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Research article

OPTIMIZATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ESSENTIAL OIL FROM *CINNAMOMUM TAMALA* ALONG WITH ITS CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY

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ABSTRACT: Essential oil was extracted from leaves of *Cinnamomum tamala* by four different methods; Traditional hydro distillation (HD), solvent extraction (SE), ultrasonication (US) and supercritical carbon dioxide extraction (SC-CO_2). The optimization process was carried out using factorial design and maximum yield (3.64%) of essential oil was obtained at optimum conditions of pressure (200 bar), temperature (55 ^{0}C) and CO_2 flow rate (15 g/min). Essential oil extracted by SC-CO_2 was found to be best in quantity and quality. Chemical composition of essential oils obtained from leaves *Cinnamomum tamala* were analysed by GC/MS and its antioxidant activity was also studied.
Key words: *Cinnamomum tamala*, Essential oil, SC-CO_2, Optimization, GC-MS

INTRODUCTION

Supercritical fluid extraction (SFE) is a good technique for the production of flavors and fragrances from natural materials and can constitute a valid alternative to conventional extaction techniques. In fact, compressed carbon dioxide, CO_2, is able to solubilize hydrocarbons and oxygenated mono- and sesquiterpenes [1], the main essential oil constituents. Cinnamon belongs to the Lauraceae family. The genus Cinnamomum includes around 250 species which are widely spread in China, India, Australia, Vietnam, Sri Lanka, Madagascar, Seychelles [2]. Cinnamon has a long history of use as a significant traditional medicine. *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 [3]. The most important use of cinnamon is as spice because of its distinct fragrant, sweet and warm odour [4]. Cinnamon has been used to cure blood circulation disturbance, dyspepsia, gastritis and inflammatory diseases in many countries since prehistoric period [5, 6]. Essential oils are of great commercial value in case of aromatherapies which provides relief 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 [7].
In this work, essential oil from dried leaves of *Cinnamomum tamala* has been extracted by conventional methods (HD, SE, US) and by innovative technique SC-CO_2 extraction. The volatile compounds in the essential oils were identified by GC/MS.

MATERIALS AND METHODS

Plant Materials

The following plant material (dried leaves) was purchased from local market of Aligarh, India. As a first step, dried cinnamon leaves were grounded in a mechanical grinder for a short but sufficient period of time (30 s) to get a uniform particle size distribution. The grounded powder was sieved.

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. Other reagents and chemicals were purchased from sigma

Design of experiment

Full factorial design (FFD)

A factorial design is type of designed experiment that lets us study the effects that several factors can have on a response. When conducting an experiment, varying the levels of all factors at the same time instead of one at a time lets us study the interactions between the factors. The variation levels for parameters as pressure (100, 150 and 200 bar), temperature (45, 50 and 55 ^{0}C) and flow rate (5, 10 and 15 g/min).

Hydro- distillation method

Hydro distillation is most commonly used traditional methods for the extraction of volatile compounds from different matrix [8]. This extraction method have certain disadvantages as there is loss of volatile compounds, extraction efficiency is low, not suitable for thermolabile compounds [9]. 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 [10]. 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.

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.

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.

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 vessel. The pressure in the extraction vessel was controlled by back pressure regulator. Heat exchangers were provided in system to maintain temperature in the extractor and separator vessel. Extraction pressure was varied from 100-250 bar, temperature 60-80 ^{0}C and CO_2 flow rate was varied from 8 to 12 g/min. Total time of extraction was 45 min. Total time of extraction was 45 min. After that oil was collected from the collecting vessel [11]. The extracted oil was collected in a glass vial and stored at 4 ^{o}C prior to analysis and its percentage yield was calculated.

Characterization of sample

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 [12].

ANTIOXIDANT ACTIVITY

Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

The samples were mixed with 95% methanol to prepare the stock solution (1 mg/ml). One hundred µL of 0.5 mM 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) in methanol was mixed with 100 µL of samples in 96 well plate at various concentrations (0.781, 1.56, 3.12, 6.25, 12.5, 25.0, 50.0 and 100.0 µg) in duplicate. The 96 well plates were allowed to stand at room temperature for 30 min in dark. The control was prepared as described above without sample or standards, whereas blank was prepared without DPPH containing sample and methanol. The changes in absorbance of all the samples and standards were measured at 540 nm in Elisa plate reader (Bio Rad 680). Radical scavenging activity was calculated using the corrected ODs (COD) of control and samples as per equations (1) & (2).

$$\text{COD control} = \text{OD control} - \text{OD control blank} \quad (1)$$

$$\text{Radical scavenging activity (\%)} = \frac{\text{COD control} - \text{COD sample}}{\text{COD control}} \times 100 \quad (2)$$

IC_{50}, which is the concentration of sample required to scavenge 50% of free radicals was calculated [13].

