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Enhanced extraction of flavonoids from *Odontonema strictum* leaves with antioxidant activity using supercritical carbon dioxide fluid combined with ethanol

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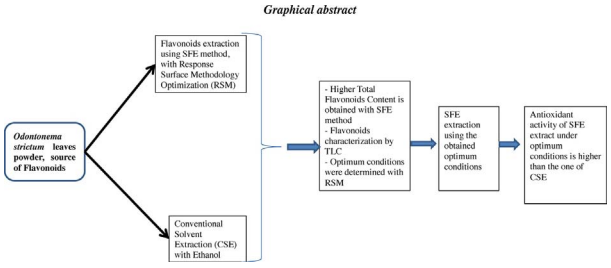
GRAPHICAL ABSTRACT



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ABSTRACT

Flavonoids were extracted from *Odontonema strictum* leaves by supercritical carbon dioxide, with ethanol. The effect of three independents variables (time, pressure and temperature) on the Total Flavonoid Content (TFC) were optimized by factorial investigation. The TFC and flavonoid recovery varies respectively from 99.33 to 247.78 mg/g of dried extract and 10.68–18.92 mg/g of dried leaves powder, while the conventional solvent extraction (CSE) yielded 36.45 mg/g and 3.13 mg/g. Analysis of the factorial identified the optimal conditions with an extraction time of 270 min and a pressure of 200 bar, the temperature had no effect within the tested ranges. The predicted TFC was 203.11 mg/g under optimal conditions and experimentally 230.48 mg/g. The antioxidant activity with 2,2-Diphenyl-1-picrylhydrazyl method of the extracts under optimum conditions was 49.21% while the CSE extract was 37.05%. The TLC analysis of the supercritical fluid extracts from the optimum conditions showed 5 majors flavonoids.

1. Introduction

Natural products represent an unparalleled reservoir of molecular diversity to drug discovery and development. *Odontonema strictum* (Acanthaceae) is one of common plants used in Burkina Faso by the traditional physicians for the treatment of hypertension, which is the most frequent cardiovascular disease in Africa. The prevalence of hypertension is particularly high in sub-Saharan Africa. In Ghana, the hypertension prevalence rate is 27% in rural areas and 29% in urban areas [1]. In Burkina Faso, the prevalence of hypertension in urban area was 23% [2].

Conventional drugs against hypertension are too expensive for the

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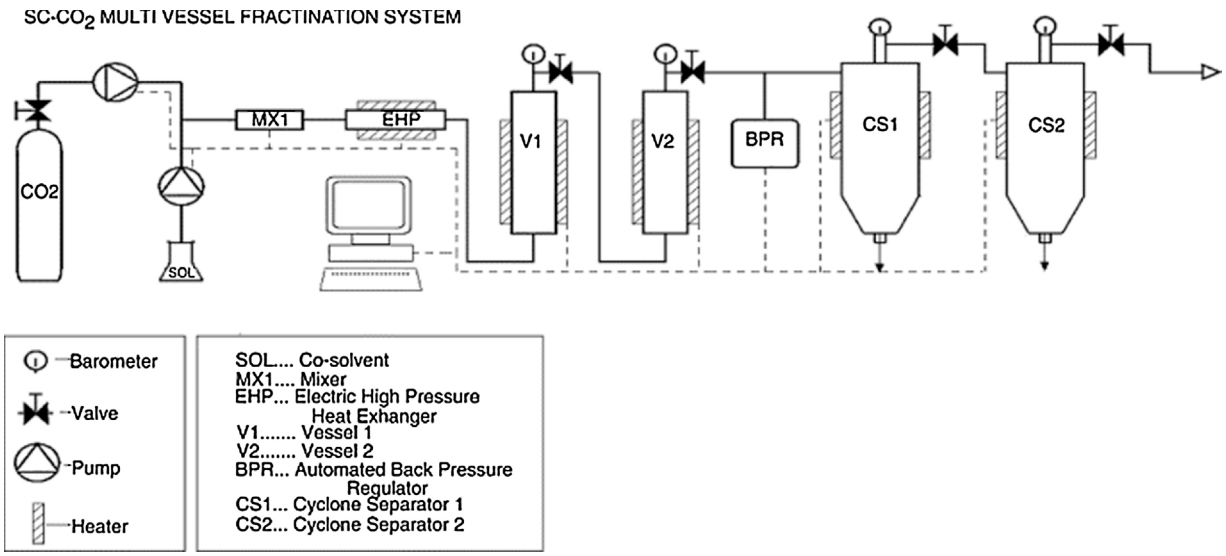

Fig. 1. Schematic diagram of the SC-CO_2 system.

population of developing countries, where around 80% people use traditional medicine for their medical care [3,4]. Cost effective valorization of active plants resulting in affordable herbal medicine represents a promising alternative, for sustainable health programs.

