Optimization of total essential oil yield of Cinnamomum zeylanicum N. by using supercritical carbon dioxide extraction

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Abstract - The present work deals with the extraction of essential oils from dried bark of Cinnamomum zeylanicum N. Essential oil was extracted by four different methods; Traditional hydro distillation (HD), solvent extraction (SE), ultrasonication (US) and supercritical carbon dioxide extraction (SC-CO2). The optimization process was carried out using factorial design and maximum yield (3.8%) of essential oil was obtained at optimum conditions of pressure (200 bar), temperature (40 0C) and CO2 flow rate (4 g/min). Chemical composition of essential oil obtained from bark of cinnamon was analysed GCMS and FTIR and its antioxidant activity was also evaluated.

Index Terms - Cinnamomum, Essential oil, FT-IR, GC-MS, Optimum, SC-CO2, Antioxidant

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1. Introduction

The genus Cinnamomum includes around 250 species which are widely spread in China, India, Australia, Vietnam, Sri Lanka, Madagascar, Seychelles [1]. Cinnamon belongs to the Lauraceae family. Cinnamon has a long history of use as a significant traditional medicine. Cinnamon bark is used as a chief medicinal substance in China [2]. Cinnamomum cassia (Chinese Cinnamon) is also named Chinese Cinnamon, which has been found both wildly and cultivated in Southeast Asia since ancient age, then introduced into Indonesia, South America and Hawaii. Cinnamomum zeylanicum or true cinnamon is the inner bark of a small tropical evergreen tree native to Sri Lanka [3]. Cinnamomum tamala (Indian cassia) moderate sized evergreen tree which is native to India and have originated from the south slopes of Himalayas and it is spread up to an altitude of 900-2500 m in tropical and sub-tropical Himalayas [4]. Cinnamomum burmannii (Indonesian Cinnamon) is usually found in West Sumatra. Cinnamomum pauciflorum is cultivated in southwest China, north-eastern India, Assam, and Khassia hills. Among the major species of Cinnamomum, C. cassia, C. burmannii, C. zeylanicum, C. tamala and C. pauciflorum are of high commercial importance and have been used in the traditional medicines of India and China [5]. The most important use of cinnamon is as spice because of its distinct fragrant, sweet and warm odour [6]. Cinnamon has been used to cure blood circulation disturbance, dyspepsia, gastritis and inflammatory diseases in many countries since prehistoric period [7], [8].

Various studies have been done by researchers and have been found to possess significant as antipyretic, antiulcerogenic, anaesthetic, antiallergic, analgesic [9], [10], antioxidant [11], anticancer[12], [13], and antibacterial [14] activities. The in vitro studies on cinnamon extract have explored that it imitates the role of insulin, which enhances the action of insulin in inaccessible adipocytes and also improves the insulin receptor function [15], [16]. Essential oils are of great commercial value in case of aromatherapies which provides relieve from anxiety, pain, irritability, tiredness etc. Essential oil obtained from the leaves of C. osmophloeum ct. contains about 90% linalool which has been found to be a commercial source for aromatherapies

[17]. In this work, essential oil from dried bark of *C. Zeylanicum* N. have been extracted by conventional methods (HD, SE, US) and by innovative technique (SC-CO_2) in which CO_2 has been used as supercritical fluid as it is being non-toxic, inert and non-flammable it is considered as a GRAS (Generally Recognized As Safe) solvent. Through extraction of essential oils by 4 methods the volatile compounds in the essential oils were identified by GC/MS, FTIR. Optimum conditions of pressure, temperature and flow rate were determined and their effects on yield as well.

2. Materials and methods

2.1. Plant Materials

The plant material (dried bark) was purchased from local market of Aligarh, India. The dried bark of *C. zeylanicum* was grounded in a mechanical grinder in order to get uniform particle size distribution. The grounded powder was sieved.

2.2. Solvents and reagents

All the ingredients taken were of pharmacopeial quality and quantity. Standards were obtained from Sami Labs Ltd., Bangalore, (India) as a gift samples and other chemicals and reagents used were of analytical grade (AR) and procured from Merck Ltd. India. Carbon dioxide from Sigma gases, New Delhi, India.