Statistical analysis

The data obtained from SC-CO_2 extraction were subjected to analysis of variance (ANOVA) to determine significant difference among all extract yields. P-value less than 0.05 were considered significant. All statistical analysis were performed using MINITAB 14 statistical software package.

RESULTS AND DISCUSSION

Different extraction techniques were carried out in order to obtain maximum yield of oil. The extract yields of essential oil of *C. tamala* (dried leaves) were 1.80%, 2.5%, 2.9% and 3.64%, v/w for HD, SE, US and SC-CO_2, respectively.

Comparative GC-MS chromatograms of essential oils of *C. tamala* extracted by traditional methods (HD, SE, US) and innovative SC-CO_2 technique as shown in Fig. 1.

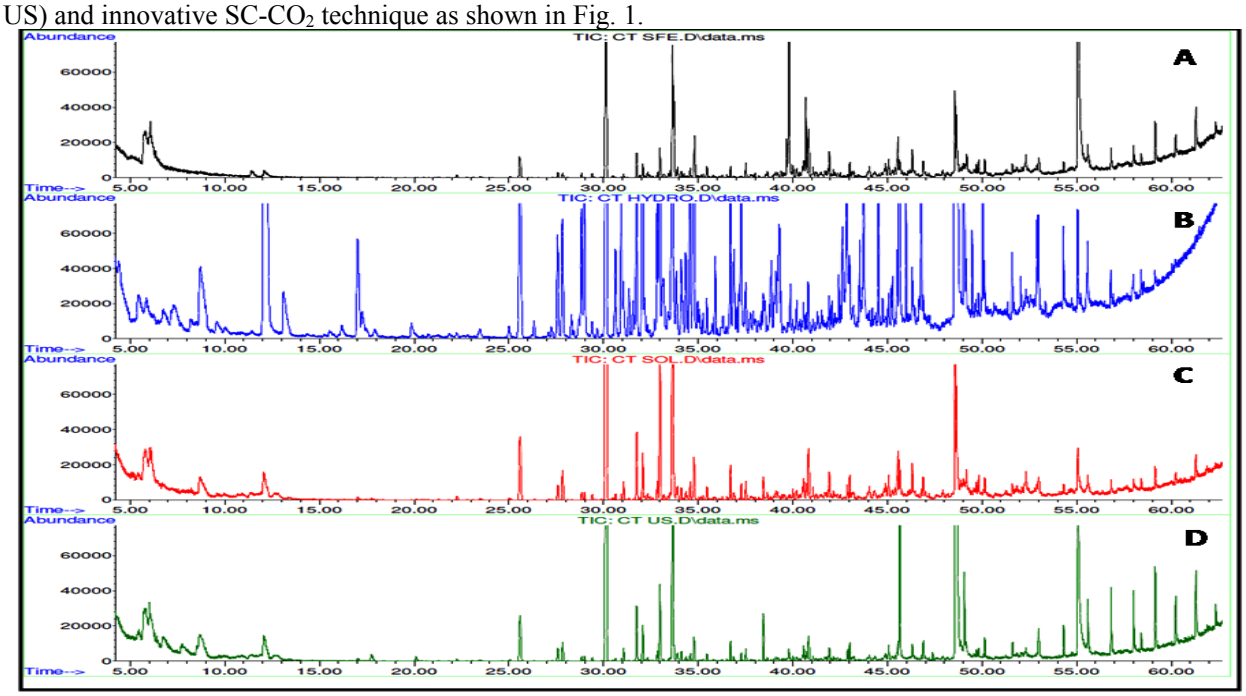

Fig. 1 Comparative GC–MS chromatograms of different oils of *C. tamala* extracted using different extraction techniques A: SC-CO_2 oil, B: Hydro distilled oil, C: Solvent extracted oil, D: Ultra sonication oil

A total of forty five compounds were identified with GC-MS analysis using their retention time and mass from library (Nist and Wiley) **(**Table 1). Among them 21 compounds were sesquiterpene hydrocarbons, non-isoprenoid compounds containing fatty acid. Transcaryophyllene was present in maximum amount, of all the identified compounds in oil extracted by hydro distillation. It was absent in oil extracted by solvent extraction and ultrasonicated oil. Germacerene D was next highest compound present in oil extracted by hydro distillation. Present in lesser quantity in solvent extracted and supercritical fluid extracted oil, absent in ultrasonicated oil.

