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Extraction of hydrolysable tannins from *Phyllanthus niruri* Linn.: Effects of solvents and extraction methods

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Abstract

Effects of solvent types and extraction methods (solvent extraction (SE), supercritical fluid extraction (SFE) and pressurized water extraction (PWE)) were investigated for effective recovery of bioactive hydrolysable tannins from *Phyllanthus niruri* Linn. Various organic and aqueous solvents screened by Soxhlet method showed that the gallic acid and ellagic acid contents increased with water content whereas corilagin yield reached a maximum value at 30% (v/v) ethanol in water. At a fixed temperature, solvent extraction by Soxhlet is the best method for gallic and ellagic acid extractions, whereas pressurized methods are better for the corilagin extraction. Even though exhaustive extraction is achieved fastest by PWE, SFE with the addition of ethanol–water cosolvent is superior in terms of low liquid solvent consumption and component fractionation produced. Solvent polarity, solvent-to-solid ratio and contact time play significant roles in determining the most efficient method for tannin extraction.

Keywords: *Phyllanthus niruri*; Solvent extraction; Supercritical fluid extraction; Sub-critical water; Ellagitannins

1. Introduction

Phyllanthus niruri (Euphorbiacea) is an herbal plant indigenous to Malaysia and is locally known as 'dukung anak'. It is commonly found in tropical regions and in other countries, it is known as 'chanca piedra' (Spanish), 'paraparai mi' (Paraguay), 'quebra pedra' (Brazil) or 'punarnava' (India). *P. niruri* is a popular folk medicine for treating kidney and gallbladder stones, liver related diseases such as jaundice and liver cancer, viral infections such as hepatitis and tuberculosis, malaria, diabetes and fever [1].

In scientific studies, *P. niruri* was found to exhibit antispasmodic, hypotensive, analgesic, antihepatotoxic, antihepatitis, antimutagenic, antiviral and antibacterial properties. The aqueous and/or alcohol extracts were found to inhibit activity of hepatitis B virus *in vitro* and *in vivo* [2–4], HIV-1 reverse transcriptase virus [5–7], enzymes processes peculiar to cancer cell's replication and growth [8] and the formation of kidney stones [9], lower the blood glucose levels [10], have the liver-protecting (antihepatotoxic) properties *in vivo* and *in vitro* [11] and produce analgesic effects in mice [12]. Other documented properties are antimalarial [13] and lipid lowering activity [14].

The medicinal effects are attributed to the active components present in *P. niruri* such as lignans, glycosides, flavonoids, alkaloids, ellagitannins, terpenes, and phenylpropanoids [1]. Common lipids, sterols, and flavonols also occur in the plant. Two tannin groups identified in *P. niruri* are hydrolysable tannins (ellagitannins) and condensed tannins (flavonoids). The final hydrolysis of ellagitannins yields ellagic acid and gallic acid [15]. Chemical structures of active hydrolysable tannins, namely geraniin and corilagin, gallic acid and ellagic acid, and condensed tannins such as flavon-3-ol and flavonol in *P. niruri* are as shown in Figs. 1 and 2, respectively [5,16,17].

Most research on *P. niruri* was on the chemical screening, identification and isolation, and the biological assay and pharmacological studies [10,18,19]. However, not much study on the effects of solvents have on the extraction of active components from *P. niruri* has been reported. Notka et al. reported the effects

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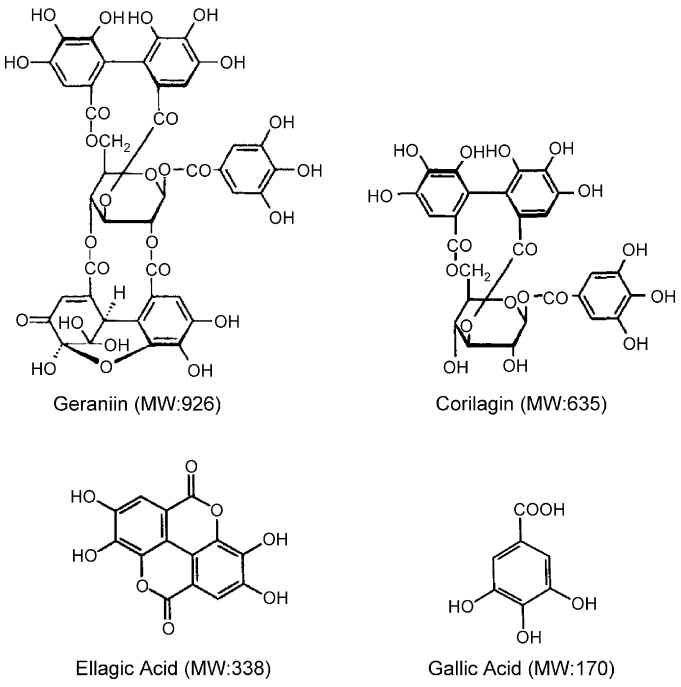

