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Characterization of pectin extracted from *Citrus reticulata* L. Blanco collected from different altitudes of Sikkim Himalaya

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Abstract
Sikkim mandarin (*Citrus reticulata*) is most important cash crops of Sikkim Himalaya, a tiny state in North East India. The fruit is usually peeled off and eaten as desert used for extraction of juiceor processed for other products. The peel is thrown as waste, though it is rich commercially important essentialoil andpectin. The pectin can be obtained from the pulp waste after extraction of essential oil. The essential oil and pectin content is the effect of the climatic functions. In Himalayas, there is abrupt change in microclimate with change in the altitude. *C. reticulata* in Sikkim Himalayas grows at the altitudinal range of 800 to 1800 metre from mean sea level. During the present studies pectin was extracted from peel waste after extraction of essential oil. The fruits were collected from five different altitude range *viz*: 800-1000m, 1000-1200m, 1200-1400m, 1400-1600m and >1600m. FTIR works on the basis of functional group showed range from 3607 cm^{-1} (O-H stretch region) to 748cm^{-1} (C-H bend) in mature stage and 3585 cm^{-1}(O-H stretch) to 883 (C-Cl stretch)cm^{-1} in immature stage. Moreover essential oil showed different compound identification. Limonene was found to be the highest at >1600m altitude (88.46%) at mature stage and (89.06%) at immature stage respectively. These variation may be due to different climatic condition and soil of the elevation. The overall results showed that the pectin can be beneficial for industrial use as well as in pharmaceutical health promotion and treatment. Further peel of the species can be evaluated for its rich content of limonene by different industries.

Keywords: Altitude, Essential oil, Pectin, Pulp waste, Sikkim mandarin (*Citrus reticulata*)

INTRODUCTION

Pectin is a biodegradable and natural gelling agent which can be used for commercial purpose (Chin *et al*., 2014) such as making jam, jellies and marmalade (Devi *et al.,* 2014) and in fruit juices and milk drink as a stabilizer and also as dietary fiber (Tobias *et al.,* 2011). Pectin is produced as bi - product after juice extraction from citrus peel or apple pomace. Apple pomace gives 10-15% pectin and that of citrus fruit peel 20-30% (Kulkarni *et al.,* 2006). Productionof pectin differs between plant to plant, various parts of the single plant, and in the same plant in different time interval (Krishnamurthi andGiri, 2003). Considering the fact, present studies envisage for extraction of pectin from the citrus (*Citrus reticulata)*peel after the extraction of essential oil.

MATERIALS AND METHODS

This research work was carried out between the months of August – December 2017 in the Department of Horticulture, Sikkim University, 6th Mile, Tadong, Sikkim, India.

Sample preparation: Fruits of mature and ripened stage of *C. reticulata* were collected from five different altitude range of Sikkim *viz:* 800-1000m, 1000-1200m, 1200-1400m, 1400-1600m and >1600m. The essential oil was extracted from the peel. After the extraction of essential oil, the leftover of peel were dried in a hot air oven at 60 º C until the weight becomes constant followed by grinding. The dried peel was then subjected to extraction of pectin. For extraction of pectin, 5 g of powdered sample was subjected for extraction with 90ml distilled water and 10ml citric acid at pH 2. The mixture was heated at 60 ° C and continuously stirred for 1 hour. The acid extract was then filtered using Whatman No. 1 filter paperand the filtrate was coagulated with an equal volume of 95% ethanol. The coagulated filtrate waskept undisturbed for 2 hours to make the pectin float on the surfaceasgelatinous jelly. The pectin jelly was filtered and washed with ethyl alcohol 2-3 times to remove impurities. The precipitate was then dried in a hot air oven at 35 - 40°C. The yield of pectin (fresh weight anddry weight basis) was calculated in digital weighing balance (Khule *et al*.,2012).

Ypec(%) *P* x100 …….Eq 1
Bi

Where, Y is the yield of pectin in (%), P is the amount of extracted pectin in gram and B is the initial weight of fruit peel powder.

Physicochemical characterization of the pectin: The dried pectin samples were subjected to the following qualitative and quantitative tests in order to characterize them.

Qualitative tests

Color: This was done by visual observation

Solubility of dry pectin in cold and hot water: To determine the solubility of dry pectin, 0.25 g of pectin powdered sample were placed in two different conical flask and 10 mL of 95%ethanol and 50 mL of distilled water was added. The mixture in one flask was kept and other was shaken vigorously toform a suspension and then heated at 85-95°C for 15 min (Fishman *et al*., 2003).

Solubility of pectin solution in cold and hot alkali (NaOH): In two different conical flasks 1 ml of 0.1 N NaOH was placed and 5ml of pectin solution was added. Further second flask was heated at 85- 90°C for 15 min (Joslyn, 1980).

Sugar and organic acids: 1 g of pectin samplewas placed in 500 mL flask and5 mL ethanol and 100 mL water was poured rapidly, shaken and then allowed to stand for 10 minutes. To this solution, 100 mL of ethanol containing 0.3 mL hydrochloric acid was mixed and then filtered using what man No1 filter. The filtrate around 2.5 mL was measured into a conical flask (25 mL), the liquid was placed in steam bath for evaporation and residue was dried in a hot air oven at 50°C for 2 h (Devanooru *et al*., 2015)

Quantitative Tests

Equivalent weight determination: Equivalent weight was determined from 0.5 g of pectin sample, to which5mL ethanol was added followed by 1 g sodium chloride and 100mL of distilled and few drops of phenol red indicator. The mixture was shaken well to avoid the clumping and titrated against0.1 M NaoH to a pink colour at the endpoint (Owens *et al*., 1952).

Following equation was used to calculate equivalent weight of pectin. (Krishnamurthi and Giri, 2003).

Equivalent Weight: (weight of pectin sample x Molarity of alkali) / *Volume of alkali* x100 ……Eq 2

Methoxyl content determination: The neutralized solution left after determining equivalent weight was placed in a conical flask (250mL). In that 25 mL of 0.25 M NaOH was added. The mixture was stirred thoroughly and allowed to stand for 30 min at ambient temperature (Norziah, *et al*., 2000). The methoxyl content was calculated using the equation below(Kulkarni *et al*., 2006).

Methoxyl content % =Volume of alkali x weight / Weight of pectin samplex100 .. . .Eq.3

Moisture content determination: An empty petri dish was kept in a hot air oven, cooled and then weighed in a digital weighing balance. 5g of the pectin sample was then transferred into the empty dish, kept in a hot air oven at 130°C for 1 h thereafter the petri dish was removed, cooled indesiccators and weighed.Moisture content was then calculated using Joye and Luzio, 2000

Moisture Content (%) = Weight of the Residue / Weight of the Sample x 100% …….Eq.4

Anhydrouronic Acid (AUA) Content: Anhydrounic acid content (AUA) was calculated by using values from equivalent weight and methoxyl content. AUA content was then estimated as per (Pagan *et al.,* 2000) (Owens *et al*., 1952)

AUA% = 176 / Z Weight of sample (mg) x100 ………...Eq.5

(where, 176 is the molecular weight of AUA) and meq of Titration A + meq of Titration B

Degree of esterification (DE): DE %was calculated by following equation (Khule *et al.,*2012), using the values from methoxyl and anhydrouronic acid content determinations (Schultz, 1976).

DE % = 176 x MeO % x 100 / 31 x AUA % …Eq.6

Ash percentage: An empty crucible was weighed in which 1 g of pectin was placed and then kept in muffle furnace at 600°C for 3-4 hours. The crucible was then cooled at room temperature in a desiccators and weigh (Ranganna, 1986)

Ash % was calculated using following equation

Ash % = Weight of ash x Weight of pectin x 100 …Eq.7

Alkalinity percent as carbonate: In 25 ml of 0.1 N HCl, ash was dissolved. It was heated to boiling and cool. Then titration was done with 0.1 N NaOH using phenolphthalein indicators. (Ranganna, 1986)

Alkalinity % as carbonate = Titre X Normality of NaOH / Weight of ash X 1000 X 60 X 100 ….Eq.8

Carbonate free ash %: It is calculated by using the following equations (Ranganna, 1986) as:

Carbonate free ash % = Ash% - Carbonate free ash% Eq.9

Spectral analysis: Subsequent to the above mentioned tests, the pectin from *C. limon* was further subjected to FTIR analysis (Shimadzu, IRAffinity-1) and the resultingspectrum was studied in order to understand the functional groups present (Kanmani *et al*., 2014

Essential oil extraction: Fresh fruits were washed to remove dirt andrind was separated. 500g of peelwas subjected to hydro distillation by adding water enough to dissolve the rind in a 2000ml round bottom flask fitted to a Clevenger apparatus. After 3 hours, oil was collected and stored in brown bottle and stored at 4 °C until analysis. Separation of oil with water was done using separating funnel, further water was removed by addition of anhydrous sodiumsulphate. All the extracted oil from Citrus germplasm was then subjected for profiling and characterization following standard procedure and protocol as follows.