2.3. Design of experiment

Full factorial design (FFD)

The most basic structures experimental design, where responses are measured at all combinations of experimental factor levels. SC-CO_2 data was then analysed and the P-value less than 0.05 were considered significant. All statistical analysis were performed using MINITAB 14 statistical software package.

2.3. *Hydro- distillation method*

Hydro distillation is most commonly used traditional methods for the extraction of volatile compounds from different matrix [18]. This extraction method have certain disadvantages as there is loss of volatile compounds, extraction efficiency is low, not suitable for thermolabile compounds [19]. The grounded powder (150 g) and 500 mL distilled water was mixed in one L round bottomed flask and heated for 5.0 h to yield essential oil in in a Clevenger-type apparatus, according to the method of Demirci, et al [20]. The extract was centrifuged at 10,000 rpm for 10 min in order to separate small water droplets present in the essential oil. The oil was kept at 4°C until further use.

2.4. Solvent Extraction

The coarse powder (50 gm) was extracted with hexane (100 mL) was kept in conical flask in 1:2 w/v, ratio for 24 hrs .The extract was filtered and concentrated under reduced pressure in rotary vacuum evaporator at 40°C. The essential oil obtained was weighed and kept at 4°C until further analysis.

2.5. Ultrasonic assisted extraction

The coarse powder (50 gm) of plant material was taken in a 250 mL conical flask along with 100 mL hexane (1:2, w/v). The flask was covered and then placed in an ultrasound water bath apparatus for 30 min (frequency 33 kHz). The temperature of the water bath was held constant at 25°C. The extract was filtered and concentrated under reduced pressure in rotary vacuum evaporator. The obtained oil was kept at 4°C until further use.

2.6. Supercritical carbon-dioxide extraction

A SFE-1000M1- 2- C50 system (Pittsburgh, PA) was used for extractions. The extraction vessel was 200 mL stainless steel. Grounded plant material (50 g) was loaded in the extractor. The CO_2 was allowed to pass through the matrix at desired pressure (100, 150 and 200 bar) and temperature (40, 45 and 50 ^{0}C) and optimised flow rate (4, 8 and 10 g min^{-1}). Total time of extraction was 30 min. After the completion of extraction, the extracted oil was collected from the collecting vessel [21].

2.7. Characterization of sample

In High performance thin layer chromatography (HPTLC) analysis, the samples were spotted in the form of band (3.0 mm) with a Camag microlitre syringe on TLC aluminium plate precoated with silica gel 60F-254 (20 x 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V sample applicator. The development was carried out mobile phase composed of hexane: ethyl acetate: formic acid (7:2:1, v/v/v). The HPTLC plates were studied at 254 nm and 366 nm as well as in visible range (580 nm) after spraying with anisaldehyde sulfuric acid reagent.

Gas chromatography-mass spectrometry (GC–MS) was equipped with a DB-5 fused silica capillary tubes column (30 m × 0.25 mm × 0.25 μm). The injection volume was 1.0 μL using auto-sampler at a carrier gas (helium) flow of 2.0 μL min^{-1} helium with a split less mode. The initial oven temperature was 65° C (3.0 min) then raised to 2.0°C min^{-1} -114°C, then to 4°C min^{-1} - 160°C, 6 °C min^{-1} - 302°C, finally ramped to 310°C at 15°C min^{-1}. Other setting of detector type was MS and its interface temperature was 250°C. The essential oil obtained by different extraction technique were diluted by adding 1998 μL of hexane to 2.0 μL oil (Hydro distilled oil) and 1990 μL hexane to 10 μL oil (other extracted oil).

By comparing with the National Institute of Standard and Technology (NIST) library compounds were detected and identified [22].

Fourier transform infrared spectroscopy (FTIR) spectral analysis was done and FTIR spectrum was obtained using shimadzu BioRad FTIR (Kyoto, Japan). The samples were dispersed and triturated with dry potassium bromide (wt 2μL of sample), grounded well in motar and pestle and potassium bromide (K Br) disk were at a pressure of 1,000 psig. The disk was placed in the FTIR sample holder, where IR spectra in absorbance mode was obtained in the spectral region 4,000 to 400 cm^{-1} using the resolution 4 cm^{-1}.

