LIQUID YIELD AND CHEMICAL CHARACTERIZATION OF *MAYTENUS ILICIFOLIA* MART. EX REISS EXTRACTS OBTAINED FROM CO_2 AT HIGH PRESSURES

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This work is focused on the chemical characterization of *Maytenus ilicifolia* extracts obtained from high-pressure CO_2 extraction. The experiments were performed in a laboratory-scale unit, where the effect of particle size (35-200mesh), CO_2 mass flow rate (1 to 3gmin^{-1}), temperature (293 to 323K), pressure (100 to 250bar), and extraction time was investigated in terms of liquid yield and chemical composition of the extracts. Results show that the particle size and CO_2 mass flow rate did not affect the liquid yield, whereas the extraction temperature and solvent density exerted a pronounced effect on both liquid yield and chemical distribution of volatile compounds. The extracts chemical analysis carried out in a a GC/MSD permitted to identify phytol, squalene, vitamin E, stigmasterol, friedelan-3-ol, friedelin, dodecanoic acid and geranyl acetate as major components in the extracts.

INTRODUCTION

The use of plants with pharmaceutical properties has received increased interest nowadays from both homeopathic and allopathic branches. Besides, these medicine plants play an important role in public health, especially in developing countries. Celastraceae family comprises 55 different genders with 850 species spread throughout the tropics and sub-tropics. The *Maytenus* gender is one of the greatest in this family, where 77 species have been found in Brazilian flora [1].

In the Brazilian context, the species *Maytenus aquifolia* and *Maytenus ilicifolia* are widely used in the popular medicine, being known as “espinheira santa”, “cancerosa” and also “cancarosa”, among others. Both species are native growing plants, with natural occurrence in South Brazil where they are used in the form of teas for stomach and ulcer illness treatment. For instance, Souza-Formigori et al.[2] have demonstrated the antiulcerogenic property of *Maytenus aquifolium* and *Maytenus ilicifolia*, which seems to be closely, related to the presence of two classes of substances, namely, phenols and triterpenes [3].

The active principles 4-O-methylepigalocatequina, friedelin and friedelan-3-ol in these species have been shown to exhibit antiulcerogenic gastric activity [4]. Indeed, the antiulcerogenic activity of aqueous extracts of *Maytenus ilicifolia* and *Maytenus aquifolium* has also been confirmed in laboratory tests with rats. Extract fractions rich in friedelin and friedelan-3-ol presented a superior activity when compared to cymetidin, the main commercially used synthetic anti-acid drug [2].

A relevant aspect that should be taken into account when dealing with extracts or essential oils in phytotherapy is related to the extraction technique employed. Of course, it is important that the method utilized does not modify the original properties and provides satisfactory yields with the lowest solvent residue. Carbon dioxide has been used as solvent in

most cases for supercritical extraction purposes in food and pharmaceutical industries since it is nontoxic, nonflammable, non-explosive, readily available, and has a low critical temperature that avoids degradation of thermo sensitive compounds. The advantages of using near critical carbon dioxide extraction prevails when small raw material amounts and high quality products are processed [5,6]. Catchpole [7] mentions that the use of compressed carbon dioxide may be an advantageous alternative for obtaining natural remedies with therapeutic interests.

Despite the great interest in the *Maytenus ilicifolia* therapeutic properties and the fact that it is an abundant and native growing plant in Brazil, to our knowledge, only one study concerning the extraction at high pressures and characterization of its extracts has been reported in the literature [8]. In that report however the investigation was focused only on the characterization of the triterpene fraction and no evaluation of the effects of process extraction parameters was provided.

In this context, this work is aimed at investigating the influence of particle size (35 to 200mesh), CO_2 mass flow rate (1 to 3g min^{-1}), temperature (293 to 323K), pressure (100 to 250bar), and extraction time on the liquid yield and characteristics of the extracts obtained from high-pressure carbon dioxide extraction of *Maytenus ilicifolia*. Chemical analyses were conducted in a GC/MSD (Shimadzu, Model QP 5050A). The liquid yield (extract/raw material, wt/wt) and extract chemical composition are reported in this work. The extracts obtained were then grouped into three classes comprising the most interesting compounds, with special attention devoted to friedelin and friedelan-3-ol.

EXPERIMENTAL

Material. Samples of *Maytenus ilicifolia* leaves were collected and dried at room temperature. Afterwards, they were crushed manually and stored under nitrogen atmosphere. The samples were classified into three different particle sizes 35, 115 and 200 mesh. Carbon dioxide (99.9% purity) was purchased from White & Martins. The analytical standards geranyl acetate, phytol, dodecanoic acid, squalene, vitamin E, stigmasterol, friedelan-3-ol, friedelin and biphenyl were from Aldrich, Palo Alto, CA, USA. For each standard, solutions were prepared (1000 mg L^{-1}) using dichloromethane (MERCK) and stored under refrigeration.

