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Process development studies for recovery of bio active isolates from spent black pepper generated from ayurvedic industry

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ABSTRACT

The present study investigates the yield and purity of piperine isolated from spent black pepper (*Piper nigrum* L.), a 'thrown away residue' from ayurvedic industry. The spent pepper generated from a major ayurvedic industry has been screened for the presence of high value volatiles, extracts and active principles and also a commercially acceptable process have been developed and optimized herewith to produce oleoresin and high purity piperine from this residue. Results obtained from the UV spectrophotometry and high performance liquid chromatography (HPLC) clearly suggested that purity of piperine from raw (92.54%) and spent (93.58%) peppers were close to that of a standard piperine (97.00%). A significantly higher yield of piperine (44.74%) was obtained by prewashing the oleoresin with hexane followed by recrystallization in ethyl acetate/*n*-hexane ratio of 60:40. The gas chromatography (GC) analysis of essential oils isolated by hydro-distillation from raw and spent pepper samples showed significant reduction in monoterpenes compared to sesquiterpenes, with an increase of β-caryophyllene content with the effect of processing. Moreover, the contents of oleoresin, piperine and essential oil in both spent and raw pepper showed that spent pepper retains 60% of valuable compounds even after industrial processing. In this way, spent pepper generated from ayurvedic industries could be utilized for value addition by development of high value oleoresin and nutraceuticals for healthcare application.

1. Introduction

Ayurveda is one of the ancient and traditional medical practices in the world (Prakash, 1961). The conventional ayurveda dealt with the preparation of herbal extracts with water, oil or milk in small scale that possesses medicinal properties to different ailments (Sekar and Mariappan, 2008), whereas modern ayurveda involves formulation of drugs in a commercial scale which generates substantial quantities of processed wastes (Abraham and Pradeep, 2000). These spent materials have gained a lot of importance to their value added components as well as the technologies to maximize their component yields.

The fruits of black pepper (*Piper nigrum* L.) have been widely used since time immemorial in the cooking practices and also in traditional ayurveda medicine (Prakash, 1961). Pepper fruits are found to possess antihelmenthic, antiasthmatic, analgesic properties in addition to the treatment of health disorders such as insomnia and epilepsy (Government of India, 2001). The fruits contain 1.0–2.5% of volatile oil, 5–9% of alkaloids among which piperine, chavicine, piperidine and piperetine are major bioactive compounds (Evans, 2010).

Piperine, {IUPAC name – 1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine} ($C_{17}H_{19}O_3N$), (Fig. 1), a major constituent of black pepper, possesses several pharmacological activities including central nervous system depressant, antipyretic, analgesic, anti-inflammatory and antioxidant properties (Khajuria et al., 1997; Lee et al., 1984). Evidenced from earlier reports, it can also enhance the bioavailability of several drugs, including sulfadiazine, tetracycline, streptomycin etc. (Atal et al., 1980; Bano et al., 1987; Umesh et al., 2011 Zutshi et al., 1985). It is also utilized to prepare insecticides (Smita et al., 2011; Vanderlucia et al., 2000). Smita et al. (2011) have shown that piperine is water insoluble, whereas ayurveda industries process several tonnes of black pepper (about 30–40 tonnes of pepper processed per year as per the data received from M/s. Arya Vaidya Sala, Kottakkal) by hot aqueous extraction for producing different formulations like khwathas,

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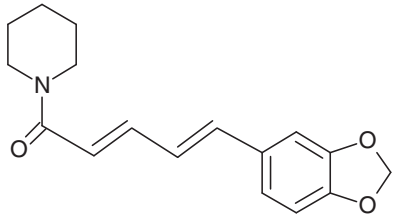

Fig. 1. Structure of piperine.

asavas etc. Hence there exist, chances for the retention of high valued volatiles, terpinoids, oleoresins and bio active piperine in the extracted materials with significant therapeutic values.

In this context, the present study investigates to develop an efficient and inexpensive process for the isolation of piperine from spent black pepper residue generated from the industry and the work is of great significance to ayurvedic sector not only by product utilization, by value addition and profit generation but also through pathway for better utilization of natural resources. This study also envisages on the chemical profiling and comparative evaluation of the essential oil isolated from both raw and spent pepper.

2. Materials and methods

2.1. Plant materials and chemicals

This study was planned in collaboration with M/s. Arya Vaidya Sala, Kottakkal, Kerala, India's leading manufacturer in ayurvedic formulations. The authentic samples of both raw pepper (RP) and spent pepper (SP) were recovered from this industry. The spent material revived was the one which has undergone aqueous extraction for the manufacture of formulations as practiced in commercial level. Standard piperine (97% purity) was purchased from Sigma–Aldrich (Missouri, USA). High performance liquid chromatography (HPLC) grade water and methanol were purchased from Merck (Darmstadt, Germany). Solvents for extraction, including *n*-hexane and ethyl acetate were purchased from SD fine chemicals (Bangalore, India).

