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GC-MS analysis of ethanolic extract of propolis from Jhajjar district in Haryana, India

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Abstract

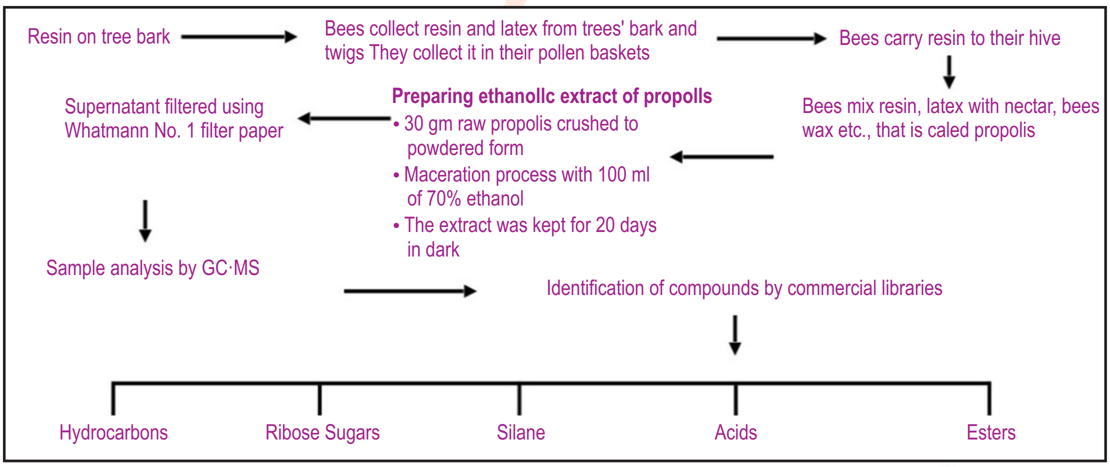
Aim: To analyse the chemical composition of ethanolic extract of propolis, a glue like resinous natural material collected by worker honey bees from Jhajjar district of Haryana.

Methodology: Chemical composition analysis of the ethanolic extract of propolis sample collected from Jhajjar district of Haryana, India was determined by Gas chromatography–mass spectrometry. Propolis sample was crushed to powdered form and extracted using 70% ethanol and kept for around 20 days. The supernatant was then filtered and thereafter, the filtered extract was dried and collected in a clean vial.

Results: GC-MS analysis revealed the presence of hydrocarbons, sugars, fatty acids, carboxylic acid, alcohols, ethers, carbamate ester and other organic compounds.

Interpretation: The results revealed the presence of diverse biochemical constituents which can be utilized in therapeutics of several ailments to enhance the quality of life by improving human health.

Key words: Chemical profile, Fatty acids, Organic compounds, Propolis



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Introduction

Propolis, commonly referred to as bee glue, is a resinous substance collected by worker honeybees. It comprises of resin and diverse substances, such as lipophilic material from leaves, mucilage, gum, and latex. Bees collect these materials from various botanical sources like plant leaves, buds and exudates (Rana *et al.,* 2022 a,b). The collected substances undergo digestion by the action of bee salivary enzymes and are combined with beeswax to form a glue like substance called propolis. It has a soft texture and can be seen in colours ranging from yellowish green to deep-brown (Asgharpour *et al.,* 2020). This natural product is a unique blend of diverse natural elements that possess distinct characteristics. It plays a crucial role in safeguarding the beehive by acting as a thermal insulator for the hive as well as an antiseptic to protect the developing brood and larvae from pathogenic microorganisms by acting as a physical barrier and immune modulator (Tosic *et al.,* 2017). It also functions as a disinfectant for bees, effectively warding off diseases and preventing their occurrence within the beehive (Simone-Finstrom *et al.,* 2017).

Propolis has been utilised in traditional medicine since ancient times and is believed to have beneficial effects on human health. Antimicrobial, antioxidative, anti-inflammatory, anticancer, cytotoxic, antiparasitic, immunomodulatory as well as leishmanicidal effects have been reported in previous studies on propolis extracts from various regions of the world (Pasupuleti *et al.,* 2017; Sena-Lopes *et al.,* 2018; Rana and Parmar, 2022; Rana and Kumar, 2022; Rana and Kumar, 2023). It has the potential to be a promising component in drug development sector for therapeutic purposes (Belmehmdi *et al.,* 2023). It roughly comprises 50% plant resins, 30% beeswax, 10% essential oils, 5% pollen and 5% other organic compounds, vitamins such as B1, B2, B6, C and E as well as amino acids (De Figueiredo *et al.,* 2015; Zaccaria *et al.,* 2017). Among bioactive constituents, flavonoids, terpenoids, phenolic acids, phenolic esters, and sugars, have been commonly found in various propolis samples (Rana and Bajwa, 2023) though, the chemical composition of propolis is influenced by different factors, such as geographic location, surrounding vegetation, bee species, plant origin, time of collection and storage conditions (Biluca *et al.,* 2016; Rana and Kumar, 2022).

