SUPERCRITICAL FLUID EXTRACTION OF *Minthostachys mollis* (Kunth) Griseb

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Minthostachys mollis (Kunth) Griseb is of great ethnobotanical, pharmacological and commercial interest because of the essential oils found in the leaves. It does not only find use, as a condiment or tea, in the traditional cuisine of the Andes, but is one of the most important plants in the folk medicine of the area. On the basis of this observation, it receives growing attention from modern pharmacology and medicine, as plant decoctions and extracted essential oils are tested for pharmacological effects. In Argentina and Peru, essential oil is extracted on a commercial scale.
The dependence of *Minthostachys mollis* (Kunth) Griseb essential oil composition, obtained by supercritical carbon dioxide (SC-CO2), with the following parameters: pressure, temperature, extraction time (dynamic), and modifier (ethanol) was studied. The results were also compared with those obtained by extraction solvent method in laboratory conditions. Regarding the percentages of menthone (19,21%) and pulegone (52.0%), the optimum SC-CO2 results were obtained at the following experimental conditions: pressure = 115 bar, T = 35 ◦C, static time = 10 min, and Vmodifier = 30ml. The results of extraction solvent that the major components of *M. pulegium* L. were pulegone (10,92%), menthone (19,21%). The evaluation of the composition of each extract was performed by gas chromatography–mass spectrometry.

INTRODUCTION

The Labiatae family has several members with a significant content of essential oils, some of them being utilized in perfumery, or as spices in foods. *Minthostachys mollis* (Kunth) Griseb that grow naturally now restricted to Andean South America[1] is one of the most important plants in the folk medicine of the area. In Argentina and Peru, essential oil is extracted on a commercial scale. *Minthostachys* from southern Peru for seasoning, tea, medicine against colds and stomach ache, and especially for the preservation of stored potatoes lists uses of *Minthostachys* like condiment for soups, stew, and even modern Pizza, medicinal infusions, and commercial production of aromatic oil. She also presents two culinary receipts containing *Minthostachys* (for “chupe verde” and “lagua de maíz”). Also one of traditional uses of *Minthostachys* in the vicinity of Cuzco and Puno: the protection of stored potato and oca tubers from pests, the application of water boiled while containing *Minthostachys* leaves or even of fresh plant parts against aphids on crop plants, and use in the house against flea infestations.[1-4]

Previous studies on the isolation of *M. mollis*(Kunth) Griseb essential oils with hydro/or steam distillations, resulted in the identification of the main constituents of the oils.[1-2]

Supercritical fluid extraction (SC-CO2) is receiving great attention in the agrochemical fields, can be considered one of the most potentially useful new methods of sample

preparation and will eventually compete successfully with conventional methods, e.g. hydrodistillation, steam distillation, and solvent extraction, in the isolation of volatile compounds from natural matrices.[6]
The solvent strength of the supercritical fluids directly related to its density and thus can be controlled by changing the extraction pressure (or to a lesser extent, the temperature). The low viscosities and high solute diffusible characteristic of supercritical fluids facilitate mass transfer during extraction. [5,6]
When volatile, reactive, and heat-sensitive terpene compounds are isolated, low critical temperature and non-polar character of the fluids are preferable. The most commonly used supercritical fluid is carbon dioxide, possesses these features and in addition provides a chemically inert, low cost, non-flammable and non-toxic environment for the extraction.[5,6]
The objective of this work was to compare the SC-CO2 with solvent extraction process. Also the influence of parameters such as temperature, pressure, modifier and yield the SC-CO2 of *Minthostachys mollis* (Kunth) Griseb essential oil was studied. This study was undertaken to address the following goals: (i) Finding suitable extraction parameters to isolate volatile oil and extraction and (ii) determination of composition of the essential oil.

MATERIAL AND METHODS

Plant Material

The plant material was collected in the town of Tarma, Junin-Peru. The drying temperature was 35 ° C, was then sent by air dried, and protected from direct light. It was sent by air to the Federal University of Santa Catarina, Florianopolis-Brazil.

Sohxlet extraction

The plant (were charged with a particle size about 0,2 um) was submits a extraction by sohxlet method for 2h using methanol as solvent. The volatile extract was collected and refrigerated till time of analysis.