Profiling of essential oil: Profiling of essential oil was done by GC/MS-QP-2010 plus Ultra (Shimadzu company) using an Rtx-5 MS fused silica capillary column (30 m × 0.25 m i.d., film thickness 0.25 μm*)*.

Gas chromatography–mass spectrometry analysis: A sample of 1 μl was used in split plot ratio of 100:1. An electron ionization system with ionization energy of 70eV was used. Helium was used as carrier gas at a flow rate of 1.5 ml/min. Mass scanning range was varied over 50-550m/z while injector and MS transfer line temperatures was set at 220 and 290°C respectively.

Compounds identification: Identification of essential oilvolatile compounds was based on the calculation of theirretention indices (RI) relative to (C_8–C_{22}) *n*-alkanes withthose of authentic compounds available in our laboratory. Further identification was made by matching their recordedmass spectra with those stored in the Wiley/NBS massspectral library of the GC-MS data systems and other published mass spectra (Adams, 2001).

RESULTS AND DISCUSSION

Physico chemical characterization: The physico chemical characterization of dried pectin from peel is presented in Table 2. The degree of esterification was found to be highest in the samples from the altitude >1600m in both mature (90.94%) and immature stages (91.79%). The data presented in the finding is higher than reported by Fakayode and Abobi (2018) with 60.4% in Nigeria at higher altitude.

Higher DE i.e.>50% is marked as high methoxyl pectin (HMP) which help pectin to form gel quickly at high temperature with effective action on the lipid profile (Brouns *et al*. 2012; Dominiak *et al.*2014).

Likewise, equivalent weight was 496.33and 444.49, respectively for rind taken from mature and immature fruits at >1600m. The data in the finding was comparatively lower than reported by Fakayode and Abobi, 2018 who mentioned 599.74 in Nigeria as the value in their result. As mentioned by Yadav *et al.* (2017), high equivalent weight showed higher level of gel forming ability

Table 1. Qualitative test of the *Citrus reticulata* at different altitudinal range.

Altitude range (m)	Colour		Solubility of dry pectin in cold water		Solubility of dry pectin in hot water		Solubility of dry pectin in cold alkali		Solubility of dry pectin in hot alkali		Sugar and organic acid	
	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature
800-1000	Dark brown	Dark brown	Insoluble	Insoluble	Sparingly dissolves in yellow colour	Dissolves with pale yellow color	Dissolves with light brown precipitates	insoluble	Dissolves with yellow color precipitate	Dissolves with yellow color precipitate	40.67± 0.89	44.33± 1.20
1000-1200	Dark brown	Dark brown	Insoluble	Insoluble	Sparingly dissolves in yellow colour	Dissolves with pale yellow color	insoluble	insoluble	Dissolves with yellow precipitate	Dissolves with yellow color precipitate	44.33± 1.20	51.33± 1.76
1200-1400	Light brown	Dark brown	Insoluble	Insoluble	Sparingly dissolves in yellow colour	Dissolves with water color	insoluble	insoluble	Dissolves with yellow precipitate	Dissolves with yellow color precipitate	45.67± 0.88	51.8± 1.33
1400-1600	Dark brown	Pale yellow	Insoluble	Insoluble	Sparingly dissolves in yellow colour	Insoluble	Insoluble	Insoluble	Dissolves with yellow precipitate	Dissolves with yellow color precipitate	49.67± 0.88	55.33± 1.76
>1600	Light brown	Dark brown	Insoluble	Insoluble	Dissolves with transparent color	Dissolves with water color	Insoluble	Insoluble	Dissolves with yellow precipitate	Dissolves with yellow color precipitate	56.53± 1.64	56.33± 0.88

Table 2. Quantitative tests of *Citrus reticulata* at different altitudinal range.

Parameters	Stages	Altitude range (m)				
		800-1000	1000-1200	1200-1400	1400-1600	>1600
% yield on dry basis	Mature	0.68±0.43	0.69±0.22	0.83±0.17	0.83±0.13	0.88±0.30
	Immature	0.53±0.31	0.58±0.32	1.46±0.14	1.50±0.15	1.53±0.03
% yield on wet basis	Mature	29.49±1.05	35.83±1.40	36.82±1.35	37.00±0.57	40.82±0.74
	Immature	10.00±0.58	13.25±0.67	15.21±0.94	15.10±0.10	15.21±0.32
Equivalent weight	Mature	395.00±2.89	412.89±1.97	451.81±2.82	494.70±2.03	496.33±1.86
	Immature	378.82±1.64	396±2.31	396.33±1.86	414.89±1.06	444.49±2.79
Methoxyl Content (MeO %)	Mature	5.07±0.07	6.29±0.04	6.57±0.05	6.64±0.15	6.93±0.03
	Immature	4.32±0.01	5.33±0.35	6.24±0.46	6.46±0.21	6.91±0.04
Anhydrouronic Acid (AUA %)	Mature	41.95±0.45	42.03±0.07	42.58±0.22	43.20±0.73	43.27±0.42
	Immature	37.79±0.21	41.43±0.98	41.86±0.95	42.31±0.05	43.59±0.22
Degree of esterification (DE %)	Mature	88.23±0.87	88.65±0.69	89.23±0.66	90.75±0.57	90.94±0.36
	Immature	85.67±0.33	88.90±0.27	89.33±0.34	90.34±0.63	91.79±0.79
Moisture	Mature	56.83±0.44	63.33±0.33	64.40±0.30	65.40±0.30	66.47±0.29
	Immature	55.00±0.58	64.00±0.58	64.33±0.33	64.96±0.50	66.3±0.15
Ash %	Mature	55.00±2.52	56.57±2.09	56.74±2.80	62.33±1.86	67.53±1.29
	Immature	44.33±0.33	46.40±0.83	54.00±0.58	56.00±0.58	72.33±0.88
Alkalinity as carbonate	Mature	4.28±0.09	5.60±0.60	5.53±0.76	6.47±0.20	6.11±0.17
	Immature	4.29±0.01	6.06±0.60	6.66±0.01	7.10±0.48	7.92±0.40
Carbonate free ash	Mature	50.72±2.48	50.98±2.26	52.05±2.53	55.87±1.88	61.42±1.16
	Immature	40.04±0.32	40.34±0.86	47.34±0.56	48.90±0.98	64.41±1.15

Table 3a. Functional groups present at mature stage in Sikkim mandarin (*Citrusreticulata)* at 800m altitude.

Frequency	Bond	Functional group
3607 (m,s)	O-H stretch	Alcohol
3029 (w,b)	C-H stretch	Aromatics
2956(m)	C-H stretch	Alkane
1727 (s)	C= O stretch	α,β unsaturated ester
1201(m)	C-N stretch	Aliphatic amines
1075 (m)	C-N stretch	Aliphatic amines
984 (s)	C-H out of plane blend	Aromatic
867 (s)	C-H out of plane blend	Aromatic

Table 3b. Functional groups presentat mature stage in Sikkim mandarin at 1000-1200m altitude.

Frequency (cm)	Bond	Functional group
3135 (w)	O-H stretch	Alcohol
2939 (m)	C-H stretch	Alkane
1721(s)	C=O stretch	Aldehyde
1625 (s)	C=C stretch	α,β unsaturated ketone
1219 (m)	C-N stretch	Aliphatic amine
1073 (m)	C-N stretch	Aliphatic amines
1002(s)	C-Hout of plane blend	Alkene

Table 3c. Functional groups present at mature stage in Sikkim mandarin at 1200-1400m altitude.

Frequency (cm)	Bond	Functional group
3537(s)	O-H stretch, H-bonded	Alcohols, phenols
3066(s)	C-H stretch	Aromatics
2957(m)	C-H stretch	Alkanes
1721(s)	C=O stretch	α, β unsaturated ester
1214(m)	C-N stretch	Aliphatic amines
1074(m)	C-N stretch	Aliphatic amines
1005(s)	C-H out of plane blend	Alkenes

Table 3d. Functional groups presentat mature stage in Sikkim mandarin at 1400-1600m altitude.

Frequency (cm)	Bond	Functional group
3048 (s)	C-H	Aromatics
1712(s)	C=O	α, β unsaturated ester
1619 (m-w)	C=C	Alkenes
1209 (vs)	C-N stretch	Aliphatic amines
1103(s)	C-H wag ($-CH_2X$)	Alkyl halides
1064 (m)	C-N stretch	Aliphatic amines
1017 (m)	C-N stretch	Aliphatic amines
748 (s)	C-Hout of plane blend	Alkene

Table 3e. Functional groups present at mature stagein Sikkim mandarinat >1600m.

