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Superficial Fluid Extraction, Identification and Characterisation of High Value Bioactive Compounds from Medicinal and Aromatic Herbs Alongwith Study of their Antimicrobial Activity

Panshu Pratik* and Prem Mohan Mishra
Department of Chemistry, M.L.S.M. College, L. N. Mithila University, Darbhanga, Bihar, India
*Corresponding author: pratikpanshu@gmail.com

Abstract: Supercritical carbon dioxide extraction is used to obtain extracts from the herbs: 1. Tulsi (Ocimum tenuiflorum), 2. Mint (Mentha), 3. Motha (Cyperus rotundus) and 4. Changeri (Oxalis corniculata). The influence of pressure and modifiers on the oil yield and content was studied. The extraction pressure ranged from 18.5 to 26.5 MPa at 48 °C. Ethanol was used as a modifier. Components in the extracts were analysed by gas-liquid chromatography and identified using an existing retention index database. Both extraction pressure and fluid modifier alter the extraction yield and its composition. The antimicrobial activity test by the Agar Well Diffusion method of the Tulsi and Mint extract.

Keywords: Anti-microbial activity, Gas chromatography, Modifier, Supercritical carbon dioxide, Superficial fluid extraction.

I. Introduction

Extraction of natural products with supercritical carbon dioxide ($SCCO_2$) has gained increasing attention from the food and perfumery industries to obtain flavour and fragrance ingredients as well as the pharmaceutical industry, namely because the dissolving power of supercritical fluid can be adjusted by regulating its pressure and temperature [1-4]. Also, by using $SCCO_2$ instead of steam distillation or extraction with organic solvents (hexane, chloroform) eliminates the problem of toxic residual solvent in the products, and permits the use lower temperatures leading to lesser deterioration of the thermally labile components in the extract. Likewise, extraction with $SCCO_2$ retains the organoleptic characteristics of the starting spice mate-rials [5]. In addition, the modifier - a small amount of polar organic solvent added to the supercritical CO_2, will increase overall extractability of target analytes [6].

The leaves of plants such as Tulsi, Mint, Motha and Changeri have been added to meat, fish and food products for seasoning. It is known that in addition to improving flavour, certain spices and essential oils prolong the storage life of foods. The antimicrobial activities of various spices [7, 8] and of essential oils obtained by hydrodistillation methods and agar well diffusion method [9] have been reported.

In the present study, $SCCO_2$ was used to extract the volatile aroma components from four herbs: Tulsi, Mint, Motha and Changeri. Since different targeted compounds have different diffusion rates inside the plant matrix [10], and the solubility of every substance in the supercritical fluid depends on extraction pressure, temperature and modifier, data about yield provide a diagnostic tool for further optimisation of the extraction process. We also report here the antimicrobial properties of supercritical extracts.

II. Experimental

A. Plant Material and Chemicals

The herbs: 1. Tulsi (Ocimum tenuiflorum), 2. Mint (Mentha), 3. Motha (Cyperus rotundus) and 4. Changeri (Oxalis corniculata) were grown in Bihar (India). The raw material was dried at room temperature (approximately 25 °C) and protected from direct light.

The carbon dioxide used in the experiments was 98.5% (w/w) pure. The ethanol as modifier used was spectroscopic grade and added according to weight of the sample (from 3.5 to 8%).

B. Extraction of Herbs

Supercritical fluid extractions from dried leaves and flowers of plants were performed on Sample Preparation Accessory apparatus (Soxlet Extractor, M.L.S.M. College, Darbhanga). Extractions were carried out at 48 °C, at pres-sures from 18.5 to 26.5 MPa. The flow rate of super-critical CO_2 was 1 ml min^{-1} and extraction time for all experiments was 60 min. The extract was collected in n - hexane. Extraction time (1 h) was chosen on the basis of previous studies [11]. There was estimated, that at this pressure range used the yield was ~75% of total yield able to achieve during 5-h extraction.

For comparison, a conventional steam distillation using a Clevenger apparatus for 4 h was utilized for the isolation of the essential oils. After steam distillation, the essential oil was isolated and kept refrigerated.

