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Evaluation of the effects of different extraction methods for main volatile compounds from *Bambusa textilis* leaves

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Abstract—*Bambusa textilis* is widely used in popular medicine to treat all kinds of wound inflammation, chronic fever, pulmonary and infectious diseases. The aim of this study was to compare the chemical composition of the extracts of *B. textilis* leaves obtained by three different extraction methods: solid/liquid extraction, Soxhlet and Clevenger system using gas chromatography with flame ionization detector (GC-FID) and gas chromatography with mass spectrometry (GC-MS) analyses. The analytical characteristics of the extracts showed some differences and the GC-MS analysis indicated the presence of higher concentrations of nitro compounds and alkalis.

Key words: *Bambusa textilis*; leaves; Soxhlet; Clevenger; GC-MS.

INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [1]. Furthermore, an increasing dependency on the use of medicinal plants in industrialized societies has been observed through the extraction and development of several drugs and chemotherapeutics from these plants, as well as from traditionally used rural herbal remedies [2]. Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments, and also on account of the increasing costs of personal health maintenance. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity [2].

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The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, the modern pharmacopoeia contains at least 25% drugs derived from plants. Many others are synthetic analogues built on prototype compounds isolated from plants. The demand for medicinal plant is increasing in both developing and developed countries, owing to the growing recognition of natural products, being non-toxic, having no side-effects, easily available at affordable prices [1].

The industrial uses of medicinal plants are countless. These range from traditional medicines, herbal teas, and health foods such as nutriceuticals to galenicals, phytopharmaceuticals and industrially produced pharmaceuticals. In addition, medicinal plants constitute a source of valuable foreign exchange for most developing countries. In several industrialized societies, plant-derived prescription drugs constitute an element in the maintenance of health. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds [3]. There has been resurgence in the consumption and demand for medicinal plants. These plants have found use as pharmaceuticals, neutraceuticals, cosmetics and food supplements.

The pharmaceutical studies of natural products are among the most interesting and active research areas. Clinical tests have indicated that certain herbal plants do contain pharmacologically active ingredients that are effective in treating some difficult diseases. Since pharmacologically active compounds in herbal plants usually are in low concentrations, a great deal of research has been done to develop more effective and selective extraction methods for recovery of these compounds from the raw materials [4, 5].

A broad spectrum of extraction procedures is currently being used. Pharmacopoeia monographs (for example, Ref. [6]), which serve as official standards for the quality control of many medicinal plants, employ a broad range of methods such as Soxhlet extraction, percolation, maceration, digestion, extraction under reflux and steam distillation [4, 5, 7].

The bamboo species called *Bambusa textilis* is native to Asia and widespread in all southern China. This is a medium-sized sympodial bamboo whose straight culms reach up to 15 m in height and 3–5 cm in diameter [8]. Several parts of the culm or stem, such as the peel, the nodes and the internodes, present astringent characteristics and therapeutic applications against epilepsy and fever [3].

The extract of some bamboo species obtained by means of distinct extraction methods, i.e., distillation methods, using solvents such as methanol, alcohol and chloroform, has shown the presence of germanium, silicon and other organic matter, when concentrated and analyzed by column chromatography, demonstrating the presence of three major composites, which have still not been identified [3].

With the fast development of modern chromatographic and spectroscopic techniques, the chemistry of natural products has made great progress during the past decades [8]. With a better understanding of natural products, an increasing number of people have become interested in studying active natural products as medicines [4], food additives [9] or natural pesticides [10].

Despite the fact that many Brazilian herbal preparations consist of crude plant extracts and are not further purified, prior to use, the isolation and structure elucidation of the leading structures is essential in order to provide a better understanding of the alleged bioactivity. However, isolation is still a key step, because most of the vegetable extracts are comprise complex mixtures. The separation of the compounds present in a crude extract is often performed by repeated processes based on adsorption column chromatography. In this work, we investigated the main compounds in the leaves of the *B. textilis*, obtained by different extraction methods such as the Soxhlet, solid/liquid extraction and Clevenger system. The extracts were analyzed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography with mass spectrometry (GC-MS) and the results were compared.

MATERIALS AND METHODS

Sample preparation

The plant material was collected in Bauru, São Paulo, and dried for 5 days at 26°C. The storage and transportation of plant material was performed in brown paper bags to ensure adequate aeration while keeping the material in the dark. The dry sample was cut into very small pieces with scissors, ground into a fine powder and homogenized.

