

Effect of Harvesting Time on Phytochemical Profile and Antioxidant Activity of Essential Oil from *Citrus medica* Leaves

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Abstract

Leaves (500 g) of *Citrus medica* L. harvested at interval of three hours from 7:00 am to 7:00 pm on a day during dry season were separately hydrodistilled for three hours. Oil yields from the leaves ranged from 0.22 - 0.34%. GC and GC-MS analyses revealed the predominance of monoterpenoids (50.5-70.3%) in the oils. The percentage composition of sesquiterpenoids was in the range of 29.7-29.5 %. The principal constituents of the oils were; β -ocimene (4.1–5.6 %), D-limonene (13.5 – 19.8 %), γ -terpinene (10.8 – 17.9 %), linalool (3.8 – 4.0 %), citronellal (2.7 – 7.5 %), terpinen-4-ol (1.2 – 8.7 %), citronellol (10.1 – 13.7 %), linalylanthranilate (2.5 – 9.3 %), δ -elemene (3.0 – 6.4 %), α -bergamotene (4.1 – 6.6 %), humulene (1.2 – 2.8 %), β -caryophyllene (7.9 – 16.5 %), β -bisabolene (3.6 – 5.2 %). The oils were of D-limonene and γ -terpinene chemotypes. Antioxidant activity assay on the oils revealed that they were biochemically active against DPPH radicals. The activity was concentration dependent with the oil from 7 am harvest having the highest activity and lowest EC₅₀. Hence, the plant may be used as an alternative to synthetic antioxidants.

Keywords: D-limonene, γ -terpinene, chemotypes, antioxidants, *Citrus medica*

1.0 Introduction

There is need for alternative therapy to allay the public concern about the safety, affordability and accessibility of synthetic antioxidants that are used in curtailing oxidative stress [1]. Earlier reports indicated that some plant extracts had radical scavenging potentials that are devoid of the shortcomings associated with the synthetic anti-oxidative agent. Notable among them are essential oils of *Origanum*, *Rosemary* and *Mentha* that demonstrated significant antioxidant properties comparable to the synthetic antioxidant [2-7]. *Melissa officinalis* leaf oil also exhibited comparable antioxidant potentials with synthetic antioxidant [8-10].

Citrus medica L. commonly known as citron is an evergreen shrub with short, thick and thorny branches cultivated majorly in the tropical regions of the world [11]. Different parts of the plant are widely used in folkloric medicine. Ripe fruits are used in treatment of sore throat, cough and asthma [12]. The juice is used as tonic, stimulant, poison expellant and correction of fetid breath [13]. The seed is useful in palpitation and fruit decoction is analgesic and sedative [14]. Also, the juice and rind had served as antidote for food poisoning [12]. Both the leaves and fruits of citron are used by a population of South Nigeria for febrile illnesses [11]. Fruit extracts also showed antioxidant activity [15]. Phytochemical investigation of extracts from different parts of *C. Medica* revealed the presence of flavonoid, steroid, alkaloid and terpenoid. Biochemical and biological activities of the plant extracts are attributable to the phytochemicals in the extracts. Hence, justifying the use of the plant as a therapeutic agent in folkloric medicine [11, 16-17].

Earlier works on essential oil from leaves of *Citrus medica* grown in Korea, China and Italy revealed that the oils were of D-limonene chemotype [18-20]. However, there were variations in the phytochemical profile of the oils. This is attributable to differences in environmental factors that affect the physiological conditions of the plants at various locations. The conditions determine the activity of the enzyme that facilitates the biosynthesis of terpenoid constituents of essential oils from their respective precursors. This will consequently affect the phytochemical profiles and biochemical activities of the oils. Changes in environmental factors could also cause variations in the physiological conditions of a plant from time to time in a day. It is on this basis that the present study was conceived to monitor the effect of harvesting time on the chemical composition and antioxidant activity of essential oil from leaf of *Citrus medica*.

2.0 Materials and Methods

2.1 Plant Material

Leaves of *Citrus medica* were collected at interval of three hours in a day (7 am, 10 am, 1 pm, 4 pm and 7 pm) from the orchard of Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria in November, 2016. The leaf was identified at the Herbarium of the Plant Biology Department, University of Ilorin, where voucher specimen (UIL/001/998) was deposited. The leaves from each of the harvests were separately pulverized prior to extraction.

2.2 Oil Isolation

Each of the pulverized sample (500g) was hydrodistilled for 3 hours using Clevenger apparatus in accordance with the British Pharmacopoeia specification [21]. The resulting oils were collected, preserved in sealed sample tubes and stored under refrigeration until analysis.

2.3 Gas Chromatography (GC) Analysis

GC analysis were performed on an Orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with Cp- Sil 5 and Cp-Sil19 (fused silica, 25 m x 0.25 mm, 0.15 μ m film thickness) and flame ionization detector (FID). The volume injected was 0.2 mL, and the split ratio was 1:30. Oven temperature was programmed from 50 – 230°C at 3°C/min using hydrogen as a carrier gas. Injection and detector temperatures were maintained at 200°C and 250°C respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factors.