Extraction methods used so far for plants in many countries are conventional solvent extraction (CSE), which is environmental unfriendly because a large amounts of organic solvents are used; these solvents are often expensive and potentially harmful [5]. Therefore, environmental friendly solvents are recommended [6,7].

A bio-guided study found that the active molecules in *Odontonema strictum* leaves were predominantly found in the ethyl acetate fraction – known to be rich in flavonoids [8,9]. In the context of sustainable development, the cost and reliability of the ethyl acetate extraction method needs to be addressed. An alternative is supercritical carbon dioxide (SC-CO_2) extraction. It is the most attractive and environmentally friendly method of choice, able to extract bioactive compounds from plants [10–13]. It constitutes an emerging technology in terms of environmental impact. SC-CO_2 is beneficial for the quality as well as for the quantity of the extracted products and does not give rise to excessive heating, which has a negative effect on the thermolabile compounds [14]. This method was successfully used for flavonoids extraction with ethanol as a co-solvent [15–17]. Among polyphenols, flavonoids constitute an important group of great importance because they help the human body to fight against diseases. They are well-known to have anti-hypertensive properties [18]. The ability of flavonoids to act as potent antioxidants are proved and it depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure [19,20]. They are abundantly found in *Odontonema strictum* leaves as glycoside [8]. In addition to flavonoids, *Odontonema strictum* leaves contain alkaloids, carbohydrates, glycosides, saponins, phytosterols and tannins [21].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of confidently predict the outcome of new experimental conditions [22,23]. This methodology has been successfully used to optimize flavonoids extraction with supercritical fluid extraction method [24,25]. As for us, no report mentioned flavonoids extraction from *Odontonema strictum* leaves by SC-CO_2 method using RSM.

The aim of this work was to optimize the effect of supercritical carbon dioxide extraction parameters, namely extraction time, temperature and pressure, to obtain flavonoids enriched extracts from *Odontonema strictum* leaves using RSM. In addition, the antioxidant capacity of the flavonoids extracts under optimum conditions were assessed in comparison with the conventional solvent extraction (CSE) extract.

2. Materials and methods

2.1. Plant material

The plant material used for this investigation was *Odontonema strictum* leaves. It was collected in the garden of the department of Traditional Medicine, Institute for Health Sciences Research at Ouagadougou (Burkina Faso). The leaves were shade dried under ventilation, powdered and stored in an air-tight container till further use.

2.2. Chemicals

The chemicals used in this study were analytical reagent grade including, Quercetin from Merck, Aluminium chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma – Aldrich, Formic acid, Ethanol 95%, Methanol and Ethyl acetate, ethanolamine diphenyl borate, polyethylene glycol. The carbon dioxide (99.998%) used for extraction was from AGA Gas (Sundbyberg, Sweden).

2.3. Conventional solvent extraction (CSE)

For CSE, 50 g of *Odontonema strictum* leaves powder with an average particle size of 0.6 mm were introduced into a 1 L Erlenmeyer flask and 500 mL of ethanol (70% v/v) was added. The mixture was stirred with a magnetic stirrer at room temperature for 24 h. The solution was then filtered and centrifuged (Centrifuge OSI, Italy) for 10 min at 2000*g* to obtain the hydro-ethanol extract which was further analyzed.

2.4. Supercritical carbon dioxide extraction (SC-CO_2)

The extraction with supercritical carbon dioxide was performed in a system, SFE 2 × 100F, from Thar Technology Inc., Pittsburgh, USA. A schematic drawing of experimental apparatus previously described is shown in Fig. 1 [26,27]. It consists of two extracting and two separation vessels with nominal volumes of 100 and 200 mL each, respectively.

For each experiment, 10 g of ground material with an average particle size of 0.6 mm were loaded in an extractor vessel (100 mL of