3. Results and Discussion

3.1. Percentage yield (% v/w)

Different extraction techniques were carried out in order to obtain maximum yield of oil. The extract yields of essential oil of *C. zeylanicum* (dried bark) were 0.70%, 0.72%, 0.78 and 3.8%, v/w for HD, SE, US and SC-CO_2, respectively.

3.2. Development of solvent system for separation of oil sample by TLC

The development of solvent system was carried out by hit and trial method using different solvents considering the results of earlier reports. The solvent system composed of hexane: ethyl acetate: formic acid (7:2:1, v/v/v) with maximum separation of constituents and compactness of bands for all the oil samples.

3.3. Detection of spots of different samples

Spotted plates were observed before derivatization at UV 254 nm and 366 nm and after derivatization by spraying with anisaldehyde sulphuric acid reagent at 254 nm Figure1.The HPTLC plates were studied at 254 nm and 366 nm as well as in visible range (580 nm) after spraying with anisaldehyde sulfuric acid reagent. A good separation of constituent was observed at 254 nm and 580 nm. The comparative results of different oils were showing a dominant constituent at R_f value 0.61 at 366 nm whereas it is observed at 254 nm in only supercritical fluid extracted oil and not observed at 580 nm (sprayed). Track 1 and 2 of hydro distilled oil showing lesser no. of compounds with major part being intense blue spot. Solvent extracted and ultrasonic assisted oil showed equal amount of separation. Supercritical carbon dioxide extracted oil showing some new spots not present in other extracted oil as shown in **Figure 1.**

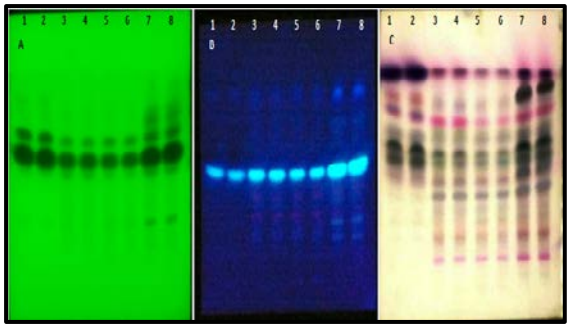

Figure 1: HPTLC fingerprint of different oils of C. zeylanicum N. extracted using different extraction techniques [track 1-2: Hydrodistilled oil, 3-4: Solvent extracted oil, 5-6: Ultra sonication oil, 7-8: SC-CO_2 oil] visualized at A: 254 nm, B: 366 nm, C: Day light after derivatization using anisaldehyde sulphuric acid reagent.

5.5. Comparative GC/MS analysis of *C. zeylanicum* oil obtained by different extraction techniques

Comparative GC-MS chromatograms of essential oils of *C. zeylanicum* extracted by traditional methods (HD, SE, US) and innovative SC-CO_2 technique as shown in (**Figure 2)**. A total of sixty compounds were identified with GC-MS analysis by using their retention time and mass from library (Nist and Wiley) (**Table 1**). The majority of the identified oil compounds consisted of sesquiterpene hydrocarbon, fatty group and aromatic compounds. The most abundant component of the bark oils was trans- cinnamaldehyde (55.97%), α-humulene (8.21%) β-phellandrene (3.63%), eugenol (3.54%) caryophyllene (3.28%). Hexadecanoic acid (5.28%), 9, 12-Octadecadienoic acid (8.89%) was the fatty acid compounds present.

