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Colour Reaction of the Chemical Components of Leaves of *Ocimum basilicum* on Thin Layer Chromatogram

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Abstract: **In the present study, leaves of Ocimumbasilicum were extracted using n-hexane and dichloromethane as solvents in the ratio 5:1.**
These crude extracts were subjected to different chromatographic techniques for isolation of pure compounds. The infra red spectrum of the isolated compounds were obtained and interpreted to know the functional groups. GC-Ms was also carried out for the identification of constituents compounds.
The results observed are strong and broad band for phenol due to O-H hydrogen bonded alcohol (3423cm-1), C-H stretching vibration for aliphatic hydrocarbon (2956cm-1) and bending vibration for alkane at lower frequency (1462cm), C-N aromatic tertiary amine (1367), C-O tertiary and primary alcohol, C-H out- of-plane bending vibration of alkene with band of 896.
We have C-H out-of-plane bending vibration of disubstituted and phenyl substitution aromatic ring. The bending in this range gives strong bands in the 800-1000cm. The absorption bands of 737, 721, 669 are assigned to these phenyl substitution on aromatic ring respectively.
There is a unique chemical characteristic of sulphur.i.e S-S formation bond on extended chain called Catenation (polysulfide and arylsulfide with bands of 470 and 418).

Keywords: **Ocimumbasilicum, aromatic tertiary amine, dichloromethane.**

1. INTRODUCTION

Ocimumbasilicum also known as sweet basil is an annual medicinal and aromatic plant of the Lamiaceae family. Its main active agent is the essential oil which accumulates during flowering in 0.5-1.5 percent. Basil belongs to the genus Ocimum derived from the Greek ‘’OZO’’ which means to smell, in reference to the strong odour of the species within the genus. Ocimum basilicum is an important symbol in the Hindu religious tradition. It is being used by the Hindus as regal medicine and it is worshipped in the morning and evening by the Hindus at large. The various basils have different scents because the herb has a number of different essential volatile oils that come together in different proportions for various breeds. It is cultivated for its herbal, culinary and medicinal applications. Based on chemical contents, basil can be grouped into four groups.

1. French; Ocimumbasilicum contains relatively high amounts of phenol
2. Exotic contains methyl chavicol 40-80%
3. Methyl cinnamate- ether 90%
4. Eugenol

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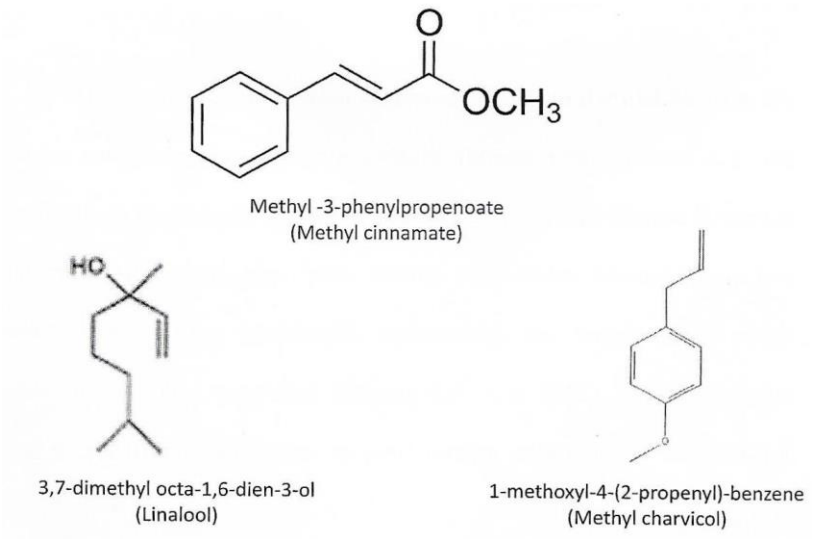

The flavour and smell of basil varieties is largely determined by their chemical components. It contains a high number of compounds in varying quantities: citronellol, cinnamate, geraniol, linalool (a flowering scent also in coriander), methyl chavicol, myrcene (bay leaf, myrcia) pinene, ocimene, terpineol. For example linalool has anti-cancer effect, improves sleep and lessen stress response. These essential oil can be used as additives, pharmaceuticals and perfumery for its spicy fragrance. They are active antioxidant agent which has been reported to show some activities against a wide range of bacteria, fungi and parasites.