The *C. tamala* oil sample showed a significant concentration-dependent antioxidant activity by inhibiting DPPH free radical with an IC_{50} values of 89.4µg mL^{-1}, whereas, IC_{50} value of ascorbic acid was found to be 28.09 µg mL^{-1} used as standard (Fig. 2). 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}).

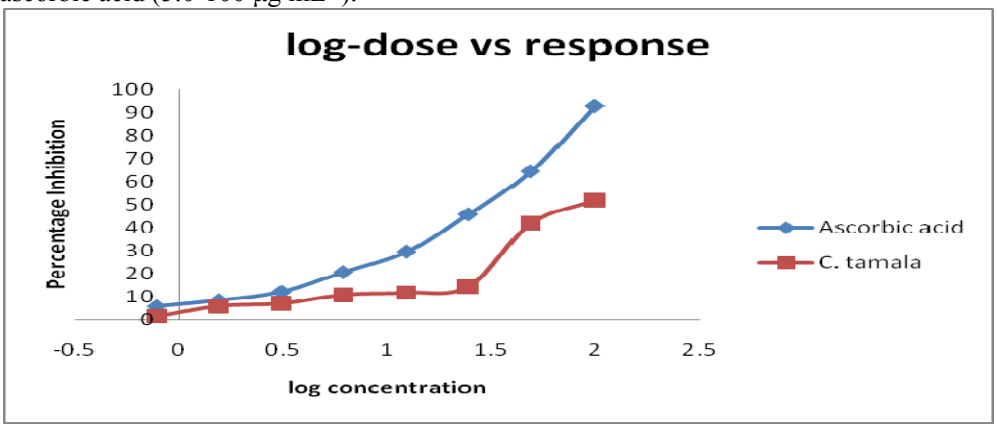

Fig. 2: Comparative dose response curve between percent inhibitions against log concentration by DPPH method

Table 1. Results of GC-MS analysis of *Cinnamomum tamala* oil extracted by different extraction techniques.

