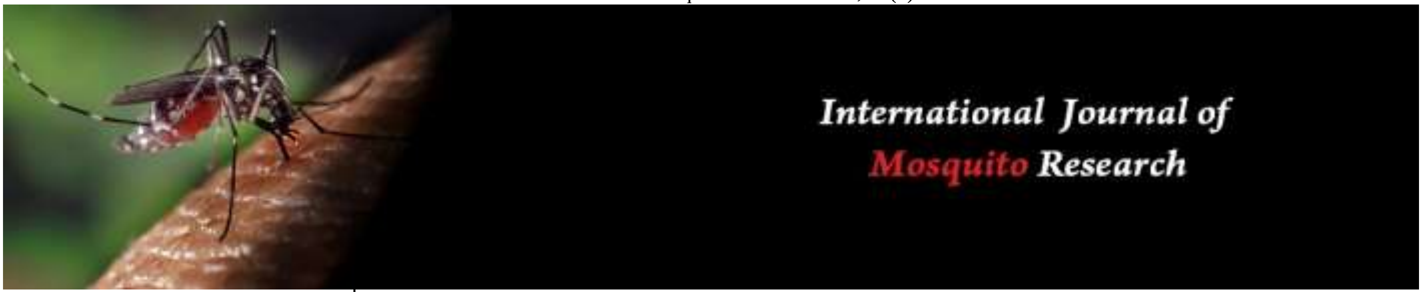

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Mosquito repellence activity of solvent extracts and activated charcoal obtained from agro waste-coconut shells

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Abstract
Burning Coconut shells have been used since ages in rural India to ward away mosquitoes; we study this traditional method in detail in our research, using modern techniques such as dry distillation, solvent extraction, chromatography and infrared spectroscopy to decipher its constitutional contents and efficacy scientifically. In our research, coconut shells in the form of activated charcoal and chemical extracts (solvent extracts of petroleum ether, chloroform and methanol) obtained from the shell itself were used to test against their mosquito repellence and knock off efficacy in a Peet Grady's chamber as per WHO guidelines to test insecticides. The petroleum ether extract was found to be most efficacious with 100% mortality rate in 30 mins. The extract mainly contained aromatic compounds, alkanes and ketonic groups deduced using the abovementioned chemical means.

Keywords: Solvent extracts, charcoal obtained, coconut shells

Introduction
Mosquitoes are considered as a pest to humankind in all parts of the world. They serve as a major vector for spreading diseases, not only through pathogens but also through parasites [2]. Mosquitoes are responsible for the propagation of diseases, specifically malaria, chikungunya, dengue, Japanese encephalitis, West Nile, yellow fever, and Zika throughout the world [3]. Around 400,000 deaths are caused per year due to malaria [2]. Even though constant developments at generating vaccines for mosquito-borne arboviral diseases are in way, either as inactivated vaccines, viral-vector vaccines, live attenuated vaccines, protein vaccines, or nucleic acid vaccines, approximately half of the world's population is expected to be at the risk of arbovirus transmission by 2050 [4]. This raises an alarming note for the need for pest control and eradication or at the least control of its propagating vector i.e., mosquitoes.

Series of literature suggest the use of natural ingredients (including *Zanthoxylum limonella, Azadirachta indica, Tinospora rumphii, Citrus grandis, Jatropha curcas, Cymbopogon nardus*, and *Cocos nucifera*) used to repel or eradicate mosquito larvae and adults [2, 5-9].

In rural India, coconut (*Cocos nucifera*) coir or shell fibres have been used in bonfires or small flames to ward away mosquitoes; a few select references suggest the use of coconut derived compounds as efficient mosquito repellents as well [5-12]. This was the very hypothesis for our research i.e. to find any active component in the traditional ways of repelling mosquitoes and whether it can be used for commercial purposes.

The coconut tree is affectionately called the *kalpavriksha*- the mythical wish fulfilling tree, by coastal Indians, as each part of the tree can be used for various purposes. Besides the fruit having nutritious value, research has also shown the use of coconut fibres, kernels, leaves and bark in production, purification, and isolation of various chemicals: it has been used as an industrial filler, in water purification to remove bacteria, organic pollutants, and heavy metals and even in the generation of nanoparticles like magnetite and palladium [12-19]. Based on our research and past literature, we assess the use of coconut shell and its isolated extracts in its efficiency as active ingredients, by part, or as whole, in mosquito repellent and mosquito-cidal activity.