Extraction methods

Solid/liquid extraction. The dried plant material was ground to a powder and 7.0 g extracted with 200 ml of methanol using a sonication bath (30°C for 8 h). The crude extracts were filtered (Whatman #1 paper filter) and completely dried so as to remove all traces of solvent before the analysis. The extract was solubilized in an appropriate volume for the analysis.

Clevenger system. Dry plant samples (7.0 g) were also submitted to steam distillation in a Clevenger-type apparatus with 250 ml of water for 8 h, to obtain the relevant extract. The extract was concentrated under nitrogen and further diluted for a suitable analysis.

Soxhlet system. For the Soxhlet extraction, 7.0 g of the sample was weighed and inserted into the thimble. The solvent was selected based on the method mentioned

in the Chinese Pharmacopoeia [6]. After extraction with 300 ml of ethanol/water (50 : 50, v/v) for 8 h, the excess solvent was evaporated with the rotary evaporator under reduced pressure at 40 °C, and the final extracts were dried under a nitrogen gas flow and diluted as appropriate for the analysis.

Analysis

GC-FID. A Hewlett–Packard model 5890 series II gas chromatograph equipped with flame ionization detector and a fused silica capillary column LM-100 cross-linked polyethylene glycol (CW-20 M; 15 m × 0.25 μm I.D.; film thickness 0.25 μm) was used. The operating conditions were as follows: initial temperature 38 °C (1 min), increased at 8 °C/min to 240 °C, kept for 5 min, injector temperature 250 °C; H_2 carrier gas; column linear velocity (μ = 45 cm/s) operated in the split mode (1 : 50); injection volume 1 μl and finally detector temperature at 300 °C with a make-up gas N_2 at a flow rate of 30 ml/min.

GC-MS. The analysis of the extract of the leaves of *B. textilis* was performed using a Hewlett Packard 5890 II gas chromatograph, equipped with a HP-5 mass spectrometry capillary column (30 m × 0.25 mm i.d., 0.25 μm) and a HP 5972 mass selective detector. For the GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and detector MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was initially kept at a 40 °C, then gradually increased to 300 °C at 8 °C/min rate and held for 2 min. Diluted samples of 1.0 μl were injected manually and in the split mode (1 : 50). The components were identified by comparing their mass spectra with NIST library data of the GC-MS system.

RESULTS AND DISCUSSION

Natural products are a major resource in the pharmaceutical industry. Historically, drug discoveries from natural products have been a time- and resource-intensive process.

The first step in the qualitative and quantitative analysis of medicinal plant constituents is the extraction that represents the separation of compounds to be analyzed from the cellular matrix. Ideally, an extraction procedure should be exhaustive regarding the constituents to be analyzed, being rapid, simple, inexpensive and — for routine analysis — amenable to automation. The primary development of extraction method required and screening of crude extracts of plants followed by fractionation, isolation and structure elucidation of novel bioactive compounds can take many months.

The first aim of this study was to estimate the extraction yield from bamboo leaves using three different methods such as Clevenger, solid/liquid and Soxhlet. Figure 1

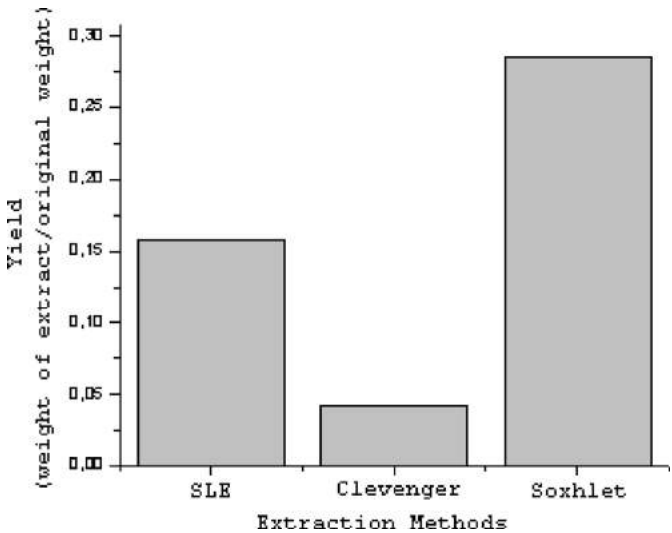

Figure 1. Comparison of extraction yield between the studied methods.

presents the obtained results for the yield expressed as the weight of extract (g) divided by the weight of the starting materials (g), multiplied by 100 (w/w). The yield obtained after an 8-hour Soxhlet extraction (28.57%), was 6.7-times higher than that produced by steam distillation (Clevenger system; yield 4.28%) performed for 8 h, too. For the solid/liquid extraction, the obtained yield was 3.7 times higher than that produced by the Clevenger system. These results indicated that the extraction efficiencies achieved by Soxhlet are higher than those attainable by solid-liquid and Clevenger extraction methods.