In past few decades, especially in Asia countries like China, Taiwan and Europe, scientists have shown much interest in plant research and it is estimated today that about 60% of the total human population relies on natural plant and herbs as drugs for treatment of diseases.

Traditionally, basil has been used as a folk remedy for an enormous number of ailments including convulsion, epilepsy, diarrhea, hiccup, insanity, nausea, sore throat, whooping cough, toothaches and cancer. It has been reported in herbal publications as an insect repellent.

In traditional system of Indian medicine, the herbs have been used in successful management of various diseases conditions like gastric, hepatic, cardiovascular and immunological disorders, also as anti-inflammatory action.

Basil is a popular culinary herb used in many cuisines including Italian and Thai. The leaves can used fresh or dried, commonly used by household and industrial producers to prepare pesto, a varying combination of basil oil, garlic, cheese and nuts.

The present analysis was undertaken in order to identify and isolate various chemical compositions in leaves of Ocimum basilicum for pharmaceutical producers using PTLC and TLC to characterise the compounds, using GC-Ms and IR Spectrometric analysis.

2. METHOD AND MATERIALS USED

The plant Ocimumbasilicum of the Lamiaceae family was plucked from the University of Ilorin at the back of Chemistry Department. The leaves were cut into smaller parts, soaked and extracted using n-hexane

Extraction procedure:

100g of Ocimumbasilicum was chopped with a sharp knife. The weighed sample was packed into a reagent bottle, soaked and decanted after soaking for 20hours. The extracted solution was concentrated in a hot water-bath by normal distillation. The colour of the extract was greenish brown.

THIN LAYER CHROMATOGRAPHY (TLC):

This is a technique used to determine the number of compounds that are present in the crude extract as well as their level of purity. It also permits the optimization of the solvent system for the separation process. The crude extract in the sample

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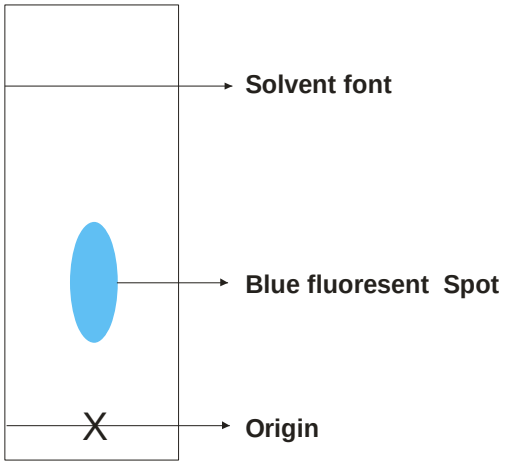
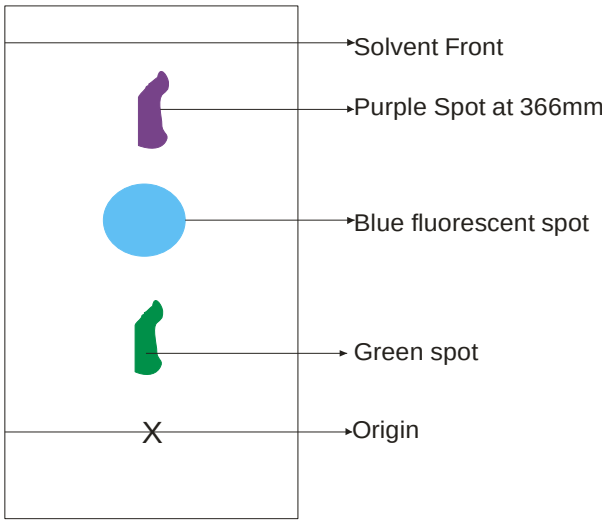
bottle was spotted and developed using a mixture of n-hexane (C6H14) and dichloromethane(CH2Cl2) in the ratio 5:1. TheTLC was placed I the tank in such a way that the spotted area was not washed into the solvent, which can contaminate the solvent used.



TLC plate showing the spot present under UV lamp

After development, the solvent front was marked and the plate was allowed to dry after which it was viewed under the UV lamp to determine the number of compounds present. There was a blue fluorescent spot and other components that were not visible. The chromatographic plate was sprayed with vanillin spray reagent and was heated for about 3 minutes at 7 degree cent. It was observed that colours developed.