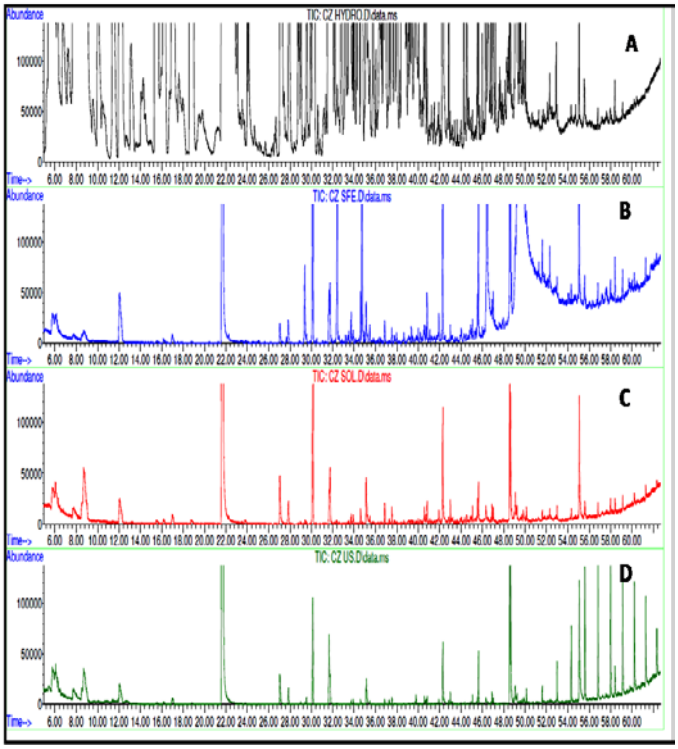

Figure 2: Comparative GC–MS chromatograms of different oils of C. zeylanicum extracted using different extraction techniques A: Hydrodistilled oil, B: SC-CO_2 oil, C: Solvent extracted oil, D: Ultra sonication oil.

5.6. FTIR analysis

The interpretation of infrared spectra of *C. zeylanicum* essential oil was done by correlating of absorption bands in the spectrum with the known absorption frequency bands. Comparative spectra of essential oil extracted by different techniques are shown in **Figure 3.** FTIR results are presented in **Table 2.**

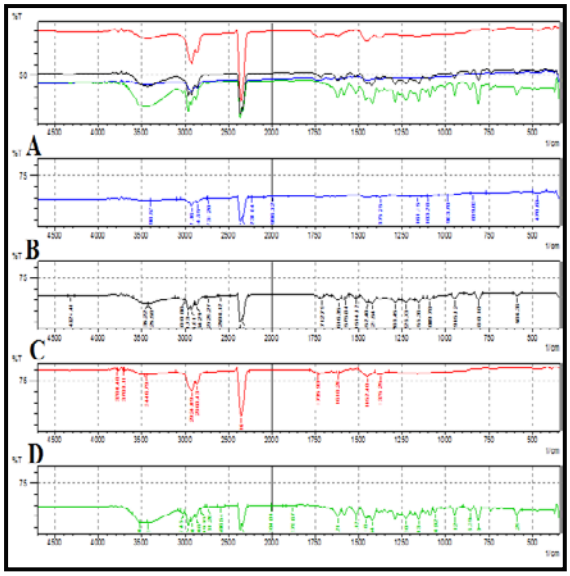

Figure 4: Comparative spectra of different oils of Cinnamomum zeylanicum N. extracted using different extraction techniques A: Hydro distilled oil, B: Ultra sonication oil, C: Solvent extracted oil, D: SC-CO_2 oil.

The typical spectra of cinnamons were analyzed and several peaks were observed such as the peak at 1727 cm^{-1}, corresponding to the aldehyde of a saturated fat, and the peaks at 1678 cm^{-1} which correspond to the stretching vibration of an aldehyde carbonyl C- O. These main peaks correspond to high levels of cinnamaldehyde and aldehydes in the volatile oil of cinnamon bark.

5.7. Antioxidant activity

The *C. zeylanicum* oil sample showed a significant concentration-dependent antioxidant activity by inhibiting DPPH free radical with an IC_{50} values of 70.55 µg mL^{-1}, whereas, IC_{50} value of ascorbic acid was found to be 28.09 µg mL^{-1} used as standard (**Figure 4)**. It was found that the oil possesses hydrogen donating capabilities about similar to ascorbic acid and acted as an antioxidant. The scavenging effect increased with increasing concentration of the extract and ascorbic acid (5.0-100 µg mL^{-1}).