2.4 Gas Chromatography – Mass Spectrometry (GC/MS) Analysis

A Hewlett Packard (HP) 5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrometer was used. Helium was used as the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization voltage 70 ev, ion source 230°C. The GC was fitted with a 25 m x 0.25 mm, fused silica capillary column coated with Cp – Sil 5. The film thickness was 0.15 μ m; the GC operating conditions were identical with those of GC analyses. The MS data were acquired and processed by on-line desktop with a computer equipped with disk memory. The percentage compositions of the oils were computed in each case from GC peak areas. The identification of the components was based on 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 [22-24].

2.5 Free Radical Scavenging Assay

The antioxidant potential of essential oils from various harvests was evaluated using DPPH radical scavenging activity. Method described by Sharififar *et al.* [25] was used for the assay. In the technique, DPPH solution (0.1 mM) was prepared in 90% v/v methanol. 2.9 ml of the DPPH solution was added to 0.1 ml solution of different concentrations of each of the oils (10-100 μ l/ml). BHT was used as standard. The resulting solutions were mixed and incubated in the dark for 30 min. The absorbance of each of the solutions was read at 517 nm against a blank. The control contains 0.1 ml of 90% methanol in lieu of the extracts. Methanol (90% v/v) was used as a blank. Analyses were carried out in triplicates. Percentage inhibition of DPPH radical was calculated as: % Radical Scavenging Activity = $(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}}) \times 100$. Mean effective concentration (EC₅₀) was also computed using regression analysis.

3.0 Results and Discussion

3.1 Percentage Yield of Essential Oils from *C. medica* Leaves

Fresh leaves of *Citrus medica* harvested at various times in a day during dry season afforded oils in the range of 0.22 - 0.34 % (w/w). The yield was 0.24 % (w/w) in 7.00 am harvest. It steadily increased from 0.30 % (w/w) in 10.00 am harvest to 0.34 % (w/w) in 1.00 pm harvest. The yield subsequently decreased from 0.26 % (w/w) in 4.00 pm harvest to 0.22 % (w/w) in 7.00 pm harvest (Fig. 1).

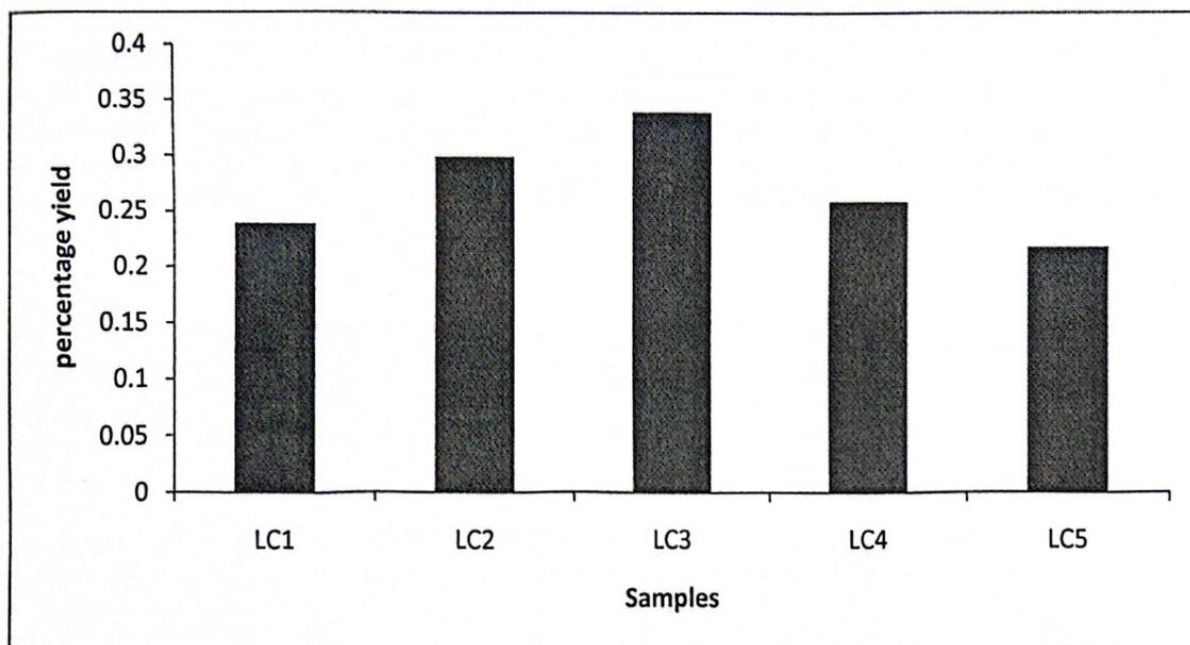


Fig. 1: Percentage Yield of Essential Oils from *C. medica* Leaves harvested at different periods of the day