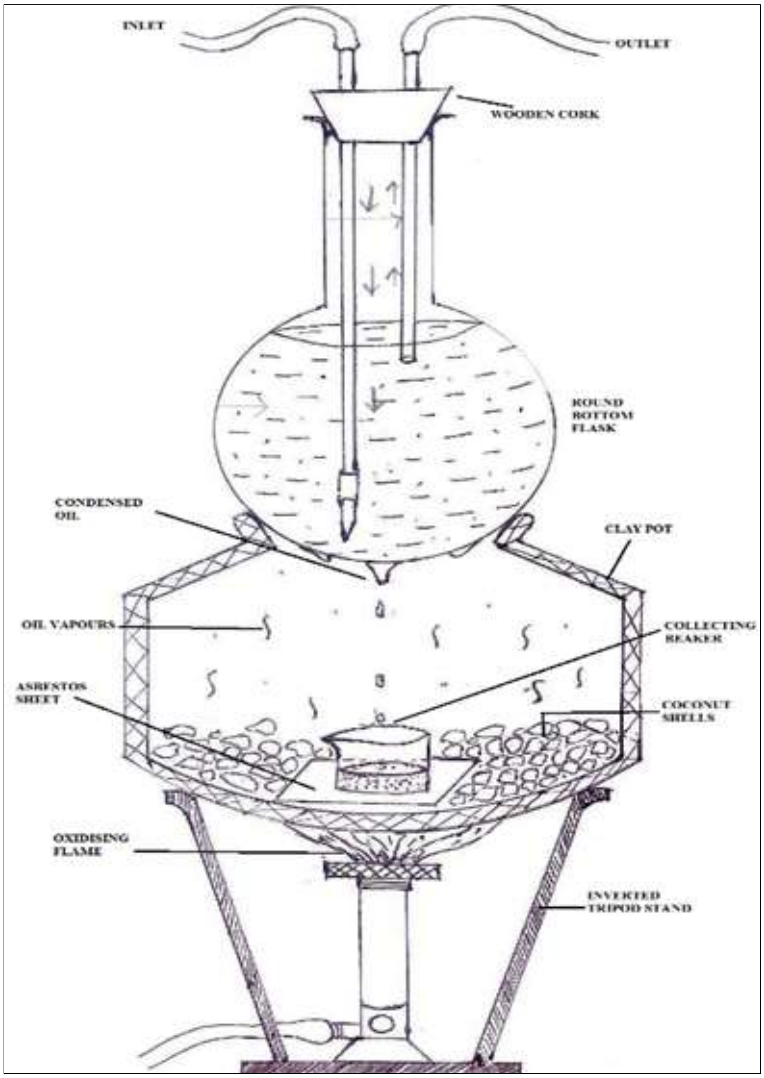

Fig 1: Fractional dry distillation of coconut shells to obtain crude coconut shell oil which is further processed by solvent extraction to obtain the solvents E1, E2 and E3.

Materials & Methods

Preparation of coconut shells extract and charcoal

Around 1kg of *Cocos nucifera* shells were sourced from local vendors in Mumbai district. They were peeled, dried and broken into large pieces of about 3 x 3 cm each. The pieces were roasted gradually in an earthen pot apparatus as described in fig 1, for 8hrs at high temperatures to remove moisture and organic oils. The organic oils were collected as (E) which would be processed and characterised through solvent extraction and other processes. The shell residue obtained was crushed into fine particles using mortar and pestle. The particles were filtered through a metal sieve to obtain very fine and uniform particle size (diameter of around 1mm).

Activation of charcoal

The charcoal obtained from the above process and wood charcoal (sourced from local vendors) crushed into fine uniform powder was soaked in methanol overnight to separate organic impurities. It was then filtered, dried, and heated at 1000 C in an oven for 3hrs. This clears off volatile substances and leaves behind pure charcoal carbon.

Solvent extraction

The crude oil obtained from the dry distillation of the shells (yield of 1 kg of coconut shells gives about 250cc of crude extract). This crude oil obtained was split for characterization and experimentation. 25cc of oil was separated by solvent extraction using three solvents with increasing polarities i.e. petroleum ether (non-polar), chloroform (polar) and methanol (highly polar). Different extracts obtained using these three solvents were collected and air dried to evaporate excess solvent. After complete drying, each extract was named E_1, E_2 and E_3 respectively.

Fractional distillation

A part of the crude oil was distilled on a sand bath using a water condenser to obtain distillates D1-D4 at temperatures 60C, 80C, 90C and 100C respectively. These distillates were then characterized further by Fourier Transform Infrared (FTIR) spectroscopy and silica gel-thin layered chromatography (TLC) to study the level of purity. (Supplementary data: D1-D4).

Column chromatography

A part of the dried petroleum ether extract E1 (which showed maximum mortality in the mortality tests) was further fractionated using silica gel column chromatography using gradual ratios of non-polar- polar solvents Petroleum ether-Chloroform-Methanol to obtain 6 fractions, which were further characterized by Fourier Transform infrared (FTIR) spectroscopy and its purity was checked using silica gel-thin layered chromatography (TLC). (Supplementary data: A-F).

Preparation of mosquito repellent sticks
To study the correlation between mosquito repellent activity, Jigat (*Machilus macrantha:* inert adhesive filler) powder, saw dust and coconut or wood charcoal powder were mixed in a ratio of 1:1:1 by weight and kneaded with water to obtain dough. This dough was then rolled onto bamboo sticks (average weight of each stick = 0.5g) to obtain sticks of uniform length, breadth and weight. They were then air dried for 24 hrs. The obtained the sticks were of average length 11 cm, breadth 0.5 cm and weight 1.5g each.

