereas it was absent in leaf oil. By GCMS analysis 13 and 15 compounds were identified respectively in which eucalyptol (24.17%) and methyl cinnamate (52.18%) were found as major compound in leaf oil while eucalyptol (38.23%), camphor (19.57%) and methyl cinnamate (22.53%) were found as major compound in fruit oil.

Keywords: *Camphor, Clevenger , Eucalyptol, GC-MS, Methyl cinnamate*

Introduction

The evergreen, *Cinnamomum tenuipile* Kosterm (syn. *Cinnamomum cecidodaphne*), part of the Lauraceae family, is native to Nepal and grows wild in the districts of Dang, Rolpa and Sallyan in the Rapti Zone (Rema et al., 2002, Adhikari , 2018). This species is a diploid and can grow to an altitude of 1300 meters (Ravindran et al., 2003). *Cinnamomum tenuipile* Kosterm is recognized as an aromatic plant, meaning it has an elevated level of essential oil (Gurung, 2015). Using steam distillation, the dried berries of *Cinnamomum tenuipile* Kosterm produce the essential oil commonly known as sugandha kokila oil (Ravindran et al., 2003), which is yellow in color and has a camphoraceous, spicy aroma (HPPCL, 2015). This product can be used as a fragrance in soaps, detergents, cosmetics, perfumes and industrial fragrances (Gurung, 2015). Sugandha kokila oil is also used in indigenous medicine as a demulcent and stimulant (Rema et al., 2002). The Nepal Trade Integration Strategy 2010, identified Medicinal and Aromatic Plants (MAPs) as one of Nepal's top twenty goods and services with export potential (Sharma, 2015).

The different parts of Sugandhakokila tree contains essential oil in different percentages which is used for the formulation of perfume as well as uesd in the form of scent (Adhikary, 2018). The nematicidal, termiticidal, mosquito larvicidal (Satyal et al., 2013), insecticidal, antifungal, antiaflatoxin, antioxidant (Prakash et al., 2013) and antibacterial (Rajendra et al., 2013) activities of essential oils have been also reported.

In the present study, essential oil is extracted from the fruits and leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) collected from Brindaban Botanical Garden, Makawanpur Nepal and oil was analysed by Schimadzu GC-MS QP 2010 Plus. The purpose of this study was to compare the chemical constituents present in the both oil samples.

Materials and Methods

Collection of plant materials

The fruits and leaves of *Cinnamomum tenuipile* Kosterm were collected from Brindaban Botanical Garden, Makawanpur district of Nepal. The fresh fruits and leaves were collected and dried in shed before extraction of essential oil.

Extraction of essential oil

A Clevenger apparatus was used for the extraction of essential oil from the fruits and leaves of *Cinnamomum tenuipile* Kosterm through hydro distillation (Waheed et al., 2011). The fruits and leaves were thoroughly washed and placed in Clevenger apparatus and subjected to hydro distillation for about 8 hours. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small glass bottles, dried with anhydrous sodium sulphate, sealed, labelled and stored in glass vials.

Gas chromatography- Mass spectrometry (GC-MS)

The chemical constituents in the essential oils were separated by using a Shimadzu Gas chromatograph Mass Spectrophotometer (GCMS QP 2010 Plus) with Rtx-5MS column (30mX0.25mmX0.25µm). 1 µL of the essential oil diluted with spectroscopic grade hexane (10:1) was injected into the GC inlet maintaining column flow rate of 0.68 mL/min and purge flow 3 mL/min in the split mode. The initial column oven temperature was set at 40°C and the injection temperature was 250°C.

The qualitative analysis of the essential oil was further continued in a Shimadzu GCMS-QP2010 Plus. During the analysis, the ion source temperature and the interface temperature was set at 250°C and 200°C respectively. The detector scanning start time was 4 min and end time was 68 min; scan speed was 666 with scanning range of m/z 40.00-350.00. Identification of compounds was done by comparing the Mass spectral data present in the mass spectral library NIST 2017 and FFNSC 1.3.