Frequency (cm)	Bond	Functional group
3062 (s)	C-H stretch	Aromatics
2959 (m)	C-H stretch	Alkane
1715 (s)	C=O stretch	α, β unsaturated ester
1613 (m-w)	C=C stretch	Alkene
1214 (s)	C-N stretch	Aliphatic amine
1108 (m)	C-H wag ($-CH_2X$)	Alkyl halides
1095 (m)	C-N stretch	Aliphatic amine
1021 (s)	C-N stretch	Aliphatic amine

Table 3f. Functional groups present at immature stage in Sikkim mandarin (*Citrus reticulata)* at 800m altitude.

Frequency (cm)48	Bond	Functional group
3364(s)	O-H stretch	Alcohol
2949(m)	C-H stretch	Alkane
1722(s)	C=O stretch	α, β unsaturated ester
1204(v,s)	C-O stretch	Phenols
1026(m-s)	C-N stretch	Amines

Table 3g. Functional groups present at immature stage in Sikkim mandarin (*Citrus reticulata)* at 1000 -1200m altitude.

Frequency (cm)	Bond	Functional group
3514 (s, sh)	O-H stretch, H-bonded	Alcohols, phenols
3096(m)	O-H bend	Carboxylic acid
2993(s)	C-H stretch	Alkanes
1692(s)	C=O stretch	Carboxylic acid
1209(s)	C-N stretch	Aliphatic amines
1096(m)	C-N stretch	Aliphatic amines
1003(m)	C-N stretch	Aliphatic amines

Table 3h. Functional groups present at immature stage in Sikkim mandarin (*Citrus reticulata)* at 1200 -1400m altitude.

Frequency (cm)	Bond	Functional group
3585(s)	O-H stretch	Alcohols, phenols, H-bonded
3048(s)	C-H stretch	Alkane
2932(m)	C-H stretch	Alkane
1714(s)	C=O stretch	α, β unsaturated ester
1229(s)	C-N stretch	Aliphatic amines
1075(m)	C-N stretch	Aliphatic amines
1018(m)	C-N stretch	Aliphatic amines

while at lower levelthere will be higher partial degradation of the pectin which is disadvantageous.
On the other hand, respective MeO was 6.93and 6.91 in mature and immature stage at>1600m altitude. The results were in corroboration with Fakayode and Abobi, 2018, who reported6.23% MeO in Nigeria at higher altitudein orange peel. In 2014, Kanmani *et al.,* illustrated MeO range from 0-12% based on source and method of extraction. MeO less than 7%, is considered to be the pectin of good quality as mentioned by (Bagde *et al*., 2017) who had done research in Yavatmal. Low MeO contributes furthermore-irreversible gel properties, which means it will remain in gelled form even if heated at melting temperature. Further, they can be used in making low sugar jam in food industry as itdoes not require high sugar for forming gel They can also be used in various aspects as gelling agent, thickening agent and fat substitute (Tiwari *et al.* 2017).
The total anhydrouronic acid content was found to be 43.27% and 43.59% at >1600m altitude while the lowest was accorded in 800m with 41.95% and 37.79% in mature and immature stages respectively. Low value of AUA means that the extracted pectin might have high amount of protein, starch and sugars in the precipitated pectin in Bangladesh (Ismail *et al*., 2012).
Moisture content of the pectin was also estimatedhighin>1600m altitude with 66.70% and 64.30%, respectively. It was higher than orange peel (50%) and lower than lemon peels (70%) as reported by Bagde *et al*., 2017in Yavatmal.
Yield of pectin on wet basis showed 40.82%,

Table 3i. Functional groups present at immature stage in Sikkim mandarin (*Citrus reticulata)* at 1400-1600m altitude.

Frequency (cm) 5	Bond	Functional group
2952(m)	C-H stretch	Alkane
2617(m)	O-H stretch	Carboxylic acid
1990(m)	C-N stretch	Aliphatic amines
1717(s)	C=O stretch	α, β unsaturated ester
1620(s)	C=C stretch	Vinyl ether
1339(m,w)	C-N stretch	Amine
1206(s)	C-N stretch	Aliphatic amines
1074(m)	C-N stretch	Aliphatic amines
883(m)	C-Cl stretch	Alkyl halides

Table 3j. Functional groups present at immature stage in Sikkim mandarin (*Citrus reticulata)* at >1600m altitude.

Frequency (cm)44	Bond	Functional group
3604(s)	O-H stretch	Alcohol, phenols
3042(b)	O-H stretch	Carboxylic acid
2957(m-s)	C-H stretch	Alkyl
1696(s)	C=O stretch	Unsaturated/aromatic carboxylic acid
1210(s)	C-O stretch	Ether
1075(often over lapped)	C-N stretch	Aliphatic amines
1019(often over lapped)	C-N stretch	Aliphatic amines

Note : s= strong , m= medium, w= weak , b= bend

15.21% in mature and immature stages while 0.88% and 1.5% in mature and immature stages on dry basis respectively. The data in the present findings was comparatively higher for wet and dry basis as illustrated by Bagde *et al*., 2017 in lemon peel and orange peel are 11, 8% while on dry basis were 1, 0.5%, respectively.
The data regarding ash % showed 72.33% and 67.53% occupying the maximum value at >1600m altitude in mature and immature stages which contradicts the finding of Bagde *et al.,* 2017 wherein, only 30% and 35% of ash content in lemon and orange peel pectin was reported. Likewise alkalinity as carbonate and carbonate free ash were studied which revealed that immature (6.92, 64.41) has high value compared to mature stage (6.11, 61.42) and was found to be higher at >1600m altitudes.
There is considerable variation in physic chemical characterization of pectin from different altitude.
It may be due to variability in soil characteristics and climatic conditions (rainfall, temperature and humidity) in the various altitudes under research. It can also be revealed that geographical location plays a major role in the alteration of these parameters.
FTIR Spectral analysis: The FTIR spectrum of pectin extracted from Sikkim mandarin is illustrated in Fig.1 and corresponding functional groups

Table 4a. Composition of essential oil compounds at 800m altitude in mature stage.

S.N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1634	922	8.449	$C_{10}H_{16}$	136
2.	α -Pinene	0.7776	929	8.735	$C_{10}H_{16}$	136
3.	Camphene	0.0044	945	9.374	$C_{10}H_{16}$	136
4.	Sabinene	0.5019	969	10.329	$C_{10}H_{16}$	136
5.	β -Pinene	0.5504	973	10.526	$C_{10}H_{16}$	136
6.	Myrcene	1.6476	988	11.068	$C_{10}H_{16}$	136
7.	Octanal	0.4249	1003	11.665	$C_8H_{16}O$	128
8.	(+)-2-Carene	0.0643	1015	12.279	$C_{10}H_{16}$	136
9.	Limonene	88.3991	1042	13.243	$C_{10}H_{16}$	136
10.	β -Ocimene	0.0448	1048	13.666	$C_{10}H_{16}$	136
11.	γ -Terpinene	4.6372	1061	14.249	$C_{10}H_{16}$	136
12.	Acetophenone	0.0160	1064	14.511	C_8H_8O	120
13.	1-Octanol	0.0653	1071	14.825	$C_8H_{18}O$	130
14.	α -Terpinolene	0.1836	1084	15.414	$C_{10}H_{16}$	136
15.	α-p-dimethylstyrene	0.0077	1088	15.649	$C_8H_{18}O$	130
16.	Linalool	1.4607	1102	16.202	$C_{10}H_{18}O$	154
17.	Nonanal	0.0243	1104	16.340	$C_9H_{18}O$	142
18.	Trans-p-Mentha-2,8-dienol	0.0136	1120	17.607	$C_{10}H_{16}O$	152
19.	Cis-p-Mentha-2,8-dien-1-ol	0.0121	1134	17.826	$C_{10}H_{16}O$	152
20.	Citronellal	0.0499	1150	18.537	$C_{10}H_{18}O$	154
21.	Terpinen-4-ol	0.1188	1178	19.893	$C_{10}H_{18}O$	154
22.	α -Terpineol	0.1518	1193	20.614	$C_{10}H_{18}O$	154
23.	Decanal	0.1630	1205	21.088	$C_{10}H_{20}O$	156
24.	Nerol	0.0148	1223	21.981	$C_{10}H_{18}O$	154
25.	Carvacryl methyl ether	0.0680	1228	22.174	$C_{11}H_{16}O$	164
26.	Neral	0.0281	1236	22.571	$C_{10}H_{16}O$	152
27.	Carvone	0.0093	1240	22.810	$C_{10}H_{14}O$	150
28.	Geranial	0.0310	1265	23.932	$C_{10}H_{16}O$	152
29.	Perillaldehyde	0.0384	1271	24.244	$C_{10}H_{14}O$	150
30.	o-Cymen-5-ol	0.1283	1292	25.262	$C_{10}H_{14}O$	150
31.	Undecanal	0.0096	1306	25.588	$C_{11}H_{22}O$	170
32.	α-Elemene	0.0320	1332	26.916	$C_{15}H_{24}$	204
33.	β -Elemene	0.0168	1386	29.316	$C_{15}H_{24}$	204
34.	Dodecanal	0.0200	1407	30.203	$C_{12}H_{24}O$	184
35.	Germacrene B	0.0094	1426	31.015	$C_{15}H_{24}$	204
36.	β -Farnesene	0.0200	1451	32.017	$C_{15}H_{24}$	204
37.	Germacrene D	0.0535	1476	33.157	$C_{15}H_{24}$	204
38.	α -Farnesene	0.0106	1502	34.154	$C_{15}H_{24}$	204
39.	γ -Elemene	0.0279	1553	36.288	$C_{15}H_{24}$	204

