Reporting effective extraction methodology and chemical characterization of bioactive components of under explored *Platycladus orientalis* (L.) Franco from semi-arid climate

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Abstract

The bioactive ingredients of many essential oils are complex and difficult to reproduce synthetically. In present study, the influence of extraction method on relative extraction of bioactive compounds of essential oil of *Platycladus orientalis* (L.) Franco (*Thuja orientalis* L. is a synonym of it) leaves gathered from semi-arid climate was studied. A higher yield of essential oil was obtained by hydrodistillation (0.1%) as compared to steam distillation (0.07%) under newly optimized conditions. Initial analysis of these oils by TLC showed the presence of different polarity groups ranging from non-polar terpene hydrocarbons to polar terpenoid alcohols. GC-MS analysis revealed that major component of hydrodistilled essential oil of *Platycladus orientalis* (L.) Franco leaves was α-pinene (17.83%) which is a bioactive monoterpene whereas steam distilled essential oil contained α-cedrol (12.44%) as a major component. The results obtained in the present study emphasize that suitable extraction technique should be used to obtain component of interest. Also, both extraction techniques should be used to study complete chemical composition of essential oil from a particular plant.

Keywords: *Platycladus orientalis* (L.) Franco, essential oil, steam distillation, hydrodistillation, α-Pinene, α-Cedrol, diterpenoid, triterpenoid

Experimental

Chemicals

All chemicals and reagents used in this study were of analytical grade and obtained from Sigma Aldrich, England.

Collection of plant material

The city Lahore is located between 74° 20′ 37″ East and 31° 32' 59″ North, latitude and longitude respectively covering an area of 1,772 km^2. It has a hot semi-arid climate with a little annual rainfall (607 mm) and an average temperature of 24.1 °C (Siddiqui et al. 2016). *Platycladus orientalis* (L.) Franco is found to be widely grown in this city as an ornamental plant

along roadsides and in gardens. The fresh and healthy branches of *Platycladus orientalis* (L.) Franco were collected from different regions of this city of Pakistan during the month of April. The plant was identified by Dr. Mansoor Hameed, Associate professor, Department of Botany, University of Agriculture, Faisalabad and was deposited to herbarium placed at Nano and Biomaterials Lab, Department of Chemistry, University of Agriculture, Faisalabad, Pakistan under the voucher specimen number 18-R-001.

Isolation of the essential oils

Fresh leaves of the plants were separated and shade dried. Two different processes were adopted for essential oil extraction from leaves. In first process, 9kg of plant material was distilled using hydrodistillation for a total time period of 8h (the distillation time was calculated from first drop of distillate in the separating funnel). The essential oil was separated from water using a separatory funnel. In 2^{nd} process called as steam distillation, water steam was passed through 9kg of plant material for a period of 8h and distillate was collected in a separatory funnel. Later, essential oil and water layers were separated easily. The oils so obtained were separated from water (due to immiscibility), dried over anhydrous sodium sulfate and filtered using syringe filters (0.45 μm, NY, Simple pure). These oils were stored in sealed glass tubes at 4 °C prior to analysis. The % yield (v/w) of each method was calculated on the basis of dried weight of the plant leaves and reported as a mean of three replications.

Measurement of the refractive index and density

Essential oils were subjected to tests of refractive index using an Abbe refractometer (Tokyo, Japan), while density was determined using density bottle (Ospina et al. 2016).

TLC (Thin layer chromatography) analysis

Essential oils (hydrodistilled and steam distilled samples) and standards including α-Cedrol, α-Pinene, Δ-3-Carene, β-Caryophyllene, α-Humulene, Limonene and (-)-Terpinen-4-ol were dissolved separately in ethanol (99%) to prepare 10% solutions. About 1.5μl of each solution was applied on silica gel 60 F_{254} aluminium sheet based TLC plates (Merck, Germany). The solvent was allowed to evaporate and plates were developed in a TLC tank using *n*-hexane/ethyl acetate (9:1, v/v) solvent system (Guleria et al. 2008). The separated spots were initially detected with UV light (254 nm) and then by spraying with a vanillin/sulfuric acid spray (VS) reagent (100 °C for 5 minutes).