Apparatus and Experimental Procedure. The experiments were performed in a laboratory scale unit, as presented in detail by Rodrigues et al. [9], which consists basically of a CO_2 reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 0.1dm^3 jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of $\pm$ 0.12 bar, a collector vessel with a glass tube and a cold trap. Amounts around 25 g of dried *Maytenus ilicifolia* leaves were charged into the extraction vessel. The CO_2 was pumped into the bed, which was supported by two 300 mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least one hour to allow the system stabilization. Afterwards, the essential oil was collected opening the micrometering valve and the CO_2 mass flow was accounted for the pump recordings. The experiments were accomplished isothermally, at constant pressure. The experimental range investigated was 293 to 323 K in temperature and from 100 to 250 bar in pressure. Triplicate extraction runs were accomplished for all conditions.

Extract Characterization. The extracts were analyzed with a gas-chromatograph interfaced with a mass selective detector – GC/MSD (Shimadzu, Model QP 5050A), using a

capillary column DB-5 (30 m x 0.25 mm x 0.25 μm); flow rate of 1 mL min^{-1}; in electronic impact mode of 70 eV; split mode (split ratio 1:20), at 310ºC interface temperature, with the following column temperature gradient programming: 65°C (3min); 3°Cmin^{-1} up to 260°C; 2.5°Cmin^{-1} up to 310°C (10 min). Standard samples of 40,000 ppm were prepared using dichlorometane (Merk, analytical grade) and injected 1 μL. The identification of geranyl acetate, phytol, dodecanoic acid, squalene, vitamin E, stigmasterol, friedelan-3-ol and friedelin were accomplished through the analytical standards and biphenyl as internal standard by comparing the mass spectra and GC retention.

RESULTS AND DISCUSSION

Extraction of *Maytenus ilicifolia* samples. Table 1 presents the liquid yield along with the experimental conditions investigated in this work. Here, the yield is defined as the weight percentage of the oil extracted with respect to the initial charge of the raw material in the extractor. From runs 1 to 6, one can see that the particle size and CO_2 flow rate, in the ranges investigated, had no significant influence on the extraction yields. For other experiments, the solvent flow rate was then fixed at 2 gmin^{-1} and no particle size classification was attempted. The general analysis of the results reveals that a rise in temperature at the lowest extraction pressure (100 bar) leads to a sharp decrease of the extraction yield while a slight increase is verified at the highest pressure (250 bar). It is also worth noticing that an enhancement in pressure at constant temperature results in an increase of extraction yield, revealing the remarkable effects of these process variables on the extraction yield.

Table 1: Extraction yield and characteristic parameters.

Run	Particle size (Mesh)	CO_2 flow rate (g min^{-1})	T (K)	P (bar)	Extraction yield (%) and standard deviation[†]
1	200	2	308	175	1.04 ± 0.031
2	115	2	308	175	0.98 ± 0.028
3	35	2	308	175	1.07 ± 0.032
4	nc[*]	1	308	175	1.04 ± 0.029
5	nc	2	308	175	1.02 ± 0.042
6	nc	3	308	175	1.08 ± 0.034
7	nc	2	293	100	0.70 ± 0.019
8	nc	2	323	250	1.11 ± 0.062
9	nc	2	323	100	0.16 ± 0.008
10	nc	2	293	250	0.83 ± 0.034

[*] samples not classified according to particle size
[†] n=3

Chromatographic Analyses of *Maytenus ilicifolia* Extracts. The extracts were analyzed with a gas-chromatograph interfaced with a mass selective detector where the peak area of each compound selected was determined in relation to the area of the internal standard (biphenyl). In order to better analyze the effect of the investigated variables, the compounds were grouped into three classes: in the first one, compounds of lower molecular weight (geranyl acetate, phytol and dodecanoic acid); compounds of higher molecular weight (squalene, vitamin E and stigmast-5-en-3-ol) were classified as group II and, in the third group, the triperpenes (friedelan-3-ol and friedelin) that present phytoterapy property. To investigate the effect of the extraction time, in all runs the extracts were collected separately in five samples.

