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PHYTOCHEMICAL PROFILING OF CRUDE PROPOLIS COLLECTED FROM BEEHIVES

Zoology

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ABSTRACT

Propolis is well known for its valuable biological activities widely used in the treatment of many diseases and beneficial effects on human. It is rich in chemical compounds which depend on the type of plant accessible to the bees. In the present study, the chemical composition of propolis ethanol extract were analysed using GCMS where 10 active phyto compounds were identified and characterized by the presence of significant amount of lipids (2.44 µg/g) and alkaloids (1.53 µg/g). In addition, compounds such as alkaloids, phenols, tannins, saponins, carbohydrates, lipids and protein were quantified. According to chemical composition of propolis, the propolis ethanol extract is a promising source of new bioactive compounds, which need identification of compounds responsible for the biological activity.

KEYWORDS

Propolis, Phytochemical Constituents And Gc-ms

INTRODUCTION

Propolis is a honeybee product with a broad spectrum of biological properties [1]. As a resinous substance, propolis is prepared by the honeybees to seal the cracks, smooth walls, and to keep moisture and temperature stable in the hive all year around. Raw propolis is typically composed of 50% plant resins, 30% waxes, 10% essential and aromatic oils, 5% pollens and 5% other organic substances. It has been reported that propolis is collected from resins of poplars, conifers, birch, pine, alder, willow, palm, *Baccharis dracunculifolia*, and *Dalbergia ecastaphyllum* [2-4].

Propolis is widely used to prevent and treat colds, wounds and ulcers, rheumatism, sprains, heart disease, diabetes [5-8] and dental caries [9] due to its diverse biological properties such as anti-inflammatory [8,10-12], antimicrobial, antioxidant, antitumor [3], antiulcer and anti-HIV activities [13]. The wide application of propolis in modern medicine has drawn growing attention to its chemical composition. Many studies have revealed that the observed effects might be the result of synergistic action of its complex constituents [14-16].

As the major constituents of propolis, flavonoids contribute greatly to the pharmacological activities of propolis. The quantity of flavonoids is used as a criterion to evaluate the quality of temperate propolis [17]. Flavonoids have a broad spectrum of biological properties, such as antibacterial, antiviral and anti-inflammatory effects [16,18]. In view of this it was thought it would be worthwhile to isolate, purify and screen and quantify the phytochemical contain of Propolis which would throw more light on its medicinal properties.

MATERIALS AND METHODS

Collection and Extraction of Propolis

Bee hives were collected from Udankudi, Tamil Nadu, India, and the collected honey combs were throughly washed with running tap water and distilled water to remove the debris and then shade dried for 10 days. The dried hives were then powdered using kitchen blender and taken for further analysis. About 20 g of powdered honey comb was soaked in 200 ml of ethanol and kept in the orbital shaker for three days and the extracts was filtered through Whatman filter paper (No.41). The filtrate was then stored in refrigerator for further study.

GC-MS Spectral Analysis

Propolis ethanol extract was taken for analysis by GC-MS technique. Before analyzing the extract using GC-MS, the temperature of the oven, the flow rate of the gas used and energy of the electron gun was programmed initially. The temperature of the oven was 100˚C. The gas used as a carrier as well as elute was helium. The flow rate of helium was set to 1ml/min. The column employed here for the separation of components was Elite 1 (100% dimethyl polysiloxane). About 2 µl of the honey comb extract was injected into the GC-MS using a micro syringe and scanning was done for 36 min. A chromatogram was obtained with retention time in the abscissa and percentage of the peak in the ordinate. Also from the mass spectrum data, the compounds present in the extract were identified.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of of propolis ethanol extract was done according to the method of Manimekalai *et al*. (2016) [19].

Quantitative Phytochemical Screening

Quantitative phytochemical screening of alkaloids, saponins, phenolics, tannins, carbohydrates and lipids was done according to the method of Van Handel (1985) [20], Chang *et al*. (2002) [21], Cunha *et al*. (2004) [22] and Yen *et al*. (2008) [23].

RESULTS AND DISCUSSION

The present study demonstrates the composition of ethanol extract of propolis by GC-MS analysis. A total of 10 major compounds belonging to different chemical groups have been identified and listed in Table 1 and Fig. 1. The result revealed that the ethanol extract of propolis was characterized by the presence of aromatic amino, plasticizer, fatty acid ester, alcohol, bromo, alkane and cyclohexane compounds.

Table 2 summarizes the qualitative and quantitative estimation of ethanolic extract of propolis in which alkaloids, phenols, tannins, saponins, carbohydrates, lipids and proteins were present. The ethanol extract showed high concentration of lipids (2.44 µg/g) and alkaloids (1.53µg/g) whereas, phenol, tannins, saponins, carbohydrates and proteins showed moderate concentration as shown in the Table 2.