36 sticks with coconut charcoal and 36 sticks with wood charcoal were prepared
The dried extracts E1- E2 and E3 obtained after solvent extraction were dissolved using methanol and the prepared sticks were dipped in their respective solutions and air-dried to evaporate the methanol. The sticks are now impregnated with extracts E1, E2 and E3. The composition is as follows:

Extract	Wood charcoal (WC)	Coconut Charcoal (CC)
E1	12 sticks	12 sticks
E2	12 sticks	12 sticks
E3	12 sticks	12 sticks

Mosquito repellence and mortality test: The mosquito repellent tests were performed at the Haffkine Institute, Mumbai under the WHO guidelines for testing the efficacy of insecticide products on mosquitoes [1]. Tests were performed on 50 non-blood-fed, 2–5-day old female *Aedes aegypti* species of mosquitoes in a Peet Grady chamber of internal volume 180cc, at 27 C ± 2 C, 80% ± 10% relative humidity (RH) and photoperiod 12:12 h (Light: dark). The repellent sticks were lit after the mosquitoes are kept captivated in the chamber, at T=0 and the tests were carried out for a total of 30 mins from being incensed. Knockdown readings were recorded for every 5 min up to T=30min and mortality of mosquitoes was calculated by the formula:

Mortality (%) = No. of mosquitoes knocked out (for 24hr)/ Total mosquitoes in chamber x 100

Results and Discussion

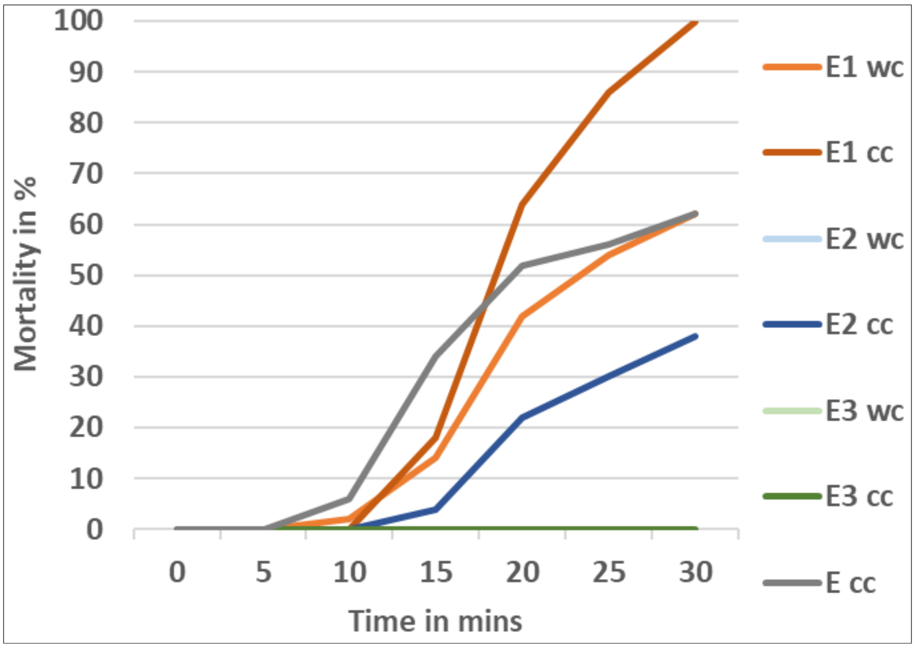

Fig 2: Mortality rates of the solvent extracts (E1 in Petroleum Ether, E2 in Chloroform and E3 in Methanol and E is crude coconut shell oil; (diluent used is Methanol) in wood charcoal (wc) or coconut charcoal (cc) sticks

As observed in Fig. 2, the mortality rates were highest in the (E1 cc) coconut charcoal sticks dipped in petroleum ether extracts compared to its wood charcoal counterparts. Coconut charcoal enhanced the ability to repel mosquitoes much more efficiently than the wood charcoal filled repellent sticks. The repellence activity between the three solvent extracts was in the order of Petroleum Ether > Chloroform >Methanol, with methanol having the least activity. Crude oil extract (E) with coconut charcoal filler, showed average mosquito repellence. Isolated Petroleum Ether (E1) extract showed repellence higher than crude extract and thus was more refined or free of any inhibitory substances. The characterization of the E1 extract showed presence of alkanes, ketonic groups and aromatic hydrocarbons (supplementary data). The fractions isolated from fractional distillation and column chromatography could be further tested against its mosquito repellence activities in future prospects.

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Supplementary Data
Typical Ranges of Frequencies in Fourier Transform Infrared Spectroscopy

Bond	Compound Type	Frequency range, cm^{-1}
C-H	Alkanes	2960-2850
		1470-1350
	CH_3 Umbrella Deformation	1380
C-H	Alkenes	3080-3020
		1000-675
C-H	Aromatic Rings	3100-3000
	Phenyl Ring Substitution Bands	870-675
	Phenyl Ring Substitution Overtones	2000-1600
C-H	Alkynes	3333-3267
		700-610
C=C	Alkenes	1680-1640
C□C	Alkynes	2260-2100
C=C	Aromatic Rings	1600, 1500
C-O	Alcohols, Ethers, Carboxylic acids, Esters	1260-1000
C=O	Aldehydes, Ketones, Carboxylic acids, Esters	1760-1670
O-H	Monomeric -- Alcohols, Phenols	3640-3160
	Hydrogen-bonded -- Alcohols, Phenols	3600-3200
	Carboxylic acids	3000-2500
N-H	Amines	3500-3300
		1650-1580
C-N	Amines	1340-1020
C-N	Nitriles	2260-2220
NO_2	Nitro Compounds	1660-1500
		1390-1260