Compared to the analytical apparatus, the solid/liquid extraction exhibited several advantages, including (i) the cost-saving factor and (ii) the lower environmental impact, due to the small solvent quantity in relation to the Soxhlet extraction.

Qualitatively, a single investigation using GC-FID in *B. textilis* extracts showed some differences in the extracts composition obtained by the three different extraction methods. Figure 2 shows the chromatograms obtained by the Soxhlet and Clevenger methods. As it can be observed, the major quantity of compounds (diversification) appears when the Soxhlet method is used. This fact can be related to extraction conditions such as: long extraction time, extraction temperature and the solvent used, which produced higher amount of compounds. However, the Clevenger system presented more volatile compounds or smaller molecular weight, due to mild extraction conditions. The higher complexity of the chromatographic patterns produced by the Soxhlet and solid-liquid methods indicates that the Clevenger system affords enhanced extraction selectivity for compounds of low molecular weight.

The analysis by GC/FID and GC/MS of extracts obtained through the respective extractions demonstrates that the analytical results were similar for the solid-liquid and Soxhlet extractions (Fig. 2, top chromatogram), presenting a composite

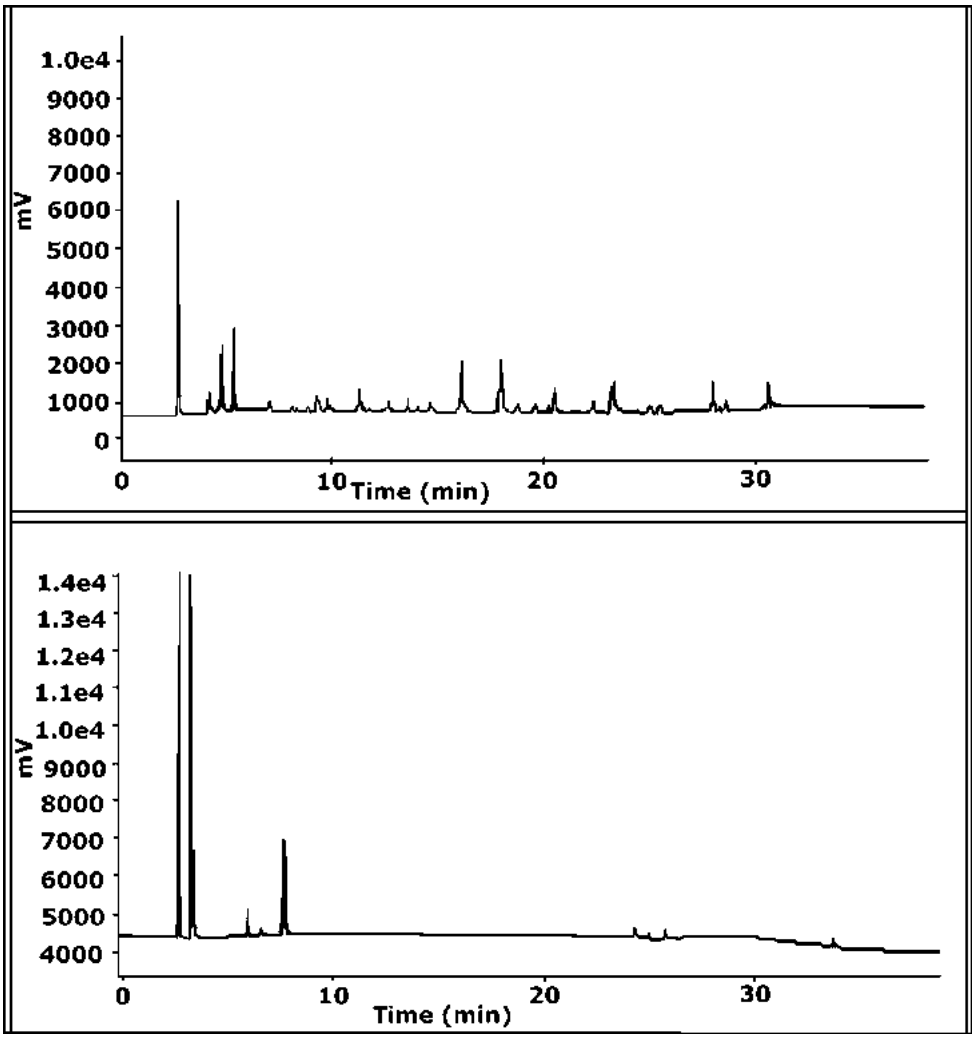

Figure 2. Chromatogram obtained for extract of *Bambusa textilis* extracted for (top) Soxhlet method and (bottom) Clevenger method.

diversity greater than that obtained by the Clevenger method (Fig. 2, bottom chromatogram).