The presence of many forms of propolis with distinctive chemical profiles has been recorded, making its standardization a major issue in propolis research (Caetano *et al.,* 2023). Variability in the total content of various phytocomponents (such as phenols and flavonoids) found in propolis collected from diverse geographical areas have been reported due to the presence of diverse flora in the vicinity of an apiary (Woźniak *et al.,* 2022; Grassi *et al.,* 2023). Propolis has been recognized as a nutritional supplement, functional food, and natural preservative in nutraceutical and other industries worldwide over the past two decades. More study is required to fully understand the potential uses of propolis from diverse regions and to establish a strong platform for its widespread use in various industries for commercial purposes (Pant *et al.,* 2023). Although, studies on chemical composition of propolis using organic solvent have been reported from all over the globe Data regarding propolis sample from Jhajjar has received little attention and are poorly documented. Hence, this study was carried out to investigate the chemical composition of ethanolic extracts of propolis from Jhajjar district of Haryana, India using the GC/MS analytical technique.

Materials and Methods

Study area and sample collection: Samples of crude propolis produced by *Apis mellifera* were collected from an apiary located in Jhajjar district (Fig. 1) of Haryana, (India) in May 2023. The vegetation around the apiary was diverse including flora such as *Dalbergia sissoo, Ocimum sanctum, Shorea robusta* and *Brassica juncea.*

Extraction Procedure: The sample extraction technique was prepared following the methods of Kumar *et al*. (2008) and Kalia *et al*. (2015). A 30 g raw propolis sample was crushed into powdered form with a mortar and extracted with 70% ethanol. The extract was kept for around 20 days in a tightly closed glass container in the dark with intermittent stirring. The supernatant was then filtered using the Whatmann No. 1 filter paper. The filtered extract was then evaporated to dryness with a rotatory evaporator under reduced pressure and was collected in a clean vial.

Gas chromatography/mass spectrometry analysis: The chemical profile of the propolis sample was analysed by GC-MS. This sample analysis was done at University Centre for Research & Development (UCRD), Chandigarh University. The GC-MS analysis was performed by using GC-MS/MS Agilent Technologies, Inc. Where carrier gas was helium flowing at a constant rate of 1 ml min^{-1}. The source temperature was 280°C and the solvent delay time was 1min. Chromatographic data were processed using the Agilent MassHunter software. Identification of the compounds was based on a comparison of mass spectra and computer searches on commercial libraries and literature data. Certain components remained unidentified due to lack of appropriate references and information.

Statistical analysis: The compounds observed were identified by means of their retention time by comparing their mass spectra with the National Institute of Standard and Technology library data. The experiments were conducted in three replicates.

Results and Discussion

Therapeutic and medicinal properties of propolis are strongly linked to their chemical composition, which mandates research on diversity of its bioactive constituents (Campos *et al.,* 2015). Now-a-days identification of chemical components in propolis has become necessary to guarantee future research so that propolis can be utilized for commercial purposes. With its rising popularity around the globe and due to its pivotal role in promoting health, propolis has recently been employed by producers, distributors and consumers in the global market,

