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• Research Article

Investigation of the nutraceutical potential of monofloral Indian mustard bee pollen

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OBJECTIVE: This study was designed to investigate the nutraceutical potential of monofloral Indian mustard bee pollen (MIMBP).
METHODS: The nutritional value of MIMBP was examined in terms of proteins, fats, carbohydrates, and energy value. Its chemical composition in terms of total polyphenol and flavonoid content was determined. MIMBP was screened for free flavonoid aglycones by developing and validating a high-performance liquid chromatography-photo diode array (HPLC-PDA) method. MIMBP was analyzed for *in vitro* antioxidant effect in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity.
RESULTS: MIMBP was found to be comprised of proteins ((182.2±5.9) g/kg), fats ((137.7±6.8) g/kg) and carbohydrates ((560.6±17.4) g/kg), which result in its high energy value ((17 616.7±78.6) kJ/kg). MIMBP was found to contain polyphenols ((18 286.1±374.0) mg gallic acid equivalent/kg) and flavonoids ((1 223.5±53.1) mg quercetin equivalent/kg). The HPLC-PDA analysis revealed the presence of kaempferol ((65.4±0.5) mg/kg) and quercetin ((51.4±0.4) mg/kg) in MIMBP, which can be used as markers for determining the quality of bee pollen. The MIMBP extract showed DPPH free radical-scavenging activity with a half maximal inhibitory concentration of 54.79 μg/mL.
CONCLUSION: The MIMBP was found to be a rich source of nutrients providing high caloric value, which makes it a candidate for a potential nutraceutical agent. The study also illustrated the high antioxidant content of MIMBP, especially in the principle polyphenols and flavonoids, which suggests its potential role in the prevention of free radical-implicated diseases. The DPPH-scavenging effect of MIMBP further confirmed its antioxidant potential. Additionally, we developed a simple, specific and accurate HPLC-PDA method for the identification and quantification of free flavonoid aglycones. This can be applied in future screenings of the quality of pollen collected by honeybees.
KEYWORDS: dietary supplements; bee pollen; flavonoids; mustard plants

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1 Introduction

Apiculture, the science of bee keeping, has contributed to the field of nutrition and medicine by providing access to apiproducts such as honey, propolis, and royal jelly, which have demonstrated beneficial properties as nutraceutical agents[1,2]. Bee pollen is plant pollen collected from different sources by the worker honeybee *Apis melliferra* feeding its larvae in the early stages of development. Bee pollen is known to be a rich source of polyphenols, flavonoids, sugars, proteins, amino acids, fatty acids, minerals and vitamins which makes it relevant and useful for humans[3–6]. Analyses of bee pollen's chemical composition have reported it to be the "only perfectly complete food"[7] that possesses a wide array of pharmacological activities including being antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, antiatherosclerotic, antianaemia, antiosteoporosis and antiallergic as shown in multiple studies[8–14]. Bee pollen has also demonstrated clinical relevancy for its anti-prostatic effect in humans[15].

Studies on the palyonology, chemical composition and benefits of bee pollen have been conducted in various regions including Australia[16,17], Brazil[18], China[19], Chile[20], Portugal[21], South Africa[22], and the Sonoran Desert, USA[23]. Bee pollen is currently used as a functional food or supplementary nutrition in some of these countries, however to our knowledge there are no studies examining the composition or benefits of bee pollen from Indian sources. Major regions for apicultural activities in India include Punjab, Jammu-Kashmir, Himachal Pradesh, Uttar Pradesh, Haryana, Bihar and West Bengal[24,25]. The forests, farms around sub Himalayan tracts, cultivated vegetation in Madhya Pradesh, Rajasthan, Eastern Ghats in Andhra Pradesh and Maharashtra are known to be the major regions for honey collection[24–26]. These regions are home to diverse flora of nectariferous and polliniferous plant species, which are prime conditions of apiculture that help produce high-quality pollen. The current study was designed with the aim of exploring the nutritional value and chemical composition of Indian bee pollen in order to assess its utility as a nutraceutical agent.

The composition of bee pollen varies with the plant source and geographic origin. Standard quality pollen with minimal variations, obtained by collecting bee pollen from single botanical taxa, is termed monofloral pollen[27]. Monofloral pollen ensures uniform organoleptic and biochemical characteristics to that of the original plant, while heterofloral pollen exhibits variable properties[27]. Among various pollen-yielding sources in India, mustard crops (Family: Brassicaceae) are one of the major sources. Worldwide, these are used as extensive dietary crops and possess economic significance[28,29]. The phenolic composition of *Brassica* vegetables has been well established, however the nutritional and chemical composition of pollen from these sources is not yet understood. Therefore, the current study was designed to recognize floral origin and nutritional value in terms of proteins, fats, carbohydrates, and energy value; to determine chemical composition in terms of total polyphenols and flavonoid content of monofloral Indian mustard bee pollen (MIMBP), *i.e.*, *Brassica juncea*; and to develop a simple, specific and accurate high-performance liquid chromatography-photo diode array (HPLC-PDA) method for identification and quantification of free flavonoid aglycones from the bee pollen.

2 Materials and methods

2.1 Bee pollen material and chemicals

The MIMBP pellets were collected from 24 Parganas district of West Bengal, India during December 2012 to January 2013. The collected fresh pollen pellets were hand-sorted by appearance to avoid possible contamination of pollen from other sources. The pollen samples were identified and authenticated by Central Bee Research and Training Institute, Pune, India (Voucher Specimen No (1/WB/2012)). The fresh pollen was dried at temperatures below 40 ℃, vacuum packed in food-grade polyethylene bags and stored in a −15 ℃ freezer throughout the study. All the analysis was performed within a period of one month after pollen collection in order to best preserve its nutritive value and free radical-scavenging capacity, and avoid possible age-induced degradation of the pollen[8]. No signs of degradation or fermentation were observed on the stored samples. The samples were sieved by 200 μ mesh before analysis. Analytical standards of gallic acid, rutin, chrysin, kaempferol and quercetin were procured from Merck, USA. Aluminum chloride, mercuric oxide, sodium carbonate, sodium hydroxide, and sodium sulfate were procured from Sigma Aldrich, USA. Folin-Ciocalteu's phenol reagent, sulfuric acid, hydrochloric acid, *O*-phosphoric acid and petroleum ether, and methanol (HPLC-grade) were procured from Merck, USA. All reagents used during the study were of analytical research grade. Distilled water was used throughout the study.

2.2 Sensory analysis and microscopic examination

The MIMBP was subjected to sensory analysis in terms of color, appearance, odor and taste. The pollen sample was observed under a scanning electron microscope, Oxford Instruments, Inca X Sight Model No. 6650-M. The pollen was scattered on a 12 mm carbon grid attached to scanning electron microscope specimen mounts and were sputter-coated with a layer of gold/palladium. The pollen was then subjected to standard acetolysis method[30] followed by microscopic examination using Nicon E800 Eclipse compound microscope in phase contrast mode with Image