Tables 2 and 3 present the effect of particle size and CO_2 mass flow rate, respectively, on the chemical characteristics of the extracts. The compounds concentrations in these tables refer to the ratio between their peak areas by the area of the internal standard. All experiments were performed at least in triplicate and the values reported are in fact the mean of these replicates. Standard deviations for each condition are also presented in these tables. It can be observed from Table 2 that the particle size from 115 to 200 mesh, as analyzed by Tukey test at 5%, had no significative effect on the concentration of the extracts. From Table 3, one can note that the CO_2 flow rate also had a weak effect on the concentration of all groups in the beginning of the extraction. On the other hand, it seems that a raise in CO_2 flow rate caused a decrease in the concentration of group I, indicating that the extraction solvent was not saturated in these compounds at the extractor end or, in other words, the equilibrium concentration had not been reached for the compounds present in group I.

Table 2: Effect of particle size on the extract chemical characteristics of grouped compounds of *Maytenus ilicifolia* obtained from CO_2 extraction at high pressures.

Group	Mesh	Mass of CO_2 (g)				
		60	120	180	240	480
I	35[a]	4.034±0.322[a]	2.182±0.208[b]	1.456±0.143[c]	0.868±0.155[d]	0.531±0.092[d]
	115[b]	2.355±0.153[a]	1.523±0.153[b]	0.885±0.0326[c]	1.117±0.106b[c]	0.686±0.016[c]
	200[b]	3.650±0.338[a]	0.708±0.060[b]	0.640±0.166[b]	0.675±0.103[b]	0.691±0.101[b]
II	35[a]	53.812±3.252[a]	37.955±4.475[b]	26.486±2.860[c]	12.939±2.304[d]	5.747±1.287[d]
	115[a]	46.481±9.928[a]	37.364±3.962[a]	22.866±0.290[b]	25.366±2.947[b]	7.221±0.613[c]
	200[a]	72.802±2.481[a]	21.750±1.166[b]	17.307±5.215[b]	19.427±2.706[b]	5.354±0.926[c]
III	35[a]	4.348±0.371[b]	4.431±0.550[b]	6.597±0.191[a]	7.467±0.978[a]	5.855±1.445[b]
	115[b]	4.512±1.074[a]	3.715±0.275[a]	4.441±0.080[a]	4.862±0.788[a]	4.652±0.216[a]
	200[b]	3.367±0.98[b]	3.940±0.160[ab]	3.943±1.042[ab]	6.005±0.668[a]	5.399±0.836[ab]

Mean ratio peak areas (n at least 3) (Tukey < 0.05).
[a, b, c , d] Values with the same superscript description are statistically similar.

Table 3: Effect of carbon dioxide flow rate on the extract chemical characteristics of grouped compounds of *Maytenus ilicifolia* obtained from CO_2 extraction at high pressures.

Group	CO_2 ($gmin^{-1}$)	Mass of CO_2 (g)				
		60	120	180	240	480
I	1[a]	3.313±0.119[a]	1.428±0.042[cd]	1.364±0.104[d]	1.801±0.981[c]	2.696±0.142[b]
	2[b]	2.509±0.257[a]	1.616±0.212[b]	1.294±0.213[b]	1.338±0.133[b]	2.256±0.119[a]
	3[c]	2.354±0.080[a]	1.208±0.082[b]	1.174±0.116[b]	1.027±0.116[b]	1.353±0.152[b]
II	1[b]	63.452±3.955[a]	29.964±2.054[b]	14.969±1.292[c]	17.767±0.799[c]	17.074±1.159[c]

	2^{a}	51.619 ± 5.106^{a}	39.057 ± 6.866^{b}	31.433 ± 6.746^{bc}	25.549 ± 2.921^{c}	28.470 ± 2.489^{bc}
	3^{a}	57.146 ± 0.982^{a}	37.188 ± 3.183^{b}	34.947 ± 3.898^{bc}	26.081 ± 2.812^{c}	26.308 ± 2.973^{bc}
III	1^{b}	3.914 ± 0.512^{b}	3.962 ± 0.386^{b}	3.709 ± 0.350^{b}	6.593 ± 0.356^{a}	8.487 ± 0.978^{a}
	2^{a}	4.893 ± 0.501^{c}	4.177 ± 0.732^{c}	7.133 ± 1.693^{b}	6.524 ± 0.189^{bc}	11.379 ± 1.522^{a}
	3^{b}	4.030 ± 0.225^{b}	4.428 ± 0.351^{b}	5.200 ± 0.637^{b}	6.109 ± 1.095^{b}	10.463 ± 0.566^{a}

One can also observe from these tables that the concentration of groups I and II are significatively reduced as the extraction takes place. This fact was not observed for the triterpenes group, indicating that the lighter compounds were extracted earlier and then the extracts were becoming enriched in the highest molecular weight compounds of group III.