TLC plate after spraying with vanillin spray reagent

PREPARATION OF VANILLIN SPRAY REAGENT:

To 1 gram of vanillin was added 18ml of ethanol (C2H6O), 1ml of concentrated H2SO4, and 6 drops of acetic acid (CH3COOH).This reagent was used for colour visualisation. The prepared vanillin can be left in the sprayer for at most a day.

ISOLATION OF THE CONSTITUENT USING PTLC:

Thinning was carried out to allow the solvent to move the loaded spot so as not to allow the extract on the plate fall in line. The solvent used was dichloromethane (CH2Cl2).The plates were developed using a mixture of n-hexane and DCM in ratio 1:5. The solvent front was marked and chromatogram was viewed under UV light at 366nm. A fluorescent zone was observed on the plate. The most important zone corresponding to the brightest fluorescent zone was scrapped, eluted with CH2Cl2, filtered and concentrated.

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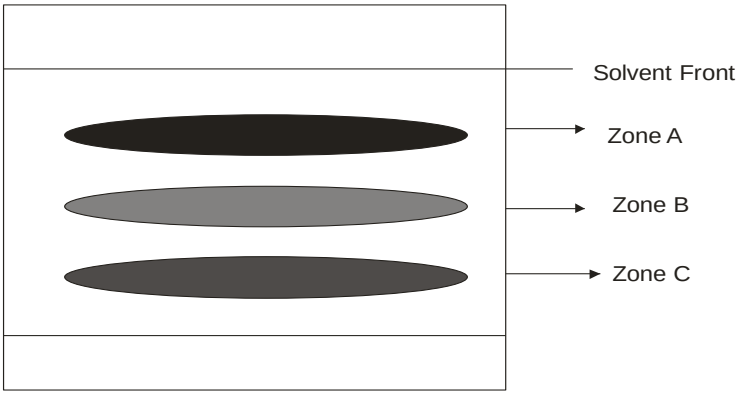
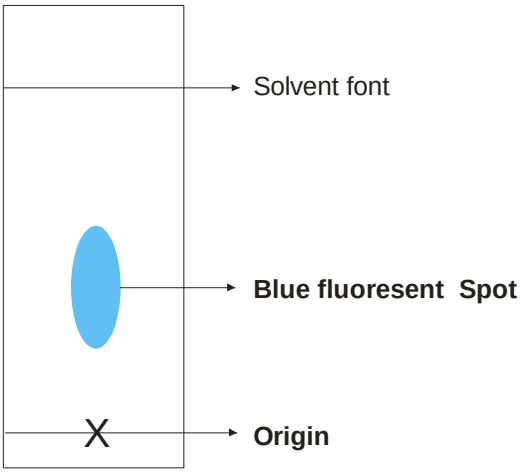


PTLC of the three zones

THIN LAYER CHROMATOGRAPHY OF ZONE B:

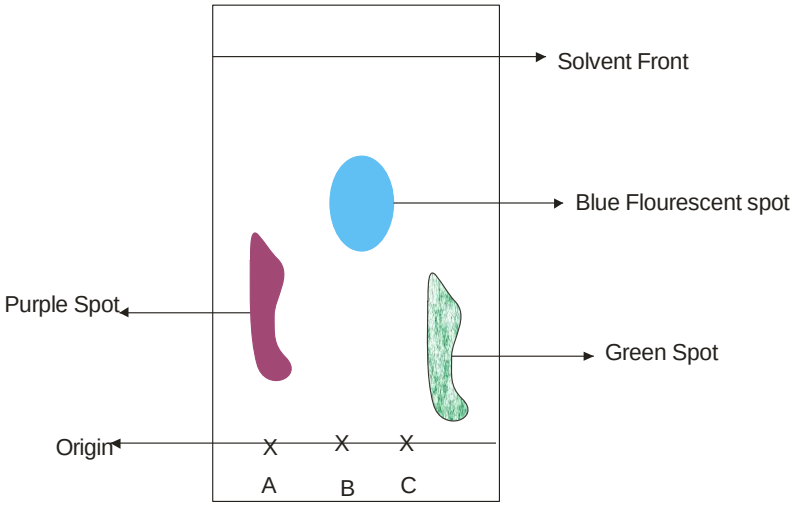
The scrapped zone was eluted with CH2Cl2, filtered and concentrated on a hot water-bath. TLC of the scrapped zone was carried out to ascertain the purity of the compound using n-hexane:DCM as the solvent system in the ratio 5:1.