Results and Discussion

The oil extracted from fruits and leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) was analyzed by GCMS instrument and the composition of various constituents present in the respective oil is tabulated below in Table 1 & Table 2 respectively which is nearly similar to the analysis of essential oil of fruits of sugandhakokila (Adhikary et al., 2011) which shows 1,8-cineole, methyl cinnamate, alpha-terpineol as major constituents.

Table 1: Chemical constituents present in the essential oil extracted from fruits of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) based on GCMS analysis.

Peak	R. Time	Area	Area%	Name of the Compounds
1	11.668	539761	1.93	Pinene <alpha->
2	13.505	909094	3.26	Sabinene
3	13.64	530067	1.9	Pinene <beta->
4	15.937	304184	1.09	Cymene <para->
5	16.287	10674502	38.23	Eucalyptol
6	17.61	168681	0.6	Terpinene <gamma->
7	19.61	355533	1.27	Linalool
8	21.826	5463814	19.57	Camphor
9	22.882	173829	0.62	Terpineol <delta->
10	23.379	1014454	3.63	Terpinen-4-ol
11	24.02	1194704	4.28	Terpineol <alpha->
12	29.339	288113	1.03	Cinnamate <methyl-, (Z)->
13	32.807	6002721	21.5	Cinnamate <(E)-, methyl->
14	38.319	151850	0.54	2-Acetylbenzoic acid
15	40.995	152191	0.55	Caryophyllene oxide

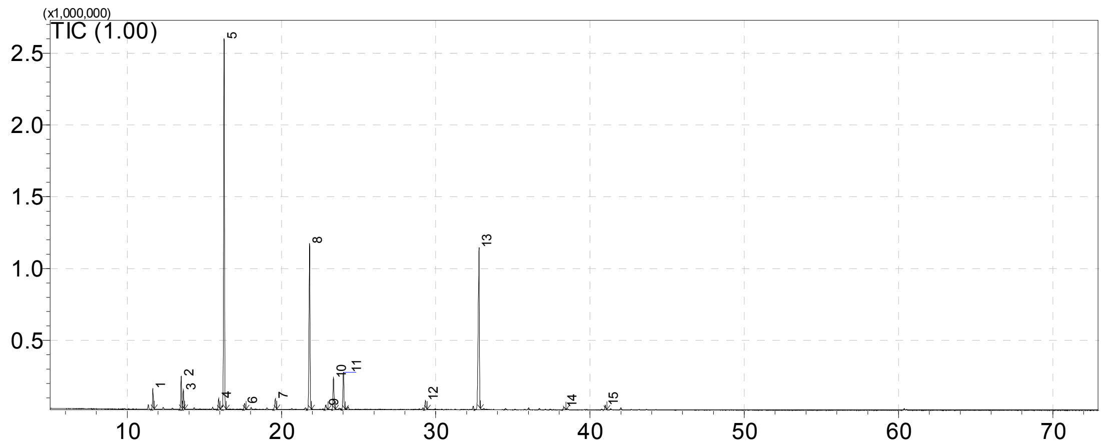

Figure 1: GCMS chromatogram of essential oil of fruits of Sugandhakokila collected from Brindaban, Makawanpur

