

Supercritical Carbon-dioxide Extraction of essential oil from leaves of *Eucalyptus globulus* L., Analysis and their Application

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Supplementary information

Fig. S1: Calibration plot for total phenolic contents using Gallic acid as standard

Fig. S2: Comparative study of phenolic contents in essential oil of *E. globulus* extracted by different extraction techniques

Fig. S3: Comparative study of anthocyanin contents in essential oil of *E. globulus* extracted by different extraction techniques

Fig. S4: Comparative spectra of different oils of *E. globulus* L. extracted using different extraction techniques A: Solvent extracted oil, B: Ultra sonication oil, C: Hydrodistilled oil, D: SFE oil

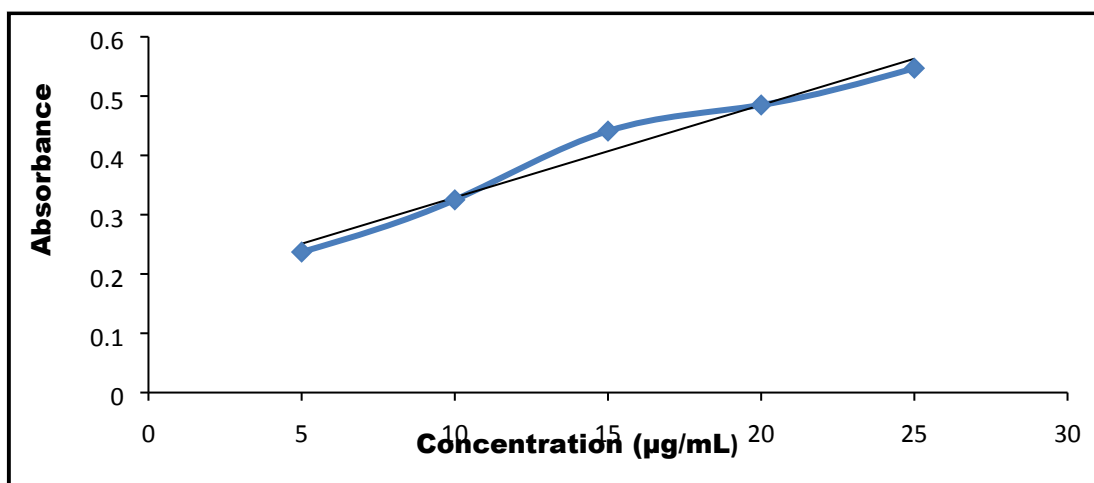


Fig. S1

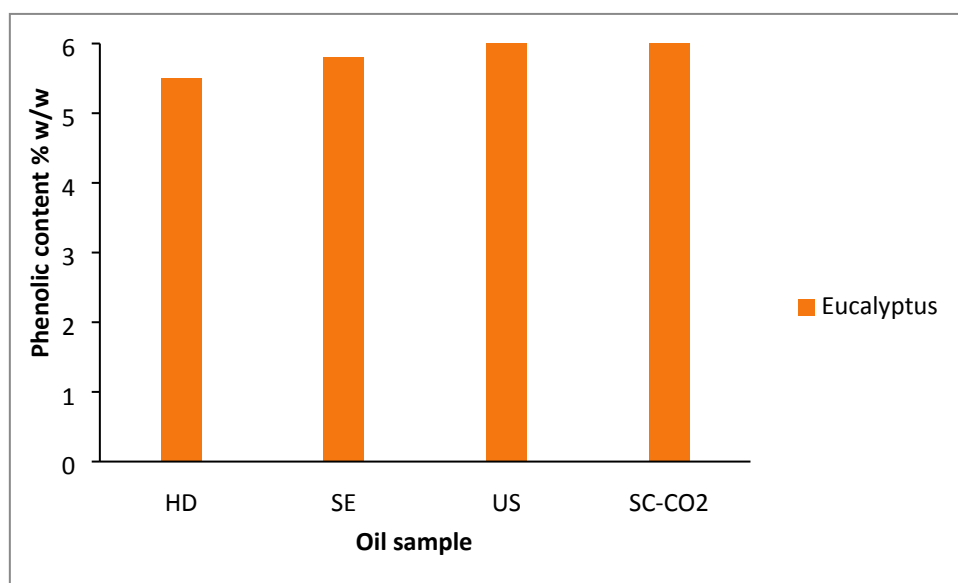


Fig. S2

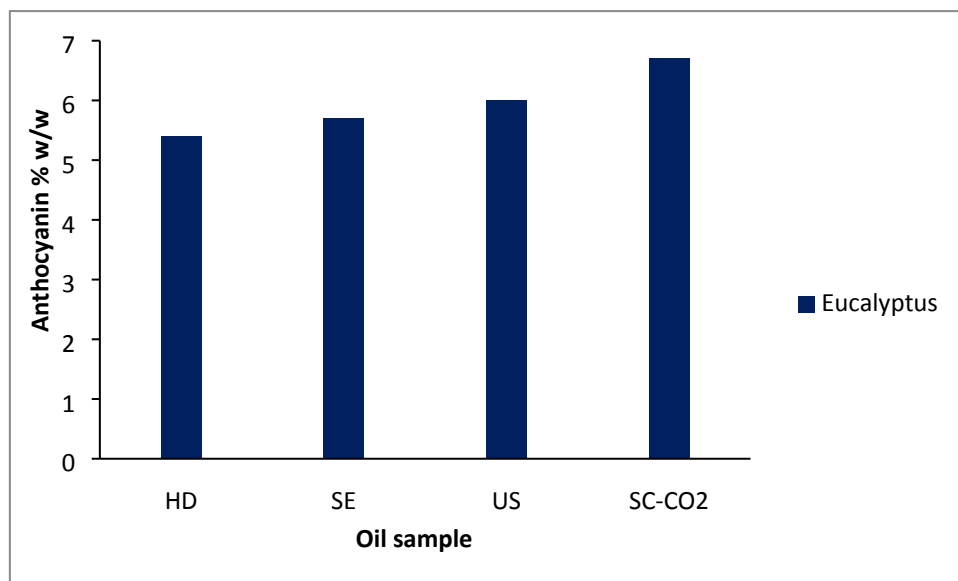


Fig. S3

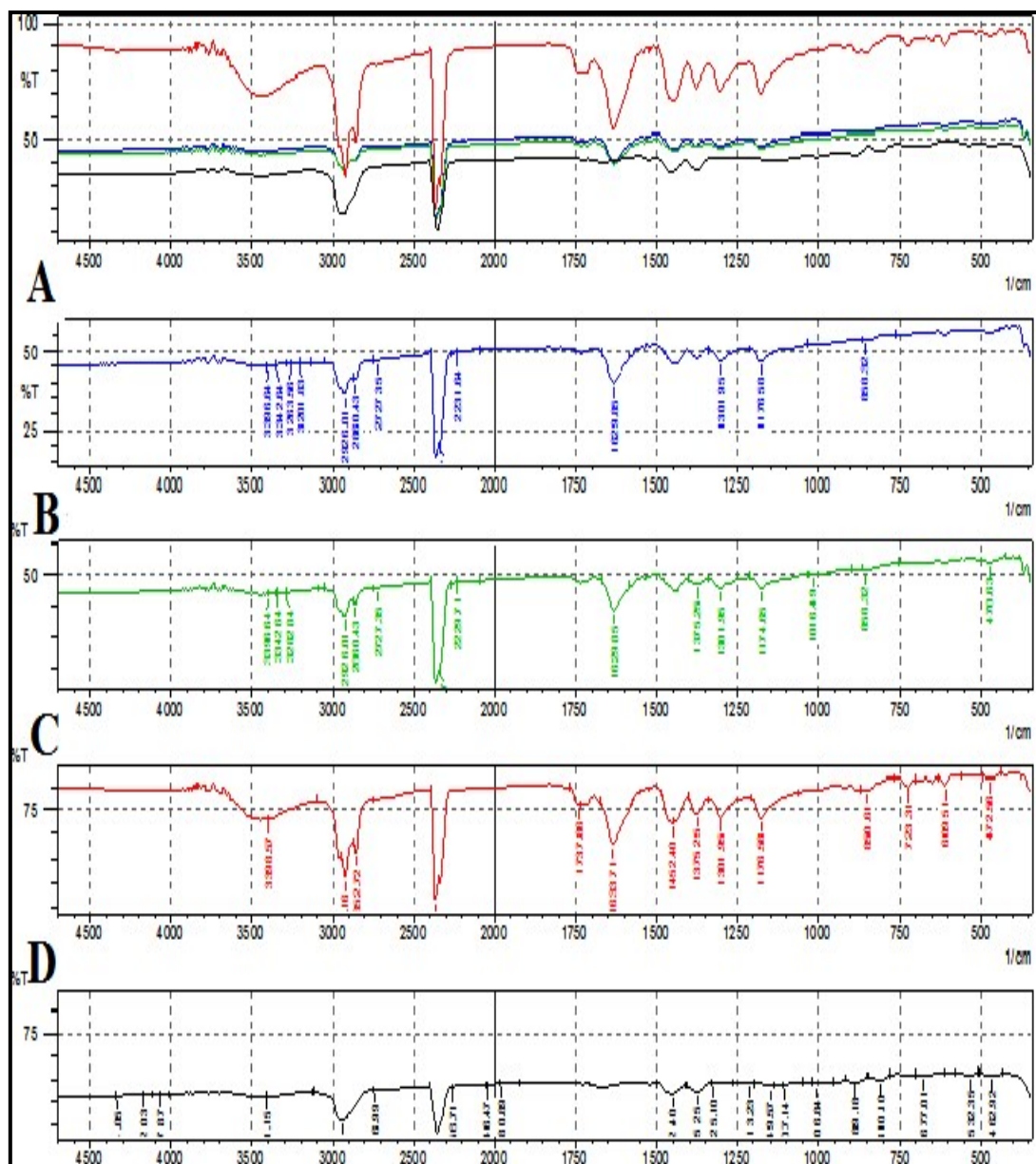


Fig. S4

Table S1. Physicochemical characterization of essential oil

<i>E. globulus</i> (dried leaves) oil	HD*	SE*	US*	SC-CO ₂ *	Reported value ³²
specific gravity (25°C)	0.899	0.880	0.878	0.899	0.906-0.925
refractive index (25°C)	1.44	1.460	1.459	1.65	1.458-1.470
Optical rotation (25°C)	-3.9°	-4°	-7.1°	-3.9°	-2° to +5°
Solubility in alcohol (% v/v ethanol)	Clearly soluble in 5.0 mL	Clearly soluble in 5.0 mL	Clearly soluble in 4.5 mL	Clearly soluble in 4.4 mL	Soluble in 4 mL of 70% ethanol
Acid value (Mean±SD*)	5.8±0.2	6.0±0.5	5.9±0.2	5.4±0.1	-
Ester value (Mean±SD*)	9.1±0.1	9.9±0.2	9.6±0.1	8.5±0.1	-
Aldehyde content (% w/v)	2.5	3	3.1	3.4	-
Phenolics in %w/w (Mean ± SD*)	5.5±0.2	5.8±0.1	6.0±0.08	6.3±0.1	-
Anthocyanin content %w/w (Mean ± SD*)	5.4±0.1	5.7±0.1	6.0±0.1	6.7±0.08	-

HD*Hydrodistillation, SE*Solvent extraction, US*Ultrasonication, SC-CO₂*Supercritical carbon dioxide extraction

Table S2. SC-CO₂ table for extraction parameters of *E. globulus* L.