KEY

- LC1=Leaves from 7.00 am harvest
- LC2=Leaves from 10.00 am harvest
- LC3= Leaves from 1.00 pm harvest
- LC4= Leaves from 4.00 pm harvest
- LC5= Leaves from 7.00 pm harvest

3.2 Chemical Composition of Essential Oils from *C. medica* Leaves

The identities, retention indices and mass spectra data of the constituents of essential oil isolated from leaves of *C. medica* harvested at different times of the day are presented in Table 1. In the Table, 19 - 24 compounds were identified from their mass spectra. The numbers represented 90.7–95.7% of the oils. Percentage composition of hydrocarbon monoterpenoids in the oils was 22.1–42.1%, while oxygenated monoterpenoids constituted 20.8–30.1% of the oils. Meanwhile 23.4–42.3% of the oils were sesquiterpenoids.

Table 1: Chemical composition (%) of essential oil from leaves of *C. medica* harvested at various times in a day during dry season

Compound ^a	RI ^b	RI ^c	% Composition					Mass spectra data
			CE1	CE2	CE3	CE4	CE5	
α -pinene	939	933	1.0	0.6	1.4	1.9	0.9	136, 121, 93, 77, 67
β -pinene	953	975	-	1.6	2.9	2.1	1.3	136, 107, 93, 79, 69
β -myrcene	991	989	-	-	0.8	-	-	136, 107, 93, 79, 69
Car-3-ene	1011	1009	-	1.4	-	-	1.0	136, 121, 93, 79, 69
β -ocimene	1020	1034	5.6	4.1	4.4	5.5	4.5	136, 105, 93, 79, 67
D-limonene	1031	1027	-	19.8	14.5	13.5	14.4	136, 121, 68, 65, 55
γ -terpinene	1040	1057	14.1	10.8	17.1	17.9	13.2	136, 121, 93, 77, 65
Terpinolene	1068	1086	1.4	0.8	0.7	1.2	0.5	136, 105, 93, 79, 65
Linalool	1098	1098	4.0	-	-	3.8	-	154, 111, 93, 71, 69
Allo-ocimene	1129	1045	-	1.1	-	-	1.2	136, 128, 121, 91, 79
Citronellal	1140	1145	7.2	2.8	7.5	4.3	2.7	154, 121, 69, 67, 55
Terpinen-4-ol	1153	1174	7.1	3.9	-	8.7	1.2	154, 136, 93, 71, 55
Citronellol	1177	1226	10.1	11.9	13.7	10.8	10.7	156, 123, 69, 67, 55
Linallylanthranilate	1238	1259	-	9.3	2.5	-	5.7	154, 121, 93, 91, 71
Geraniol	1248	1250	-	2.2	-	-	-	154, 136, 93, 69, 53
δ -elemene	1340	1337	6.4	3.1	-	3.0	3.3	204, 161, 121, 93, 77
Citronellyl acetate	1354	1352	-	-	-	-	0.5	156, 123, 95, 81, 69
β -elemene	1375	1391	1.4	0.9	-	-	0.5	204, 147, 93, 79, 69
β -guaiene	1378	1437	0.5	-	-	-	-	204, 189, 161, 133, 105
γ -elemene	1433	1554	1.7	-	3.7	0.9	-	204, 161, 121, 107, 93
α -bergamotene	1436	1435	6.6	4.9	4.4	4.1	4.3	204, 119, 93, 79, 69
Humulene	1440	1452	2.8	1.3	1.3	1.2	2.0	204, 175, 93, 80, 67
α -himachalene	1447	1445	-	-	0.5	0.6	-	204, 161, 93, 79, 69
γ -muurolene	1453	1475	1.4	1.3	0.6	0.5	1.5	204, 189, 161, 119, 93
β -caryophyllene	1454	1417	16.5	8.7	8.5	7.9	14.8	204, 161, 133, 93, 79
β -cadinene	1472	1448	0.6	-	-	-	-	204, 161, 134, 119, 91
γ -gurjunene	1473	1471	0.6	1.2	1.2	1.0	1.2	204, 161, 121, 105, 93
β -bisabolene	1509	1508	-	4.0	4.1	3.6	5.2	204, 161, 93, 69, 55
Caryophylleneoxide	1529	1574	1.0	-	-	-	-	220, 159, 119, 69, 55
Spathulenol	1576	1574	0.6	-	-	-	-	220, 159, 119, 69, 55
α -cadinol	1653	1651	0.8	-	-	-	-	222, 161, 94, 79, 71
α -bisabolol	1683	1685	0.9	-	-	-	-	204, 119, 109, 69, 43
α -sinensal	1753	1758	0.5	-	-	-	-	218, 133, 93, 79, 67
Ledene oxide	1890	1896	-	-	0.9	0.6	0.6	220, 159, 91, 81, 69
Phytol	1949	1948	0.8	-	-	-	-	278, 123, 95, 81, 71
TOTAL			93.6	95.7	90.7	93.1	91.2	

^a:- compounds are listed in order of elution from silica capillary coated on cp-sil 5;^b:- Retention indices on fused silica capillary column coated with cp-sil5;^c:- Retention indices from literature;

CE1:- Essential oil from 7.00 am harvest;

CE2:- Essential oil from 10.00 am harvest;

CE3:- Essential oil from 1.00 pm harvest;

CE4:- Essential oil from 4.00 pm harvest;

CE5:- Essential oil from 7.00 pm harvest.