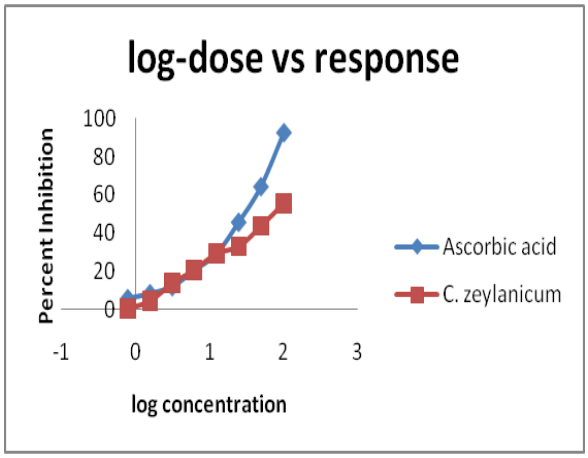

Figure 4: Comparative dose response curve between percent inhibitions against log concentration by DPPH method.

The superoxide scavenging activity of the drug was increased markedly with the increase in concentrations. A comparison of the antioxidant activity of the extracts and ascorbic acid is shown in **Figure 5**. The IC_{50} value of the essential oils of *C. zeylanicum* was found to be 32.34µg mL^{-1}, whereas the IC_{50} value of ascorbic acid was 20.14µg mL^{-1}.

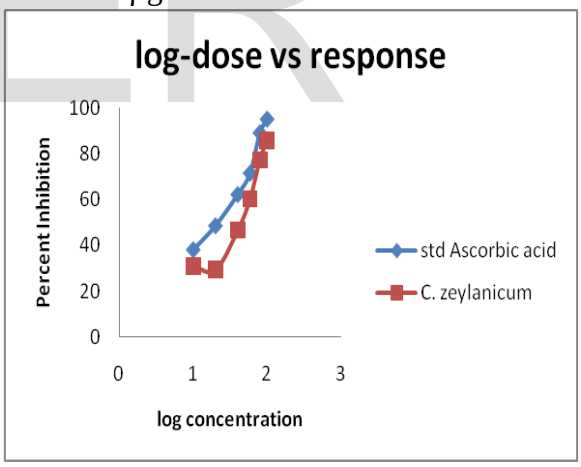

Figure 5: Comparative dose response curve between percent inhibitions against log concentration by superoxide scavenging method.

5.8. Factorial Design

An experiment was run to study the effect of three factors (Pressure, temperature, flow rate) with three levels on response (Yield). The results were analyzed and the results are presented in **Table 3**. It can be seen that the pressure and flow rate have

significant effect directly on the total yield of essential oil. The selected factors explained 86% fit model for the given data. The contour and surface plots have been shown in Figure 6 & 7.

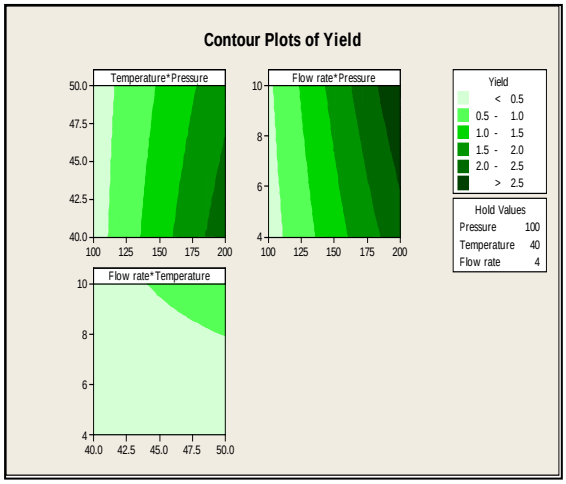

Figure 6: Contour plots of yield vs pressure, temperature and flow rate.