C. Gas Chromatography (GC)

Chemical composition of extracts was determina-tion by gas-liquid chromatography method using a fused silica capillary column with a bonded stationary phase NB 25 (GCM015, Darbhanga), and flame ioni-sation detector. The identification was performed by comparing their Kovats relative Retention Indexes (RI) with those of authentic compounds and from database collected from literature.

D. Assay for Antimicrobial Activity

The microorganisms: aggregatibacter actinomycetemcomitans, prevotella intermedia and porphyromonas gingivalis were used in this study. Laboratory strains, kept on the solid agar at 5 °C, were used. Antimicrobial activity was tested by the agar well diffusion method [12]. A 6.5-mm sterile paper disc was impregnated with a test material and set on agar using a micropipette. Plates were then inverted and incubated for bacteria at 40 °C for 58 h, for moulds at 15 °C for 86 h. Following incubation, the zones of inhibition were measured (in mm s).

III. Results and Discussion

A. Effect of Pressure on the Extract Yield

Preferable extraction conditions were 18.5 MPa for Tulsi and Changeri, and 26.5 MPa for Mint and Motha (Table I). The total maximum yield of extract for 2-h extraction time for Tulsi was 23.2 mg/g, for Changeri 8.2 mg/g, for Mint 6.4 mg/g, and for Motha 6.1 mg/g. A decrease in yields of the extracts for Tulsi and Changari at increased pressures is hard to explain. It was found in all experiments made at the same conditions but in different time. The similar results, where system behaviour cannot be solely attributed to the effects of either temperature or pressure, have been reported in the previous study on $SCCO_2$ extraction of herbs [13], where it was found to be specific for Tulsi only. Later from the literature, the same kind of unusual behaviour was found in case of extraction of stevia leaves [14]. Possibly, here could be some structure changes or textural transformations in the plant matrix at the higher extraction pressure, which would hinder the diffusion, and practically it means that longer extraction times are needed. Another explanation could be in some cumulative effect of extract components solubility changes, but this needs further study on solubility data in supercritical CO_2 of the main components in Changeri extract, and estimation of phase equilibrium data is necessary for the system.

The important conclusion is that one must be very careful with extrapolating data from other experiments, and must check the yield dependence from extraction time at higher pressures for certain plants.

B. Effect of Ethanol as Modifier

Although carbon dioxide is a relatively good solvent for the extractions, it has some limitations for the extraction of polar substances from plant matrix. Ethanol was used as modifier in this study, because it is environmentally benign and relatively safe to human health. The amount of ethanol added initially com-posed 2.6, 5 and 7.5% of the total sample weight.

Table I: Total Yield (mg/g) and Relative Peak Area (%) from GC Analysis of Main Constituents of Plant Extracts at Different Pressures (18.5 and 26.5 MPa, at 48 °C, Extraction Time 60 min, Each Experiment Repeated Three Times)

Tulsi		*Mint*		*Motha*		*Changer*	
18.5 MPa	26.5 MPa	18.5 MPa	26.5 MPa	18.5 MPa	26.5 MPa	18.5 MPa	26.5 MPa
Total yield (mg/g)							
13.0±1.5	7.5±0.8	4.8±1.4	4.5±0.5	33±0.3	4.2±0.3	5.4±0.2	4.5±0.2
Main Constituents (area %)							
Camphene		Menthol		α-cyperone		Malic acid	
65	53	52.5	42.4	7.0	11.8	64.4	41.2
β-Elemene		Methyl acetate		Cyperene		Tartanic acid	
9.1	0.5	22.0	15.2	8.2	10.3	12.1	0.2
1,8, copane – 8 – 01		Menthone		Sugeonol		Citric acid	
9.5	12.5	5.5	4.5	3.3	5.5	2.5	0.5
α- Copane		Menthofuran		Kobusons		Itexin	

Tulsi		*Mint*		*Motha*		*Changer*	
27.3	18.9	5.5	10.4	0.5	12.5	7.4	5.5
Combrenene							
15.1	18.6						
Other minor constituents (area %)							
32.5	44.2	14.5	27.5	81.0	59.9	13.6	52.6

C. Comparative Analysis of Plant Components at Different Extraction Pressures

The chemical composition of the $SCCO_2$ extracts from these plants was determined by GC. The total yield of extracts and relative content of main com-pounds are presented in Table I. It confirms that every plant has its own unique extract composition.

