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ScienceDirect

Resource-Efficient Technologies ■■ (2017) ■■–■■

www.elsevier.com/locate/reffit

Research paper

Optimization studies on extraction of phytocomponents from betel leaves

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Received 12 October 2016; received in revised form 31 January 2017; accepted 27 February 2017

Available online

Abstract

The present study aims at finding out the optimum parameters for the extraction of components from Betel leaves possessing medicinal applications using ethanol solvent by Soxhlet apparatus. The optimum conditions for the extract were calculated based on the extract yield by varying four parameters: material quantity (A: 2–4 g), solvent quantity (B: 250–300 ml), mantle temperature (C: 65–75 °C) and extraction time (D: 1–3 hours) and optimized using a four factor three level Box–Behnken response surface design (BBD) coupled with desirability function methodology. Results showed that temperature and extraction time had significant effect on yield of extract. Optimum conditions for highest yield of extract (10.94%) are as follows: material quantity (2 g), solvent quantity (281.4 ml), temperature (72 °C) and time (3 hours). The extract at the maximum yield condition was analyzed for phytocomponents by FTIR and GC–MS. The results indicated the presence of Hydroxy chavicol (69.46%), 4-Chromanol (24%) and Eugenol (4.86%), which possess wide application including as antioxidant, anti-inflammatory, anti-platelet and antithrombotic, antibacterial and antifungal agents.

Keywords: Betel leaves; Box–Behnken response surface design (BBD); Phytocomponents; Antioxidants

1. Introduction

Piper betel, belonging to the *piperaceae* family, is one of the precious medicinal herbs found in central and eastern Malaysia, Southeast Asia. In India, it is commonly known as Paan, which is second to tea and coffee based on daily consumption. In spite of its alienness, the plant is much more popular in India than in any other country in the world since antiquity. This would be evident from the numerous citations laid down in the ancient literature, particularly the Indian scriptures [1,2]. Betel leaves are very nutritive and contain substantial amount of vitamins and minerals [3]. The leaves also contain the enzymes like diastase and catalase besides a significant amount of several essential amino acids including lysine, histidine and arginine [1,2,4–6]. Most of the previous studies on Betel leaf concentrated more on the components present in betel leaf extract [7–12]. There is a large scope in determining an appropriate extraction technique that extracts the phyto-components from betel leaves. Selection of solvent is an important aspect in any extraction; in general, solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate, have been commonly used for the extraction of phenolics from fresh products [13]. Appropriate extraction technique coupled with the optimization of the parameters involved in extraction and using a right optimization technique proves essential in order to capture medicinal components for further processing in pharmaceutical industries.

Scientific research on betel leaf reveals that it possesses many beneficial bioactivities and its extract has a great potential to be used in developing commercial products [14–19]. Though research has been done extensively on the components of betel leaf, its medicinal properties and potential applications, few research studies focused on the maximization of the yield of extract for various parameters.

The present study mainly concentrates on maximizing the yield of the extract by varying the parameters such as quantity of material fed, quantity of solvent, heating temperature in the mantle and extraction time. Further, the optimization of parameters using right optimization tools would reduce number of experimentation focusing on maximum medicinal components of betel leaf for further processing. The prediction of number of experiments to be performed was governed by using an appro-

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http://dx.doi.org/10.1016/j.reffit.2017.02.007

Please cite this article in press as: Muruganandam L, Anantha Krishna, Jashwanth Reddy, G.S. Nirmala, Optimization studies on extraction of phytocomponents from betel leaves, Resource-Efficient Technologies (2017), doi: 10.1016/j.reffit.2017.02.007

priate response surface model as in Design Expert 9.0.4.1 software. Sirisomboon and Kitchaiya [20] reported that the total amount of oil extracted using Soxhlet from jatrohpa kernels depends mainly on the extraction time and temperature. Ahmad et al. [21] reported the effects of extraction time and solvent type on the extracted oil of Herba leonuri, a medicinal plant. Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated. Selection of right extraction procedure considering the pros and cons of other extraction alternatives is a key step in extraction. Soxhlet extraction has been the most used extraction technique worldwide for a number of decades, surpassing the performance of other extraction alternatives and being used as an efficiency reference for the comparison of its conventional and new counterparts [21]. Being a continuous–discrete technique, it shows some important advantages. Specifically, in Soxhlet extraction, the sample is repeatedly brought into contact with fresh portions of solvent facilitating the displacement of the transfer [22].