Chemical Analysis of each fraction
Fraction D_1
1. Organic spotting
a. **Boiling range:** <60 ºC
b. **Colour:** Light yellow
c. **Odour:** Pleasant
d. **Saturation test:** Saturated compound
e. **Elemental analysis:** C, H, (O)
f. **Determination of functional group:** Ketonic group

2. Chromatographic study
TLC: Single spot

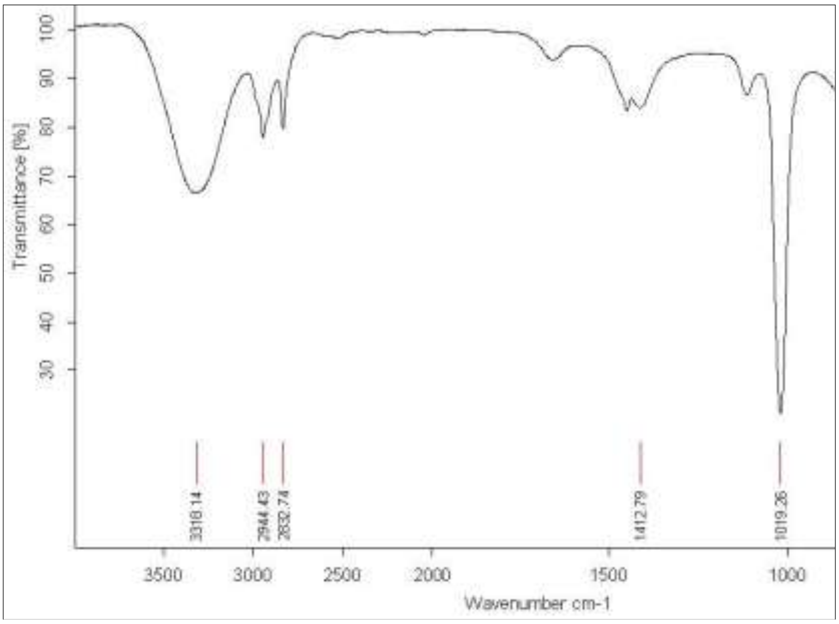
Fraction D_2
Organic spotting
a) **Boiling range:** 60-80 ºC
b) **Colour:** Light brown
c) **Odour:** Pungent
d) **Saturation test:** Saturated compound
e) **Elemental analysis:** C, H, (O).
f) **Determination of functional group:** Carboxylic group present.

Chromatographic separation
TLC: shows two distinct spots. Therefore, further separation was done by column chromatography.

Chromatographic separation of B
Fraction B was adsorbed on 1gm activated silica gel and loaded on a silica gel column of 30 cm in length. Various fractions collected and monitored using TLC techniques are showed in tabular form below.

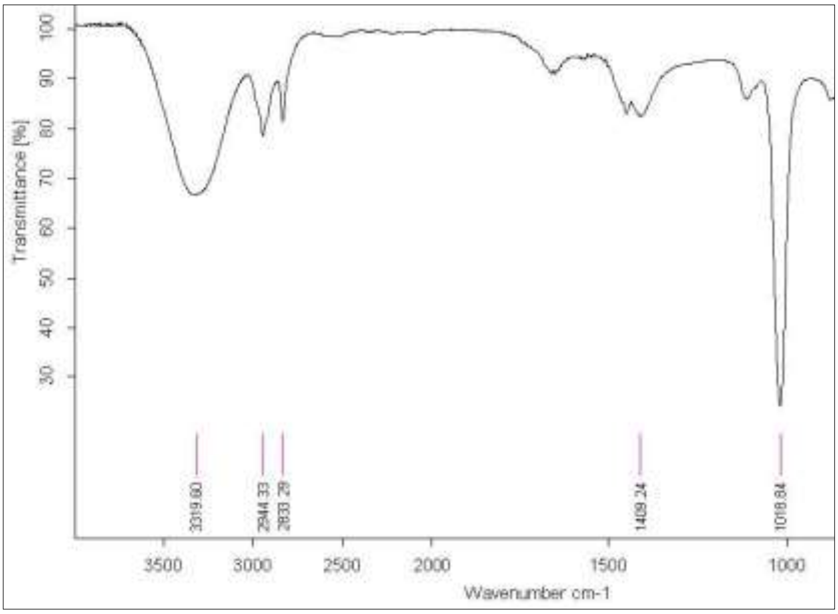
Test tube nos.	Composition of Mobile Phase	TLC results
1-18	Pet. Ether (100%)	-
19-20	Pet. Ether: Chloroform (9:1)	-
21	Pet. Ether: Chloroform (8:2)	-
22	Pet. Ether: Chloroform (7:3)	-
23	Pet. Ether: Chloroform (6:4)	-
24-26 (Distilled)	Pet. Ether: Chloroform (5:5)	Single spot
27	Pet. Ether: Chloroform (3:7)	-
28	Chloroform: Methanol (7:3)	-
29	Methanol (100%)	Two distinct spots