S. No.	Pressure (bar)	Temperature (°C)	Flow rate (g min ⁻¹)	Amount (gm)	Time (min)	Yield (% v/w)
1.	350	80	12	80	60	3.6
2.	350	40	8	50	60	2.8
3.	100	40	8	80	60	0.04
4.	350	40	12	50	120	2.5
5.	100	40	12	80	120	0.065
6.	350	80	12	50	120	3.24
7.	350	40	12	80	60	2.91
8.	350	40	8	80	60	1.52
9.	200	80	10	70	90	1.81
10.	350	80	12	80	120	3.32
11.	100	80	8	50	60	0.064
12.	350	80	8	50	60	1.56
13.	200	60	10	70	90	1.32
14.	350	40	12	50	60	2.52
15.	100	40	8	80	120	0.31
16.	200	60	10	70	90	2.93
17.	100	80	12	80	120	0.09
18.	350	80	12	50	60	3.04
19.	100	40	12	80	120	0.14
20.	350	40	12	80	120	2.58
21.	350	80	8	80	120	1.13
22.	100	80	8	50	120	0.03
23.	100	80	12	50	60	0.06
24.	100	40	8	50	120	0.021
25.	100	40	12	50	120	0.05
26.	200	60	10	70	90	1.62
27.	100	40	8	50	60	0.04
28.	100	80	8	80	120	0.034
29.	100	80	8	80	60	0.051
30.	100	80	12	50	120	0.09
31.	350	80	8	80	120	1.2
32.	350	40	8	80	120	1.32
33.	350	80	8	50	120	1.04
34.	100	40	12	50	60	0.031
35.	100	80	12	80	60	0.065
36.	350	40	8	50	120	1.21

Table S3. Solvent system tried for separation of phytoconstituents of essential oil

S.no	Solvent system	Ratio (v/v/v)	Observation
1.	Hexane : dichloromethane	95 : 5	No separation
2.	Toulene : diisopropyl ether : ethyl acetate	80 : 10 : 10	No separation
3.	Toulene : diisopropyl ether	95 : 5	No separation
4.	Petroleum ether : ethylacetate : formic acid	95 : 5 : 1	No separation
5.	Cyclohexane : ethylacetate	90 : 10	No separation
6.	Chloroform : methanol : formic acid	90 : 2 : 1	Little separation
7.	Chloroform : methanol : formic acid	90: 3:1	Little separation
8.	Chloroform : methanol : formic acid	90: 1:1	Little separation
9.	Toulene : methanol : formic acid	90 : 7.5 : 1	Little separation
10.	Toulene : methanol : formic acid	90:6:1	Little separation
11.	Toulene : chloroform : formic acid	90 : 2 : 1	Little separation
12.	Toulene : acetone	95 : 5	No separation
13.	Toulene : ethyl acetate	97 : 5	Fair
14.	Toulene : ethyl acetate	97 : 3	Fair
15.	Toulene : ethyl acetate: formic acid	95 :5:1	Fair
16.	Hexane : ethyl acetate : formic acid	90:1:1	Good separation
17.	Hexane : ethyl acetate : formic acid	70: 30 : 1	Good separation
18.	Hexane : ethyl acetate : formic acid	70: 20 : 1	Best separation

Table S4. Results of FTIR analysis of *E. globulus* L. oil extracted by different techniques

S.no	Peak	Functional group	Intensity	HD*	SE*	US*	SC-CO ₂ *
1	858.32	Aromatic p-disubstituted benzene	Strong	-	+	+	-
2	1174.65	C-F(fluoroalkane)	Strong, broad	-	+	+	+
3	1301.95	C-O(ether)	Strong,stretch	-	+	+	+
4	1375.95	N-O(nitro,aliphatic)	weaker	+	+	-	+
5	1452.4	C=C(aromatic)	Stretch,medium - strong	+	-	-	+
6	1629.85	C-C(conjugated diene)	Strong	-	+	+	-
7	2229.71	-C=-C(alkyne)	Stretch,strong	-	+	+	-
8	2926.43	methylene	Medium - strong	-	+	+	-
9	3342.64	O-H(alcohol)	Strong, broad	-	+	+	-

HD*Hydrodistillation, SE*Solvent extraction, US*Ultrasonication, SC-CO₂* Supercritical carbon dioxide extraction