Area percent (%)						
S.no	**Component Name**	**R_T***	**HD***	**SE***	**US***	**SC-CO_2***
Monoterpene hydrocarbon						
1	α-Ylangene	27.57	0.74	-	-	
Oxygenated monoterpene						
2	Linalool	12.04	8.83	-	-	-
3	Apiol	38.45	-	0.77	0.63	-
Sesquiterpene hydrocarbons						
4	Bicycloelemene	25.59	2.1	4.29	-	-
5	Copaene	27.84	0.82	1.77	-	-
6	β- elemene	28.83	0.81	-	-	-
7	Caryophyllene	30.12	5.11	26.3	9.05	-
8	trans-Caryophyllene	30.13	26.2	-	-	13.4
9	Germacrene B	30.94	0.74	-	-	-
10	Aromadendrene	31.4	1.12	2.09	1.3	-
11	α.-Humulene	31.74	1.54	3.44	1.4	-
12	α.-Caryophyllene	31.75	-	3.44	1.04	-
13	β- cubene	31.8	0.49	-	1.3	-
14	Bicyclogermacrene	33.64	6.17	20.9	5.48	-
15	α.-Amorphene	32.84	0.64	-	-	-
16	Germacrene D	32.98	13.5	8.32	-	1.61
17	γ-Cadinene	34.35	0.49	-	-	-
18	δ.-Cadinene	34.6	0.54	2.11	0.41	1.63
19	β.-Sesquiphellandrene	34.81	-	-	-	2.4
20	Germacrene B	35.91	0.37	-	-	-
21	(-)-Spathulenol	36.69	1.11	-	-	-
Non isoprenoid						
	Isolongifolene	36.71	-	1.44	-	-
23	1-Dodecene	17.01	0.94	-	-	-
24	Cyclotetradecane 3-Hexadecene, (Z)-	28.98	1.72	-	-	-
25	Phenol,2,4-bis(1,1-dimethylethyl)	34.57	4.61	-	-	-
26	1-Hexadecene	37.27	2.64	-	-	-
27	Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene-	38.88	0.45	-	-	-
28	1-Nonadecene	42.85	2.44	-	-	-
29	Docosane	44.69	-	1.96	0.36	1.59
30	Nonacosane	45.07	-	0.81	-	-
31	Hexadecanoic acid, methyl ester	45.61	3.6	-	-	-
32	Methyl palmitate	45.62	-	1.72	3.97	-
33	3-Eicosene, (E)-	46.79	1.34	-	-	-
34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	48.56	14.2	-	49.4	3.94
35	9-Octadecenoic acid (Z)-, methyl ester	48.64	13.2	5.08	13	-
36	10,13-Octadecadienoic acid	48.81	-	10.3	1.04	-
37	Octadecanoic acid, methyl ester	49.05	1.68	-	1.04	-
38	Diisooctyl maleate	49.47	0.28	-	-	-
39	1-Docosene Behenic alcohol	50.04	0.57	-	-	-
40	Dotriacontyl pentafluoropropionate	52.91	0.31	-	-	-
41	Triacontane	52.98	0.34	-	-	-
42	Butyl pthalate	55.06	0.36	1.55	6.19	30.2
43	Eicosane	55.32	-	1.8	1.17	2.1
44	Hexatriacontane	55.59	-	0.76	-	-
45	Heneicosane	59.13	-	-	-	2.12

HD*Hydrodistillation, SE*Solvent extraction, US*Ultrasonication, SC-CO_2*Supercritical carbon dioxide extraction

An experiment was run to study the effect of with three factors (Pressure, temperature, flow rate) with three levels on response (Yield). The results were analyzed and are presented in Table 2.

Table 2. Estimated Effects and Coefficients for Yield

Term	Effect	Coef	SE Coef	T	P
Constant		1.22614	0.07399	16.57	0.000
Pressure	2.17500	1.08750	0.07845	13.86	0.000
Temperature	0.07817	0.03909	0.07728	0.51	0.617
Flow rate	0.73625	0.36812	0.07845	4.69	0.000
Pressure*Temperature	0.03712	0.01856	0.07845	0.24	0.815
Pressure*Flow rate	0.72000	0.36000	0.07845	4.59	0.000
Temperature*Flow rate	0.31938	0.15969	0.07845	2.04	0.051
Pressure*Temperature*Flow rate	0.31188	0.15594	0.07845	1.99	0.057

It can be seen that the selected factors showed significant effect either directly or through the interaction with other factors. The selected factors explained 89.69% fit model for the given data. The contour and surface plots have been shown in Fig. 3 & 4.