Table 4 presents the influence of temperature in the chemical characterization of *Maytenus* essential oil obtained from carbon dioxide at high pressures. In this table, the CO_2 density was kept constant around 0.84 gcm^{-3}. It can be observed that higher temperatures produce extracts with the highest contents of all compounds in the extracts. This fact can be explained in terms of the vapor pressure increase of the components as the temperature is raised. The extraction time also produces a significant effect on the concentration of the extracts, since all compounds showed a decrease in concentration as the extraction occurs.

Table 4: Influence of extraction temperature on the distribution of chemical compounds present in the *Maytenus ilicifolia* extracts. CO_2 density constant at around 0.84 gcm^{-3}.

Compound	T(K)	Mass of CO_2 (g)				
		60	120	180	240	480
geranyl acetate	293^{a}	0.320 ± 0.095^{a}	0.164 ± 0.004^{b}	0.033 ± 0.002^{c}	0.008 ± 0.001^{c}	0.0005 ± 0.0001^{c}
	308^{a}	0.341 ± 0.019^{a}	0.124 ± 0.004^{b}	0.025 ± 0.006^{c}	0.013 ± 0.002^{c}	0.005 ± 0.0008^{c}
	323^{a}	0.482 ± 0.013^{a}	0.053 ± 0.009^{b}	0.026 ± 0.007^{b}	0.015 ± 0.004^{b}	0.006 ± 0.0007^{b}
phytol	293^{c}	5.781 ± 0.196^{a}	4.251 ± 0.204^{a}	1.155 ± 0.149^{b}	0.370 ± 0.040^{b}	0.021 ± 0.001^{b}
	308^{b}	6.729 ± 0.591^{a}	4.170 ± 0.482^{b}	1.722 ± 0.301^{c}	1.373 ± 0.144^{c}	0.688 ± 0.037^{c}
	323^{a}	17.104 ± 1.576^{a}	5.725 ± 0.712^{b}	4.625 ± 0.705^{bc}	3.661 ± 0.449^{cd}	2.905 ± 0.155^{d}
dodecanoic acid	293^{a}	1.124 ± 0.997^{a}	0.784 ± 0.074^{b}	0.183 ± 0.034^{c}	0.050 ± 0.013^{c}	0.003 ± 0.0001^{c}
	308^{a}	1.230 ± 0.296^{a}	0.587 ± 0.160^{b}	0.137 ± 0.021^{c}	0.075 ± 0.019^{c}	0.029 ± 0.005^{c}
	323^{a}	1.715 ± 0.238^{a}	0.220 ± 0.027^{b}	0.128 ± 0.025^{b}	0.069 ± 0.013^{b}	0.030 ± 0.007^{b}
squalene	293^{a}	95.273 ± 14.156^{a}	81.936 ± 6.303^{a}	21.980 ± 3.059^{b}	7.179 ± 0.933^{bc}	0.343 ± 0.018^{c}
	308^{a}	115.900 ± 9.623^{a}	66.585 ± 10.033^{b}	15.444 ± 3.292^{c}	8.310 ± 0.982^{c}	2.695 ± 0.141^{c}
	323^{a}	165.973 ± 14.687^{a}	25.017 ± 2.758^{b}	10.335 ± 1.622^{bc}	5.313 ± 0.716^{bc}	2.135 ± 0.147^{c}
vitamin E	293^{b}	25.725 ± 1.215^{ab}	29.919 ± 5.314^{a}	19.050 ± 2.708^{b}	8.204 ± 0.899^{c}	0.343 ± 0.019^{c}
	308^{a}	36.148 ± 5.181^{a}	34.366 ± 7.474^{a}	17.201 ± 3.792^{b}	9.211 ± 1.055^{bc}	2.682 ± 0.282^{c}
	323^{ab}	45.323 ± 5.614^{a}	23.005 ± 1.886^{b}	9.690 ± 1.367^{c}	4.983 ± 0.587^{c}	2.314 ± 0.264^{c}
stigmasterol	293^{c}	17.271 ± 0.244^{a}	16.898 ± 0.879^{a}	9.802 ± 1.386^{b}	6.368 ± 0.891^{b}	0.367 ± 0.031^{c}
	308^{b}	19.122 ± 2.503^{a}	17.831 ± 3.406^{ab}	13.121 ± 2.739^{bc}	10.378 ± 1.165^{c}	3.733 ± 0.391^{d}
	323^{a}	29.276 ± 1.977^{a}	20.741 ± 1.852^{b}	15.058 ± 2.533^{c}	8.437 ± 1.311^{d}	3.331 ± 0.345^{d}
friedelan-3-ol	293^{c}	6.457 ± 1.440^{a}	5.937 ± 0.430^{a}	3.748 ± 0.487^{ab}	1.785 ± 0.061^{bc}	0.0940 ± 0.006^{c}
	308^{b}	8.290 ± 0.899^{a}	7.037 ± 1.193^{a}	6.167 ± 1.348^{ab}	3.634 ± 0.306^{bc}	1.891 ± 0.280^{c}
	323^{a}	10.799 ± 1.330^{a}	8.902 ± 1.118^{ab}	8.707 ± 1.322^{ab}	8.167 ± 1.243^{ab}	7.526 ± 0.941^{b}
friedelin	293^{c}	4.912 ± 0.953^{a}	4.576 ± 0.341^{a}	2.366 ± 0.303^{ab}	1.186 ± 0.079^{b}	0.074 ± 0.006^{b}
	308^{b}	7.936 ± 1.875^{a}	5.577 ± 1.019^{ab}	4.218 ± 1.119^{bc}	3.490 ± 0.227^{bc}	1.750 ± 0.209^{c}