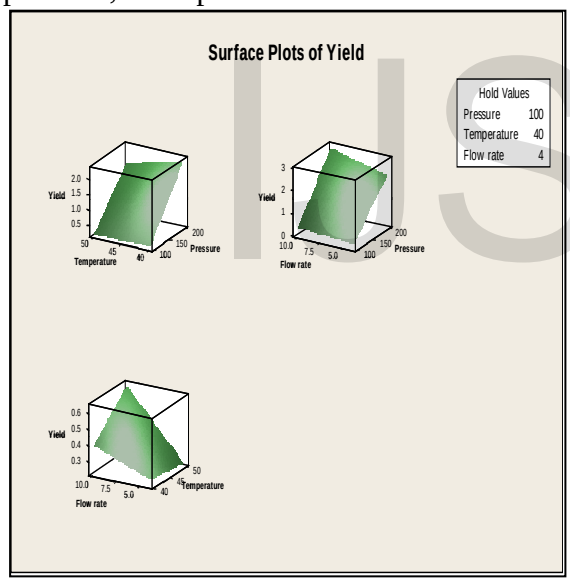

Figure 7: Surface plots of yield vs pressure, temperature and flow rate.

6. Conclusion

In present work, essential oil was extracted via Hydro distillation, Solvent extraction, Ultrasonic assisted extraction and Supercritical Carbon dioxide extraction (SC-CO_2). Among all these methods SC-CO_2 gave the highest percentage yield at optimum conditions of pressure (200 bar), temperature (40 ^{0}C) and flow rate of CO_2 (4 g/min). Furthermore evidences from GC-MS as well as FTIR analysis showed that SC-CO_2 is best technique for extraction of essential oils. Essential oil obtained from leaves of *C. zeylanicum* has shown to possess significant antioxidant activity which was investigated by DPPH and Superoxide anion scavenging methods and found to be 70.55 and 32.34 µg mL, respectively.

Acknowledgment

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Table 1: Results of GC-MS analysis of *Cinnamomum zeylanicum* N. oil extracted by different techniques

Area percent (%)						
S.no	**Component Name**	**R_T***	**HD***	**SE***	**US***	**SFE***
Monoterpene hydrocarbon						
1	α.-pinene,	5.43	0.48	-	-	-
2	l-Phellandrene	7.71	1.08	-	0.09	-
3	α-Terpinene	8.2	0.71	-	-	-
4	m-Cymene	8.53	0.71	-	0.4	-
5	β-Phellandrene	8.7	3.63	5.07	3.08	-
6	α-terpinolene	11.45	0.2	-	-	-
Oxygenated monoterpene						
7	Linalol	12.11	1.8	-	0.48	-
8	4-Terpineol	16.15	0.37	-	-	-
9	**trans-anethole**	**23.07**	**0.13**	**-**	**-**	**-**
10	**Carvacrol**	**24**	**0.03**	**-**	**-**	**-**
11	**Eugenol**	**27.12**	**3.54**	**1.6**	**1.04**	**0.3**
12	Geranyl acetate	28.77	0.04	-	-	-
13	Methyleugenol	29.92	0.04	-	-	-
14	trans-Cinnamyl acetate	31.88	7.35	-	1.58	-
15	Cinnamyl alcohol, acetate	31.67	-	0.62	-	-
Sesquiterpene hydrocarbons						
16	α-Copaene	27.85	0.44	0.61	0.45	0.32
17	Caryophylline-(I_3)	29.56	0.17	-	-	-
18	Caryophyllene	30.18	3.28	6.16	2.73	3.34
	α-Humulene	31.77	8.21	1.34	0.66	-
19	l-β-Bisabolene	34.24	0.06	-	-	-
	β-Selinene	33.2	0.24	-	-	-
	ValencenE	33.53	1.07	-	-	-
20	Longiborn-9-ene	34.38	0.26	-	-	-
21	(-)-.alpha.-Panasinsen	34.47	0.59	-	-	-
22	δ-Cadinene	34.77	0.1	-	-	-
23	Alloaromadendrene	38.71	0.11	-	-	-
24	γ-Gurjunene	39.38	0.16	-	-	-
25	β tumerone	39.66	0.08	-	-	-
26	Tumerone	39.77	0.24	-	-	-
27	Curloneα-tumerone	40.68	0.06	-	-	-
Oxygenated sesquiterpenes derivatives						
31	(-)-Caryophyllene oxide	36.86	0.15	-	-	-
32	Eudesm-4(14)-en-11-ol	39.12	0.04	-	-	-
Aromatic compounds						
33	Benzylacetaldehyde	15.44	0.51	-	-	-
34	Cinnamaldehyde, (E)-	20.94	0.18	55.9	52.6	22.4