Table 4b. Composition of essential oil compounds at 1000-1200m altitude in mature stage.

S. N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1009	937	8.450	$C_{10}H_{16}$	136
2.	α -Pinene	0.5270	945	8.734	$C_{10}H_{16}$	136
3.	Camphene	0.0057	961	9.376	$C_{10}H_{16}$	136
4.	Sabinene	1.1344	986	10.332	$C_{10}H_{16}$	136
5.	β -Pinene oxide	0.7572	991	10.527	$C_{10}H_{16}O$	152
6.	Myrcene	1.5293	1004	11.061	$C_{10}H_{16}$	136
7.	Octanal	0.2853	1016	11.651	$C_8H_{16}O$	128
8.	(+)-2 Carene	0.0797	1030	12.267	$C_{10}H_{16}$	136
9.	Limonene	87.6443	1057	13.190	$C_{10}H_{16}$	136
10.	β -Ocimene	0.1376	1063	13.652	$C_{10}H_{16}$	136
11.	γ –Terpinene	4.3163	1076	14.224	$C_{10}H_{16}$	136
12.	1-Octanol	0.2077	1087	14.829	$C_8H_{18}O$	130
13.	Terpinolene	0.1891	1100	15.407	$C_{10}H_{16}$	136
14.	p-Cymenene	0.0170	1104	15.637	$C_{10}H_{12}$	132
15.	Linalool	1.9045	1117	16.200	$C_{10}H_{18}O$	154

Contd…….

16.	Nonanal	0.0365	1120	16.330	$C_8H_{16}O$	128
17.	Citronellal	0.1046	1166	18.533	$C_{10}H_{18}O$	154
18.	Terpin-4-ol	0.1650	1194	19.890	$C_{10}H_{18}O$	154
19.	α -Terpineol	0.1844	1209	20.609	$C_{10}H_{18}O$	154
20.	Decanal	0.1722	1220	21.081	$C_{10}H_{20}O$	156
21.	Thymol methyl ether	0.1356	1244	22.168	$C_{11}H_{16}O$	164
22.	Neral	0.0207	1251	22.551	$C_{10}H_{16}O$	152
23.	α -Citral	0.0308	1281	23.924	$C_{10}H_{16}O$	152
24.	2-Decyn-1-ol	0.0323	1287	24.234	$C_{10}H_{18}O$	154
25.	Thymol	0.0909	1307	25.251	$C_{10}H_{14}O$	150
26.	α-Elemene	0.0269	1349	26.914	$C_{15}H_{24}$	204
27.	β -Elemene	0.0151	1403	29.304	$C_{15}H_{24}$	204
28.	Dodecanal	0.0198	1424	30.191	$C_{12}H_{24}O$	184
29.	Bicyclogermacrene	0.0180	1443	31.004	$C_{15}H_{24}$	204
30.	β -Farnesene	0.0192	1467	32.005	$C_{15}H_{24}$	204
31.	Germacrene D	0.0539	1493	33.143	$C_{15}H_{24}$	204
32.	α -Farnesene	0.0179	1519	34.139	$C_{15}H_{24}$	204
33.	Germacrene B	0.0201	1571	36.271	$C_{15}H_{24}$	204

Table 4c. Composition of essential oil compounds at 1200-1400m altitude in mature stage.

S. N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1335	922	8.449	$C_{10}H_{16}$	136
2.	α -Pinene	0.6222	929	8.735	$C_{10}H_{16}$	136
3.	Camphene	0.0321	945	9.376	$C_{10}H_{16}$	136
4.	Sabinene	0.5046	969	10.331	$C_{10}H_{16}$	136
5.	β -Pinene	0.4074	973	10.527	$C_{10}H_{16}$	136
6.	Myrcene	1.5654	988	11.071	$C_{10}H_{16}$	136
7.	Octanal	0.3272	1003	11.668	$C_8H_{16}O$	128
8.	α -Terpinene	0.0902	1015	12.281	$C_{10}H_{16}$	136
9.	Limonene	87.2034	1042	13.263	$C_{10}H_{16}$	136
10.	β -Ocimene	0.0837	1048	13.674	$C_{10}H_{16}$	136
11.	γ -Terpinene	4.8204	1062	14.265	$C_{10}H_{16}$	136
12.	1-Octanol	0.0993	1072	14.832	$C_8H_{18}O$	130
13.	α -Terpinolene	0.2396	1085	15.423	$C_{10}H_{16}$	136
14.	α –p-dimethylstyrene	0.0324	1089	15.654	$C_{10}H_{12}$	132
15.	Linalool	1.5009	1101	16.209	$C_{10}H_{18}O$	154
16.	Nonanal	0.0347	1104	16.340	$C_9H_{18}O$	142
17.	trans-p-Mentha-2,8-dienol	0.0080	1120	17.164	$C_{10}H_{16}O$	152
18.	Cis-Mentha-2,8-dien-1-ol	0.0237	1130	17.834	$C_{10}H_{16}O$	152
19.	Citronellal	0.1021	1135	18.546	$C_{10}H_{18}O$	154
20.	Terpinen-4-ol	0.1905	1138	19.904	$C_{10}H_{18}O$	154
21.	α -Terpineol	0.2335	1146	20.627	$C_{10}H_{18}O$	154
22.	Decanal	0.1800	1151	21.096	$C_{10}H_{20}O$	156
23.	Trans-Carveol	0.0209	1178	21.762	$C_{10}H_{16}O$	152
24.	Nerol	0.0141	1186	21.990	$C_{10}H_{16}O$	152
25.	Thymol methyl ether	0.5752	1194	22.200	$C_{11}H_{16}O$	164
26.	Neral	0.0254	1205	22.570	$C_{10}H_{16}O$	152
27.	Carvone	0.0345	1219	22.823	$C_{10}H_{14}O$	150
28.	Geraniol	0.0183	1231	23.200	$C_{10}H_{18}O$	154
29.	2,6-Octadienal, 3,7-dimethyl-, (E)-	0.0496	1238	23.946	$C_{10}H_{16}O$	152
30.	Perillaldehyde	0.0470	1242	24.250	$C_{10}H_{14}O$	150
31.	Bornyl acetate	0.0455	1251	24.638	$C_{12}H_{20}O_2$	196
32.	Carvacrol	0.1657	1266	25.270	$C_{10}H_{14}O$	150
33.	Undecanal	0.0190	1272	25.818	$C_{11}H_{22}O$	170
34.	α-Elemene	0.0373	1289	26.925	$C_{15}H_{24}$	204
35.	Geranyl acetate	0.0077	1293	28.928	$C_{12}H_{20}O_2$	196
36.	β -Elemene	0.0197	1306	29.322	$C_{15}H_{24}$	204
37.	Dodecanal	0.0232	1319	30.214	$C_{12}H_{24}O$	128
38.	Caryophyllene <(E)->	0.0191	1332	30.580	$C_{15}H_{24}$	204
39.	γ-Elemene	0.0110	1338	31.021	$C_{15}H_{24}$	204
40.	Cis- α -Bergamotene	0.0283	1344	31.179	$C_{15}H_{24}$	204
41.	β -Farnesene	0.0361	1349	32.025	$C_{15}H_{24}$	204
42.	Germacrene D	0.0679	1415	33.168	$C_{15}H_{24}$	204
43.	Bicyclogermacrene	0.0119	1430	33.772	$C_{15}H_{24}$	204
44.	α-Farnesene	0.0173	1476	34.161	$C_{15}H_{24}$	204
45.	β -Bisabolene	0.0406	1502	34.296	$C_{15}H_{24}$	204
46.	Trans- β-Bergamotene	0.0086	1505	34.932	$C_{15}H_{24}$	204
47.	Germacrene B	0.0385	1553	36.294	$C_{15}H_{24}$	204
48.	α-Bisabolene	0.0107	1624	38.788	$C_{15}H_{24}O$	220
49.	α-Cadinol	0.0723	1659	39.328	$C_{15}H_{26}O$	222
50.	α—Bisabolol	0.0334	1668	41.475	$C_{15}H_{26}O$	222
51.	α-Sinensal	0.0665	1684	43.519	$C_{15}H_{22}O$	218

Table 4d. Composition of essential oil compounds at 1400-1600m altitude in mature stage.