TLC of blue florescent zone (zone B)

THIN LAYER CHROMATOGRAPHY OF ZONES A, B, C:

The zone above the most fluorescent zone and the one below was scrapped. After elusion with CH2Cl2, the TLC of these zones were also carried out. The sample of zone B was then preserved in sample bottle for spectroscopic analysis



TLC of the scrapped zones

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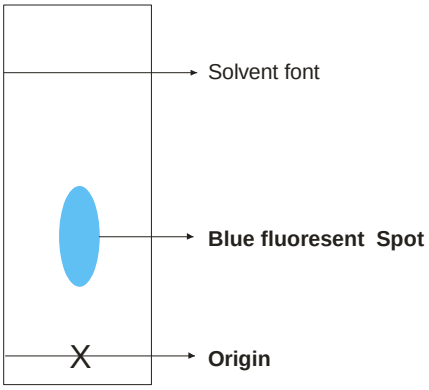
3. RESULTS AND DISCUSSION

The plant materials used were collected fresh. The crude extract obtained from the plant was greenish brown.

Thin Layer Chromatography of the Crude extract:

The TLC of the crude extract was carried out. The solvent used was a mixture n-hexane and dichloromethane in ratio 5:1.



Crude extract TLC plate showing blue florescent spot.

Under the U.V lamp at 366 TLC main spot showed blue fluorescence and other components were not visible.

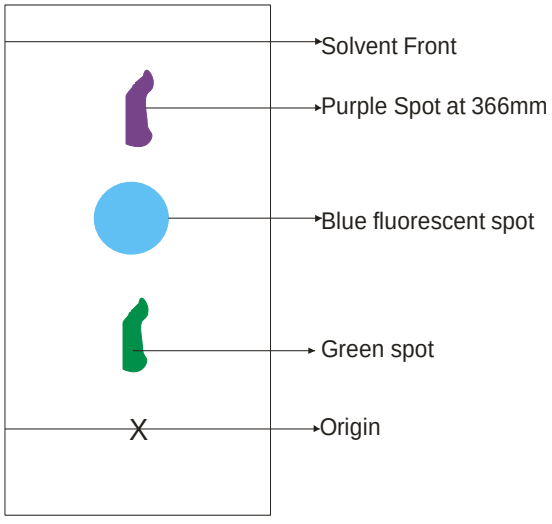
From this result the R_F Value was calculated

R_F = Distance moved by the solute

Distance moved by the solvent

= 2.5 =0.6
4.0

The chromatographic plate was sprayed with vanillin spray reagent and was heated at 700c about 3 minutes, after which it was observed that purple and green spot.



TLC plate after spraying with vanillin

R_F for the sprayed TLC

R_F for green spot = 1.0 = 0.25
4.0

R_F for purple spot =2.7 = 0.6
4.0

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The TLC served as a guide towards isolation of the components. The concentrated extract was carefully transferred into samples bottles. The percentage yields of the various components were calculated.

Weight of *Ocimumbasilicum* soaked = 100g

Volume of *Ocimumbasilicum* crude extract = 120ml

Weight of empty bottles = 25.06g

Weight of crude and bottle (2ml) = 25.238g

Weight of crude (2ml) = 0.178g

Weight of crude (120ml) = 10.08g

% Yield of crude extract = $\frac{10.08}{100} \times \frac{100}{1}$

=10.08%

The comparative TLC of the extract showed that the blue component is the major component present in the crude extract of the leaves and stem of the plant.