Supercritical Fuids apparatus

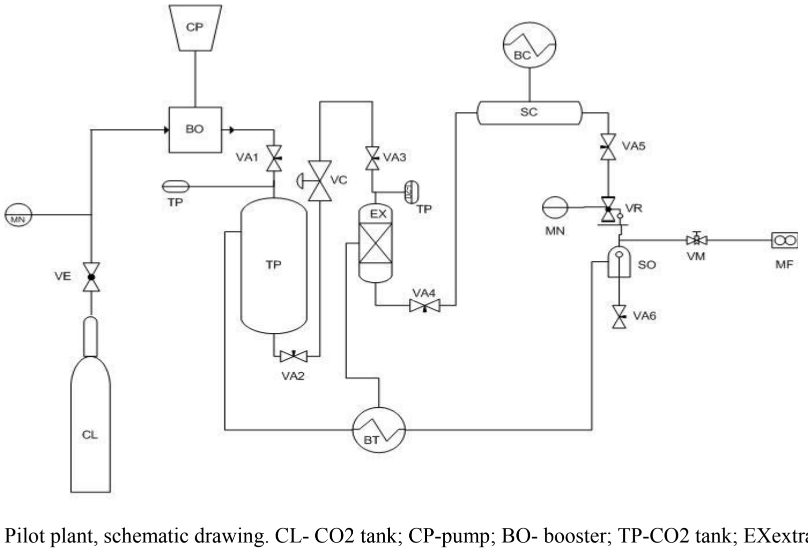

Figure 1 – Pilot plant, schematic drawing. CL- CO2 tank; CP-pump; BO- booster; TP-CO2 tank; EXextractor; SO- oil separator; SC- wax separator; VC- control valve; VR- back pressure valve; VAneedle valves; VE- ball valve; VM- regulation valve; MF- flow meter; BT-heat exchanger (hot water); BC- heat exchanger (cold water); TP- flow rate measurement; MN- manometer.

GC and GC/MS analysis

The oil was analyzed by gas chromatography-mass spectrometry (GC/MS)and flame ionization detector. The compounds were identified by comparison of retention time by Kovatz indexes with authentic compounds, as well as comparison of mass spectra with authentic or published data.

Supercritical fluid extraction (SC-CO2) procedure

Nine extractions were made to temperatures 35, 45, and 55 ◦ C, and pressures of 100, 115 and 125 bar according to Table 1. shows. Carbon dioxide was used as supercritical solvent with flow rate of 1,2 L/min and 30 ml of ethanol as modifier. The static time was 60 minutes for all extractions. In each extraction was used 70g of powdered plant material.

Table 1.
Conditions extractions of SC-CO2: each extraction has 60 minutes of static time and 30 ml of ethanol as modifier.

Ext. No	**Pressure (bar)**	**Temperature (◦C)**
1	100	35
2	100	45
3	100	55
4	115	35
5	115	45
6	115	55
7	125	35
8	125	45
9	125	55

Results and discussion

The sohxleth extraction is one of traditionally methods for essential oils in laboratory. In this study, we intend to compare the efficiency of this process with the relationship to the volatile compounds of the extracts of *Minthostachys mollis* obtained by SC-CO2
As show the table 2 than 22 compounds consisting over than 58,16% of the total solvent extraction of essential oil were The major components were pulegone (20.3%), menthone (19,21%), Veratril (13,35%), iso-Menthone (4,21%), Copaene (3,86%), . The yield of the extraction was 0,6%.