GC analyses revealed that the main constituent of the extract from Tulsi at 18.5 MPa is a-copane, but its relative content decreases at 26.5 MPa, as well as its b-Elemene content. a-cyperone and cyperene are the main components in the Motha extract. Changeri extract is characterized by the relatively high content of Maleic acid and Tataric acid. Menthol is the main constituent in the mint extract. Despite the higher total yield of Mint at 26.5 MPa compared to pressure 18.5 MPa, the percentage of the major components (except 1.8-copaene-8-01), is less. It means that at higher pressures the additional components are extracted and content of extract is changing substantially. Some of them are difficult to analyse by GC methods normally used for essential oils, and additional chromatographic methods might be instructive.

In conclusion, the compositions of these extracts depends on the extraction conditions, hence $SCCO_2$ extracts cannot be identified with the essential oils from the plants. Some researchers have recommended temperatures between 40 °C and 50 °C and extraction pressures lower than 100 bar (9.9 MPa) for obtaining extracts that are similar to essential oils from plant materials [15]. Our results confirm that for each aro-matic plant, specific extraction conditions are needed to obtain, especially for an extract similar to that essential oil by conventional methods, and higher extraction pressures and addition of modifier increase the yield of the extract with changed proportion of components.

D. Antimicrobial Activity of Supercritical Extract

There is not very much data about the antimicrobial activity of supercritical extracts from herbs, and those that exist concern extracts obtained using traditional methods, such as hydrodistillation [10] and also with Agar well diffusion method. In this study, the antimicrobial activity was tested with extracts from Tulsi and Mint; the studies with extracts from other plants are ongoing. Here Tulsi showed the highest inhibitory effect against aggregatibacter actinomycetemcomitans, prevotella intermedia and porphyromonas gingivalis. However, the anti-microbial activity of Mint is against some Gram-positive and Gram - Negative bactaria. It was also evaluated by agar well diffusion method. It was found that the distilled concentration of essential oil inhibit the growth of micro organism and the results were comparasable with those of antibiotic Gentamycin. Inspite of good antibacarial it is found to be good antioxidant too.

E. Comparision

For Tulsi it was possible to compare the activity of SFE extract and that from the essential oil obtained using steam distillation/extraction method. One explanation for this different behaviour is the difference in composition of extracts, as shown for Tulsi in Table II.

There is more camphene; b - Element trans-caryophyllene murrola diene – 8 - b - ol in the Tulsi $SCCO_2$ extract, than in the essential oil. It may be supposed that these compounds have higher antimicrobial activity against the bacteria, or that there is some synergy between the extract com-ponents resulting in the observed antimicrobial activity.

Table II: Comparison Table of Chemical Composition of Tulsi Essential Oil and SFE Extract (%)

Component	*Essential Oil*	*SFE Extract (18.5 Mpa, 4.8 °C)*
Ecigenol	5.9	5.2
Camphene	4.0	6.2
β-elemene	5.0	9.6
1,8,copaene-8-01	10.5	9.6
Transcarryo-phyllene	20.6	27.5
β - selinin	5.0	4.0
(R) – combrenene	20.6	15.2
α-copaene	5.4	4.5
Sabiene	3.5	1.9
Other	8.0	7.6
Total	99.6	96.4

IV. Conclusions

Extraction conditions: CO_2 pressure and amount of modifier should be optimised to obtain extracts with attractive properties.

Extraction pressure is a very sig-nificant parameter; however, an increase of the pres-sure and addition of a modifier does not always has a positive effect on the yield of extracts (over the same extraction time). Specific studies on the extract of Tulsi showed a difference between the $SCCO_2$ extract and the native essential oils concerning antimicrobial activity. Further research is needed in order to obtain more reliable results on determination of antimicrobial ac-tivity of supercritical extracts.

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