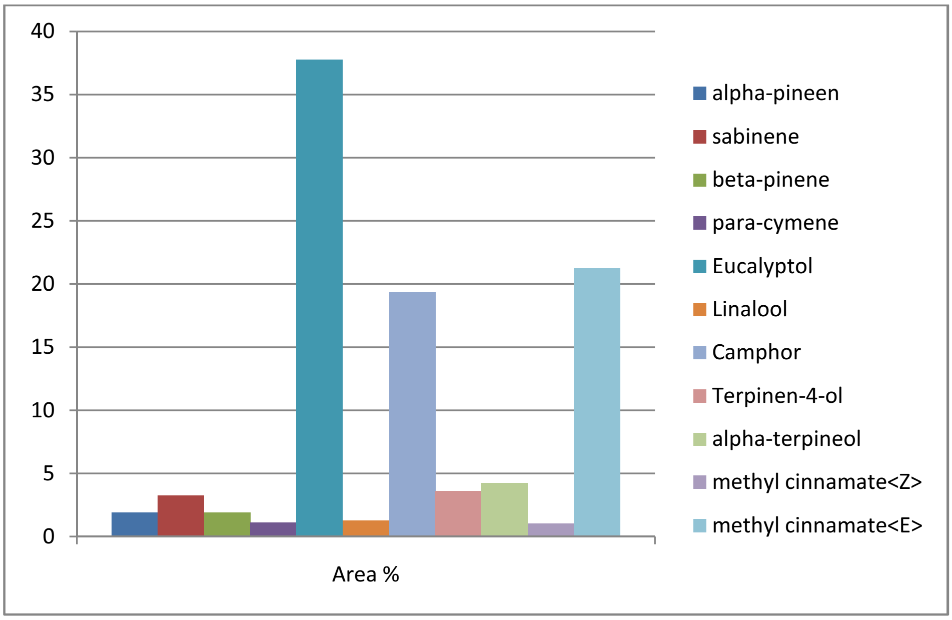

Figure 2: Graphical representation for the major chemical constituents present in the essential oil of fruits of *Cinnamomum tenuipile* Kosterm analyzed by GCMS.

Table 2: Chemical constituents present in the essential oil extracted from leaves of *Cinnamomum tenuipile* Kosterm (Sugandhakokila) based on GCMS analysis.

Peak	R. Time	Area	Area%	Name of the Compounds
1	11.689	732795	2.49	Pinene <alpha->
2	13.529	2061207	6.99	Sabinene
3	13.664	985045	3.34	Pinene <beta->
4	14.362	216021	0.73	Myrcene
5	16.166	205549	0.7	Limonene
6	16.298	7126612	24.17	Eucalyptol
7	23.396	430624	1.46	Terpinen-4-ol
8	24.038	1286930	4.36	Terpineol <alpha->
9	29.361	563838	1.91	Cinnamate <methyl-, (Z)->
10	32.89	14824373	50.27	Cinnamate <(E)-, methyl->
11	34.354	505255	1.71	trans-.alpha.-Bergamotene
12	38.588	231513	0.79	Cadinene <delta->
13	43.681	317663	1.08	Cadin-4-en-10-ol

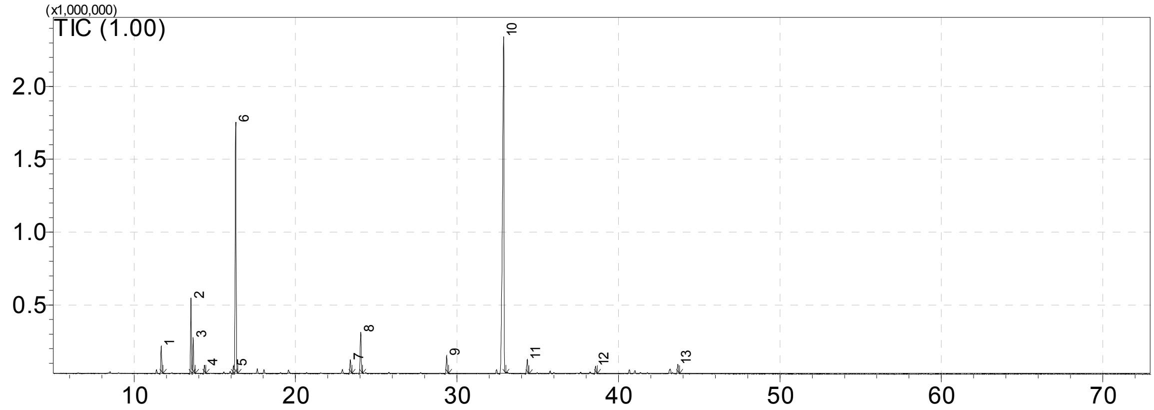

Figure 3 : GCMS chromatogram of essential oil of leaves of Sugandhakokila collected from Brindaban, Makawanpur