Constituents of the oils that occurred in significant quantities were; α -pinene (0.6-1.9%), β -pinene (1.3-2.9%), car-3-ene (1.0-1.4%), β -ocimene (4.1-5.6%), D-limonene (13.5-19.8%), γ -terpinene (10.8-17.9%), terpinolene (0.5-1.4%), linalool (3.8-4.0%), allo-ocimene (1.1-1.2%), citronellal (2.7-7.5%), terpinen-4-ol (1.2-8.7%), citronellol (10.1-13.7%), linalylanthranilate (2.5-9.3%), geraniol (2.2%), δ -elemene (3.0-6.4%), β -elemene (0.5-1.4%), γ -elemene (0.9-3.7%), α -bergamotene (4.1-6.6%), humulene (1.2-2.8%), α -himachalene (0.5-0.6%), γ -muurolene (0.5-1.5%), β -caryophyllene (7.9-16.5%), γ -gurjunene (0.6-1.2%), β -bisabolene (3.6-5.2%), ledene oxide (0.6-0.9%). Others were; β -myrcene (0.8%), citronellylacetate (0.5%), β -guaiane (0.5%), β -cadinene (0.6%), caryophyllene oxide (1.0%), spathulenol (0.6%), α -cadinol (0.8%), α -bisabolol (0.9%), α -sinensal (0.5%), phytol (0.8%). Comparison of the oils revealed that there were variations in the composition patterns of oils.

Quantitatively, β -ocimene, terpinolene, linalool, δ -elemene, β -elemene, α -bergamotene, humulene and β -caryophyllene were detected in appreciable quantities in oil of 7.00 am harvest than other oils. Furthermore, car-3-ene, D-limonene and linalylanthranilate were more abundant in oil of 10.00 am harvest than oils of other harvests. β -pinene, citronellal, citronellol, γ -elemene and ledene oxide were of greater abundance in oil of 1.00 pm harvest than oils of other harvests. Meanwhile, higher quantities of α -pinene, γ -terpinene, terpinen-4-ol and α -himachalene were detected in the oil of 4.00 pm harvest than oil of other harvests. Allo-ocimene, γ -muurolene and β -bisabolene were found in appreciable quantities in oil of 7.00 pm harvest than oils of other harvests. Quantities of β -caryophyllene were more abundant in oils of 7.00 am and 7.00 pm harvests than oils of other harvests.

3.3 Radical Scavenging Activity

The DPPH radical scavenging activities of essential oil from *C. medica* leaves harvested at different times of the day during dry season is presented in Fig. 2. The anti-radical capacity of the oils was concentration dependent and increases as the concentration increases. At the highest concentration (100 μ l/ml), there was no significant difference in the activity of the oils harvested at different times of the day, whereas at the least concentration (10 μ l/ml), oils extracted from leaves harvested at 10.00 am had the highest radical scavenging activity (~35.07%) while oil from 7pm harvest had the lowest (7.67%). Activities of the essential oils at 10, 20 and 100 μ l/ml were significantly lower than that of BHT used as reference. This is reflected in the EC_{50} values of the oils (42.66 – 52.55 μ l/ml) which were significantly higher than that of BHT (40.19 μ l/ml) as revealed in Table 2.

