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Original Article

Comparative study of Bee Propolis from different Geographical location

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ABSTRACT

Cancer is now one of the leading causes of death in developing countries, killing more people globally each year than any other. By 2020 there are expected to be 15 million new cases of cancer every year globally, 70% of which will be in developing countries. International Status: It is estimated that about 9 million new cancer cases are diagnosed every year and over 4.5 million people die from cancer each year in the world. National Status: The estimated number of new cancers in India per year is about 7 lakhs and over 3.5 lakhs people die of cancer each year. Bangalore tops the list of metros in the country with the highest number of women suffering from cancer and breast cancer continues to be the killer, according to the Population Based Cancer Registry (PBCR). Propolis, a bee hive product rich in natural flavonoids can be used as a drug for cancer. A lot of research is being carried out worldwide directed toward finding natural antioxidants and antitumor activity in propolis. Methanolic extract of Indian Propolis was screened for flavonoid content responsible for antitumor activity. Materials and Methods-Aluminum chloride method for total flavonoid content and GCMS for Quantification of flavonoids. Conclusions: Our findings provide evidence that methanolic extract of propolis is a potential source of Lupeol which can be isolated and used for cancer therapy.

Key words: Propolis, Flavonoids, GCMS

INTRODUCTION

Bees in the hive produce unique most complete natural food and protection against illness. There is a growing interest in beehive products in Nutrition, Medicine and Cosmetics. The products of bee hive are honey, pollen, royal jelly, propolis, beeswax, bee-poison etc. Propolis is a wax-like resinous substance collected by honey bees from tree buds or other botanical sources that is used as cement and to seal cracks or open spaces in the hive creating an antiseptic environment.

Propolis is a complex resinous material produced by honeybees from plant exudates, beeswax, and bee secretions [1] and is responsible for the sterility of honeycombs [2]. The chemical composition of Apis mellifera propolis and its wide spectrum of biological activities (hepato-protective, anti-tumour, anti-oxidative, anti-microbial, and anti-inflammatory properties) have attracted the attention of researchers [3] Propolis is another medicinal marvel from the beehive [4]. The chemical composition of propolis varies depending on the season, bee species and geographic location [5]. More than 160 compounds have been identified [6]. Colors range from golden brown to brownish, green to reddish brown to blackish brown. A broad analysis reveals approximately 50% resinous compounds and balsam, 30% beeswax, 10% ethereal and aromatic oils, 5% bee pollen, 5% various other organic and inorganic compounds .The chemical composition of propolis includes flavonoids, aromatic acids, esters, aldehydes, ketones, fatty acids, terpenes, steroids, amino acids, polysaccharides, hydrocarbon, alcohols, hydroxybenezene and several other compounds in trace amounts [7-8].

Flavonoids, phenolic acids and their esters are major active constituents .The flavonoids in propolis are considered to be responsible for its inhibitory effect. Flavonoids, one of the main groups of phenolic compounds in propolis, are the key compounds for estimation of propolis quality. Flavonoids in propolis are aglycones (without sugar component). The concentration of flavonoids in propolis depends on the geographic origin and eco-system (plant sources) [9]. Flavonoids play a role in the destruction of infecting organisms, they strongly affect the connective tissue [10]. Triterpenoids including β-amyrin, β-amyrone,

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lupeol, and lupenone, and polyprenyl benzophenones such as 7-epi-nemorosone, 7-epi-clusianone, xanthochymol, and gambogenone have been detected in propolis samples from the Brazilian Amazon [11]. Lupeol and some related compounds have demonstrated antitumor activities

Because of the phytogeographic dependence of the flavonoid content in raw propolis, our aim was to determine the concentration of flavonoids in raw propolis samples collected from six different localities of South India and Quantify Flavonoids by GCMS to be used as a source of potential drug for cancer

MATERIALS AND METHODS

Collection of sample: Samples of propolis produced by Apis mellifera were obtained from. from Apiry centers in Karnataka and Tamilnadu. The collected propolis samples were kept desiccated in the dark until analysis. The appearance, form, color and smell of collected raw propolis samples are described in Table I. Propolis extract (PEE) was prepared as follows. One gram of raw propolis was extracted with 25 mL of 95% ethanol (V/V) for 24 h at 37 °C and the filtrate was adjusted to25 mL with 80% ethanol (V/V).

Total Flavonoid Contents: Total flavonoid contents were determined by the aluminum chloride method. Calibration curves were made using quercetin (aluminium chloride method) as reference. Analyses were performed in triplicates.

Quantification of Flavonoids by GCMS: GC-MS analysis of various crude organic extracts of propolis was performed on a PerkinElmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m×0.25 mm inner diameter, ×0.25 μm film thickness; maximum temperature, 350 °C), coupled to a Perkin Elmer Clarus 600C MS. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 60 °C (hold for 2 min) to 280 °C at a rate of 3 °C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 μL) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The percentage composition of the crude extract constituents was expressed as a percentage by peak area.

The identification and characterization of chemical compounds in various crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries. A lot of known flavonoid constituents were identified, however, several high molecular weight compounds including lupeol alkanoates were identified.