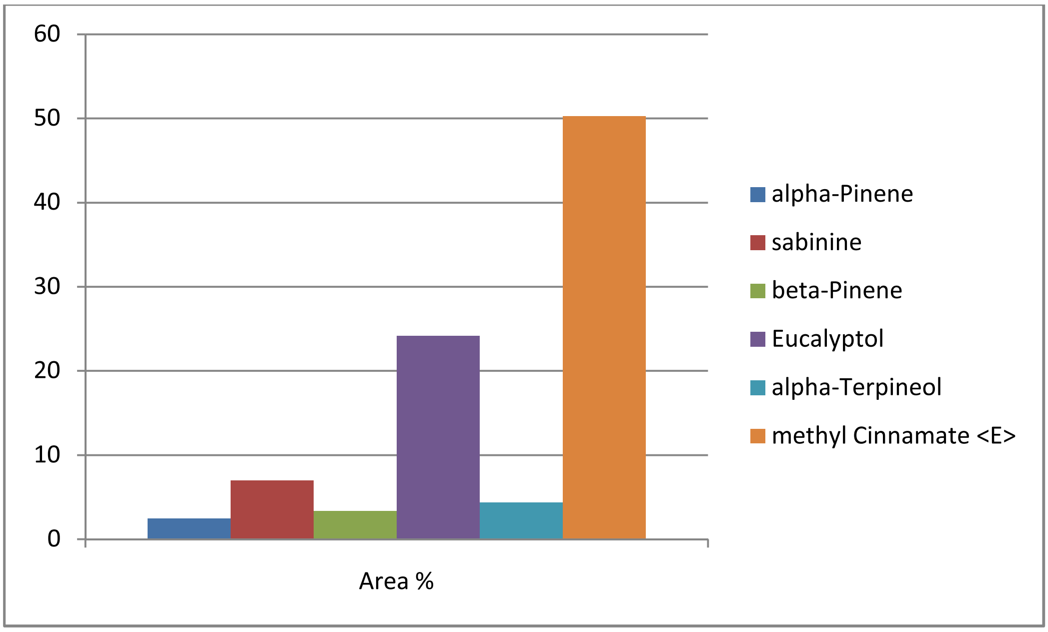

Figure 4: Graphical representation for the major chemical constituents present in the essential oil of leaves of *Cinnamomum tenuipile* Kosterm analyzed by GCMS.

Mass fragmentation pattern of various compounds that are identified by GCMS analysis