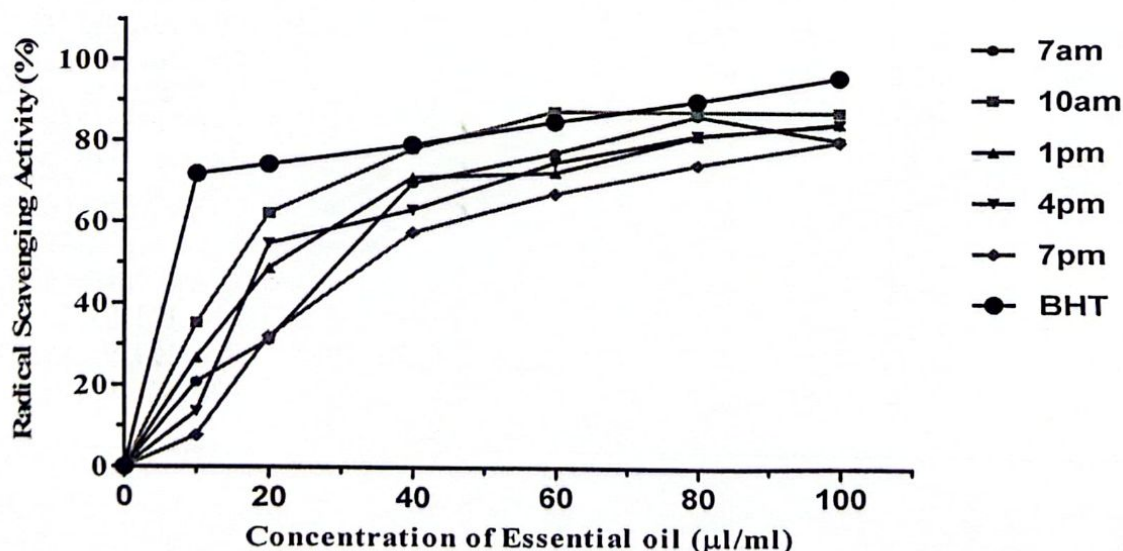


Fig. 2: DPPH radical scavenging activity of oils from fresh leaves *Citrus medica* harvested at different times of the day during the dry season

Table 2: Mean effective DPPH radical scavenging concentration (EC_{50}) of essential oils from *C. Medica* leaves harvested at different times of the day during dry season

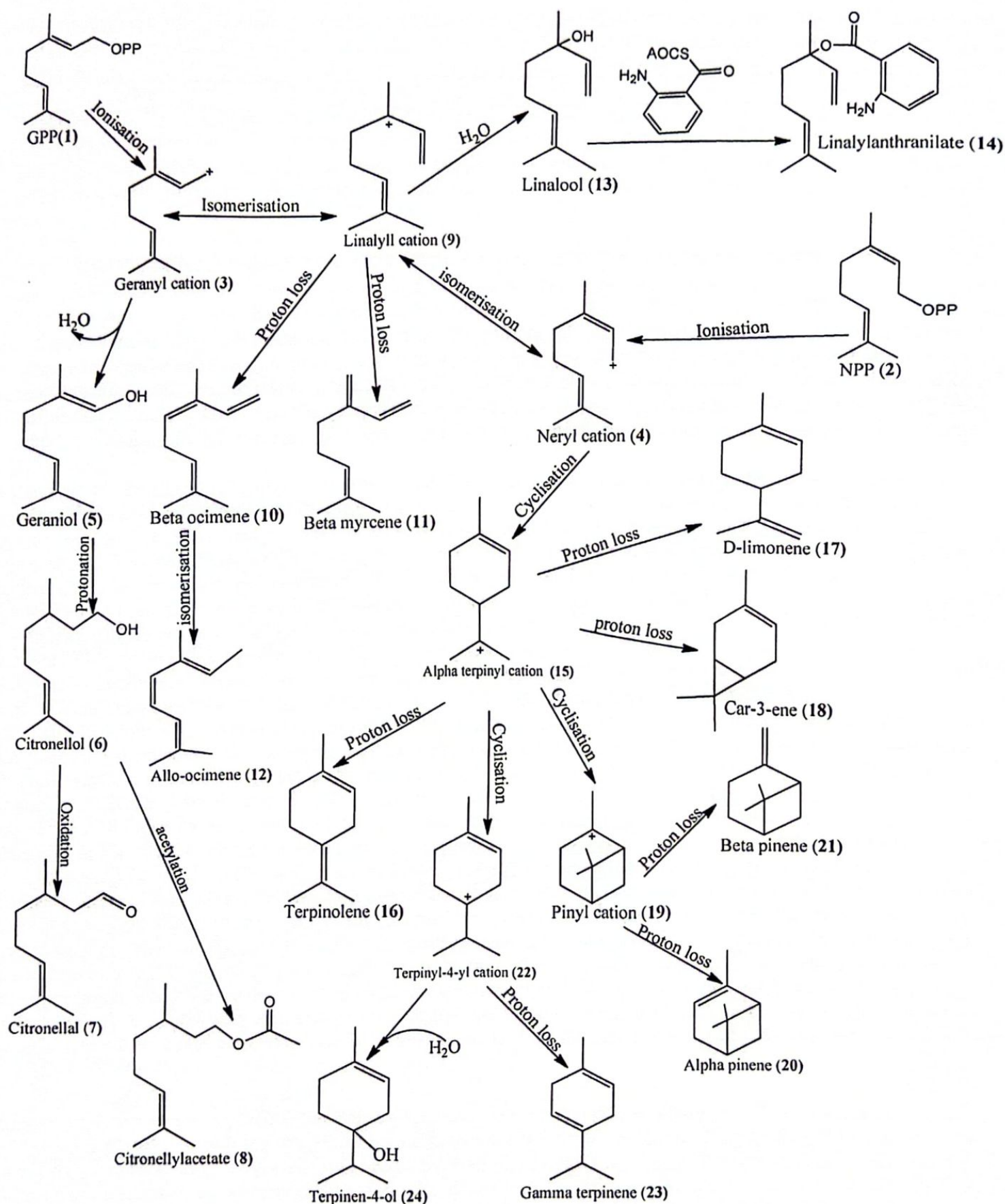
Time of Harvests	EC_{50}
7.00 am	47.26
10.00 am	42.66
1.00 pm	46.86
4.00 pm	47.26
7.00 pm	52.25
BHT	40.19

4.0 Discussion

Variations in the composition patterns of the oils are attributable to difference in the activities of terpene synthases that mediated the formation of the terpenoids from their respective precursors. According to previous findings, the synthase of the most abundant mono- and sesquiterpenoids normally mediates the transformation of their precursors to cationic intermediates [26, 27]. The intermediates subsequently undergo series of cyclizations, hydride and methyl shifts until the reaction is terminated by deprotonation or addition of nucleophile to give various terpenoids via cationic mechanisms.