35	Cinnamaldehyde	33.7	0.65	1.43	0.76	0.95
36	Myristyl aldehyde	37.96	0.11	-	-	-
Non isoprenoid						
37	Linalyl propionate	16.97	0.69	-	-	-
38	3-Hexadecene, (Z)-	29.03	0.05	-	-	-
39	Octadecane	33.73	-	0.2	-	-
40	Phenol, 2,4-bis(1,1-dimethylethyl)	34.62	0.19	-	-	-
41	1-bromo-2-methyl-decane	36.95	0.15	-	-	-
42	Docosane	40.83	-	0.35	-	-
43	Eicosane	41.31	-	0.28	2.48	0.29
44	Tricosane	41.94	-	0.17	-	-
45	Benzoic acid, phenylmethyl ester	42.29	1.55	1.96	1.36	1.23
46	1-Nonadecene	42.93	0.08	-	0.17	-
47	Pentacosane	45.26	-	0.3	0.18	0.23
48	Methyl palmitate	45.62	0.02	0.63	0.8	-
49	Pentadecanoic acid, 14-methyl-, methyl ester	45.61	-	-	-	0.99
50	Hexadecanoic acid	46.43	-	-	-	5.28
51	Trifluoroacetoxy hexadecane	46.78	0.04	-	-	-
52	Nonacosane	46.91	-	-	0.15	-
53	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	48.55	0.02	5.33	8.89	6.03
54	9-Octadecenoic acid (Z)-, methyl ester	48.64	-	2.13	3.19	2.5
55	Octadecanoic acid, methyl ester	49.06	-	-	0.3	-
56	1-Docosanol	50.03	0.02	-	-	-
57	Nonadecane	51.6	-	-	0.2	-
58	n-Octadecane	52.99	-	-	0.59	-

59	Butyl phthalate	55.05	0.02	2.61	3.33	1.76
60	Squalene	58.4	-	-	-	0.36

HD*Hydrodistillation, SE*Solvent extraction, US*Ultrasonication, SFE*Supercritical fluid extraction

Table 2: Results of FTIR analysis of *C. zeylanicum* N. oil obtained by different extraction techniques

S.no	Peak	Functional group	Intensity	HD*	SE*	US*	SFE*
1	686	C-Cl(chloroalkane)	Stretch, strong	+	-	+	+
2	974	=C-H(alkene)	Bending, strong	+	+	+	+
3	1124	C-O(alcohol)	Stretch, strong	+	+	+	+
4	1246	C-O(acid)	Stretch, strong	+	+	+	+
5	1384	N-O(nitro)	Stretch, strong	+	-	+	-
6	1450	C=C(aromatic)	Stretch, medium	+	-	-	+
7	1678	C-O(aldehyde)	Stretch, variable	+	+	+	+
8	2268	-C=-C(alkyne)	Stretch, variable	-	+	-	+
9	3059	=C-C(alkene)	Stretch, variable	+	+	-	-
10	3408	O-H(alcohol,)	Strong, broad	-	-	+	+

HD*Hydrodistillation,SE*Solvent extraction, US*Ultrasonication, SFE*Supercritical fluid extraction

Table 3. Estimated Effects and Coefficients for Yield

Term	Effect	Coef	SE Coef	T	P
Constant		1.43791	0.07888	18.23	0.000
Pressure	2.10000	1.05000	0.08360	12.56	0.000
Temperature	-0.07750	-0.03875	0.08360	-0.46	0.647
Flow rate	0.54777	0.27389	0.08309	3.30	0.003
Pressure*Temperature	-0.17750	-0.08875	0.08360	-1.06	0.297
Pressure*Flow rate	0.28250	0.14125	0.08360	1.69	0.102
Temperature*Flow rate	0.16750	0.08375	0.08360	1.00	0.325
Pressure*Temperature*Flow rate	0.03750	0.01875	0.08360	0.22	0.824