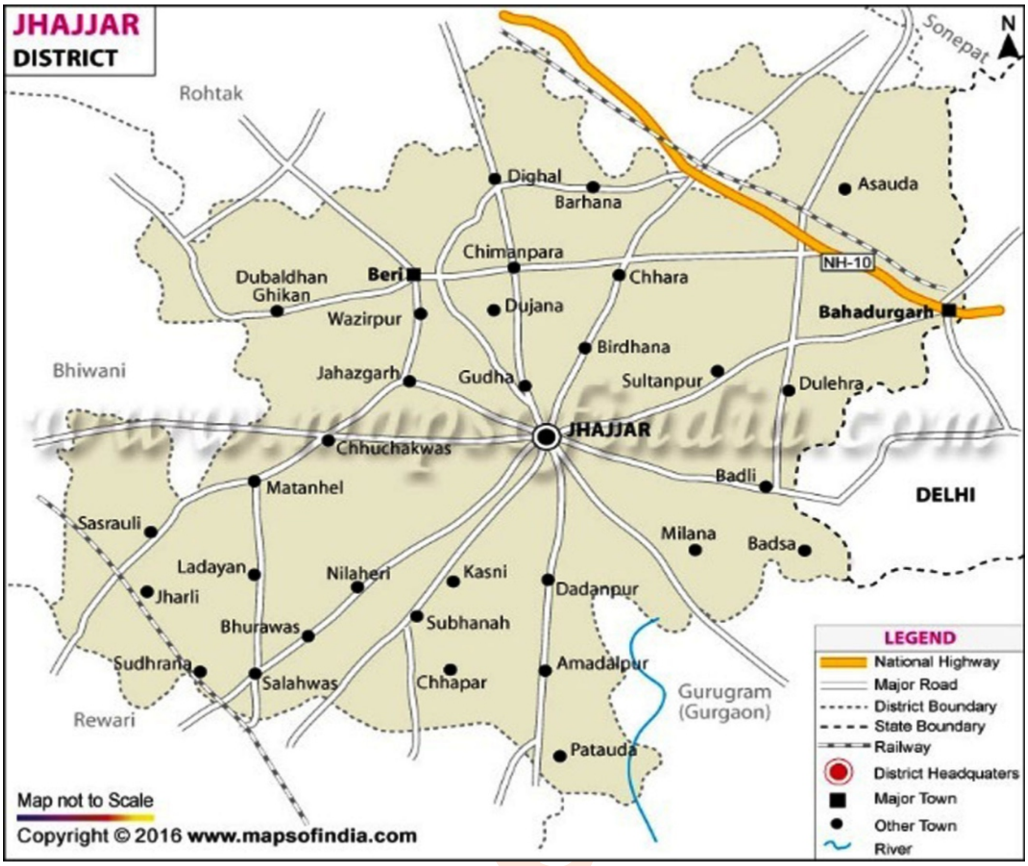

Fig. 1: Map of study area.

particularly in the medical field, food industry and cosmetic industry (Stan *et al.,* 2011; Bozkus *et al.,* 2021; Smriti *et al.*, 2024).

In the present study, GC-MS analysis of ethanolic extract of propolis showed the presence of 21 chemical compounds with quality > 60% which belonged to the class of hydrocarbons, fatty acids, sugars, carboxylic acid, alcohols, ethers, carbamate ester and other organic compounds (Table 1, 2). One of the main component found in propolis was hydrocarbons, including 1,2-Bis (methyl silyl) ethane at RT value ranging between 3.196-10.527 and ethane, 1- isothiocyanato-2-methoxy at RT value ranging between 1.179-10.984. This result corroborates with the previous studies which revealed the presence of hydrocarbons (alkanes, alkenes, alkyne), monoesters, diesters and aromatic esters, fatty acids, and steroids in propolis from various geographical regions (Popova *et al.,* 2011; Ahangari *et al.,* 2018). In this study, the presence of isothiocyanates was also reported that are naturally occurring tiny molecules formed by glucosinolate precursors of cruciferous vegetables. Other than this, Silane methylenebis [methyl- (at RT values1.822, 3.606 and 11.840), methylpropylsilane (at RT value 4.741) and Silane, [(methylsilyl) methyl](silyl methyl) at RT value 2.465 and11.473 were also identified. The use of silane in propolis as a bio-friendly hydrophobic agent has been shown to improve wood protection. The presence of silane in ethanolic extract of propolis is also supported by the results of Salleh *et al.* (2021). Silane found in propolis extracts works well as an eco-friendly hydrophobic agent to protect wood (Woźniak *et al.,* 2018).

GC-MS analysis of the test samples revealed that sugars are not typically major components of propolis; small amounts of sugars can be present due to the collection and processing of plant materials by bees. During the collection and processing of plant resins by honeybees, enzymatic activity can lead to the breakdown of complex polysaccharides present in the resins, resulting in the release of monosaccharides like sugars. Beta-D-Ribopyranoside, methyl,cyclic 2, 3, 4 phosphorothioate (RT value ranging between 6.144-11.083) and Beta,-D-Glucosyl oxyazoxy methane (RT value 4.386 and 8.912) were sugars present in the samples that were derivatives of ribose and glucose which are common type of sugars usually found in propolis and their primary role is to provide nutrition and energy to the developing brood (Kartal *et al.,* 2002; Xu *et al.,* 2020). This result is also in line with the study of Abdullah *et al.* (2019), who revealed that ethanolic extract of propolis usually contains a low amount of carbohydrate making up 0.17-0.48% of the total chemical constituents and might change depending on the bee floral preferences, seasonality as well as availability around their hives. Lim *et al.* (2021) also noted that stingless bee honey samples obtained