Fraction 24-26



Sr. No.	Peaks	Functional	Group
		Structural Form	**Names**
1.	3318.14	O-H	Alcohols
2.	2944.43	C-H	Alkanes
3.	2832.74	C-H	Alkanes
4.	1412.79	C-H	Alkenes
5.	1019.26	C-O	Ethers, Carboxylic Acids

Fraction 29



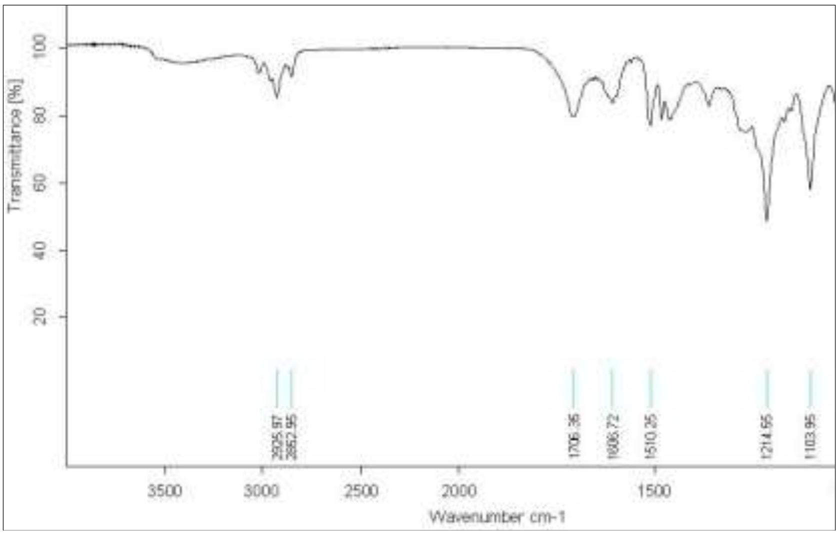
Sr. No.	Peaks	Functional	Group
		Structural Form	**Names**
1.	3319.60	O-H	Alcohols
2.	2944.33	C-H	Alkanes
3.	2833.29	C-H	Alkanes
4.	1409.24	C-H	Alkenes
5.	1018.84	C-O	Ethers, Carboxylic Acids

Fraction D_3

1. Organic spotting

a) Boiling range: 80-90 ºC
b) Colour: brown
c) Odour: pungent
d) Saturation test: saturated compound
e) Elemental analysis: C, H, (O).
f) Determination of functional (since the quantity remaining was not suitable for classical methods so further information about functional group was collected using IR spectroscopy)

2. Chromatographic separation



TLC: Two distinct spots. (Fraction size was too small so column chromatography was not implemented)

3. IR Spectrum

Sr. No.	Peaks	Functional	Group
		Structural Form	**Names**
1.	2925.97	C-H	Alkanes
2.	2852.95	O-H	Carboxylic Acids
3.	1606.72	C-H	Phenyl Ring Substitution
4.	1510.25	NO_2	Nitro Compound
5.	1214.55	C-O	Alcohol, Ester, Carboxylic Acid
6.	1103.95	C-O	Alcohol, Ester, Carboxylic Acid

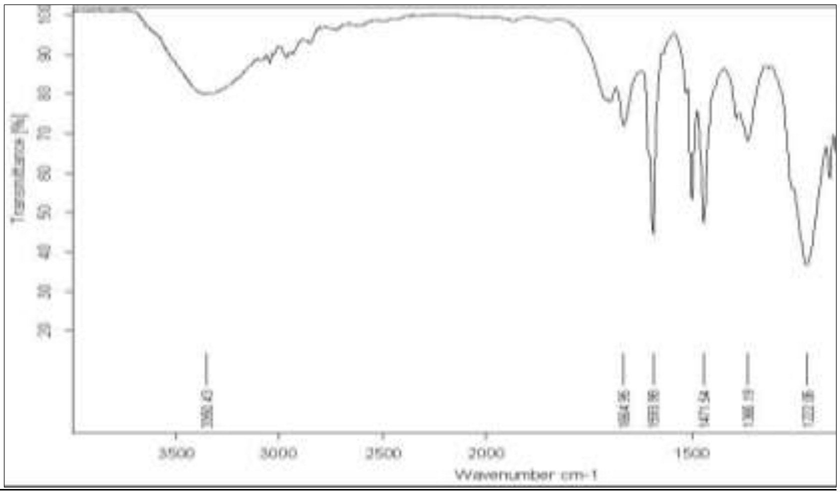
Fraction D_4

1. Organic spotting

a) Colour: brown
b) Odour: pungent
c) Saturation test: saturated compound.
d) Elemental analysis: C, H, (O).
e) Determination of functional group: OH
f) Boiling range: 90-98ºC

2. Chromatographic separation

(Fraction size was too small so column chromatography was not implemented)



