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Turpentine as an alternative solvent for the extraction of gutta-percha from *Eucommia ulmoides* barks

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ABSTRACT

Turpentine was used in the extraction of gutta-percha from *Eucommia ulmoides* barks. The Box-Behnken experimental design was used to optimize the extraction parameters. The optimized conditions were 25 mL/g liquid to solid ratio, 82.6 °C reaction temperature, and 1.0 h heating time, which resulted in a practical gutta-percha extraction efficiency of 80.46 ± 2.55 mg/g. The obtained samples of gutta-percha were analyzed by Fourier transform infrared spectroscopy (FTIR), gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR). The GPC results showed the molecular weight distribution (MWD) of gutta-percha from the barks was between 2.9×10^3 and 3.8×10^5 using turpentine as the extraction solvent, and the MWD was between 1.7×10^3 and 9.7×10^5 when petroleum ether was used as the extraction solvent. Turpentine showed promise as an alternative extraction solvent compared to traditional organic solvents for the extraction of gutta-percha from *Eucommia ulmoides* barks.

1. Introduction

Eucommia ulmoides is a well-known gummiferous deciduous tree belonging to the family of Eucommiaceae with only one species and one genus (*Eucommia*) (Zhang et al., 2008). Its bark, leaves and samara (especially bark) are taken as an abundant source of rubber raw materials (gum). The gum is also named gutta-percha or balata (Fig. 1) (Takeno et al., 2010, 2008). Natural rubber (polyisoprene) is divided into *trans*-1,4-polyisoprene (TPI) and *cis*-1,4-polyisoprene (CPI) based on the three-dimensional molecular structure. Gutta-percha exists in a trans configuration, which can change formation at room temperature. Gutta-percha has the same flexibility and plasticity of natural rubber. Natural CPI has distinct properties such as elasticity, abrasive performance, good thermal dispersion and impact resistance. In some areas, gutta-percha has been applied to materials such as root canal fillings, golf balls, chutty, conveyor belts and submarine cables (Subbiya et al., 2013; Shan et al., 2011). Meanwhile, barks of *E. ulmoides* are one of the most famous Chinese folk medicines (Pharmacopoeia of the People's Republic of China, 2015) and have been used extensively as food additives (Kemp and Xie, 2006). Modern pharmacology studies indicate that the barks of *E. ulmoides* have multiple pharmacological actions, and the bark products have been commonly used for treating hypertension (Greenway et al., 2011), diabetic nephropathy (Niu et al., 2016), osteoarthritis (Xie et al., 2015), cognitive deficits (Kwon et al., 2013), and neurodegenerative diseases (Kwon et al., 2012). In recent years, several active target components have been identified in the barks of *E. ulmoides*, among which geniposide (GP) and pinoresinol diglucoside (PD) are the two primary active components of the pharmacological effects of *E. ulmoides* barks (Lee et al., 2013; Song et al., 2015). Both GP and PD have iridoid glycoside and lignan glycoside-type structures, which are shown in Fig. 1.

GP has been reported to possess an important and wide variety of pharmacological functions including hepatoprotective (Chen et al., 2016a,b), cholagogic (Tan et al., 2016), anti-inflammation (Song et al., 2014), anti-diabetic (Liu et al., 2017), neuroprotection (Zhang et al., 2016a,b), and immunomodulation effects (Wang et al., 2017). PD has attracted significant research in pharmacology due to its various biological and pharmacological activities, such as a protective effect for osteoporosis (Zhang et al., 2016a,b), alleviation of oxLDL-induced dysfunction (Yao et al., 2016), and hypotensive (Sih et al., 1976) and anti-diabetic activities (Kwon et al., 2014). In view of these biological and pharmacological activities, it is critical to obtain an efficient technology to simultaneously extract gutta-percha, GP and PD from the barks of *E. ulmoides*. The traditional methods for obtaining gutta-percha, GP and PD are as follows: 1) barks of *E. ulmoides* are mixed with conventional petroleum-based solvents, such as trichloromethane, petroleum ether, toluene or benzene; 2) gutta-percha in the barks of *E. ulmoides* is extracted by hot reflux; 3) the remaining suspension of barks

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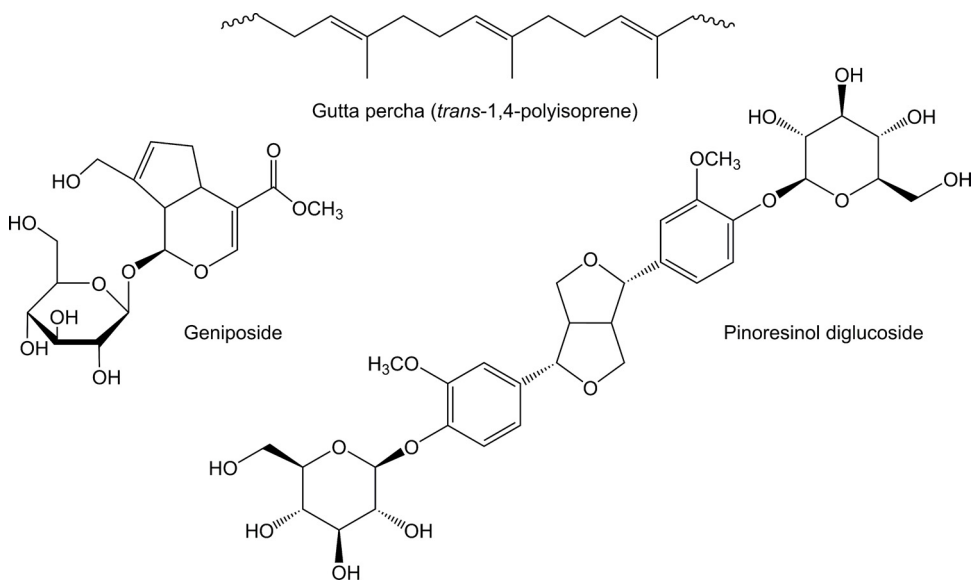