S.N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1375	923	8.443	$C_{10}H_{16}$	136
2.	α -Pinene	0.6000	930	8.727	$C_{10}H_{16}$	136
3.	Camphene	0.1883	945	9.369	$C_{10}H_{16}$	136
4.	Sabinene	0.6698	969	10.323	$C_{10}H_{16}$	136
5.	β -Pinene	0.7190	974	10.516	$C_{10}H_{16}$	136
6.	Myrcene	1.4426	988	11.053	$C_{10}H_{16}$	136
7.	α -Terpinene	0.0737	997	11.450	$C_{10}H_{16}$	136
8.	Octanal	0.6826	1003	11.665	$C_8H_{16}O$	128
9.	α -Humulene	0.1104	1015	12.258	$C_{10}H_{16}$	136
10.	Limonene	73.0895	1035	13.122	$C_{10}H_{16}$	136
11.	Phenylacetaldehyde	0.0218	1041	13.505	C_8H_8O	120
12.	β -Ocimene	0.1086	1046	13.633	$C_{10}H_{16}$	136
13.	γ -Terpinene	4.4602	1058	14.205	$C_{10}H_{16}$	136
14.	Cis-Linalool oxide	0.0590	1069	14.721	$C_{10}H_{18}O_2$	170
15.	1-Octanol	0.2106	1073	14.848	$C_8H_{18}O$	130
16.	α -Terpinolene	0.2570	1083	15.400	$C_{10}H_{16}$	136
17.	Linalool	9.1389	1105	16.305	$C_{10}H_{18}O$	154
18.	Trans-p-Mentha-2,8-dien-1-ol	0.0379	1121	17.279	$C_{10}H_{16}O$	152
19.	(Z) Sabinene Hydrate	0.0242	1124	17.608	$C_{10}H_{18}O$	154
20.	Cis-p-Mentha-2,8-dien-1-ol	0.0398	1135	17.843	$C_{10}H_{16}O$	152
21.	β -Terpineol	0.0371	1141	18.125	$C_{10}H_{18}O$	154
22.	Citronellal	0.0562	1151	18.536	$C_{10}H_{18}O$	154
23.	Borneol	0.1012	1170	19.529	$C_{10}H_{18}O$	154
24.	Terpinen-4-ol	1.8127	1180	19.942	$C_{10}H_{18}O$	154
25.	p-Cymen-9-ol	0.0972	1187	20.294	$C_{10}H_{18}O$	154
26.	α -Terpineol	1.3312	1196	20.662	$C_{10}H_{18}O$	154
27.	Decanal	0.2439	1206	21.093	$C_{10}H_{20}O$	156
28.	Trans-Carveol	0.0317	1218	21.763	$C_{10}H_{16}O$	152
29.	2,6-Octadien-1-ol	0.1005	1224	21.986	$C_{10}H_{18}O$	154
30.	Carvacryl methyl ether	0.2813	1228	22.170	$C_{11}H_{16}O$	164
31.	Neral	0.0747	1237	22.566	$C_{10}H_{16}O$	152
32.	Carvone	0.0458	1241	22.814	$C_{10}H_{14}O$	150
33.	Geraniol	0.1200	1251	23.205	$C_{10}H_{18}O$	154
34.	Geranial	0.1439	1267	23.945	$C_{10}H_{16}O$	152
35.	Perillaldehyde	0.1053	1272	24.247	$C_{10}H_{14}O$	150
36.	Bornyl acetate	0.3379	1282	24.637	$C_{12}H_{20}O_2$	196
37.	Limonen-10-ol	0.0472	1290	25.063	$C_{10}H_{16}O$	152
38.	Thymol	1.2160	1295	25.295	$C_{10}H_{14}O$	150
39.	1,3-Dioxolane, 2,2-dimethyl-4,5-dipropenyl	0.0342	1304	25.686	$C_{11}H_{18}O_2$	182
40.	Cyclohexene	0.1087	1322	26.430	$C_{11}H_{20}O$	168
41.	Neoisopulegol hydrate	0.0431	1339	27.264	$C_{10}H_{20}O_2$	172
42.	Limonene glycol	0.1286	1346	27.566	$C_{10}H_{18}O_2$	170
43.	Carvotanacetone<8-hydroxy->	0.0410	1400	29.974	$C_{10}H_{16}O_2$	168
44.	2-Cyclohexen-1-one	0.0404	1423	30.984	$C_{10}H_{16}O_2$	168
45.	β -Farnesene	0.0650	1452	32.012	$C_{15}H_{24}$	204
46.	Germacrene D	0.1128	1476	33.161	$C_{15}H_{24}$	204
47.	2 Methyl isoborneol	0.0874	1492	33.808	$C_{11}H_{20}O$	168
48.	β -Bisabolene	0.0812	1505	34.288	$C_{15}H_{24}$	204
49.	α -Elemol	0.0282	1545	35.940	$C_{15}H_{26}O$	222
50.	Spathulenol	0.0246	1572	37.057	$C_{15}H_{24}O$	220
51.	trans-Valerenyl acetate	0.0227	1618	38.786	$C_{17}H_{26}O_2$	262
52.	α -Muurolol	0.4692	1633	39.332	$C_{15}H_{26}O$	222
53.	Cadin-4-en-10-ol	0.0720	1651	40.066	$C_{15}H_{26}O$	222
54.	α -Bisabolol	0.1471	1685	41.304	$C_{15}H_{26}O$	222
55.	Zierone	0.1438	1689	41.468	$C_{15}H_{22}O$	218
56.	α–Sinensal	0.0948	1746	43.511	$C_{15}H_{22}O$	218

Table 4e. Composition of essential oil compounds at >1600m altitude in mature stage.

S. N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1417	923	8.458	$C_{10}H_{16}$	136
2.	α -Pinene	0.6977	930	8.751	$C_{10}H_{16}$	136
3.	Camphene	0.0053	945	9.384	$C_{10}H_{16}$	136
4.	Sabinene	0.7037	969	10.351	$C_{10}H_{16}$	136
5.	β -Pinene	0.4439	974	10.545	$C_{10}H_{16}$	136
6.	Myrcene	1.6085	989	11.096	$C_{10}H_{16}$	136
7.	Octanal	0.3542	1003	11.698	$C_8H_{16}O$	128
8.	(+)-2-Carene	0.0214	1015	12.280	$C_{10}H_{16}$	136
9.	γ -Terpinyl acetate	0.0559	1022	12.661	$C_{12}H_{20}O_2$	196
10.	Limonene	88.4631	1042	13.604	$C_{10}H_{16}$	136
11.	β -Ocimene	0.0691	1048	13.818	$C_{10}H_{16}$	136
12.	γ -Terpinene	3.9952	1061	14.422	$C_{10}H_{16}$	136
13.	1-Octanol	0.1646	1072	14.908	$C_8H_{18}O$	130
14.	α-Terpinolene	0.1899	1085	15.482	$C_{10}H_{16}$	136
15.	α-p-Dimethylstyrene	0.0138	1089	15.698	$C_{10}H_{12}$	132
16.	Linalool	1.6725	1103	16.321	$C_{10}H_{18}O$	154
17.	trans-p-Mentha-2,8-dienol	0.0067	1121	17.196	$C_{10}H_{16}O$	152
18.	Cis-Limonene oxide	0.0148	1131	17.637	$C_{10}H_{16}O$	152
19.	Trans-Limonene oxide	0.0247	1135	17.852	$C_{10}H_{16}O$	152
20.	Citronellal	0.0819	1151	18.561	$C_{10}H_{18}O$	154
21.	1-Nonanol	0.0113	1172	19.567	$C_9H_{20}O$	144
22.	Terpinen-4-ol	0.1563	1179	19.921	$C_{10}H_{18}O$	154
23.	m-Cymen-8-ol	0.0067	1186	20.292	$C_{10}H_{14}O$	152
24.	α -Terpineol	0.2111	1194	20.649	$C_{10}H_{18}O$	150
25.	Decanal	0.2305	1206	21.116	$C_{10}H_{20}O$	156
26.	Nerol	0.0106	1224	21.992	$C_{10}H_{18}O$	154
27.	Thymol methyl ether	0.1379	1228	22.189	$C_{11}H_{16}O$	164
28.	Neral	0.0144	1236	22.566	$C_{10}H_{16}O$	152
29.	Carvone	0.0160	1241	22.818	$C_{10}H_{14}O$	150
30.	Geranial	0.0270	1266	23.939	$C_{10}H_{16}O$	152
31.	Perillaldehyde	0.0527	1272	24.247	$C_{10}H_{14}O$	150
32.	Thymol	0.1871	1293	25.273	$C_{10}H_{14}O$	150
33.	Undecanal	0.0049	1307	25.752	$C_{11}H_{22}O$	170
34.	α-Elemene	0.0131	1333	26.921	$C_{15}H_{24}$	204
35.	β -Elemene	0.0138	1387	29.312	$C_{15}H_{24}$	204
36.	Dodecanal	0.0238	1408	30.199	$C_{12}H_{24}O$	180
37.	γ -Elemene	0.0156	1426	31.011	$C_{15}H_{24}$	204
38.	β -Farnesene	0.0200	1451	32.012	$C_{15}H_{24}$	204
39.	Germacrene D	0.0375	1476	33.155	$C_{15}H_{24}$	204
40.	α-Farnesene	0.0189	1502	34.149	$C_{15}H_{24}$	204
41.	Germacrene B	0.0134	1553	36.284	$C_{15}H_{24}$	204
42.	β -Sinensal	0.0169	1690	41.474	$C_{15}H_{24}$	204
43.	Trans- α-Bergamotene	0.0322	1747	43.512	$C_{15}H_{24}$	204

