ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF PROPOLIS

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ABSTRACT

BACKGROUND

In the present scenario of increasing bacterial drug resistance where the selection of antibiotics available becomes a limiting factor, botanicals (natural products) with antioxidant properties which can stimulate the immune system to kill the pathogen are considered best for oral diseases. Propolis is one such natural product which exhibits a broad spectrum of biological and pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, antitumor, anticancer, antiulcer, hepatoprotective, cardioprotective, and neuroprotective actions. The chemical composition and beneficial properties of propolis vary greatly depending on the phytogeographical areas, seasonal collection time, and botanical source. Very few studies on propolis are reported from Karnataka. This invitro study therefore aimed to evaluate efficacy of propolis against oral microorganisms. Propolis used in the study is procured from Sullia, Dhakshina Kannada district, Karnataka state, India.

Aims and Objectives- This invitro study evaluates the antioxidant and antimicrobial activity of water extract propolis procured from Sullia taluk, Dhakshina Kannada district, Karnataka state, India. It also determines the total phenolic and total flavonoid contents of the sample responsible for these properties.

MATERIALS AND METHODS

Propolis was extracted using distilled water by maceration and refluxing method. Total phenolic and flavonoid contents were determined by using Folin-Ciocalteu spectrophotometric method and aluminium chloride colorimetric method respectively. Antibacterial activity was determined by using standard agar disc diffusion method against four oral microbes (*S. aureus*, *S. mutans*, *A. actinomycetemcomitans*, *Candida albicans*).

RESULTS

Total phenolic content & total flavonoid content of pure water extract sample was 18 mg/g of gallic acid equivalent (GAE) and 32.86 mg/g of quercetin equivalent (QE) respectively. The sample showed significant antimicrobial activity against tested microbes.

CONCLUSION

Propolis collected from bee hive of *Apis mellifera* at Sullia taluk, Dhakshina Kannada District, Karnataka State, India is a potential natural antioxidant source and is a promising antimicrobial drug for various bacterial infections related to dental diseases.

KEY WORDS

Propolis, *Apis mellifera*, Karnataka, Phenolic Content, Flavonoids Content, Antioxidant Activity, Antimicrobial Activity.

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BACKGROUND

Microorganisms that are present in the dental plaque are an important aetiological factor for the development of most of the oral diseases. However, a variety of chemical means, like

antibiotics and antiseptics, have been introduced as an adjunct to mechanical therapy for control of microorganism in dental plaque.^{1,2} The administration of antimicrobial agents as a quick, with least side effect and inexpensive means of supporting mechanical periodontal debridement is therefore need of the hour.³ Bee products, like honey and propolis, have a great potential to be used as adjuncts to mechanical periodontal treatment and infection control since they include the hyperosmolarity effect (>80% sugar content), acidic pH, hydrogen peroxide, methylglyoxal, bee defensin-1, various proteinaceous compounds, flavonoids and phenolic compounds.^{4,5,6}

Propolis is a resinous honeybee product collected from plants and mixed with the enzyme, beta-glycosidase, present in the bee's saliva, partially digested and added to beeswax to

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form the final product, which is used to cover hive walls and fill gaps. Bees collect the resin-like product from cracks in the bark of trees and leaf buds.^{7,8} Thereby, propolis does not only act as a structural compound, but is mainly responsible as a chemical agent for the safety of honeycombs, especially against microorganisms.^{9,10,11} The chemical composition of propolis is highly variable and depends on the local flora at the site of collection and on the season of collection.^{12,13,14-17}

WHO supports the idea of integrating conventional and complementary practice in order to reach the best result for patients and society. Hence the development of natural form of therapies for the treatment of diseases of the oral cavity is of great relevance. However, evaluation on the antimicrobial effects of propolis on bacterial species relevant to oral diseases and dentistry is still lacking. Very few studies are reported from Karnataka. This invitro study therefore aimed to evaluate efficacy of propolis against four microorganisms. The propolis used in the study is procured from Sullia, Dhakshina Kannada district, Karnataka state, India.

MATERIALS AND METHODS

Chemicals and Instruments

This is invitro study all the chemicals and reagents used were of analytical grade. Aluminium trichloride (AlCl₃), Folin Ciocalteu's phenol reagent, sodium carbonate (Na₂CO₃), gallic acid, DPPH (2, 2-diphenyl-1-picryl hydrazyl) and ascorbic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). UV-visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu) was used for absorbance measurements.

Sample Collection

Sample of propolis was collected from the region of Sullia, Dhakshina Kannada district, Karnataka state, India. The sample was kept in a freezer so that propolis could be handled easily. 15 gms. of Propolis was cut in to small pieces and was grounded well. It was dissolved in 150 ml (1:10) of a distilled water. The solution was macerated for 24 hrs. and refluxed for 2 hrs. and then filtered through Whatman 41 filter paper twice. It was then evaporated to dryness on the water bath. 1 mg/ml stock solution was prepared. This stock solution was used for analysing the total phenolic content, total flavonoid content and antimicrobial activity.

Determination of Total Phenolic Content

A total phenolic content of extract was determined according to the method of Kujala¹³ with minor modifications, using gallic acid as standard (0-100 µg). 1 ml of extracts was taken, and volume was made up to 1 ml with distilled water. To this 1ml of FC reagent was added and the mixture was allowed to stand for 10 minutes followed by the addition of 2 ml of 8% sodium carbonate. After 10 minutes of incubation at ambient temperature the absorbance of supernatant was measured at 730 nm in visible UV-Spectrophotometer. Gallic acid was used as standard to produce the calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of Gallic acid equivalents (GAE/100 g) of propolis.