	323[a]	10.148±0.637[a]	7.656±0.945[ab]	6.797±1.231[b]	6.225±0.813[b]	5.478±0.622[b]

Table 5 presents the effect of solvent density on the concentration of the extracts at constant temperature of 323K. From this table it can be noted that the density presented a remarkable influence on the concentration of the extracts when it was increased from 0.41 to 0.84 gcm^{-3}. This fact is attributed to the increase of the solvent power with density. Also, as the extraction takes course, the concentration of the extracts in these compounds were decreased, indicating again that a possible fractionation could be possible in relation to the extraction time.

Table 5: Influence of solvent density on the distribution of chemical compounds present in the extracts of Maytenus ilicifolia obtained from CO_2 extraction at high pressures. Extraction temperature constant at 323K.

Compound	CO_2 density	Mass of CO_2 (g)				
	(gcm^{-3})	60	120	180	240	480
geranyl acetate	0.408[b]	0.156±0.004[a]	0.079±0.001[b]	0.057±0.001[c]	0.070±0.005[bc]	0.033±0.002[d]
	0.835[a]	0.482±0.013[a]	0.053±0.009[b]	0.025±0.007[c]	0.015±0.004[cd]	0.006±0.001[d]
phytol	0.408[b]	2.022±0.220[a]	2.451±0.104[a]	2.550±0.218[a]	2.583±0.212[a]	2.732±0.158[a]
	0.835[a]	17.104±1.576[a]	5.725±0.712[b]	4.625±0.705[bc]	3.661±0.449[c]	2.905±0.155[c]
dodecanoic acid	0.408[b]	0.353±0.037[a]	0.278±0.021[a]	0.232±0.025[a]	0.245±0.046[a]	0.144±0.012[a]
	0.835[a]	1.715±0.238[a]	0.220±0.027[b]	0.128±0.025[b]	0.068±0.013[b]	0.030±0.007[b]
squalene	0.408[b]	11.403±0.365[a]	5.121±0.159[a]	7.666±0.230[a]	10.323±0.647[a]	9.865±0.661[a]
	0.835[a]	165.973±14.687[a]	25.017±2.758[b]	10.335±1.622[c]	5.313±0.717[c]	2.135±0.147[c]
vitamin E	0.408[b]	2.333±0.284[a]	0.469±0.064[a]	1.436±0.050[a]	1.778±0.324[a]	1.425±0.132[a]
	0.835[a]	45.323±5.614[a]	23.005±1.886[b]	9.690±1.367[c]	4.983±0.587[c]	2.314±0.264[c]
stigmasterol	0.408[b]	2.723±0.146[a]	1.669±0.156[a]	1.870±0.185[a]	1.859±0.241[a]	1.393±0.066[a]
	0.835[a]	29.176±1.977[a]	20.741±1.852[b]	15.058±2.533[c]	8.437±1.311[d]	3.331±0.345[e]
friedelan-3-ol	0.408[b]	1.663±0.261[a]	0.919±0.090[a]	1.250±0.104[a]	1.058±0.186[a]	0.525±0.032[a]
	0.835[a]	10.799±1.330[a]	8.902±1.177[ab]	8.707±1.321[ab]	8.167±1.242[b]	7.526±0.941[b]
friedelin	0.408[b]	1.013±0.0.32[a]	0.603±0.051[a]	0.880±0.070[a]	0.762±0.208[a]	0.431±0.033[a]
	0.835[a]	10.1482±0.637[a]	7.655±0.945[b]	6.797±1.231[bc]	6.224±0.813[bc]	5.478±0.622[c]

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