are mentioned in Table2. It showed sharp and strong peaks at 3607 at 800m altitude with O-H stretch. C-H stretch was found to be 2956cm^{-1} at 800-1000m altitude. Further C=O bond was found at 1727cm^{-1} at 800-1000m altitude was observed the highest value which showed α, β unsaturated ester. C-N stretch with aliphatic amines was recorded in 1075, 1201 at 800m. Likewise, 1073, 1219 (1000-1200m). Further (1074cm^{-1}, 1214cm^{-1}); (1017cm^{-1}, 1064cm^{-1}) and (1021cm^{-1}, 1095cm^{-1}) were observed in 1200-1400m, 1400-1600m and >1600m altitudes, respectively. In addition alkyl halides was noted only in 1400-1600m (1103 cm^{-1}) and >1600m (1108cm^{-1}) as C-H wag.It is compared with Silva *et al*., 2012 who mentioned the frequency as carbohydrate ring. The result in the present finding can be compared with Kanmani *et al.,* 2014 who performed research in *C. limon*. In their research sharp and strong peaks at 3595.31 cm^{-1} as O-H stretch, C-H stretch in the frequency 2830-2695 cm^{-1}and strong C=O - unsaturated esters and aliphatic amine functional stretch occurring at 1710-1665 cm^{-1}

Instrumental analysis: GCMS analysis chromatograms and table are presented in Table 5a,5b,5c,5d,5.Inmature stage, five different altitudes showed compound identification as 39,33,51, 56, 43 at 800m, 1000-1200m, 1200-1400m, 1400-1600m and >1600m altitudes. Limonene content was found to be the highest in the altitudes *viz:*800m, 1000-1200m, 1200-1400m, 1400-1600m and >1600m (88.40, 87.64, 87.20,

Table 5a. Composition of essential oil compounds at 800m altitude in immature stage.

S.N.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1189	922	8.433	$C_{10}H_{16}$	136
2.	α -Pinene	0.6590	929	8.718	$C_{10}H_{16}$	136
3.	Sabinene	0.1778	968	10.306	$C_{10}H_{16}$	136
4.	β -Pinene	0.3613	973	10.503	$C_{10}H_{16}$	136
5.	Myrcene	1.6191	988	11.042	$C_{10}H_{16}$	136
6.	Octanal	0.1210	1002	11.632	$C_8H_{16}O$	128
7.	Nonanal	0.0373	1004	11.749	$C_8H_{16}O$	128
8.	α -Terpinene	0.1092	1014	12.248	$C_{10}H_{16}$	136
9.	γ -Terpineol acetate	0.1288	1023	12.661	$C_{10}H_{16}$	136
10.	Limonene	87.9324	1045	13.163	$C_{10}H_{16}$	136
11.	β -Ocimene	0.0367	1050	13.628	$C_{10}H_{16}$	136
12.	γ -Terpinene	5.3748	1063	14.207	$C_{10}H_{16}$	136
13.	1-Octanol	0.0580	1071	14.786	$C_{10}H_{16}$	136
14.	α -Terpinolene	0.2604	1085	15.385	$C_{10}H_{16}$	136
15.	Linalool	1.5219	1103	16.144	$C_9H_{18}O$	142
16.	Nonanal	0.0167	1105	16.298	$C_9H_{18}O$	142
17.	Citronellal	0.0120	1150	18.511	$C_{10}H_{18}O$	154
18.	Terpinen-4-ol	0.2172	1177	19.851	$C_{10}H_{18}O$	154
19.	α -Terpineol	0.2697	1192	20.565	$C_{10}H_{18}O$	154
20.	Decanal	0.1916	1204	21.057	$C_{10}H_{20}O$	156
21.	Trans-Carveol	0.0120	1216	21.696	$C_{10}H_{16}O$	152
22.	2,6-Octadien-1-ol	0.0215	1222	21.941	$C_{10}H_{18}O$	154
23.	Thymol methyl ether	0.0870	1227	22.137	$C_{11}H_{16}O$	164
24.	Neral	0.0143	1235	22.530	$C_{10}H_{16}O$	152
25.	Geraniol	0.0140	1248	23.157	$C_{10}H_{18}O$	154
26.	Geranial	0.0271	1264	23.910	$C_{10}H_{16}O$	152
27.	Perillaldehyde	0.0474	1270	24.204	$C_{10}H_{14}O$	150
28.	Carvacrol	0.2050	1289	25.159	$C_{10}H_{14}O$	150
29.	Undecanal	0.0072	1305	25.724	$C_{11}H_{22}O$	170
30.	α-Elemene	0.0331	1331	26.891	$C_{15}H_{24}$	204
31.	β-Elemene	0.0176	1385	29.276	$C_{15}H_{24}$	204
32.	Dodecanal	0.0320	1406	30.169	$C_{12}H_{24}O$	184
33.	γ -Elemene	0.0060	1425	30.975	$C_{15}H_{24}$	204
34.	β -Farnesene	0.0259	1450	31.985	$C_{15}H_{24}$	204
35.	Germacrene D	0.0613	1474	33.111	$C_{15}H_{24}$	204
36.	α -Farnesene	0.0224	1500	34.113	$C_{15}H_{24}$	204
37.	α-Cadinene	0.0115	1512	34.660	$C_{15}H_{24}$	204
38.	Germacrene B	0.0418	1551	36.239	$C_{15}H_{24}$	204
39.	β -Sinensal	0.0353	1687	41.422	$C_{15}H_{22}O$	218
40.	α-Sinensal	0.0537	1743	43.455	$C_{15}H_{22}O$	218

Table 5b. Composition of essential oil compounds at 1000-1200m altitude in immature stage.

S. No.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α -Pinene	0.3816	928	8.709	$C_{10}H_{16}$	136
2.	Sabinene	0.6908	967	10.298	$C_{10}H_{16}$	136
3.	β -Pinene	0.4758	972	10.492	$C_{10}H_{16}$	136
4.	Myrcene	1.5575	986	11.025	$C_{10}H_{16}$	136
5.	Octanal	0.2045	1001	11.632	$C_8H_{16}O$	128
6.	α -Terpinene	0.0395	1013	12.226	$C_{10}H_{16}$	136
7.	p-Cymene	0.1511	1021	12.641	$C_{10}H_{14}$	134
8.	Limonene	88.5797	1030	13.065	$C_{10}H_{16}$	136
9.	β -Ocimene	0.0482	1043	13.595	$C_{10}H_{16}$	136
10.	γ -Terpinene	1.4282	1054	14.124	$C_{10}H_{16}$	136
11.	Ethanone	0.0264	1059	14.440	C_8H_8O	120
12.	Cis- Linalool oxide	0.3537	1067	14.684	$C_{10}H_{18}O_2$	170
13.	Trans-Linaool oxide	0.2340	1083	15.417	$C_{10}H_{16}O$	152
14.	Linalool	0.9189	1098	16.098	$C_{10}H_{18}O_2$	170
15.	trans-p-Mentha-2,8-dienol	0.1082	1118	17.095	$C_{10}H_{16}O$	152

Contd…….