Fig. 1. The molecular structures of *trans*-1,4-polyisoprene, geniposide and pinoresinol diglucoside.

of *E. ulmoides* is distilled to remove petroleum-based solvents; 4) the GP and PD are extracted with an ethanol solution; 5) the ethanol solution is separated from the barks of *E. ulmoides*; 6) the ethanol solution is removed from the extract by rotary evaporation to obtain an extract containing GP and PD. The yields of the target analytes obtained using this method are low due to the prolonged heating and complicated separation process. These petroleum-based solvents are toxic, non-environmentally friendly and nonrenewable. If used as a pharmaceutical raw material, a small amount of solvent residue after purification can cause damage to the human body.

Organic solvent extraction method is generally used for gutta percha extraction, in which many toxic, environmentally unfriendly, and non-renewable chemicals (such as toluene, petroleum ether, and chloroform) are employed as extraction solvents. Some natural essential oils have been reported as gutta percha solvent in dentistry, such as eucalyptus oil, orange peel oil, and turpentine, and these natural essential oils are safe (Zhang et al., 2008). Turpentine is an ambient fluid obtained by the distillation of resin obtained from live trees, mainly the Pinaceae plant. The chemical composition of turpentine is comparatively simple and mainly consists of monoterpene components, α-pinene and β-pinene, with lesser amounts of camphene, carene, dipentene and terpinolene (Bannister and McDonald, 1983; Sadeghi et al., 2013; Sama and Bandopadhyay, 2001). It is mainly used as a solvent and source of materials for organic synthesis with some biological uses (defense against microorganisms and predators, mosquito repellent, inhibition of the growth and propagation of bacteria and fungi (Bannister and McDonald, 1983; Lucia et al., 2007; Basim and Basim, 2013; Iliev and Papanov, 1969; Li et al., 2015). Turpentine enjoys the advantages of low-toxic, renewable, and easily degradable (Charoo et al., 2008; Pandhare and Padalkar, 2013), and hence which has great potential to replace traditional industrial plant extraction solvents. In summary, turpentine tends to reduce environmental damage and holds promise as a newly developed extraction solution. Here we tried to use turpentine to replace petroleum-based solvents (such as trichloromethane, petroleum ether, toluene or benzene) as the extraction solvent of gutta-percha. At present, the main components of turpentine, such as α-pinene, β-pinene and dipentene, have been reported as extraction solvents. (Sicaire et al., 2015; Bundeesomchok et al., 2016; Breil et al., 2016; Aissou et al., 2017). Nevertheless, to our best knowledge, there are no reports on using turpentine as a solvent to extract the natural products from plant materials.

To our best knowledge, the extraction of gutta-percha with turpentine as the solvent has not yet been reported and is of interest to investigate. Taking these considerations into account, the goal of this work was to select a renewable, nontoxic and environmentally friendly extraction solvent for the extraction of gutta-percha from *E. ulmoides* barks and to compare the yields with a traditional organic solvent. The parameters affecting the extraction included the reaction temperature, heating time, soaking time and the liquid to solid ratio, and these parameters were optimized systematically. Moreover, the effects on the small molecules GP and PD in residues of the gutta-percha extraction were evaluated. Gutta-percha was extracted by turpentine and characterized by GPC, NMR and FTIR.

2. Material and methods

2.1. Materials and chemicals

E. ulmoides barks were obtained from Leshan (Sichuan, China) and authenticated by Prof. Kailin Mo from the Sichuan Academy of Forestry in China. Fresh barks were dried in the shade for one month at room temperature, pulverized to suitable size using a pulverizer and sifted through an 80-mesh screen stencil.

The GP (97% purity) reference substance was purchased from DASF Biological Technology Co., Ltd. (Nanjing, China), and PD (98% purity) was obtained from Yuanye Biological Technology Co., Ltd. (Shanghai, China). Chromatographic grade acetonitrile for the HPLC analysis was purchased from Thermo Fisher Scientific (Shanghai, China). Turpentine (Pharmaceutical grade) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China) and analyzed by gas chromatography (components included α-pinene 77.23%, β-pinene 13.08% and dipentene 8.92%). The GC analysis system (7890A, Agilent Technologies, Santa Clara, USA) of gas chromatograph is composed of a flame photometric detector and an Agilent HP-5 capillary column (30 m × 0.32 mm, 0.25 μm of film thickness). GC analysis was applied to determine the composition of turpentine. The turpentine was dissolved with *n*-hexane (1:20, v/v) before GC analysis. GC analysis was carried out under the following detection conditions: initial temperature 40 °C (1 min hold) to 200 °C at a rate of 10 °C min. 200 °C was maintained for 5 min 200 °C of temperature to 240 °C and 240 °C was held 10 min. Nitrogen was used as a carrier gas at a flow rate of 20 mL/min. The sample (1 μL) of split ratio is 1:10. The calibration equations of the α-pinene, β-pinene, and dipentene were carried out by standards addition method. The standards of α-pinene (purity 99%), β-pinene (purity ≥ 99%) and