Table 2. Composition of solvent extraction essential oil *M. mollis*

No	**Compound**	**%**
1	Menthone	19,21
2	Pulegone	10,92
3	Espatulenol	0,94
4	Cariophylene	0,79
5	iso-Menthone	4,21
6	Copaene	3,86
7	Menthol	1,11
8	Eremophylene	-
9	Cariophylene oxid	1,73
10	Cariophylene(9-epi-E)	-
11	Timol	-
12	Carvacrol	-
13	Durenol	-
14	Linalool	-
15	Isopulegol	-
16	2-Hydroxy-3-isopropyl-6-methyl-2-cyclohexen-1-one	-
17	Germacrene D	-
18	Pulegone oxide	1,48
19	Cadinene	0,56
20	Limonene	-
21	Carvone	-
22	Veratril	13,35
Total		58,16

The results of GC/MS analyses of the nine SC-CO2 extractions are shown in Table 3. These results revealed great differences between each set of SFE conditions. We can conclude according to Table 3 that the best extraction conditions in terms of the number of Menthona and Pulegone was extraction No 4 (T = 35 ◦C, pressure = 115 bar, dynamic time = 60 min, and modifier volume = 30 ml) because we have an attractive rate of Pulegone which is active in major. We can also say that the extraction No 9 (T = 55 ◦C, pressure = 125 bar) with 95% extracted of components showed high values of Menthone, however if the percentage of low Pulegone 26.17%. The best yield was presented in extraction No 7 (T = 55 ◦C, pressure = 125 bar), showing that it has a good proportion of Menthone (34,19%) and Pulegone (41,43 %).

Table 3.
The results of GC/MS analysis and each yield of the nine SC-CO2 extractions of *M.mollis*

No	Compound	Ext. 1	Ext. 2	Ext. 3	Ext. 4	Ext. 5	Ext. 6	Ext. 7	Ext. 8	Ext. 9
1	Menthone	21,87	23,08	25,79	24,77	24,85	24,17	34,19	33,95	38,96
2	Pulegone	40,02	45,21	45,16	49,93	46,25	41,78	41,43	38,38	26,17
3	Espatulenol	16,18	12,22	8,71	5,00	8,71	9,39	3,10	3,82	4,23
4	Cariophylene	3,06	3,07	3,60	3,29	3,31	2,93	3,10	2,96	2,17
5	iso-Menthone	4,01	4,25	4,81	4,57	4,48	4,15	4,75	6,33	6,19
6	Copaene	0,80	0,76	0,65	0,58	0,82	0,87	4,5	-	-
7	Menthol	1,24	1,35	1,47	1,48	1,60	1,38	1,95	1,98	2,38
8	Eremophylene	0,84	1,95	1,81	0,96	-	1,92		-	-
9	Cariophylene oxid	0,58	0,54	0,53	0,78	0,87	1,47	2,40	-	-
10	Cariophylene(9-epi-E)	0,56	0,89	0,58	0,93	0,93	-	-	-	-
11	Timol	-	-	-	-	-	-	-	-	-
12	Carvacrol	-	-	-	-	-	-	-	-	-
13	Durenol	-	-	-	-	-	-	-	-	-
14	Linalool	-	-	-	-	-	-	-	-	-
15	Isopulegol	-	-	-	-	-	-	-	-	-
16	2-Hydroxy-3-isopropyl- 6-methyl-2-cyclohexen-1-one	-	-	-	-	-	-	-	-	-
17	Germacrene D	-	-	-	-	-	-	-	-	-
18	Pulegone oxide	-	-	-	-	-	-	-	5,39	-
19	Cadinene	-	-	-	-	1,66	-	-	-	-
20	Limonene	-	-	-	-	-	-	-	-	-
21	Carvone	-	-	-	-	-	-	-	-	-
22	Veratril	-	-	-	-	-	-	-	-	-
	Total	89,16	93,32	93,11	92,29	93,48	88,06	95,42	92,81	80,1
	Yield	43,36	43,99	43,77	46,5	45,33	47,27	47,98	45,93	45,5

In a comparison between the solvent extraction method, and the nine extractions of SC-CO2, there was less percentage of constituents in the supercritical extractions. Probably the reason is the low affinity of methanol as the solvent polarity of the Pulegone.

Conclusions

The flexibility in the management of the variables involved in the SCF process allows one to optimize the experimental conditions considering the selectivity of a substance or classes of substances of interest. The selectivity of SC-CO2 allowed one to maximize the concentration of the selected compounds, with this process being more

advantageous than the solvent extraction, demonstrated for the low yield 0,6% of extraction and lows percentages of compounds menthone and pulegone.
The best percentage of pulegone and menthone was given by the condition of T = 35 ◦C, pressure = 115 bar.

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