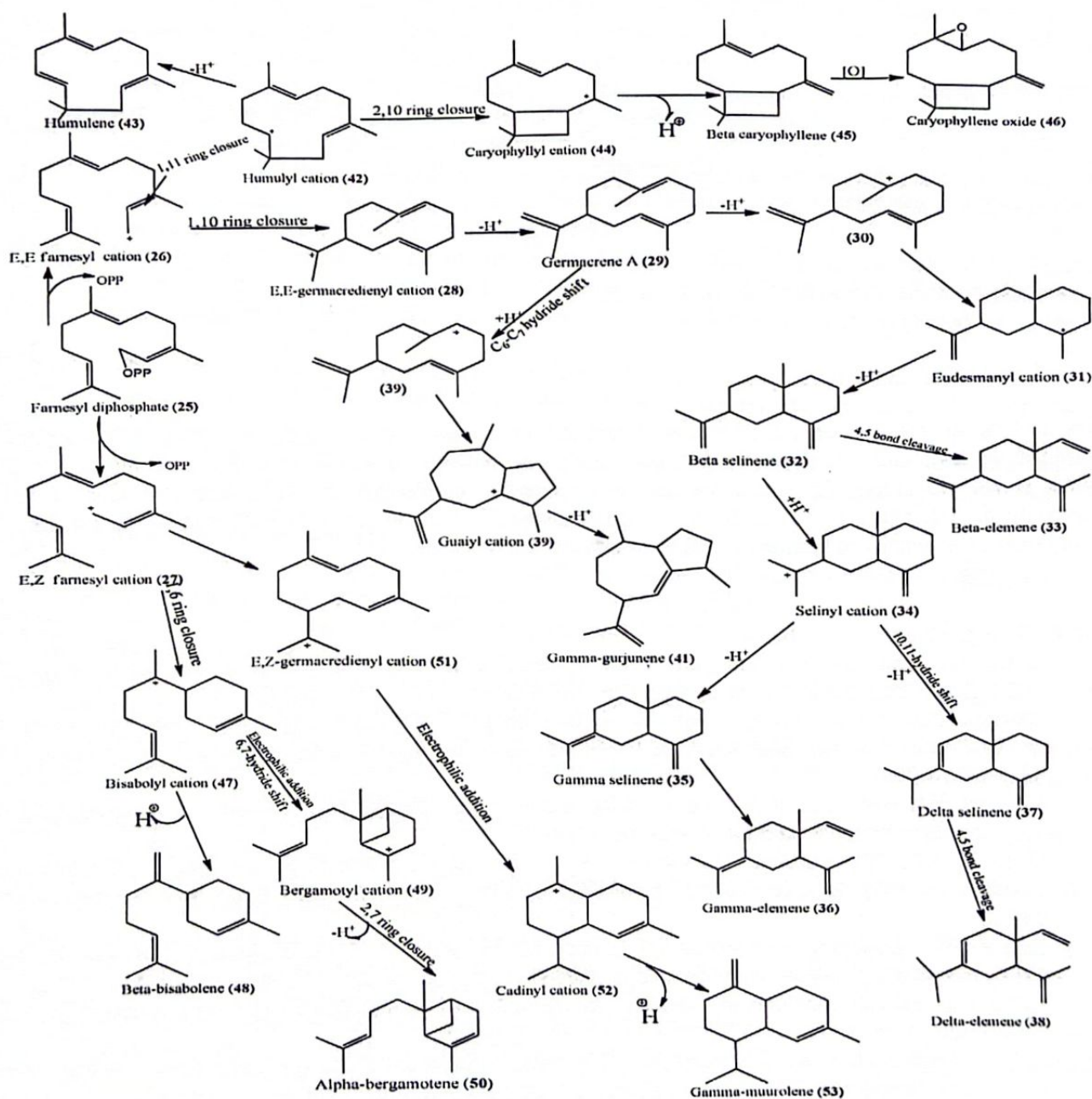
The predominance of γ -terpinene and D-limonene in oils indicates that their synthases mediated the biosynthesis of monoterpenoids in the oils where each of the compounds predominated (**Reaction scheme 1**). In the scheme, the synthases facilitated the ionization of geranyl and neryl pyrophosphates (**1, 2**) to geranyl and neryl cations (**3, 4**). The ions subsequently isomerized to cis-and-trans linalyl cations (**5 and 6**). Hydration of geranyl cation (**3**) was mediated by D-limonene synthase to produce geraniol (**7**) in oil of 10 am harvest. The same enzyme aided the hydrogenation of the compound (**7**) to citronellol (**8**) in the oils of 10.00 am and 7.00 pm harvests. Formation of the compound (**8**) in the oils of 7.00 am, 1.00 pm and 4.00 pm harvests was initiated by γ -terpinene synthase. D-limonene synthase also facilitated the oxidation of citronellol (**6**) to citronellal (**7**) in the oils of 10.00 am and 7.00 pm harvests respectively. Similarly, γ -terpinene synthase facilitated the conversion of citronellol to citronellal in the oils of 7.00 am, 1.00 pm and 4.00 pm harvests. Acetylation of citronellol (**6**) with acetyl CoA that produced citronellyl acetate (**8**) in the oil of 7.00 pm harvest was initiated by D-limonene synthase. Deprotonation of linalyl cation (**9**) at C_2 and C_{10} gave β -ocimene (**10**) in the oils and β -myrcene (**11**) in oil of 1.00 pm harvest. The transformations were initiated by D-limonene and γ -terpinene synthases. Isomerisation of β -ocimene (**10**) produced alloocimene (**12**) in oils of 10.00 am and 7.00 pm harvests. The conversion was aided by D-limonene synthase. Hydration of linalyl cation (**9**) to linalool (**13**) in oils of 7.00 am and 4.00 pm harvests was aided by γ -terpinene synthase. Acetylation of the compound (**13**) with anthranilyl-S-CoA to linalylanthranilate (**14**) in oils of 10.00 am, 1.00 pm and 7.00 pm harvests was facilitated by D-limonene and γ -terpinene synthases. The synthases also facilitated the electrophilic attack of neryl cation (**4**) on C_6 - C_7 double bond via C_6 to give α -terpinyl cation (**15**). Deprotonation of the ion (**15**) at C_6 and C_8 were initiated by the enzymes to produce terpinolene (**16**) in the oils and D-limonene (**17**) in oils of 10.00 am to 7.00 pm harvests. Deprotonation of the ion (**15**) at C_5 followed by its electrophilic attack on the deprotonated carbon that produced car-3-ene (**18**) in oils of 10.00 am and 7.00 pm harvest were initiated by D-limonene synthase. Folding of the ion (**15**) toward C_2 - C_3 double bond and its electrophilic attack via C_2 produced pinylation (**19**). Loss of proton at C_4 and C_{10} by the ion (**19**) to give α -pinene (**20**) in the oils and β -pinene (**21**) in oils of 1.00 pm to 7.00 pm harvests was aided by the enzymes. 6,7 hydride shifts in the ion (**15**) produced terpinen-4-yl cation (**22**). Deprotonation of the ion (**22**) at C_5 gave γ -terpinene (**23**) in the oils. Hydration of the ion formed terpinen-4-ol (**24**) in the oils except oil of 1.00 pm harvest. The synthases were aided by the two monoterpene synthases.