Fig. 1. Chemical structures and molecular weights of hydrolysable tannins.

of three solvents (water, methanol, 50% ethanol) for the pharmacological study on anti-HIV [5]. The researchers found that the 50% ethanol extract was the most active in inhibiting the replication of the reverse transcriptase virus. The 50% ethanol extract was assayed to consist of 1.10% geraniin and 2.28% (w/w) corilagin. De Souza et al. had reported on the quantity of active gallic acid on the water extract using high performance liquid chromatography (HPLC) technique, but no study on other solvent or analysis of other components besides gallic acid were carried out [20].

Therefore, the study of the solvent effects is very important for the screening and solvent selection of the extraction, fractionation and purification steps in the herbal processing. By understanding the solvent properties, component (solute) properties and solvent–solute interaction, rapid fractionation and isolation of desired components can be achieved. This paper presents the results on the effects of various organic and aqueous solvents with different polarities on the extract yield and the content of three hydrolysable tannins, namely gallic acid, ellagic acid and corilagin. Qualitative and quantitative effects of solvents using different extraction methods were investigated. The extraction methods utilized are the solvent extraction and the high-pressure extraction (supercritical fluid extraction and pressurized water extraction).

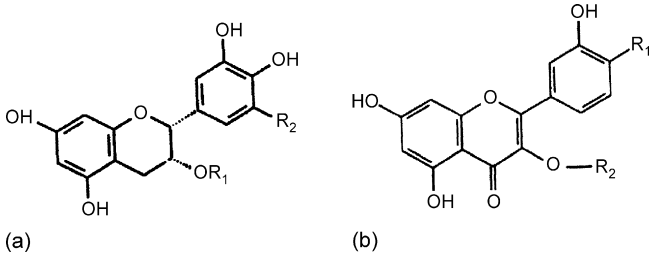

Fig. 2. Basic chemical structures of: (a) flavon-3-ol and (b) flavonol.

2. Experimental method

2.1. Chemicals and standards

The reference standards (gallic acid and ellagic acid) were both purchased from Sigma Chemicals (USA) at purity of 98%. Isolated geraniin (unknown purity) was supplied by Prof. H. Wagner (University of Munich, Germany). Analysis of corilagin was carried out by Nova Laboratories Sdn. Bhd. (Malaysia) using their isolated and patented standard (purity of 98%). The commercial *P. niruri* product, HEPAR-P^{TM} (standardized to 4% corilagin and 18% total flavonoid content) was obtained from the same company.

All chemical reagents for the extraction and component analysis (*n*-hexane, petroleum ether, dichloromethane (DCM), chloroform, acetone, methanol, ethanol, acetonitrile and phosphoric acid) were of analytical grades. Ultra-pure water obtained using ultra-filtration system (USF ELGA, UK) was utilized as the extraction solvent, solvent mixtures and HPLC mobile phase. Pure and industrial grade liquid CO_2 (99.8%) was purchased from Gas Pantai Timur (Malaysia).

2.2. Plant material

Dried and ground *P. niruri* samples were obtained from Nova Laboratories Sdn. Bhd. (Malaysia). The sample contains stems and aerial parts of the plant and has been used for the commercial production of *P. niruri* product of HEPAR-P^{TM}. The particle size distribution (% w/w) determined by sieving was in the range of 45–212 μm (8%), 212–600 μm (35%), 600 μm–1.18 mm (43%) and 1.18–3.35 mm (14%).

2.3. Solvent extraction

2.3.1. Soxhlet extraction (SE)

Five grams (±0.05) of plant sample was placed in a Whatman 25 mm × 100 mm cellulose thimble. The extraction using standard Sohxlet method (BÜCHI Laboratechnik, Model B-811, Switzerland) was carried out using 150 mL of solvent. The heating power was set to two (2) cycles per hour so that six (6) cycles of extraction were achieved within 3 h of extraction time. Various organic (7) and organic-aqueous solvents (6) with different polarities were investigated as listed in Table 1. For a mixture of organic-aqueous solvent, the percentage indicates the volume percentage of the organic solvent in the mixture (% volume/volume or v/v).

The crude extract solutions obtained were concentrated and dried using vacuum rotary evaporator (BÜCHI Laboratechnik, Model R-144, Switzerland) at temperature 80 °C or less to remove the solvents. Higher temperatures were avoided to minimize component degradation. All extracts were placed in a room temperature condition before weighing gravimetrically to determine the yields.