Gas chromatography/mass spectrometry (GC/MS)

Gas chromatography/mass spectrometry analysis was carried out using a Shimadzu GC/MS-QP 2010 system with Rtx-5 column (30 m × 0.25 mm, 0.25 μm film thickness). The initial column oven temperature was raised from 60 to 120 ^{o}C which was increased to 220 at a rate of 4 ^{o}C/min. and finally held at this temperature for 5min. The carrier gas (helium) was used with a flow rate of 0.94 mL/min and 1 µL of each essential oil (diluted with hexane) was injected in splitless mode. The temperature of injector was set at 250 ^{o}C and transfer line temperature was set at 240 ^{o}C. The ionization potential was 70 eV and mass range was between *m/z* 40 to 600. Library search was carried out by using in-house Wiley 7 and NIST27 libraries of the instrument. For quantitative analysis of diverse constituents, the method of peak area was followed and the percentages were calculated relatively (Adams 1989).

Table S1. Chemical composition of leaves essential oil of *Thuja orientalis*

Sr. #	Ret. Time	Common Name	% age composition		Identification method
			*HD essential oil	**SD essential oil	
1	1.45	Hexane	0.31	-	MS, RI
2	1.96	Pentane, 2,2,3,4-tetramethyl-	1.10	-	MS, RI
3	2.06	Bineopentyl	-	0.52	MS, RI
4	3.98	1R-α-Pinene	6.89	0.68	MS, RI
5	4.50	α-Pinene	17.72	-	MS, RI
6	4.58	(+)-Camphene	1.94	-	MS, RI
7	5.02	α-Phellandrene	1.54	-	MS, RI
8	5.13	β-Pinene	3.21	-	MS, RI
9	5.23	(-)-β-Pinene	-	1.09	MS, RI
10	5.29	β-Myrcene	4.51	1.42	MS, RI
11	5.35	β-Phellandrene	-	0.52	MS, RI

12	5.48	Cyclofenchene	-	0.93	MS, RI
13	5.73	Δ-3-Carene	12.52	7.08	MS, RI
14	6.23	Limonene	7.35	6.33	MS, RI
15	7.05	γ-Terpinene	-	0.63	MS, RI
16	7.65	Terpinolene	4.81	3.71	MS, RI
17	8.13	13-Tetradece-11-yn-1-ol	-	0.21	MS, RI
18	9.38	Verbenol	-	0.92	MS, RI
19	9.97	α-Phellandren-8-ol	-	0.89	MS, RI
20	10.28	Terpinen-4-ol	-	0.55	MS, RI
21	10.77	p-Cymen-8-ol	-	1.86	MS, RI
22	11.15	cis-Verbenone	-	0.30	MS, RI
23	13.09	Acetic acid, bornyl ester/bornyl acetate	0.48	2.14	MS, RI
24	14.89	α-Terpineol acetate/α-Terpinyl acetate	1.96	4.30	MS, RI
25	16.05	β-Elemene	0.35	2.21	MS, RI
26	16.82	β-Caryophyllene	8.79	5.09	MS, RI
27	17.11	Thujopsene	0.22	-	MS, RI
28	17.37	Cedrene	1.10	7.19	MS, RI
29	18.14	α-Caryophyllene/Humulene	8.76	10.71	MS, RI
30	18.45	Germacrene D	1.37	-	MS, RI
31	18.56	γ-Muurolene	0.26	5.11	MS, RI

32	18.59	α-Selinene	0.23	-	MS, RI
33	18.86	α-Muurolene	0.45	-	MS, RI
34	18.90	β-Selinene/β-Eudesmene	-	1.43	MS, RI
35	19.09	α-Guaiene	-	3.06	MS, RI
36	19.65	δ-Cadinene	1.14	5.12	MS, RI
37	21.46	β-Caryophyllene oxide	0.81	7.23	MS, RI
38	22.23	α-Cedrol	10.73	12.44	MS, RI
39	22.58	τ-Muurolol	0.25	-	MS, RI
40	22.73	Cubenol	-	2.63	MS, RI
41	23.16	α-Cadinol	0.22	1.26	MS, RI
42	23.35	Spiro[4.5]dec-8-en-7-ol, 4,8-dimethyl-1-(1-methylethyl)-	-	0.17	MS, RI
43	23.55	Iso-aromadendrene epoxide	0.84	0.52	MS, RI
44	23.85	Ledene oxide-(II)	-	0.46	MS, RI
45	26.72	3,5,6,12-Tetrahydroxyergostan-25-yl acetate #	-	0.40	MS, RI
46	31.58	Abieta-8,11,13-triene	-	0.31	MS, RI
47	35.34	14-Isopropyl-13-methoxypodocarpa-6,8,11,13-tetraene	-	0.63	MS, RI

*HD stands for hydrodistillation

**SD stands for steam distillation

**MS stands for Mass spectra comparison and RI for retention indices