IR Spectrum

Sr. No.	Peaks	Functional Group	Names
1.	3350.43	O-H	Monomeric - Alcohols, Phenols
2.	1664.95	C=C	Alkenes
3.	1593.98	N-H	Amines
4.	1471.54	C=C	Aromatic Ring
5.	1366.19	NO_2	Nitro Compounds
6.	1222.06	C-O	Alcohol, Ester, Carboxylic Acid

Silica gel Column Chromatography of Petroleum Ether Extract E1

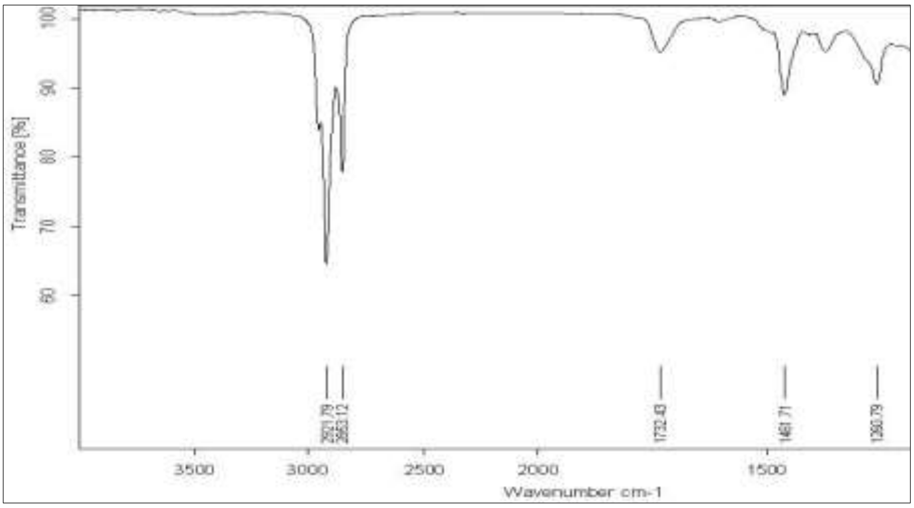
Test tube no.	Composition of Mobile phase	TLC results	Fractions
1-5	Pet. Ether (100%)	--	--
6-17	Pet. Ether: Chloroform (98:2)	--	--
18-25	Pet. Ether: Chloroform (95:5)	--	--
26-28	Pet. Ether: Chloroform (93:7)	--	--
29-35	Pet. Ether: Chloroform (92:8)	--	--
36-42	Pet. Ether: Chloroform (90:10)	--	--
43-54	Pet. Ether: Chloroform (90:10)	Single spot	A (light yellow)
55-64	Pet. Ether: Chloroform (90:1)	--	--
65-73	Pet. Ether: Chloroform (87:13)	--	--
74-81	Pet. Ether: Chloroform (85:15)	--	--
82-83	Pet. Ether: Chloroform (83:17)	--	--
84-86	Pet. Ether: Chloroform (80:20)	--	--
87-91	Pet. Ether: Chloroform (70:30)	--	--
92-113	Pet. Ether: Chloroform (50:50)	--	--
114-127	Pet. Ether: Chloroform (40:60)	--	--
128 – 133 (distilled)	Pet. Ether: Chloroform (40:60)	Single spot	B (faint green)
134-151	Pet. Ether: Chloroform (40:60)	--	--
152 – 169 (distilled)	Pet. Ether: Chloroform (30:70)	--	--
170 – 182 (distilled)	Pet. Ether: Chloroform (20:80)	--	--
183-199(distilled)	Pet. Ether: Chloroform (20:80)	Single spot	C (yellow)
200 – 210 (distilled)	Pet. Ether: Chloroform (10:90)	--	--
211 – 232 (distilled)	Pet. Ether: Chloroform (5:95)	--	--
233 – 247 (distilled)	Pet. Ether: Chloroform (97:3)	--	--
248 – 253 (distilled)	Pet. Ether: Chloroform (99:1)	--	--
254 – 256 (distilled)	Chloroform (100%)	--	--
257 – 262 (distilled)	Chloroform: methanol (90:10)	Single spot	D (orange)
263 – 265 (distilled)	Chloroform: methanol (85:15)	--	--
266 – 268 (distilled)	Chloroform: methanol (85:15)	Single spot	E (light orange)
269 – 271 (distilled)	Chloroform: methanol (85:15)	--	--
272 – 281 (distilled)	Chloroform: methanol (85:15)	Single spot	F (yellow)
282 – 284 (distilled)	Chloroform: methanol (70:30)	--	--
285 – 287 (distilled)	Chloroform: methanol (70:30)	--	--
288 – 292 (distilled)	Chloroform: methanol (60:40)	--	--
293 – 297 (distilled)	Chloroform: methanol (50:50)	--	--
298 – 302 (distilled)	Chloroform: methanol (30:70)	--	--
303 – 307 (distilled)	Chloroform: methanol (20:80)	--	--
308 – 320 (distilled)	Methanol (100%)	--	--