Isolated Component

Weight of isolate and bottle = 25.03g

Weight of empty bottle = 25.011g

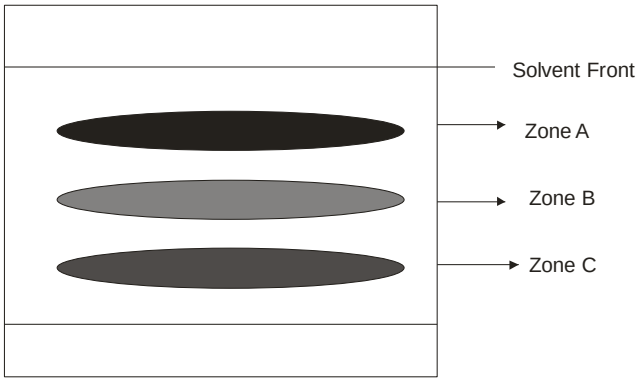
Weight of isolate = 0.024

% Yield of isolate = $\frac{0.024}{0.575} \times \frac{100}{1}$

=0.04173 *100%

∴Yield of isolate =4.17%

The preparative thin layer chromatography was performed on extract using n-hexane and dichloromethane in the ratio 1:5



PTLC plate showing the three zones

To calculate the R_F of each zone, we have

1) For Zone A (Purple Zone)

$\frac{9.2}{10.1}$ = 0.91cm

2) For Zone B (Blue Fluorescent Zone)

$\frac{8.9}{10.1}$ = 0.88cm

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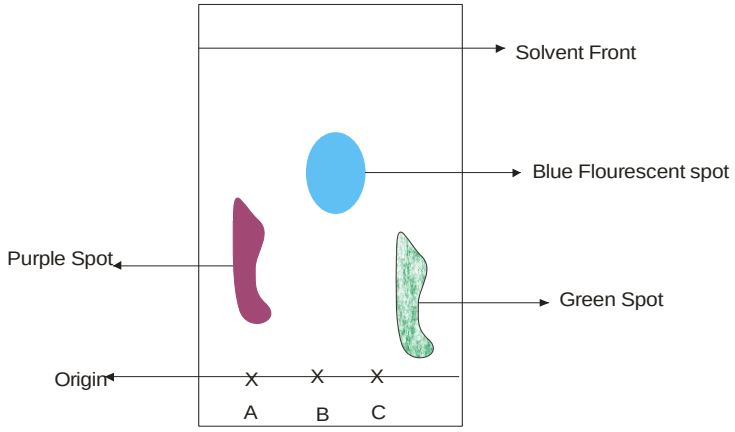
3) For Zone C (Greenish Brown Zone)

$\frac{7.9}{10.1} = 0.78\text{cm}$

The blue fluorescent zone was scrapped, eluted. The eluted used was dichloromethane. After elution from the silica-gel, the zone was isolated and its purity was confirmed using TLC. The chromatography plate was sprayed with vanillin reagent and heated. The chromatogram did not give a positive reaction on vanillin spray. Two other zones and the one below. The purity test was carried out on the isolated compounds using TLC plate. There were no component visible on Zone A and C. The chromatogram was sprayed and heated, it gave purple and green colour reaction respectively.



TLC of the scrapped zones

Infrared spectroscopic analysis of isolated compound:

The table below shows the observed peaks and functional group attached

Peak	Wavelength. Absorption (Cm^1)	Mode of Vibration	Functional Group
1	3423	Stretching	O-H phenols
2	2956	Stretching	C-H alkanes
3	1462	Bending	C-H alkanes
4	1367	Stretching	C-N amine
5	1244	Bending	C-O ether
6	1168	Stretching	C-O alcohol
7	1041	Stretching	C-O alcohol
8	896	Bending	C-H alkene
9	737	Bending	C-H aromatics
10	721	Bending	C-H aromatics
11	669	Bending	C-H aromatics
12	586	Stretching	O-H alcohol
13	470	Stretching	Polysulfide
14	418	Stretching	Arylsulfide