Variables such as temperature, extraction time, material and solvent quantity on the extraction yield of extract were optimized using a four factor three-level Box–Behnken response surface design coupled with desired function methodology. Box–Behnken design has proven to be an extremely valuable tool, it not only helps in determining the accurate optimum values of experimental parameters but also provides the possibility to evaluate the interaction between variables with a reduced number of experiments [23]. The optimized controlled conditions determined in this study should offer important reference values for any subsequent studies. The solvent used for extraction was ethanol, a polar molecule due to the presence of OH group, which attracts non-polar substances because the ethyl (C_2H_5) in ethanol is non-polar. Thus, it can dissolve both polar and non-polar substances.

During Soxhlet extraction, the system remains at a relatively higher temperature by effect of the heat applied to the distillation flask reaching the extraction cavity to some extent. In addition, no filtration is required after leaching and sample throughput can be increased by performing several simultaneous extractions in parallel, which is facilitated by the low cost of the basic equipment [24]. The sample corresponding to the maximum yield was analyzed for functional groups and the corresponding compounds and their quantity were analyzed by FTIR and GC–MS respectively. FTIR is certainly one of the most important analytical techniques available for identifying the types of chemical bonds (functional groups) present in compounds. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. GC–MS technique was used in this study to identify the components present in the extract. The structures of the components were identified using a mass spectrophotometer.

The objectives of the study include setting up different experimental parameters for Soxhlet extraction and performing selective experiments as per Design of Experiments (DOE) 9.0.4.1 Box–Behnken methodology; finding out optimum conditions for Betel leaf extract based on the effect of various parameters like solvent quantity, leaf mass fed, mantle heating temperature and contact time; and the influence of these parameters on the mass of extract obtained. The FTIR and GCMS analysis was done for the sample at the optimum condition to the components present in the extract corresponding to maximum yield.

2. Experimental methods

2.1. Materials

The plant materials *Piper betle leaves* used for performing extraction was purchased from local market of Vellore, India. The instruments used for analysis of components in extract are as follows: FTIR (Fourier Transform Infrared radiation) Spectrometer – Shimadzu IR affinity-1, and GC–MS (Gas Chromatography and Mass spectroscopy): GC – Perkin Elmer GC Clarus 680 system and MS – Clarus 600 system (GC–MS).

2.2. Extraction parameters

The parameters include material quantity: 2 g, 3 g, 4 g; solvent quantity: 250 ml, 275 ml, 300 ml; mantle temperature: 65 °C, 70 °C, 75 °C and extraction time: 1 hour, 2 hours and 3 hours. The 4 factors (parameters) with 3 levels gave a total of 29 selective experiments (in randomized order), as per Design Expert software 9.0.4.1, Box–Behnken response surface design. The final mass of extract after vaporization of solvent corresponding to each experiment was calculated and the respective percentage yield of extract was governed. Crude extract after the evaporation of the solvent is shown in Fig. 1.

The extraction yield is a measure of the solvent's efficiency to extract specific components from the original material and it was defined as the amount of extract recovered in mass compared with the initial amount of whole plant. It is presented in percentage (%) and was determined for each technique tested. Further, the extract corresponding to maximum percentage yield was analyzed for components by FTIR and GCMS analysis.

2.3. FTIR analysis

FTIR spectroscopy utilizes ceramic light source with DLATGS detector and an interferometer. The sample was placed between interferometer and detector. Ceramic rods, used to produce infrared light source, produce corresponding interferogram in the detector when they fall on the sample. This interferogram obtained from the spectroscopy was Fourier transformed and the resultant spectrum was analyzed using chemometric Technique.

2.4. GC–MS analysis

GC–MS analysis of the extract was performed using a Perkin Elmer GC Clarus 680 system and gas chromatograph interfaced to a Mass Spectrometer Clarus 600 system (GC–MS) equipped with Elite-1 fused silica capillary column (30 m × 1 μl was Mdf, composed of 100% Dimethyl poly siloxane). For GC–MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 1 μl was employed (Split ratio of 10:1).

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