Raymond *et al*. (2010) [24] stated that alkaloids are naturally occurring chemical compounds and have a wide range of pharmacological activities including anti-malaria, anti-asthma, anticancer, analgesic and anti-bacterial potential and are found to be used in traditional and modern medicine. According to Fortmann *et al*. (2013) [25] Indian propolis contains aminoacids, ketones, volatile oils and vitamins

(ascorbic acid and coumatetralylin isomers) they are beneficial in treating cold, heart diseases and strengthening immune systems in human beings.

Cowan [26] reported that the propolis is rich in various types of secondary metabolites such as phenolic compounds, tannins, terpenoids, alkaloids and flavonoids, which contain antimicrobial properties.

The most popular species of bee is the European Bee, Apis Melifera. It has been shown that the varieties of bee affect the antibacterial activity of the propolis collected from the same episode; a. *Melifera carnica* hives showed weak antibacterial activity compared to *A. Melifera* anatolica and *A. melifera* cocaine. Three bee races used neither one nor one plant source [27]. Another type of propolis, geologic, produced by uninterrupted bee species, *Melipona scutellaris*, benzephonones, but no flavonoids have been identified as major compounds [28]; However, geopropolis produced by *Melipona fasiclet* contains high concentrations of polyphenols, flavonoids, triterenoids, saponin and even tannins [29].

Propolis has received the attention of current researchers due to its diverse chemical constituents, pharmacological activities and low toxicity. From the result it is concluded that, propolis ethanolic extract have significantly high amount of aromatic compounds and their derivatives followed by alkaloids, phenols, tannins, saponins, carbohydrates, lipids and protein constituents. Thus, propolis ethanolic extract being a rich source of natural compounds may determine the best application as a promising natural medicine to prevent various diseases and can be used for different pharmacological applications.

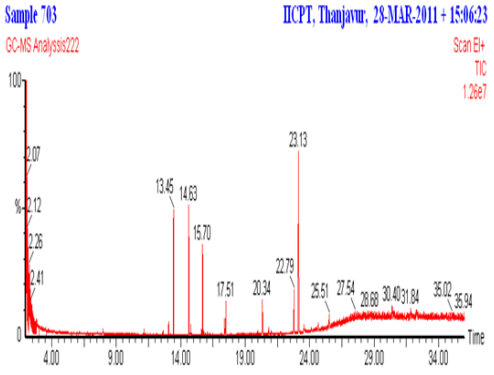

Fig. 1: GC-MS chromatogram of propolis ethanol extract

Table 1: GC-MS spectral analysis of propolis ethanolic extract

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Nature of compound	Activity
1.	7.97	Desmethyldeprenyl	C12H15N	173	0.45	Aromatic amino compound	Antimicrobial
2.	13.07	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	C18H24O4	304	0.45	Plasticizer compound	Antimicrobial Antifouling
3.	13.45	Undecanoic acid, 2-methyl-, methyl ester	C13H26O2	214	63.39	Fatty acid ester	No activity reported
4.	14.63	1-Heptadecanol	C17H36O	256	7.59	Alcoholic compound	Antimicrobial
5.	15.70	2-Octyn-1-ol	C8H14O	126	4.91	Alcoholic compound	Antimicrobial
6.	17.51	2-Bromononane	C9H19Br	206	1.79	Bromo compound	Antimicrobial
7.	20.34	Dodecane, 2,6,10-trimethyl-	C15H32	212	2.23	Alkane compound	No activity reported
8.	22.79	5-Eicosene, (E)-	C20H40	280	3.13	Alkene compound	No activity reported
9.	23.13	Tridecanol, 2-ethyl-2-methyl-	C16H34O	242	15.18	Alcoholic compound	Antimicrobial
10.	25.51	Cyclohexane, 1,1'-(2-methyl-1,3-propanediyl)bis-	C16H30	222	0.89	Cyclohexane compound	No activity reported

Table 2: Qualitative and quantitative Phytochemical screening of propolis ethanolic extract

S.No	Phytochemicals	Presence(+)/Absence(-)	Quantity (µg/g)
1	Alkaloid	+	1.53 ± 0.02
2	Phenols	+	0.056±0.005
3	Tannins	+	0.05±0.001
4	Cardiac Glycosides	-	-
5	Flavonoids	-	-
6	Saponins	+	0.35±0.0025
7	Carbohydrates	+	0.375±0.0012
8	Lipids	+	2.44±0.002
9	Proteins	+	0.44±0.0025

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