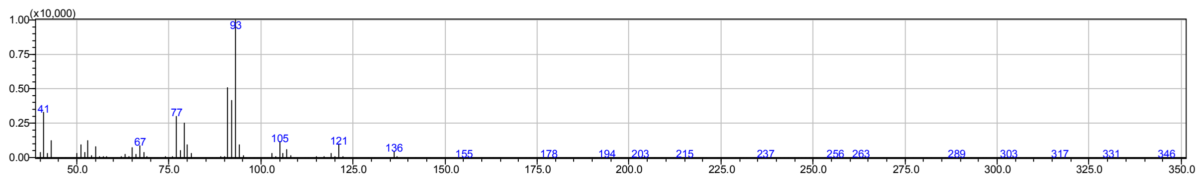

Figure 5: Mass Fragmentation of Pinene <alpha->

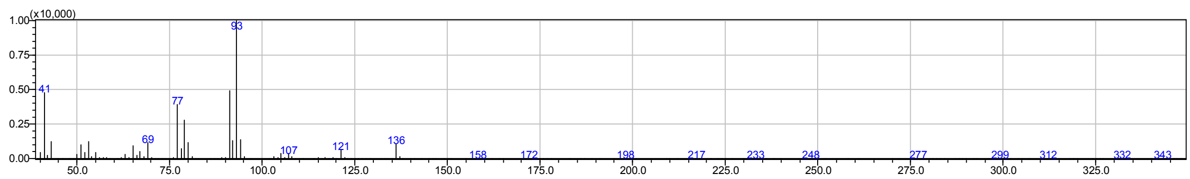

Figure 6: Mass Fragmentation of Sabinene

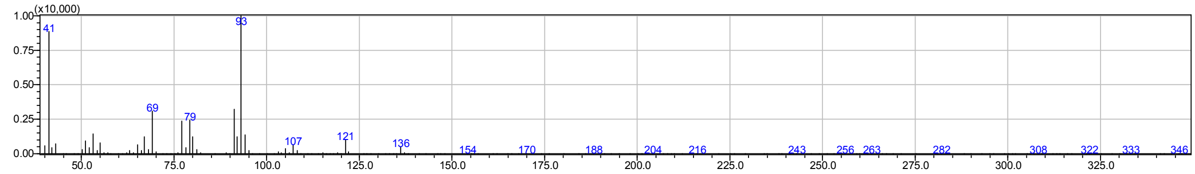

Figure 7: Mass Fragmentation of Pinene <beta->

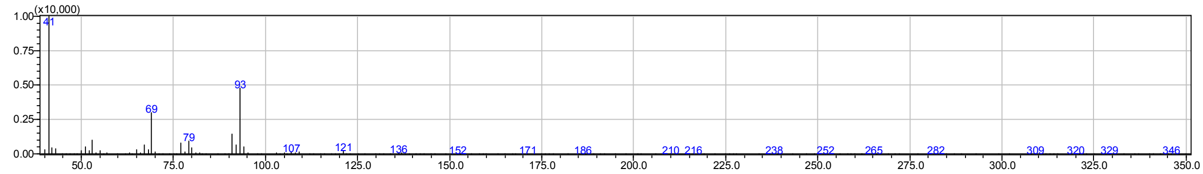

Figure 8: Mass Fragmentation of Myrcene

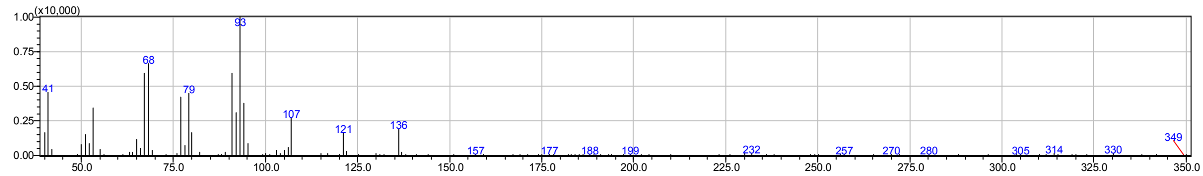

Figure 9: Mass Fragmentation of Limonene

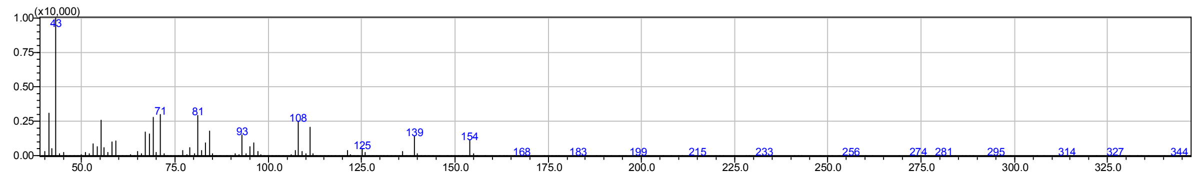

Figure 10 : Mass Fragmentation of Eucalyptol

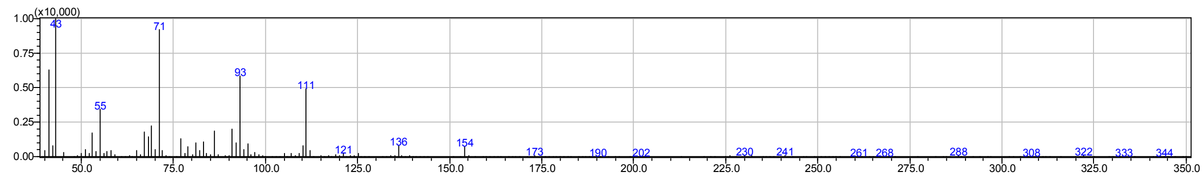

Figure 11: Mass Fragmentation of Terpinen-4-ol

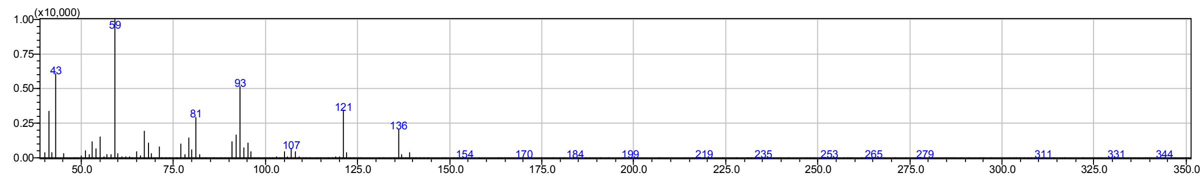

Figure 12: Mass Fragmentation of Terpineol <alpha->

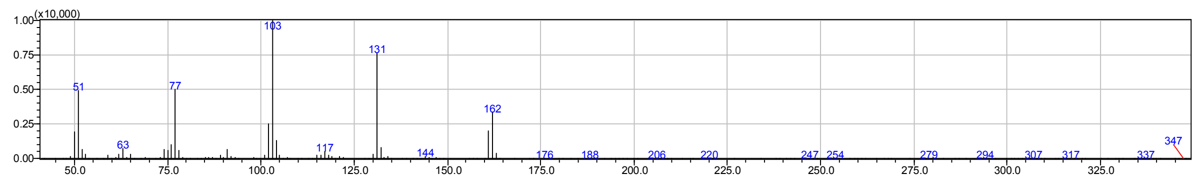