The main components in the extracts of *B. textilis* have been detected by GC-MS (Fig. 3) based on the NIST library. The results found by the GC-MS analysis in the leave extracts of the *B. textilis* presented some hypotheses in the determination of main compounds, such as nitro compounds, ketones and esters. The nitric oxide has been one of the most rapidly growing areas in biology. This simple free radical gas can regulate an ever-growing list of biological processes, even in humans, controlling the endothelium process. In most instances, the nitric oxide mediates their biological effects by activating the guanylyl cyclase and increasing the cyclic

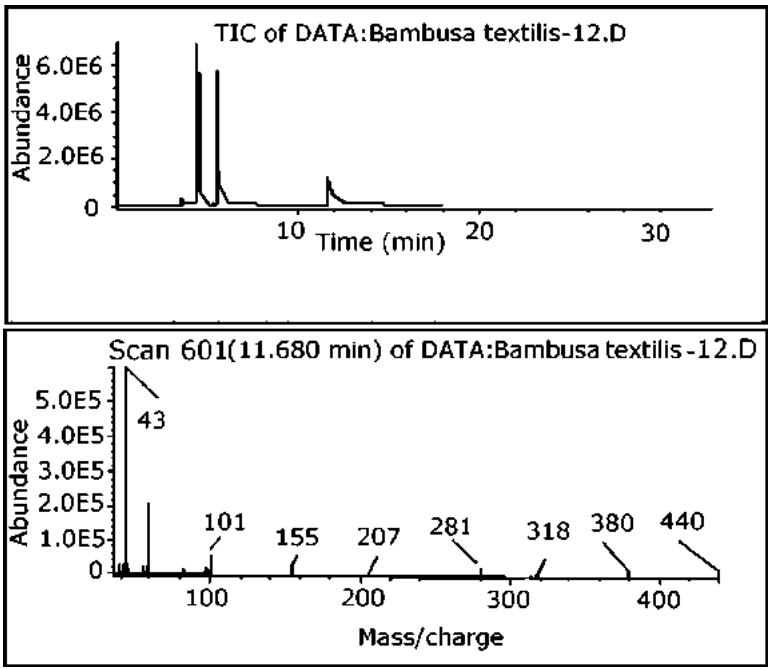

Figure 3. Chromatogram of the total ion and spectrum of masses.

CMP synthesis. However, the identification of effects of the nitric oxide that are independent of the cyclic CMP is also growing at a rapid rate. The effects of the nitric oxide can mediate important physiological regulatory events in cell regulation, cell–cell communication and signalling. Nitric oxide can function as an intracellular messenger, a neurotransmitter and hormone stimulator. Current research on *B. textilis* extracts has basically focused on active nitrocompounds, allowing the development of an expanded therapeutic armamentarium for the physician to manage, effectively, a number of important disorders. These expectations have undoubtedly fuelled the vast research interests in these simple molecules.

CONCLUSIONS

The results presented here are, to our knowledge, the first data published on a comparative evaluation of classical extraction methods of the extract of *B. textilis* leaves.

The evaluation of the extraction yield from bamboo leaves, using three different methods, Clevenger, solid/liquid and Soxhlet, showed that the yield obtained by the Soxhlet extraction was 6.7-times higher than that produced by steam distillation and 1.8-times higher than the solid/liquid extraction.

The different extraction methods tested enable the initial qualitative evaluation of the extract obtained from *B. textilis* leaves. The chromatographic profile obtained

by GC-FID showed that the Soxhlet method presents a greater efficiency in relation to the biggest extracted composite number. The characterization of extracts for GC-MS pointed as identification possibility the following composites: ketones, indacen and probably a class of nitro compounds. Further studies, using spectroscopy infrared ray and nuclear magnetic resonance are necessary, aiming at complementing the chemical characterization of main compounds in the extracts.

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