2.2. Determination of moisture content

Moisture estimation was carried out through the gravimetric method (Black, 1965). Samples (10 g) were powdered, homogenized and dried in oven at 100 °C for 6 h to determine the moisture content. Analysis repeated in triplicate.

2.3. Essential oil isolation

Samples (100 g each) were dried, pulverized and subjected to hydro-distillation in a Clevenger distillation (Ying et al., 2009) apparatus for 6 h to isolate the essential oil. The obtained essential oils were dried using anhydrous sodium sulfate, collected in sample tubes, stored at −20 °C and subjected to gas chromatography (GC).

2.4. Oleoresin extraction

Oleoresin extraction was carried out through the industrial percolation process (Govindarajan and Stahl, 1977). Oleoresin was extracted by suspension of powdered pepper samples (1 kg) in ethyl acetate and *n*-hexane (60/40 ratio) for 24 h. The extraction process was repeated by three time solvent washings to ensure the complete extraction. The extracted miscella was concentrated in wiped film evaporator (Pope Scientific Inc., USA) and further evaporated to dryness in a rotary evaporator (Buchi, Switzerland). Extraction of oleoresin was also carried out by Soxhlet apparatus (Mathai, 1988) with the same solvent mixture for 6 h to verify the efficiency of the industrial percolation method.

2.5. Crystallization of piperine from oleoresin

Piperine was isolated from oleoresin by recrystallization process. Oleoresin was first washed with *n*-hexane to remove volatiles and other fatty matter, then the resulting residue was dissolved in medium polar solvent of ethyl acetate and *n*-hexane (60/40 ratio) with solvent to residue ratio of 3/5 (v/w) and kept in refrigerator at 4 °C overnight. Piperine deposited as crystals, was filtered and washed with ethyl acetate and *n*-hexane (40/60 ratio). This process for purification of piperine through crystallization was carried out in varying ratios of solvents (Ethyl acetate: Hexane) and optimized based on maximum recovery of piperine. The percentage purity of the piperine was determined by UV spectroscopy method.

2.6. UV spectroscopic analysis of oleoresin and crystallized piperine

Piperine content in oleoresin and crystallized piperine was estimated by UV spectrophotometric method (American Spice Trade Association, 1997). The analysis was performed using Shimadzu (UV-2450) UV–vis spectrophotometer (Kyoto, Japan). About 5 mg of the oleoresin or crystallized piperine was accurately weighed and transferred into a 25 ml volumetric flask and made up to the mark using a mixture of ethyl alcohol and methanol (95/5 ratio). From this stock solution, 0.5 ml was transferred into a 25 ml volumetric flask and made up with the same solvent. The absorbance of this diluted solution was measured at 343 nm and the percentage of the piperine was calculated in the oleoresin and crystallized piperine with respect to standard piperine by the following formula:

Percentage of piperine = (concentration × dilution factor × 100)/(10^6 × sample weight).

2.7. HPLC analysis of crystallized piperine

HPLC analysis of crystallized piperine was performed by standard method (Santosh et al., 2005; Shingate et al., 2013). An analytical high performance liquid chromatography (Shimadzu, LC 20A HPLC series, Kyoto, Japan) with variable wavelength, programmable photo diode array detector, column oven and reverse phase 5 μm C_{18}Supelco column (250 × 4.6 mm) (Sigma–Aldrich, USA) was used. Methanol and water (80/20 ratio) were used as mobile phase and a constant flow rate of 1 ml/min was maintained. The column temperature was maintained at 30 °C. 20 μg of piperine was dissolved in 1 ml of methanol of which 20 μl was injected using a syringe. All analyses were performed in triplicate.

2.8. Gas chromatography analysis

Essential oils were analyzed on a Shimadzu GC 2010 model gas chromatograph (Ravi et al., 2013). Helium was used as the carrier gas at a flow rate of 1 ml/min. A capillary column (60 m × 0.22 mm i.d. × 1.00 μm df) was used for this study (BPX-5, SGE Ltd. Melbourne) and a flame ionization detector (FID) was used. The split ratio was 1:50. The oven temperature was programmed from 80 °C (hold time 1 min) to 220 °C at 5 °C/min and held for 10 min. The injector temperature was 250 °C and the detector temperature was 300 °C. Each compound identification by retention indices of authentic reference compounds where possible and also by published data (El-Ghorab et al., 2010). All quantified constituents are reported as peak area percentages.