Figure 13: Mass Fragmentation of Cinnamate <methyl-, (Z)->

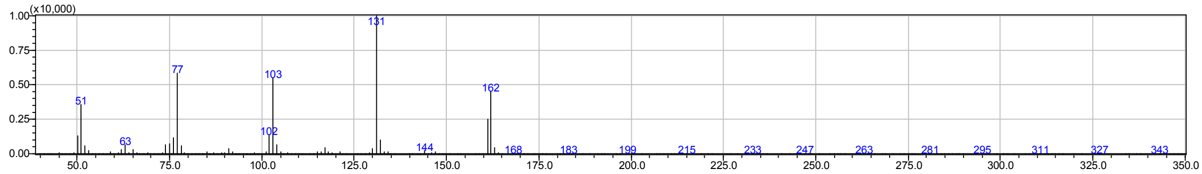

Figure 14: Mass Fragmentation of Cinnamate <(E)-, methyl->

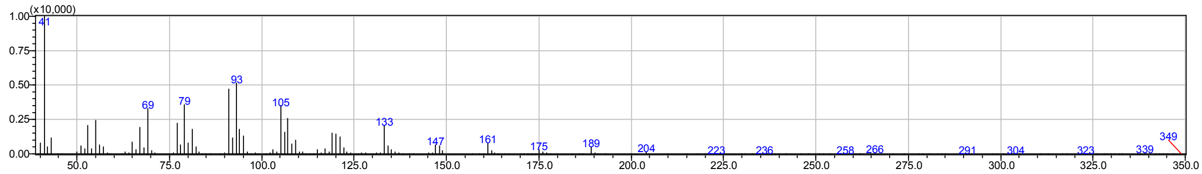

Figure 15: Mass Fragmentation of trans-.alpha.-Bergamotene

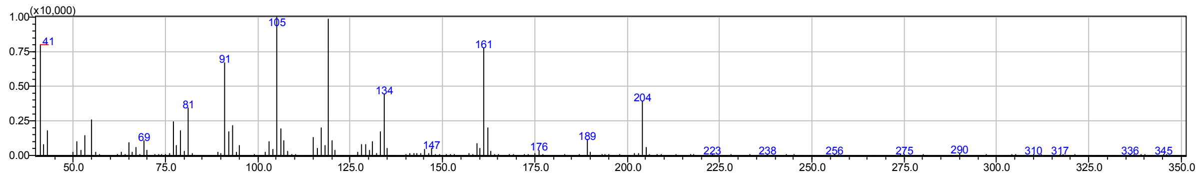

Figure 16: Mass Fragmentation of Cadinene <delta->

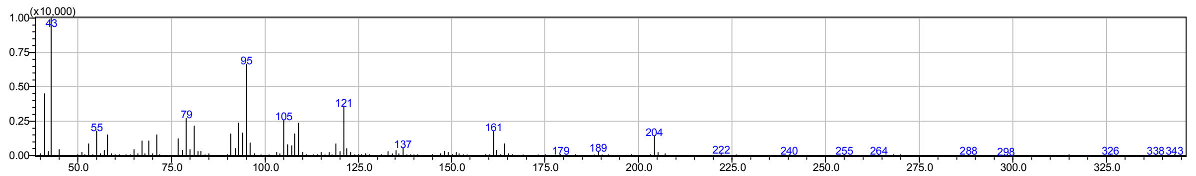

Figure 17: Mass Fragmentation of Cadin-4-en-10-ol

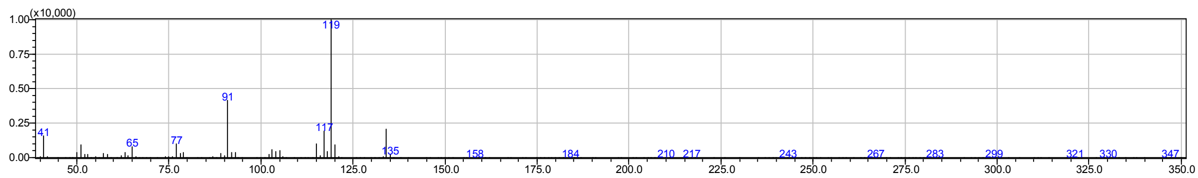

Figure 18: Mass Fragmentation of Cymene <para->

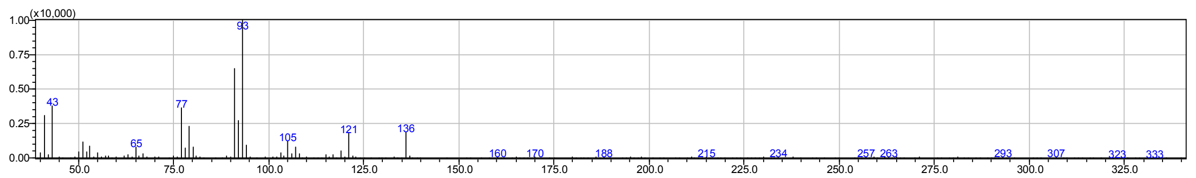

Figure 19: Mass Fragmentation of Terpinene <gamma->

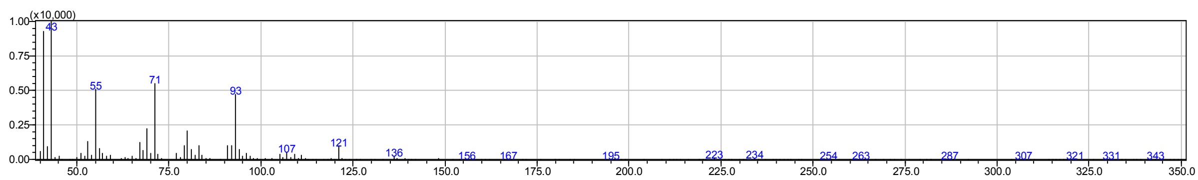

Figure 20: Mass Fragmentation of Linalool

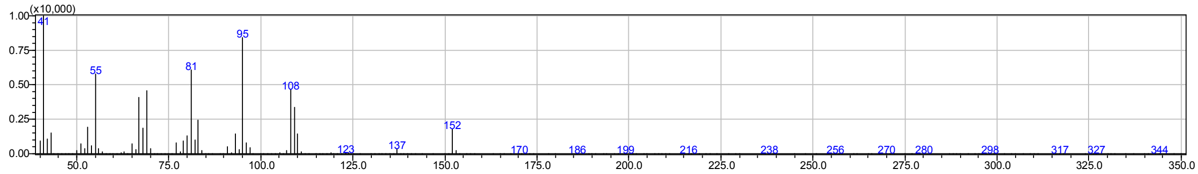

Figure 21: Mass Fragmentation of Camphor