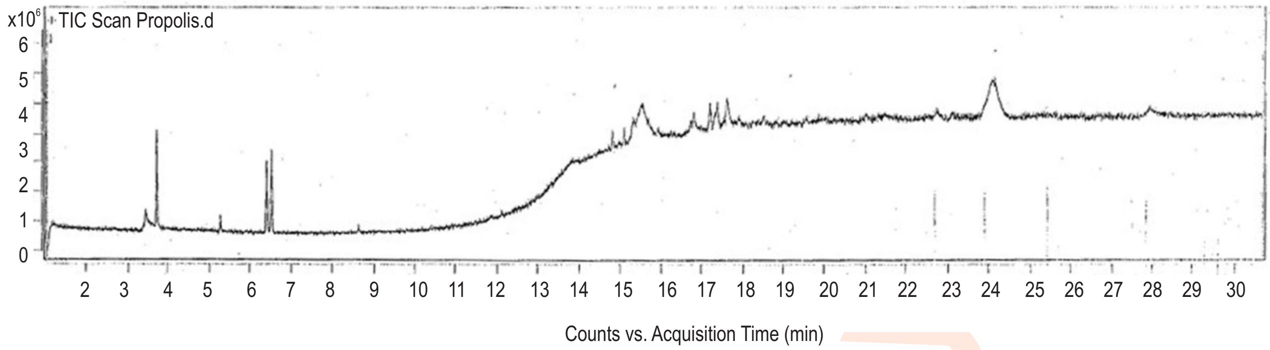

Fig. 2: Chromatographic analysis of ethanolic extract of propolis

Table 1: Biochemical compounds identified in the ethanolic extract of *Apis mellifera* propolis

Compounds	Classification	Formula	RT
Ethane, 1- isothiocyanato-2-methoxy	Hydrocarbon	C_4H_7NOS	1.179
Silanemethylenebis[methyl-	Silane	$C_3H1_2Si_2$	1.822
Silane,[(methylsilyl)methyl](silyl methyl)	Silane	$C_3H_{14}Si_3$	2.465
1,2-Bis(methylsilyl)ethane	Hydrocarbon	$C_4H_{14}Si_2$	3.196
1-Butaneboronic acid	Boronic acid	$C_4H_{11}BO_2$	3.333
1,2-Bis(methylsilyl)ethane	Hydrocarbon	$C_4H_{14}Si_2$	3.449
Silanemethylenebis[methyl-	Silane	$C_3H_{12}Si_2$	3.606
Dimethyl ether	Ether	C_2H_6O	3.711
beta.-D-Glucosyloxyazoxymethane	Sugar Derivative	$C_8H_{16}N_20_7$	4.386
Methylpropylsilane	Silane	$C_4H_{12}Si$	4.741
3-Azonia-5-hexene-1-ol, N,N-dimethyl-carbamateester,bromide	Alcohol, Carbamate ester	$C_8H_{17}N_2O_2$	5.294
1-Butaneboronic acid	Boronic acid	$C_4H_{11}BO_2$	5.888
beta-D-Ribopyranoside,methyl,cyclic 2,3,4 phosphorothioate	Sugar Derivative	$C_6H_9O_5PS$	6.144
2,4-Pentanedione,ion(1-),lithium	Organometallic compound	$C_5H_7LiO_2$	6.415
1,2-Oxaborolane,2-ethyl-4,5-dimethyl-	Boronic compound	$C_7H_{15}BO$	6.534
Acetic acid,2-[(1-methyl-1H-1,2,3,4tetrazole-5-yl) thio]	Carboxylic acid derivative	$C_4H_6N_4O_2S$	7.486
Thiepane, 1-oxide	Cyclic ether derivative	$C_6H_{12}OS$	8.644
beta.-D-Glucosyloxyazoxymethane	Sugar Derivative	$C_8H_{16}N_20_7$	8.912
1,3,2-Dioxaphosphorinane-2-methanol, 2oxo.alpha.-phenyl	Alcohol derivative with a carbonyl and a phenyl group	$C_{10}H_{13}0_4P$	9.814
1,2-Bis(methylsilyl)ethane	Hydrocarbon	$C_4H_{14}Si_2$	10.187
Ethane, 1- isothiocyanato-2-methoxy	Hydrocarbon	C_4H_7NOS	10.455
1,2-Bis(methylsilyl)ethane	Hydrocarbon	$C_4H_{14}Si_2$	10.527
Trimethylsilane	organosilicon compound	$C_3H_{12}Si_2$	10.917
Ethane, 1- isothiocyanato-2-methoxy	Hydrocarbon	C_4H_7NOS	10.984
beta-D-Ribopyranoside,methyl,cyclic 2,3,4 phosphorothioate	Sugar Derivative	$C_6H_9O_5PS$	11.083
Silane, [(methylsilyl)methyl](silyl methyl)	Silane	$C_3H_{14}Si_3$	11.473
Silanemethylenebis[methyl-	Silane	$C_3H_{12}Si_2$	11.840
1,3,4-Trimethoxy-butan-2-ol	Alcohol	$C_7H_{16}O_4$	17.382
2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol	Alcohol	$C_{16}H_{34}O_9$	19.569
3,6,9,12-Tetraoxatetradecan-1-ol,TBDMS derivative	Alcohol	$C_{15}H_{34}O_5Si$	28.616
15-Crown-5	Crown ether	$C_{10}H_{20}O_5$	29.073
Pentaethylene Glycol	Alcohol	$C_{10}H_{22}O_6$	30.014