16.	Cyclopentan-1,2-dione	0.0677	1123	17.493	$C_7H_{10}O_2$	126
17.	Cis-p-Mentha-2,8-dien-1-ol	0.1178	1132	17.782	$C_{10}H_{16}O$	152
18.	Citronellal	0.0304	1149	18.492	$C_{10}H_{18}O$	154
19.	Terpinen-4-ol	0.3719	1176	19.837	$C_{10}H_{18}O$	154
20.	α-Terpineol	0.3013	1190	20.549	$C_{10}H_{18}O$	154
21.	(-)-trans-Isopiperitenol	0.0884	1195	20.776	$C_{10}H_{16}O$	152
22.	Decanal	0.0967	1203	21.040	$C_{10}H_{20}O$	156
23.	Trans-carveol	0.2577	1215	21.691	$C_{10}H_{16}O$	152
24.	2,6 Octadien-1-ol	0.1550	1221	21.934	$C_{10}H_{18}O$	154
25.	Cis-Carveol	0.1412	1228	22.300	$C_{10}H_{16}O$	152
26.	Carvone	0.2026	1238	22.760	$C_{10}H_{14}O$	150
27.	Geraniol	0.1882	1248	23.142	$C_{10}H_{18}O$	154
28.	α-Citral	0.0798	1263	23.891	$C_{10}H_{16}O$	152
29.	Perillaldehyde	0.0624	1269	24.196	$C_{10}H_{14}O$	150
30.	2-Cyclohexen-1-one	0.0573	1288	25.153	$C_{10}H_{14}O$	150
31.	p-Mentha-2,8-diene, 1-hydroperoxide	0.2524	1302	25.743	$C_{10}H_{16}O_2$	168
32.	α-Sinensal	0.2997	1315	26.333	$C_{15}H_{22}O$	218
33.	Nerolidol	0.1489	1326	26.804	$C_{15}H_{26}O$	222
34.	Limonene-1,2-diol	0.4984	1341	27.487	$C_{10}H_{18}O_2$	170
35.	Patchenol	0.1011	1348	27.774	$C_{11}H_{18}O$	166
36.	α-Farnsesene	0.2574	1355	28.109	$C_{15}H_{24}$	204
37.	*2,6,6-Trimethyl-1-cyclohexene-1-carboxylic acid*	0.0346	1363	28.450	$C_{10}H_{16}O_2$	168
38.	α-Sinensal	0.2250	1372	28.836	$C_{15}H_{22}O$	218
39.	Germacrene D	0.4537	1474	33.101	$C_{15}H_{24}$	204
40.	Trans- α-.Bergamotene	0.0514	1502	34.215	$C_{15}H_{24}$	204
41.	Germacrene B	0.0333	1550	36.215	$C_{15}H_{24}$	204
42.	Spathulenol	0.0660	1569	36.972	$C_{15}H_{24}O$	220
43.	Cadin-4-en-10-ol	0.0242	1648	39.996	$C_{15}H_{26}O$	222
44.	Cis Thijopsenic acid	0.0460	1819	46.136	$C_{15}H_{22}O_2$	234
45.	Torulosol	0.0912	1851	47.380	$C_{20}H_{34}O_2$	306

Table 5c. Composition of essential oil compounds at 1200-1400m altitude in immature stage.

1246m S.No.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.2244	928	7.437	$C_{10}H_{16}$	136
2.	α -Pinene	0.9593	935	7.710	$C_{10}H_{16}$	136
3.	Sabinene	1.5533	977	9.263	$C_{10}H_{16}$	136
4.	β -Pinene	0.5822	979	9.370	$C_{10}H_{16}$	136
5.	Myrcene	1.6852	996	10.000	$C_{10}H_{16}$	136
6.	Octanal	0.2687	1010	10.587	$C_8H_{16}O$	128
7.	Limonene	83.6993	1057	12.667	$C_{10}H_{16}$	136
8.	β -Ocimene	0.1582	1060	12.800	$C_{10}H_{16}$	136
9.	γ-terpinene	6.4489	1074	13.400	$C_{10}H_{16}$	136
10.	1-Nonanol	0.0450	1082	13.743	$C_8H_{18}O$	130
11.	Terpinolene	0.3123	1093	14.263	$C_{10}H_{16}$	136
12.	Linalool	2.0481	1113	15.160	$C_{10}H_{18}O$	154
13.	β -Terpineol	0.0176	1131	15.983	$C_{10}H_{18}O$	154
14.	Cosmene	0.0095	1145	16.647	$C_{10}H_{14}$	134
15.	Citronellal	0.0455	1157	17.220	$C_{10}H_{18}O$	154
16.	4-Terpineol	0.4371	1186	18.577	$C_{10}H_{18}O$	154
17.	α -terpineol	0.2537	1201	19.270	$C_{10}H_{18}O$	154
18.	Decanal	0.1306	1211	19.740	$C_{10}H_{20}O$	156
19.	p-cymene	0.0935	1234	20.757	$C_{11}H_{16}O$	164
20.	Neral	0.0266	1242	21.153	$C_{10}H_{16}O$	152
21.	Cis-citral	0.0471	1272	22.527	$C_{10}H_{16}O$	152
22.	Perillaldehyde	0.0303	1278	22.780	$C_{10}H_{16}O$	152
23.	Thymol	0.4106	1302	23.877	$C_{10}H_{14}O$	150
24.	α -Terpinolene	0.0457	1337	25.390	$C_{15}H_{24}$	204
25.	β -Elemene	0.0156	1390	27.733	$C_{15}H_{24}$	204
26.	Do-decanal	0.0169	1414	28.723	$C_{12}H_{24}O$	184
27.	Germacrene B	0.0076	1430	29.403	$C_{15}H_{24}$	204
28.	β -Farnescene	0.0445	1455	30.457	$C_{15}H_{24}$	204
29.	Germacrene-D	0.0650	1480	31.500	$C_{15}H_{24}$	204
30.	γ-elemene	0.0064	1494	32.077	$C_{15}H_{24}$	204
31.	α -bergamotene	0.0530	1506	32.583	$C_{15}H_{24}$	204
32.	Bicyclogermacrene	0.0778	1557	34.577	$C_{15}H_{24}$	204
33.	β -eudesmol	0.0112	1656	38.357	$C_{15}H_{26}O$	222
34.	β -sinensal	0.0496	1697	39.890	$C_{15}H_{22}O$	218
35.	α -bergamotol	0.1197	1754	41.930	$C_{15}H_{22}O$	218

Table 5d. Composition of essential oil compounds at 1400-1600m altitude in immature stage.

1420 m S.No.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.2138	922	8.429	$C_{10}H_{16}$	136
2.	α -Pinene	0.8962	929	8.713	$C_{10}H_{16}$	136
3.	Sabinene	0.7482	969	10.301	$C_{10}H_{16}$	136
4.	β -Pinene	0.5529	973	10.495	$C_{10}H_{16}$	136
5.	Myrcene	1.6019	988	11.028	$C_{10}H_{16}$	136
6.	Octanal	0.2708	1002	11.624	$C_8H_{16}O$	128
7.	α -Terpinine	0.1640	1014	12.231	$C_{10}H_{16}$	136
8.	Hordenine	0.5371	1023	12.641	$C_{10}H_{15}NO$	165
9.	Limonene	84.1305	1041	13.083	C_7H_{12}	96
10.	β -Ocimene	0.1352	1047	13.603	$C_{10}H_{16}$	136
11.	γ -Terpinene	6.3683	1061	14.182	$C_{10}H_{16}$	136
12.	1-Octanol	0.0522	1070	14.774	$C_{10}H_{16}$	136
13.	α-Terpinolene	0.3016	1084	15.369	$C_{10}H_{16}$	136
14.	Linalool	2.2776	1102	16.133	$C_9H_{18}O$	142
15.	Citronellal	0.0292	1150	18.497	$C_{10}H_{18}O$	154
16.	Terpinen-4-ol	0.4155	1177	19.842	$C_{10}H_{18}O$	154
17.	α-Terpineol	0.2363	1192	20.548	$C_{10}H_{18}O$	154
18.	Decanal	0.1494	1204	21.042	$C_{10}H_{20}O$	156
19.	Thymol methyl ether	0.1399	1227	22.124	$C_{11}H_{16}O$	164
20.	Geraniol	0.0220	1248	23.144	$C_{10}H_{18}O$	154
21.	Geranial	0.0347	1264	23.889	$C_{10}H_{18}O$	154
22.	Perillaldehyde	0.0370	1269	24.193	$C_{10}H_{14}O$	150
23.	p-Cymene	0.2239	1289	25.145	$C_{10}H_{14}$	134
24.	α-Elemene	0.0332	1331	26.874	$C_{15}H_{24}$	204
25.	γ-Elemene	0.0211	1385	29.258	$C_{15}H_{24}$	204
26.	Dodecanal	0.0213	1406	30.153	$C_{12}H_{24}O$	184
27.	β -Farnesene	0.0401	1450	31.961	$C_{15}H_{24}$	204
28.	Germacrene D	0.0893	1474	33.092	$C_{15}H_{24}$	204
29.	α -Farnesene	0.0715	1501	34.093	$C_{15}H_{24}$	204
30.	Germacrene B	0.0663	1551	36.218	$C_{15}H_{24}$	204
31.	β -Sinensal	0.0450	1687	41.401	$C_{15}H_{22}O$	218
32.	α -Sinensal	0.0738	1743	43.435	$C_{10}H_{16}O_2$	168

Table 5e. Composition of essential oil compounds at >1600m altitude in immature stage.