The predominance of β -caryophyllene (**38**) in the oils from various harvests indicated that its synthase mediated the biosynthesis of sesquiterpenoids in the oils (**Reaction Scheme 2**). In the scheme, Farnesyl diphosphate (**25**) ionized to E, Z-farnesyl (**26**) and E, E-farnesyl (**27**) cations. Electrophilic attack of the ion (**27**) on the C_{10} - C_{11} double bond via C_{10} produced E, E-germacradienyl cation (**28**). Deprotonation of the ion (**28**) at C_{11} formed germacrene A (**29**) that subsequently protonated at C_6 - C_7 double bond to form a cationic intermediate (**30**). Electrophilic attack of the ion (**30**) on the C_2 - C_3 double bond formed eudesmanyl cation (**31**). Deprotonation of the ion at C_{14} formed β -selinene (**32**). Copes rearrangement in the compound (**32**) that led to C_4 - C_5 bond cleavage formed β -elemene (**33**) in the oils of 7.00 am, 10.00 am and 7.00 pm harvests. Protonation of the C_{11} - C_{12} double bond of the compound (**32**) formed selinyl cation (**34**). Deprotonation of the ion (**34**) at C_{10} formed γ -selinene (**35**). C_4 - C_5 bond cleavage of the compound (**35**) via Copes rearrangement gave γ -elemene (**36**) in the oils of 7.00 am, 1.00 pm and 4.00 pm harvests. 10, 11- hydride shift of the ion (**34**) and subsequent deprotonation at C_9 formed δ -selinene (**37**).