RESULTS

Six samples of raw propolis were collected from hives situated on 6 different locations in Karnataka and Tamil Nadu. The samples studied varied in consistency, colour and smell (Table I). Geographic position of the hives did not influence the physical characteristics of propolis. Appearance and form did not influence the colour and smell of propolis samples. The chemical formula of Lupeol is $C_{30}H_{50}O$ and its melting point is 215–216 °C. Properties computed from the structure of Lupeol show that it has a molecular weight of 426.7174 [g/mol]

Table 1: Physical characteristics and flavonoid concentration in raw propolis sample from South India

Sample No	Locality	Appearance	Color	Total Flavonoids Mg/ml	Lupeol
Sample-1	Bangalore	Waxy	Brown	3.4	Present
Sample-2	Mysore	Solid	Dark Brown	1.4	Absent
Sample-3	Coorg	Waxy	Black	2.32	Present
Sample-4	Chennai	Gummy	Brown	1.2	Absent
Sample-5	Madurai	Solid	Brown	0.73	Absent
Sampl;e-6	Coimbatore	Waxy	Yellowish Brown	2.61	Present

Table 2: GC-MS Summary report

Sl.No	Sample Details	Major peak details		Compound match with NIST library
		Retention time	% Area	
1	S1	Four major peaks RT 56.209	44.33	**Major peak details** **Compound match with NIST library**
2	S1	54.881	17.63	4, 4, 6a, 6b, 8a, 11, 11,14-b octamethyl-1,4, 4a
3	S1	59.393	13.41	URS-12-ene
4	S1	55.543	8.20	Lupeol

Table 3: GC-MS Summary report

Sl.No	Sample Details	Major peak details		Compound match with NIST library
		Retention time	% Area	
1	S3	Four major peaks RT 55.569	53.65	6 beta bicycle(4.3.0)nonane
2	S3	54.256	14.37	4, 4, 6a, 6b, 8a, 11, 11,14-b octamethyl-1,4, 4a, 5
3	S3	53.834	7.48	9, 19-cycloergost-24(28)en-3-ol
4	S3	55.972	7.28	9, 19-cycloanost-24(28)-en-3-ol, acetate

Table 4: GC-MS Summary report

Sl.No	Sample Details	Major peak details		Compound match with NIST library
		Retention time	% Area	
1	S6	Five major peaks RT 34.653	26.10	1-methyl -1-(3-phenylprop-2-enyl)oxy-1-
2	S6	56.177	9.13	Lupeol
3	S6	37.240	8.75	6-[1-[4-methylphenyl]ethyl]-1,3-
4	S6	43.395	8.75	Eicosane, 7—hexyl-
5	S6	41.109	8.03	4H-1-Benzopyran-4-one, 2,3-dihydro-5,7

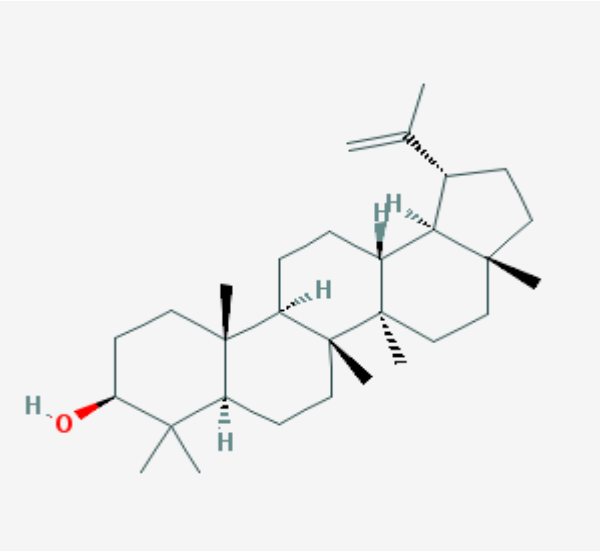

Figure 1.Chemical Structure of Lupeol

DISCUSSION

The total flavonoid content in Sample -1, Sample-3 and Sample-6 was found to be high ranging between 2.32mg/ml to 3.4mg/ml. The highest concentration of flavonoid was found in Sample-1 from Bangalore with flavonoid concentration of 3.4mg/ml, Sample-6 from Coimbatore with the flavonoid concentration of 2.61mg/ml and Sample-3 from Coorg with 2.32mg/ml. Sample2, Sample 4 and Sample 5 showed comparatively low flavonoid content ranging between 0.73 to 1.23mg/ml. GCMS results revealed that samples with slightly high concentration of flavonoid contain Lupeol. Samples with low concentration of flavonoids the Lupeol was altogether absent

Comparison of the concentrations of flavonoids and Lupeol showed that there were significant differences in Flavonoid concentration in different samples between different cities of Karnataka and Tamil Nadu, but a marked difference in Lupeol concentration. The high concenteration of flavonoids was seen in Bangalore, Coorg and Coimbatore. The presence Lupeol content was directly proportional to the concentration of flavonoid. High Flavonoid concentration led us to conclude that this will affect the biological activity.

CONCLUSION

Our results showed varied concentrations of flavonoids. Significant differences in the concentration of flavonoids between localities and differences in specific groups of flavonoids will affect biological activities and our future research will be focused on investigating the influence of concentration of the flavonoids, on the anticancerous activity. The floral diversity on which the bees foraged provided a predominance of flavonoids mainly lupeol in propolis in the samples analyzed. Propolis Sample with high concentration of flavonoids can be targeted as a potential drug. The Flavonoid concenteration in propolis is very important drug for biological activity. Lupeol a potiential compound for antitumor activity can be isolated and used by the Pharamaceutical Industry as a promising drug for Cancer.

Future scope- The bioassay guided isolation of lupeol is required for future research.

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