1650m S. No.	Compound name	Area %	RI	R. Time	Molecular formula	Molecular weight
1.	α-Thujene	0.1540	922	8.439	$C_{10}H_{16}$	136
2.	α -Pinene	0.7063	929	8.725	$C_{10}H_{16}$	136
3.	α -Fenchene	0.6163	944	10.314	$C_{10}H_{16}$	136
4.	Sabinene	0.4138	969	10.510	$C_{10}H_{16}$	136
5.	β –Pinene	1.6467	973	11.047	$C_{10}H_{16}$	136
6.	Myrcene	0.2711	988	11.643	$C_{10}H_{16}$	136
7.	Octanal	0.0752	1002	12.254	$C_8H_{16}O$	128
8.	Limonene	89.0555	1045	13.162	$C_{10}H_{16}$	136
9.	β -Ocimene	0.0577	1050	13.632	$C_{10}H_{16}$	136
10.	γ -Terpinene	4.7598	1063	14.204	$C_{10}H_{16}$	136
11.	1-Octanol	0.0414	1071	14.793	$C_8H_{18}O$	130
12.	α -Terpinolene	0.2124	1085	15.390	$C_{10}H_{16}$	136
13.	Linalool	1.0887	1102	16.133	$C_{10}H_{18}O$	154
14.	Nonanal	0.0239	1104	16.301	$C_9H_{18}O$	142
15.	Cis Limonene oxide	0.0083	1130	17.588	$C_{10}H_{16}O$	152
16.	Trans Limonene oxide	0.0078	1134	17.798	$C_{10}H_{16}O$	152
17.	Citronellal	0.0390	1150	18.515	$C_{10}H_{18}O$	154
18.	Terpinen-4-ol	0.1200	1177	19.853	$C_{10}H_{18}O$	154
19.	α -Terpineol	0.1238	1192	20.562	$C_{10}H_{18}O$	154
20.	Decanal	0.1349	1204	21.060	$C_{10}H_{20}O$	156
21.	Methyl thymol ether	0.0958	1227	22.142	$C_{11}H_{16}O$	164
22.	Neral	0.0234	1235	22.541	$C_{10}H_{16}O$	152
23.	Geranial	0.0245	1264	23.906	$C_{10}H_{16}O$	152
24.	Perillaldehyde	0.0234	1270	24.211	$C_{10}H_{14}O$	150
25.	Carvacrol	0.0657	1289	25.164	$C_{10}H_{14}O$	150
26.	Isoascaridole	0.0151	1299	25.739	$C_{10}H_{16}O_2$	168
27.	α -Sinensal	0.0105	1316	26.335	$C_{10}H_{16}O_2$	168
28.	α-Elemene	0.0279	1332	26.888	$C_{15}H_{24}$	204
29.	Nerolidol	0.0091	1356	28.112	$C_{10}H_{18}O$	154
30.	β – Elemene	0.0094	1385	28.844	$C_{15}H_{24}$	204
31.	Dodecanal	0.0130	1406	29.273	$C_{15}H_{24}$	204
32.	γ-Elemene	0.0170	1425	30.172	$C_{15}H_{24}$	204
33.	β – Farnesene	0.0218	1450	31.981	$C_{15}H_{24}$	204
34.	Germacrene D	0.0382	1475	33.108	$C_{15}H_{24}$	204
35.	α -Farnesene	0.0098	1501	34.114	$C_{15}H_{24}$	204
36.	Germacrene B	0.0387	1551	36.234	$C_{15}H_{24}$	204

73.09, 88.46), however, it was highest at >1600m lowest at 1400-1600m. Likewise, γ-Terpinene was found to be 4.82% being maximumin the samples from 1200-1400m altitude while 3.99% at >1600m was the lowest content. In addition myrcene was found maximum (1.64%) at800-1000m altitude followed by 1.61% at >1600m altitude. While at 1400-1600m only 1.44%myrcenewas recorded lowest. Further, linalool (9.13%) was accorded maximum at 1400-1600m altitude and minimum of 1.46% at 800-1000m altitudeThe result were in corroboration with the finding of Javed *et al*., 2014. They had mentioned that limoneneas the highest which was in the range of 58.50% (mandarin essential oil) to 89.84% (grape fruit essential oil).

Likewise in immature stage,40,45, 35, 32 and 36 compounds were found in 800, 1000-1200, 1200-1400, 1400-1600 and >1600m altitudes respectively (Table 5a,5b,5c,5d). Amongst this, 87.93 %, 88.58%, 83.70%, 84.13%, 89.06% of limonene were present in 800,1000-1200, 1200-1400, 1400-1600 and >1600m altitudes. It showed >1600m altitude with highest content of limonene. Thereafter, highest γ–Terpinene was found in 1200-1400m (6.45%) followed by 1400-1600m (6.37%) and of the least was found at1000-1200m (1.42%).Myrcene content was lowest at >1600m (0.27%) and highest at 1200-1400m1.68%. Linalool was found in the range of 0.92% (1000-1200m) to 2.28% (1400-1600m). Whereas,α–Pinene on was found highest at at1200-1400m altitude (0.96%) with highest value and that of 0.38% at 1400-1600m as the lowest content. Earlier worker (Wu *et al*., 2014) reported chemical content of oil from Taiwan in limonene upto57.71%, 86.05% in lemon and orange oil and α–Pineneup to 2.27%,0.58%, Myrcene upto1.44%, 2.22%. d-limoneneof Moro blood orange oil was reported upto93.32%and in Mayer lemon it was75.50%, while Interdonat lemon was noted with 66.58%. The other main components of oils were determined to be α-pinene, sabinene, β-pinene, β-myrcene, linalool, m-cymene and 4-terpineol in addition to d-limonene.

Kamal *et al*. (2011) performed research in *C. reticulata, C sinensis and C.paradisii* in fresh, ambient and air dried condition in Pakistan. It revealed that *C.reticulata*constituted 27, 27 and 16 compounds, representing upto99.98, 99.50 and 97.25% of the total oil. The major compound werelimonene (69.9, 64.1and 71.1%), followed by*β*-myrcene (3.27, 4.05 and 4.02%) and decanal (2.33, 7.71 and 5.80%) in fresh, ambient-driedand oven-dried peel oil.

Conclusion

Quantitative analysis of the peel of Citrus showed increase in altitude increase in all the parameters like percent yield on dry basis, percent yield on wet basis, equivalent weight, methoxyl content, degree of esterification, moisture content, ash percent, alkalinity as carbonate, carbonate free ash. In FTIR analysis, highest altitude (>1600m) showed C-H stretch and that of O-H bond was found at 800m altitude respectively Moreover mature stage showed 39,33,51, 56, 43 at 800m, 1000-1200m, 1200-1400m, 1400-1600m and >1600m altitudes. Limonene content showed 88.46% at >1600m altitude. Likewise γ-Terpinene was found to be 4.82% being maximum in the samples from 1200-1400m altitude while 3.99% at >1600m was the lowest content. In addition, myrcene was found maximum at 800-1000m altitude (1.64%) while 1400-1600m (1.44%) was recorded with lowest value. Immature stage on the other hand was found with 89.06% at >1600m altitude. Likewise γ–Terpinene was found in 1200-1400m (6.45%) followed by 1400-1600m (6.37%) and of the least was found at1000-1200m (1.42%).Myrcene content was lowest at >1600m (0.27%) and highest at 1200-1400m1.68%. The present research showed significant amount of component present and in which limonene was found to be the highest followed by γ-Terpinene, myrcene. Hence the essential oil has significance as a natural source of antimicrobials for therapeutic purposes. The identification of compound may be helpful in the study as well as medicinal properties.

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