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• Research Article

Marker–based standardization and investigation of nutraceutical potential of Indian propolis

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ABSTRACT

OBJECTIVE: Propolis, a resinous material collected by honey bees from various plants, has been explored globally for its medicinal and nutritional properties. However, research over Indian propolis is at infancy. This study was designed to investigate nutraceutical potential of Indian propolis.

METHODS: In the present study, propolis extract was standardized with respect to markers caffeic acid phenethyl ester, caffeic acid, galangin, luteolin, curcumin, apigenin, pinocembrin and quercetin by new high-performance thin-layer chromatographic (HPTLC) methods. The physico-chemical analysis, residues analysis and *in vitro* antioxidant activity analysis were performed. Nutraceutical value was examined in terms of fats, fibers, minerals, proteins, polysaccharides, total carbohydrates, and energy value.

RESULTS: The developed HPTLC methods were found to be simple, reliable accurate, and the validation parameters were within the limits of *the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use* guidelines. Macerated ethanolic extract of propolis (MEEP) was found to have polyphenolic content of (20.99 ± 0.24) mg/g and flavonoids content of (8.39 ± 0.04) mg/g. MEEP was found to comprise of (283.33 ± 51.31) g/kg fats, (30.07 ± 7.30) g/kg fibers, (102.56 ± 2.84) g/kg proteins and (389.36 ± 57.50) g/kg carbohydrate with a calorie value of (38 409.33 ± 6 169.80) kJ/kg. It was found that Indian propolis exhibited high nutraceutical value and showed absence of pesticides and heavy metals. The MEEP showed *in vitro* antioxidant activity with inhibitory concentration of (12.24 ± 4.64) μg/mL.

CONCLUSION: The present work explores Indian propolis as a potential nutritious candidate. The proposed analytical methods can be applied in future screening of the quality of Indian propolis.

Keywords: propolis; high-performance thin-layer chromatography; nutraceutical value; antioxidant activity

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1 Introduction

Propolis, a complex resinous material, is a mixture of wax and other substances collected from various plant species by stingless honey bees.[1–3] It is used as a sealant for unwanted spaces in the bee hive. Propolis is composed of resin and balsams (50%–60%), pollen (5%–10%), polyphenols, flavonoids, phenolic acids and their esters, terpenoids, steroids and other constituents, including amino acids, minerals and vitamins A and B complex.[4,5]

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Due to its high content of various amino acids, vitamins and minerals propolis is believed to have nutraceutical value. More than 200 compounds have been reported in propolis, including rutin, galangin (GAL), quercetin (QUR), caffeic acid phenethyl ester (CAPE),[6] caffeic acid (CA), apigenin (API), pinocembrin (PINO),[7] luteolin (LUT) and curcumin (CUR).[8] Many biological activities have been attributed to chemical constituents found in propolis. For example, propolis is extensively studied for potential antitumor effects from the activity attributed to components such as CAPE, artepillin C, p-coumaric acid and ferulic acid.[9–11] Among the various chemical constituents, CAPE is one of the main biologically active components in propolis. Huang et al.[12] showed CAPE's ability to suppress the growth of human tumor cells in their *in vitro* experiments. CAPE is reported to inhibit nuclear factor-κB (NF-κB) activation through multiple immunomodulatory and anti-inflammatory pathways.[13] It has also been used for cytotoxicity on oral submucosal fibroblast, neck metastasis of gingival carcinoma, tongue squamous cell carcinoma cells and treatment in oral cancer.[14] GAL and API were identified as the principal monoamine oxidase (MAO)-inhibitory constituents.[15] Cotherapies of CAPE and LUT, as well as CAPE and QUR, isolated from propolis, have been reported to have antibacterial activity.[16] The highly variable composition of propolis may thus influence its medicinal activity, so there is need for standardization of propolis for clinical use.

A literature survey identified that there are reports for nutraceutical value of bee products from different sources,[17–20] including Indian mustard bee pollen, Italian pollen,[18,21] Argentinean propolis and Spanish propolis.[22,23]

However, the study of Indian propolis has just begun and is not yet extensively reported. A handful of studies have reported the chemical composition of Indian propolis,[24] or its medicinal properties, such as anti-atherosclerotic,[6] anti-inflammatory,[25,26] antioxidant,[27,28] anticancer,[1] antimicrobial, antifungal,[29–31] antiplatelet[32] and pro-oral health[33] activities. Some methods have been reported for the analysis of Indian propolis including high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC/MS)[24,34,35] and gas chromatography-flame ionization detection (GC-FID)[25] methods.

However, no studies have been reported for standardization of Indian propolis in terms of marker compounds such as CA, CAPE, GAL, LUT, CUR, API, PINO and QUR. Furthermore, no attempt has been made to investigate the nutraceutical potential of Indian propolis yet.

Hence, the aim of the present work was to optimize a method for extraction, standardization and analysis of physicochemical compounds and their activities, to determine the heavy metal and pesticide content and to investigate the nutraceutical potential of Indian propolis.

2 Materials and methods

2.1 Materials and reagents

Indian propolis, made by bee primarily visiting poplar trees, was collected from a local bee keeper in Bharatpur, Rajasthan, India, in the month of December. It was authenticated from the Central Bee Research and Training Institute, Pune, Maharashtra, India. The raw materials were packed in plastic bags and stored in a domestic freezer at –10 °C.

Analytical standards of LUT, GAL, CUR, API and QUR were procured from Natural Remedies Pvt. Ltd, Bangalore, Karnataka, India. PINO was procured from Mira Biotechnology, China. The pure CAPE and CA were procured from Sigma Aldrich, Bangalore, Karnataka, India. All chemicals and solvents used were of analytical grade (E. Merck, Ltd, Mumbai). Double distilled water was used throughout the study.

2.2 Preparation of extracts

2.2.1 Maceration

An accurately weighed quantity of 50 g of crude propolis was macerated with 150 mL of ethanol and kept for 10–15 d. The maceration was filtered, evaporated and the extract obtained was designated as macerated ethanolic extract of propolis (MEEP).[36]

2.2.2 Microwave-assisted extraction

An accurately weighed quantity of 30 g of crude propolis was extracted in 200 mL of ethanol and heated in a microwave at maximum power consumption (1 200 Watts, voltage 230–240 V, 3 min, 50 Hz). This solution was frozen, filtered and the extract obtained was designated as microwave assisted ethanolic extract of propolis (MWEEP).[36]

2.2.3 Ultrasound extraction

An accurately weighed quantity of 30 g of crude propolis was mixed with 200 mL of ethanol and sonicated for 1 h at room temperature (100% duty cycle and at constant frequency and power). The resulting mixture was filtered, evaporated and the extract obtained was designated as the sonicated ethanolic extract of propolis (SNEEP).

2.2.4 Soxhlet extraction

An accurately weighed quantity of 30 g of crude propolis was extracted using 200 mL of ethanol and a Soxhlet apparatus (Standard Scientific Glass I Industries, Mumbai, consists of borosilicate glass extractor, condenser and round bottom flask). The obtained extract was filtered and evaporated.[37] It was designated as soxhlet ethanolic extract of propolis (SXEEP).