Determination of Total Flavonoid Content

The Aluminium chloride colorimetric method was modified from the procedure reported by Woisky and Salatino.¹⁷ Standard calibration curve was prepared using Quercetin. 10 mg of Quercetin was dissolved in 80% ethanol, different

aliquots of Quercetin (0-100 µg) and 100 µl of extracts were taken and was made up to 1 ml with 80% water. To this 10% of Aluminium chloride, 0.1 ml of Potassium acetate and 2.8 ml of distilled water were added and allowed to stand for 30 minutes at room temperature and the absorbance was measured at 415 nm using UV-spectrophotometer. The total flavonoid content was calculated according to the standard Quercetin calibration curve. The mean of three readings was used and expressed as mg of Quercetin equivalents (QE)/100 g of propolis.

Antimicrobial Activity Test

A total of 4 microorganism *S. aureus* (ATCC No 12598), *S. Mutans* (ATCC No 25175), *A. actinomycetemcomitans* (ATCC No 43718), *Candida albicans* (ATCC No 2091) were tested. Agar disc diffusion method of sensitivity susceptibility test was performed according to the National committee for clinical laboratory standards (NCCLS).¹⁸

RESULTS

The percentage yield of dry propolis extract procured from Sullia, Dhakshina Kannada district, Karnataka state, India was 6.69% W/W.

Place	Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)
Sullia (Pure Water Extract)	18.6 mg/ ml of Gallic Acid Equivalent	32.86 mg/g of Quercetin Equivalent

Table 1. Total Phenolic Content & Total Flavonoid Content of Propolis From Sullia, Dhakshina Kannada District, Karnataka State, India.

Total phenolic content & total flavonoid content of the water extract sample was 18 mg/g of Gallic acid equivalent (GAE) and 32.86 mg/ml of Quercetin equivalent (QE) respectively.

Microorganism	1mg	0.5mg	0.25mg	0.12mg	0.062mg
<i>S. aureus</i>	25 mm	24 mm	20 mm	18 mm	18 mm
<i>S. mutans</i>	12 mm	R	R	R	R
<i>Aa</i>	12 mm	10 mm	R	R	R
<i>C. albicans</i>	23 mm	20 mm	18 mm	17 mm	16 mm

Table 2. Susceptibility of Microorganism Against Propolis and The Diameter of Growth of Zone of Inhibition Values

Among the strains tested, the most sensitive was *S. aureus* followed by *Candida* then *S. mutans* and *A. actinomycetemcomitans*.

DISCUSSION

Antibiotic resistance among microbes urgently necessitates the development of novel antimicrobial agents, especially from safe, harmless natural products.

Propolis is one of the natural bee products. It is known that presence of flavonoid and phenolic acid is responsible for antimicrobial activity of propolis. But these contents vary in samples from different geographic areas and are based on local flora in the region from which propolis was collected. Hence the concentration of these active substances (Flavonoid and phenolic acid) in propolis samples procured from Sullia was evaluated in this study along with antimicrobial activity (Table 1).

Results showed that total flavonoid content was more compared to the total phenolic content. These results are in

agreement with that found by another author¹⁹. A positive correlation was observed between the total phenolic and flavonoid contents of the sample with its free radical scavenging activity. Thus, the propolis extract is a potential natural antioxidant source and can be used to treat diseases associated with oxidative stress.

The studies on antimicrobial activity of propolis have been carried in general and among them very few have investigated its activity towards oral pathogens. There is lack of studies on the antibacterial activity of the propolis extracts from Sullia. We investigated its antimicrobial activity using the agar-disc diffusion method. The antimicrobial susceptibility test (Agar disc diffusion) was measured in term of diameter of inhibitory zones. An inhibitory zone with diameter less than 10 mm corresponded to lack of activity. The entire sample showed inhibitory zone above 10 mm and propolis exhibited the maximum inhibitory zone of 25 mm for *S. aureus* followed by *C. albicans* 23 mm and then *S. mutans* and *Aa*.

In the present work it is evident that oral pathogens are susceptible to the propolis collected from Sullia. And our results are in concern with an in vitro investigation demonstrating the antimicrobial activity of Brazilian propolis against various oral pathogens²⁰. While some authors found propolis sample active only against gram positive bacteria, fungi and less active or no activity over gram negative,²¹ our study on propolis extract from Sullia showed active on both gram positive, gram negative and fungi.

In general, the results of the above study showed that, the propolis from Sullia region of Karnataka was active against oral bacteria. The antibacterial effect towards the entire organism tested increased with increase in the concentration of the sample, at slightly higher concentration level, the natural extract of *Apis mellifera* bee propolis sample can be a very good substitute for synthetic antibiotic as it is quick, with least side effect and inexpensive means in supporting mechanical periodontal debridement and thereby good agent in treating the dental diseases in future.

CONCLUSION

Propolis (Sullia, Dhakshina Kannada District, Karnataka) extracted with water as solvent, had more flavonoid content than phenolic content. The entire sample showed inhibitory zone above 10 mm and propolis exhibited the maximum inhibitory zone of 25 mm for *S. aureus* followed by *C. albicans* 23 mm and then *S. mutans* and *Aa*. Hence propolis from Sullia, Dhakshina Kannada district, Karnataka can be used as a potential natural antioxidant source and as a promising antimicrobial drug for various dental infections.

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