Table 2: Major compounds observed through GCMS analysis

Hydrocarbons	1,2-Bis (methyl silyl) ethane Ethane, 1- isothiocyanato-2-methoxy
Sugars	beta-D-Ribopyranoside,methyl,cyclic 2,3,4phosphorothioate Beta,-D-Glucosyloxyazoxymethane
Silane	Methylpropylsilane Silane, [(methylsilyl)methyl](silyl methyl) Silanemethylenebis[methyl-
Acids	Acetic acid,2-[(1-methyl-1H-1,2,3,4-tetrazole-5- yl)thio] 1-Butaneboronic acid
Carbamate esters	3-Azonia-5-hexene-1-ol,N,N-dimethyl-,carbamate ester, bromide
Alcohols	3-Azonia-5-hexene-1-ol,N,N-dimethyl-,carbamate ester,bromide
	1,3,2-Dioxaphosphorinane-2-methanol,2-oxo.alpha.-phenyl
	1,3,4-Trimethoxy-butan-2-ol
	3,6,9,12-Tetraoxatetradecan-1-ol,TBDMS derivative
	Pentaethylene Glycol 2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol
Ethers	Dimethyl ether
	Thiepane, 1-oxide
	15-Crown-5

from various botanical sources located in the southern region of Peninsular Malaysia contained about 68.33 to 72.25 g 100 g^{-1} of total carbohydrate. Besides, it has also been reported that total carbohydrate of bee bread or pollen from stingless bees ranged between 25 and 55% (Mohammad *et al.,* 2019; Rana, 2021; Khakhlary and Rana, 2023).

In this study, the GC-MS analysis of our propolis sample also showed presence of fatty acids such as Acetic acid,2-[(1-methyl-1H-1,2,3,4-tetrazole-5- yl)thio] (at RT value 7.486), which is the second simplest carboxylic acid after formic acid. It is an important chemical reagent and industrial chemical used primarily in the production of cellulose acetate for photographic films, polyvinyl acetate for wood gluing and synthetic fibres and fabrics. It is also widely used in the food industry, as a preservative, flavouring agent, and pH regulator (Deshmukh *et al.,* 2021). A number of alcohols like 1,3,4-Trimethoxy-butan-2-ol(at RT value 17.382), 3,6,9,12-Tetraoxatetradecan-1-ol, TBDMS derivative (at RT value 28.616), Pentaethylene glycol (at RT value 30.014), 2-[2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy) ethoxy] ethoxy] ethoxy] ethoxy] ethoxy] ethoxy]ethanol (at RT value 19.569) and 1,3,2-Dioxaphosphorinane-2-methanol,2-oxo.alpha.-phenyl (at RT value 9.814) were also identified. Apart from these, few ethers such as dimethyl ether (at RT value 3.711), 15-Crown-5 (at RT value 29.073) and Thiepane, 1-oxide (at RT value 8.644) were also found in the test samples along with some carbamate esters (3-Azonia-5-hexene-1-ol,N,N-dimethyl-,carbamate ester, bromide at RT value 5.924) and minerals. The chemical components found in previous studies on propolis from various regions demonstrate variability in the findings since its composition varied based on factors such as source, origin and storage conditions (Alvear *et al*., 2021).

It can be concluded from this study that ethanolic extract of *A. mellifera* propolis contains a significant amount of diverse of biochemical compounds, including hydrocarbons, sugars, silane, acids, carbamate esters, alcohols and ethers that can be used as natural products against several ailments and can be utilized to improve the quality of life. It is hopeful that this study will be able to encourage further investigations and studies on the biochemical constituents of propolis in order to enhance the understanding and applications of propolis in near future.

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