Qualitative Analysis of various Fractions

1) Fraction A

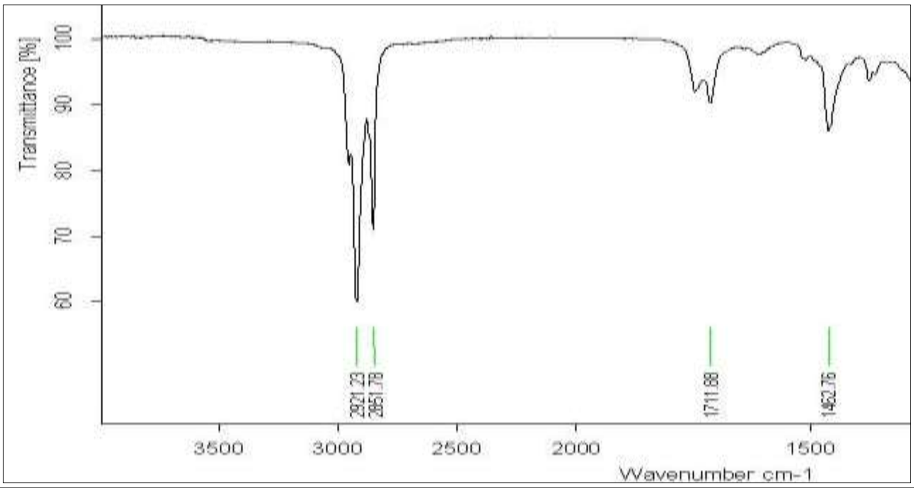
a. **IR Spectrum**

Sr. No.	Peaks	Functional	Group
		Structural form	Names
1.	2921.23	C-H	Alkanes and aliphatic molecules
2.	2851.78	C-H	Alkanes and aliphatic molecules
3.	1711.88	C=O	Ketone
4.	1462.76	C=C	Aromatic ring



2) Fraction B

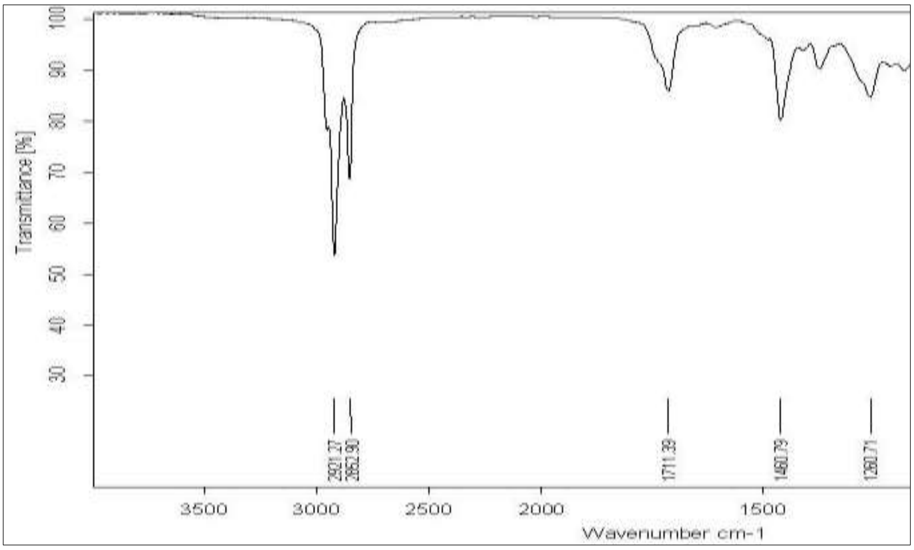
a. **IR Spectrum**



Sr. No.	Peaks	Functional	Group
		Structural form	**Names**
1.	2921.79	C-H	Alkanes and aromatic molecules
2.	2853.12	C-H	Alkanes and aromatic molecules
3.	1732.43	C=O	Ketones
4.	1461.71	C=C	Aromatic ring
5.	1260.79	C-O	Alcoholic

3) Fraction C

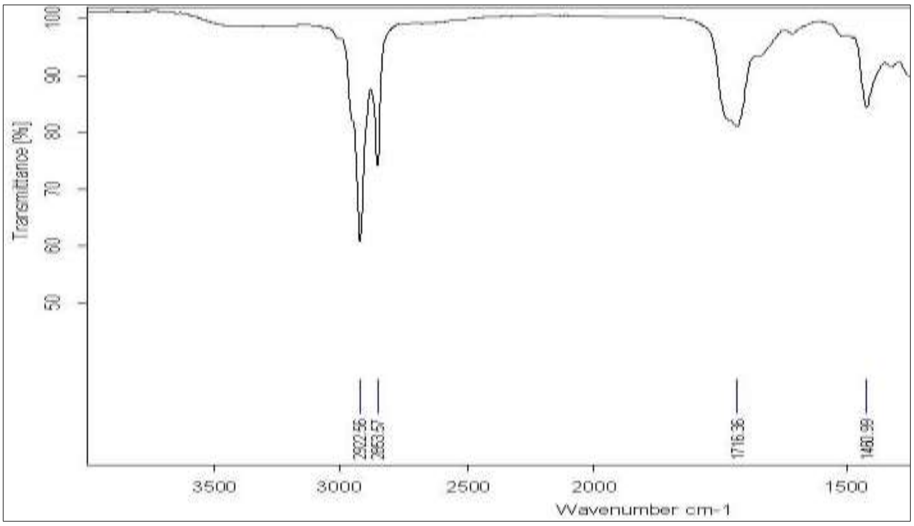
a. **IR Spectrum**



Sr. No.	Peaks	Functional	Group
		Structural form	**Names**
1.	2921.27	C-H	Alkanes and aromatic molecules
2.	2852.90	C-H	Alkanes and aromatic molecules
3.	1711.39	C=O	Ketone
4.	1460.79	C=C	Aromatic ring