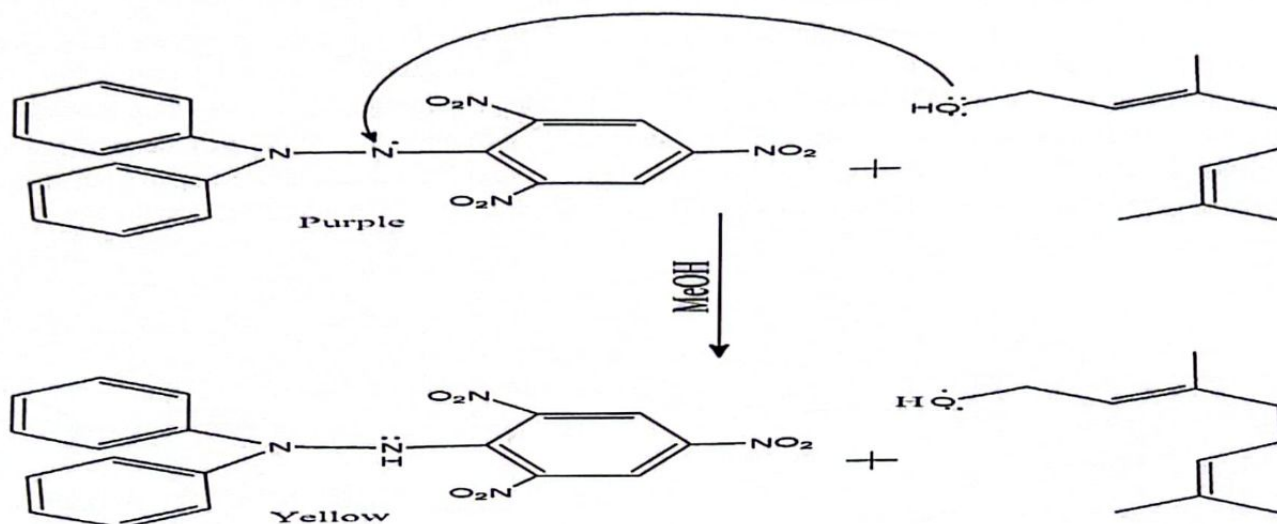
Reaction Scheme 1: D-limonene and γ -terpinene synthases mediated biosynthesis of monoterpenoids in essential oil from leaves of *C. medica* L. from various harvests in a day during dry season

Copes rearrangement in the compound (37) by C₄-C₅ bond cleavage formed δ -elemene (38) in the oils, except oil of 1.00 pm harvest. Protonation of germacrene A (29) at C₆-C₇ double bond followed by C₇-C₆ hydride shift formed a cationic intermediate (39). Electrophilic addition of the ion (39) on C₂-C₃ double bond formed guaiyl cation (40). Deprotonation of the ion (39) at C₁ produced γ -gurjunene (41) in the oils. Electrophilic attack of E, E-farnesyl cation (26) on C₁₀-C₁₁ double bond via C₁₁ produced humulyl cation (42). Deprotonation of the ion (42) at C₉ produced humulene (43) in the oils. Electrophilic addition of the ion (42) to C₂-C₃ double bond produced caryophyllyl cation (44). Loss of proton by the ion at C₁₅ (44) formed β -caryophyllene (45) in the oils. Epoxidation of the compound (45) at C₆-C₇ double bond gave caryophyllene oxide (46) in the oil of 7.00 am harvest. Electrophilic addition of E, Z-farnesyl cation (27) to C₆-C₇ double bond produced bisabotyl cation (47). Loss of proton by the ion (47) at C₁₀ formed beta-bisabolene (48) in the oils except that of 7.00 am harvest. Electrophilic attack of the ion (47) on the C₂-C₃ double bond produced bergamotyl cation (49). Deprotonation of the ion at C₄ gave α -bergamotene (50) in the oils. Electrophilic addition of E, Z-farnesyl cation on C₁₀-C₁₁ double bond formed E, Z-germacradienyl cation (51). 10,11- and 10,1-hydride shifts of the ion (51) and subsequent electrophilic addition on the C₆-C₇ double bond gave cadinyl cation (52). Deprotonation of the ion (52) at C₁₅ gave γ -muurolene (53) in the oils.



Reaction Scheme 2: β -caryophyllene synthase-mediated biosynthesis of sesquiterpenoids in essential oil from leaves of *C. medica* from various harvests in a day during dry Season

The apparent relative activity of the oils could be attributed to the high percentage of oxygenated monoterpenoids and the relative abundance of β -caryophyllene, since these components had been [28, 29]. The abundance of these components in oil from 10 am harvest than oils from other harvests may justify why the oil had the highest activities. More so, geraniol which was found only in the 10.00 am harvest has been shown to exhibit antioxidant activity (Reaction Scheme 3) by electron transfer from its hydroxyl functional group to the DPPH radical [30].



Reaction Scheme 3: Reduction of DPPH by Geraniol

5.0 Conclusion

The phytochemical profiles of the oils isolated from the leaves of *C. medica* varied significantly with period of the day. This implied that the environmental factors that determine the enzymes required for the biosynthesis of the compounds change at different period of harvest. The conditions caused variations in chemotypic and antioxidant potentials of the oils. Interestingly, the antioxidant activity of the oils compared favourably with the standard (BHT) used for the test. Hence, the oils may serve an alternative to synthetic antioxidants in foods and cosmetics.

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