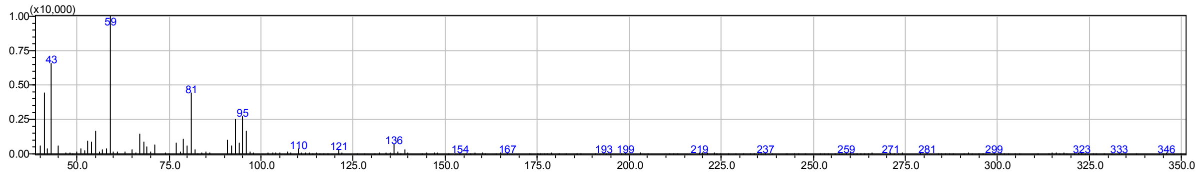

Figure 22: Mass Fragmentation of Terpineol <delta->

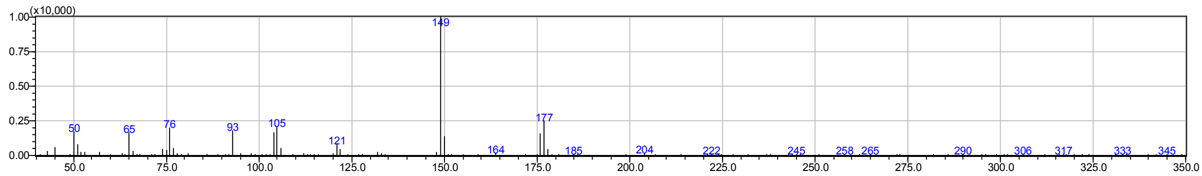

Figure 23: Mass Fragmentation of 2-Acetylbenzoic acid

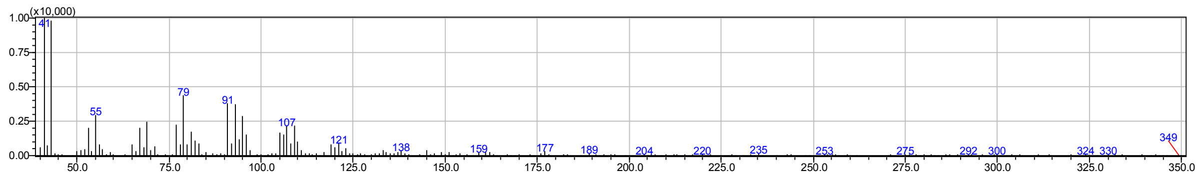

Figure 24: Mass Fragmentation of Caryophyllene oxide

Conclusion

The results of chromatographic analysis of essential oil of fruits and leaves have shown that they are somewhat similar but some compounds were present only in fruit oil and some were present in leaf oil only. Camphor is present in fruit oil while in leaf oil it was absent. By GCMS analysis 13 compounds were identified in leaf oil where as 15 compounds were identified in fruit oil. The major compounds were Eucalyptol (38.23%), Camphor (19.57%) and Methyl cinnamate (22.53%) in fruit oil and Eucalyptol (24.17%) and Methyl cinnamate (52.18%) in leaf oil.

Acknowledgments

The authors would like to thank Mr. Sanjeev Kumar Rai, Director General and Mr. Mohan Dev Joshi Deputy Director General, Department of Plant Resources for providing resources and encourages for research work. We would like to thank Plant Research Centre, Makawanpur especially Mr. Raj Kishor Pandit, Assisstant Scientific Officer for his co-operation during sample collection. The authors would like to thank Department of Plant Resources for giving an opportunity for research work in a very much friendly and sound environment and for financial support without which the research work was not possible.

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