4) Fraction D

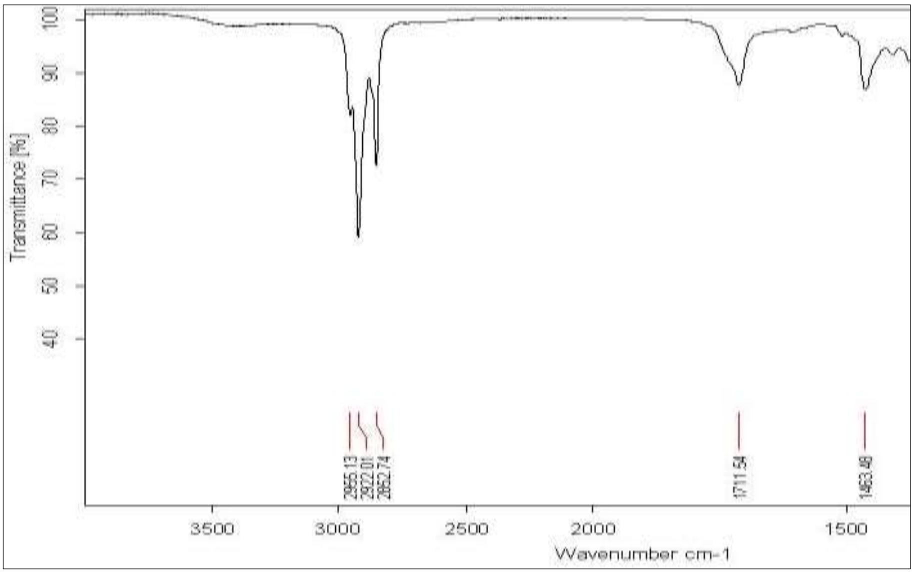
a. **IR Spectrum**



Sr. No.	Peaks	Functional	Group
		Structural form	**Names**
1.	2922.56	C-H	Alkanes and aromatic molecules
2.	2853.57	C-H	Alkanes and aromatic molecules
3.	1716.36	C=O	Ketone
4.	1460.99	C=C	Aromatic ring

Fraction E

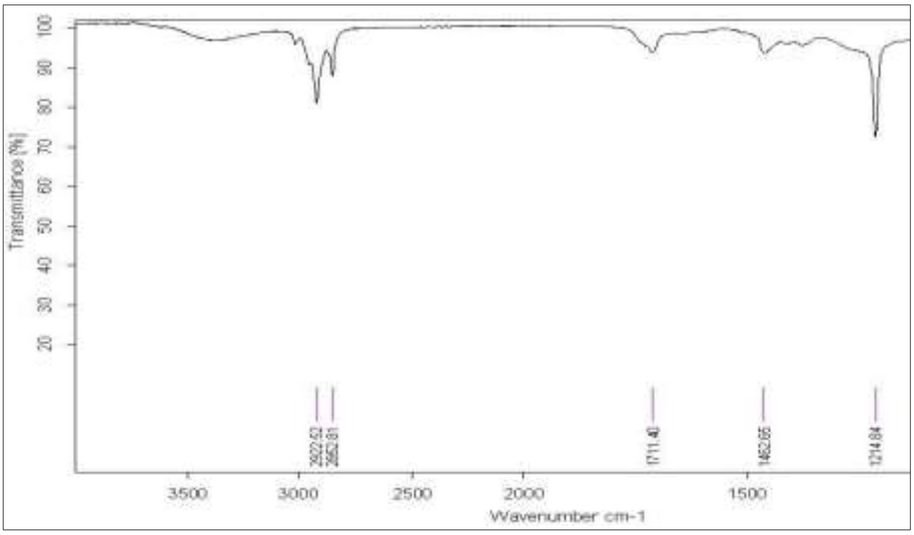
a. **IR Spectrum**



Sr. No.	Peaks at	Functional	Group
		Structural form	Names
1.	2955.13	C-H	Alkanes and aromatic molecules
2.	2922.01	C-H	Alkanes and aromatic molecules
3.	2852.74	C-H	Alkanes and aromatic molecules
4.	1711.54	C=O	Ketone
5.	1463.48	C-H	Alkanes

Fraction F

a. IR Spectrum



Sr. No.	Peaks	Functional	Group
		Structural form	Names
1.	2922.52	C-H	Alkanes and aromatic molecules
2.	2852.81	C-H	Alkanes and aromatic molecules
3.	1711.40	C=O	Ketone
4.	1462.65	C=C	Aromatic ring
5.	1214.84	C-O	Alcoholic