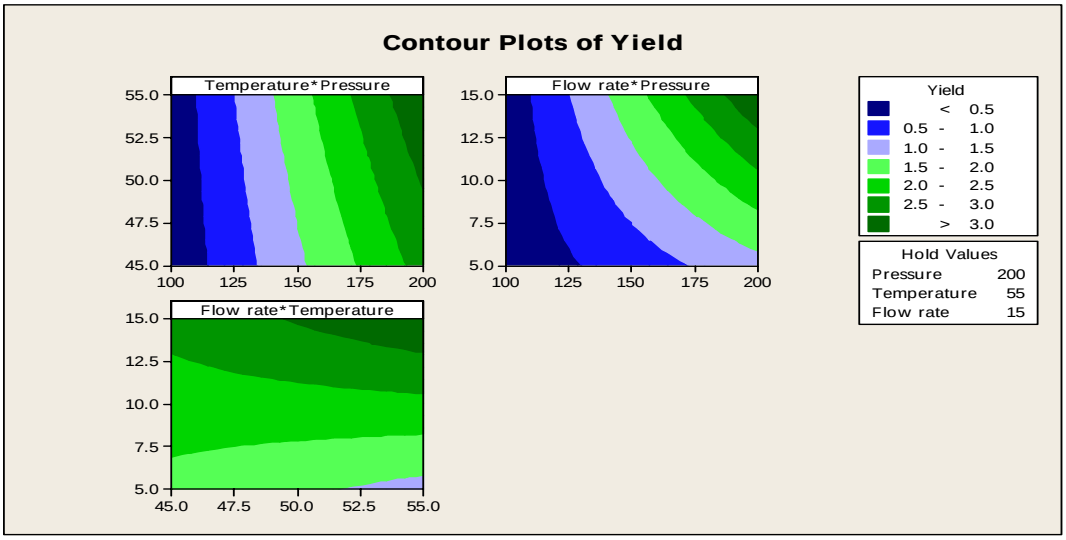

Fig. 3 Contour plots of yield vs pressure, temperature and flow rate

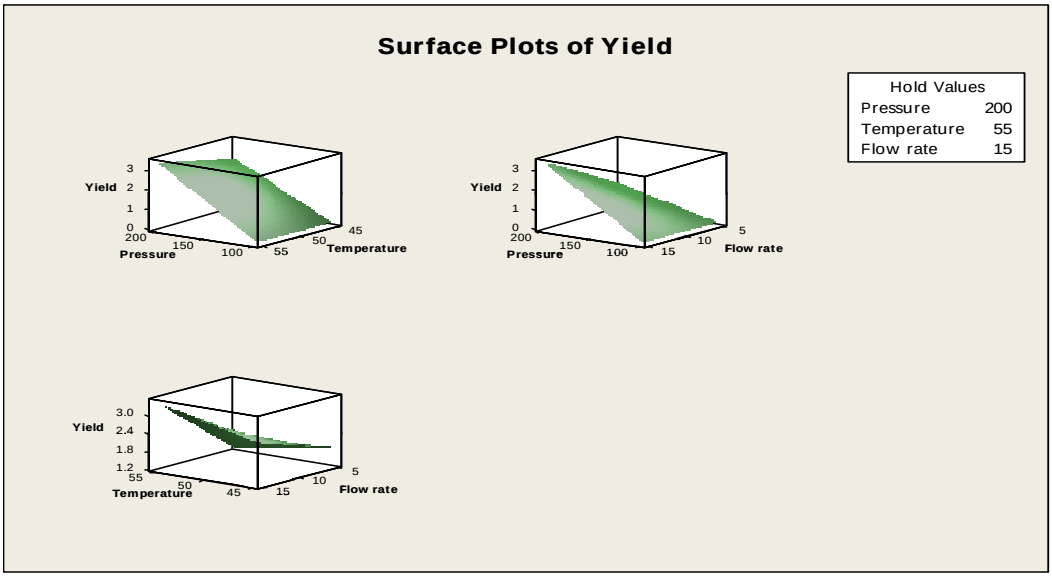

Fig. 4 Surface plots of yield vs pressure, temperature and flow rate

Pressure, flow rate and combination of pressure-flow rate have shown to have direct effect on yield as $p<0.05$, hence pressure, flow rate and combination of pressure-flow rate are significant factors for influencing yield. While temperature, combination of pressure-temperature, temperature- flow rate and pressure-temperatute-flow rate have no significant effect on yield as $p>0.05$.

The optimum operating conditions for SC-CO_2 extraction of essential oils from leaves of *C. tamala* for high yield were pressure (200 bar), temperature (55 ^{0}C) and flow rate of CO_2 (15 g/min)**.** A confirmation experiment was run using the optimum conditions and the highest yield obtained was 3.64 %.

CONCLUSIONS

In present work, essential oil from rhizome of *C. tamala* was extracted via HD, SE, US and SC-CO_2, out of all these methods SC-CO_2 gave the highest percentage yield (3.64%) using FFD with optimum conditions of pressure (200 bar), temperature (55 ^{0}C) and flow rate of CO_2 (15 g/min).The analysis shows that pressure, flow rate and interaction of pressure-flow rate have significant effect on oil yield while temperature, combination of pressure-temperature, temperature- flow rate and pressure-temperatute-flow rate have no significant effect on yield as $p>0.05$. SC-CO_2 is a green, environmentally friendly and sustainable extraction technique. Forty five compounds were identified by GC/MS. Essential oil obtained from leaves of *C. tamala* has shown to possess significant antioxidant activity which was investigated by DPPH method and was found to be 89.4 μg mL^{-1}.

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