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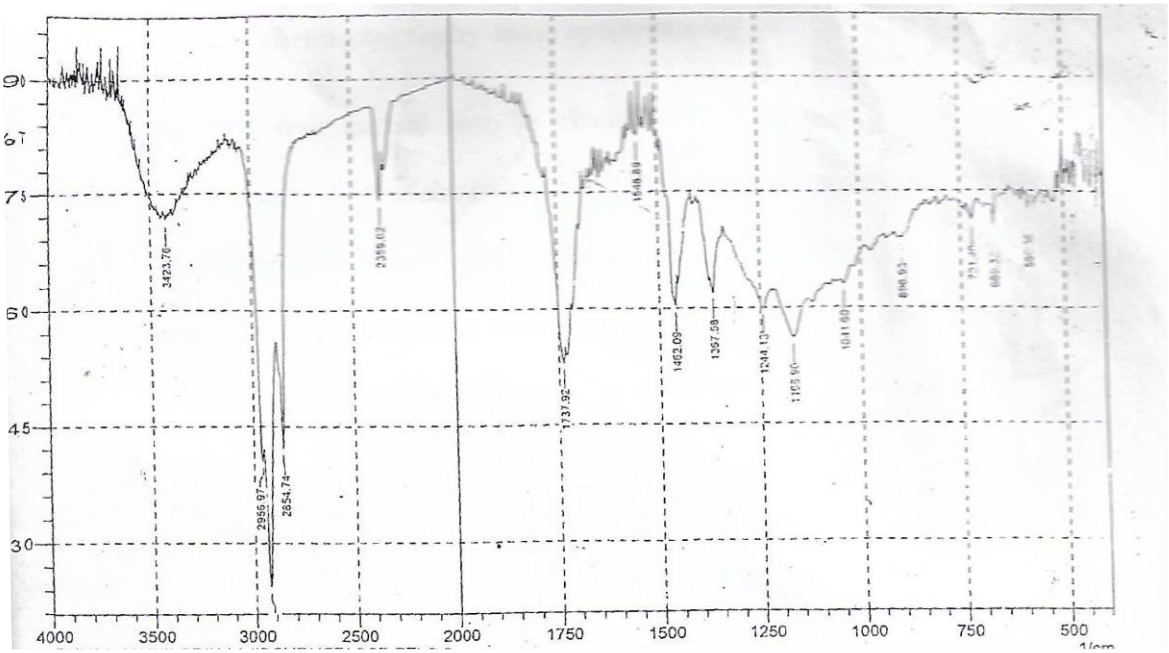

RUN0912/UNILORIN/ADENIRAN/IFE/OSB PTLC 3

	Peak	Intensity	Corr. intensity	Base(H)	Base(L)	Area	Corr. Area
1	418.57	73.01	10.05	424.35	412.78	1.24	0.31
2	470.65	75.55	4.68	474.5	466.79	0.86	0.12
3	586.38	72.78	1.96	592.17	578.66	1.77	0.06
4	669.32	70.39	3.82	675.11	665.46	1.35	0.09
5	721.4	71.3	2.31	734.9	713.69	2.98	0.16
6	896.93	69.02	0.17	898.86	885.36	2.15	0
7	1041.6	62.97	0.74	1045.45	1022.31	4.51	0.08
8	1168.9	56.16	5.4	1209.41	1132.25	17.82	1.56
9	1244.13	59.97	3.83	1319.35	1220.98	19.51	1.3
10	1367.58	61.92	3.31	1375.29	1338.64	6.55	0.19
11	1462.09	60.38	5.83	1477.52	1458.23	3.59	0.49
12	1548.89	82.27	1.8	1541.82	1541.18	0.71	0.08
13	1737.92	53.04	4.36	1772.64	1734.06	7.69	0.53
14	2359.02	74.23	7.71	2389.88	2349.38	4	0.8
15	2854.74	42.15	21.76	2881.75	2798.8	17.03	3.39
16	2926.11	24.43	22.18	2947.33	2883.68	26.78	6.85
17	2956.97	40.48	5.02	3032.2	2949.26	17.82	0.54
18	3423.76	71.7	0.76	3437.26	3419.9	2.44	0.03

Gas chromatography –mass spectrometry:

GC-MS was carried out to elucidate the structure of the isolated components. Out of the entire peaks shown in the spectrum, peak four is the major peak which contains pthalic acid esters while other peaks are minor. Library matching of peak one and two revealed the presence of 9-Hexadecenoic acid ($C_{16}H_{30}O_2$) and 13-Docosenoic acid ($C_{22}H_{42}O_2$).

4. CONCLUSION

Ocimumbasilium leave was extracted with n-hexane which produce a greenish brown extract. Compounds were isolated by means of chromatography and identification by both UV and colour reaction using vanillin spray reagent. R_F values were established. Also, infra-red spectral data made it possible to postulate the functional groups present in the compound isolated. GC-MS analysis was carried out to predict and elucidate the structure of some of the isolated compounds.

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