ABSTRACT

Piper betle L. var. Bangla, belongs to the *Piperaceace* family. It has good nutritive value contributed by high mineral and vitamin content. Public interest for bioactive compounds with a high antioxidant capacity is increasing day by day. Therefore, it is a major challenge for the researchers in the food and pharmaceutical industries to find out the most efficient extraction method as well as characterization of bioactive compounds from the leaves. The present study was focused on to optimize the extract and essential oil (EO) yield from fresh as well as cured betel leaves using different extraction methods. Moreover, physicochemical characterization, separation of compounds from EO, kinetic study and food application were also studied.

The leaf extract yield, antioxidant activities (AA), total phenolics (TPC), and flavonoid content (TFC) of fresh as well as cured betel leaf, obtained by soxhlet extraction (SE) and ultrasound assisted extraction (UAE) were analysed. The optimum conditions for the extract were calculated based on the extract yield by varying three parameters: extraction time (15-35 min), temperature (40-50°C) and ethanol to leaf powder ratio (15-25 ml/g), and were optimized using CCD coupled with RSM. Results showed that extraction time and ethanol to leaf powder ratio had a significant effect on the yield of the extract. The highest leaf extract yield (14.62%) obtained by UAE showed the highest TPC and AA using DPPH assay with optimum extraction parameters, viz. temperature (45°C), time (41.8 min) and ethanol to leaf powder ratio (20 ml/g). The quantitative analysis showed a very significant positive correlation between the assays in relation to the amount of total phenolic compounds.

The extraction of EO from betel leaves is of high interest for industrial application. Thus, the EO of both the fresh and the cured betel leaves, were also optimized by BBD using three independent variables such as extractions time (40-140 min), temperature (80-100°C) and leaf to water ratio (1-2 g/l). The results showed that the highest EO yield (0.48%) was obtained from the cured betel leaves using hydro-distillation (HD) method whereas it was only 0.35% from the fresh leaves with optimized extraction time (140 min), temperature (100°C) and leaf to water ratio (1.5 g/l). The FT-IR analysis of both the leaf EOs proved the presence of chemical groups like alkenes, alcohols, ethers, esters, phenols, etc. The GC-MS results revealed the presence of 33 and 30 components representing 96.4% and 93.96% of total EOs extracted from the fresh and the cured betel leaves, respectively. The three compounds which distinguished EO of fresh leaves from the cured leaves were: α-pinene, β-phellandrene and oxabicycloheptane. Chemical characterization of cured betel leaf EO (CLEO) revealed the presence of major compounds like eugenol (42.23%), estragole (18.12%), linalool (10.24%), α-coapene (8.54%), anethole (2.65%), caryophyllene (1.13%) and 3-methoxy cinnamaldehyde (0.66%) in different percentages. Three compounds were successfully separated from the CLEO by column chromatography method and confirmed as respective compound on the

basis of ^{1}H and ^{13}C NMR data. The separated and purified compounds that was confirmed by column chromatography and NMR analysis were eugenol, estragole and 3-methoxy cinnamaldehyde. For extraction of EO, equilibrium-time relationship was also determined by studying the extraction kinetics using three mathematical kinetics models: first order kinetic model, Ponomarev model and Peleg model. The Peleg model was found to be the best fit representing the HD extraction kinetics.

Antibacterial activity was also evaluated against *Mycobacterium smegmatis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the results revealed that CLEO showed significantly higher antimicrobial activity against *M. smegmatis* than fresh leaf EO. This is because most of the antimicrobial activity is attributed to oxygenated terpenoids present in the CLEO. Moreover, CLEO exhibited strong antibacterial activity that might be due to the presence of linalool and eugenol compound in higher percentage.

In addition, morphological study was also carried out to investigate the extraction mechanism of the fresh and the cured betel leaves with the help of scanning electron microscopy (SEM). SEM images confirmed that exhausted fresh leaves showed less porous, fibrous and smooth surface than the exhausted cured leaves which showed more porous and rough surface that improved extraction.

The potential of EO of betel leaf as a natural food preservative was also studied to find out an organoleptically acceptable concentration of CLEO that can be employed in sapota juice (*Manilkara zapota* L.). This was followed by determination of the changes in physicochemical parameters in EO treated sapota juice for enhancing the shelf life under refrigerated condition of 4°C. The results showed that the sapota juice could be preserved by adding the organoleptically acceptable concentration (0.3 µl/ml) of CLEO which could efficiently extend the shelf life of the juice without alteration of quality parameters for about one month compared to untreated and other treated samples.

Keywords: *Piper betle* L., Cured betel leaf, Leaf extract, Essential oil, Optimization, FT-IR, GC-MS, Antimicrobial activity, SEM, Column chromatography, NMR, Kinetics, *Manilkara zapota* L., Physicochemical characterization, Sensory analysis, Natural preservative.