Evaluation of the Cytotoxic and Antioxidant Effects of Propolis and Its Potential Application in Dental Caries Prevention

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ABSTRACT

Background: Propolis is a natural resinous substance produced by honeybees and is known for its diverse biological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. Due to its rich content of phenolic compounds and flavonoids, propolis has attracted considerable attention as a potential therapeutic agent for biomedical and oral healthcare applications. **Objective:** This study aimed to evaluate the antioxidant activity and cytotoxicity of propolis using DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and zebrafish (Danio rerio) embryo toxicity model. **Methods:** Antioxidant activity was assessed at concentrations of 25, 50, 75, and 100 µg/mL using DPPH and hydrogen peroxide scavenging assays, with ascorbic acid serving as the standard reference. Cytotoxicity was evaluated by determining the viability of zebrafish embryos exposed to the same concentrations of propolis. **Results:** The antioxidant activity of propolis increased in a concentration-dependent manner. In the DPPH assay, radical scavenging activity ranged from 20.5% at 25 µg/mL to 57.2% at 100 µg/mL, while hydrogen peroxide scavenging activity increased from 24.5% to 65.6% across the tested concentrations. Although lower than the activity of ascorbic acid, propolis demonstrated considerable antioxidant potential. Cytotoxicity assessment showed a gradual decrease in zebrafish embryo viability from 84.2% at 25 µg/mL to 56.4% at 100 µg/mL, indicating moderate concentration-dependent toxicity. **Conclusion:** Propolis exhibited significant antioxidant activity and moderate cytotoxic effects in a concentration-dependent manner. The findings suggest that propolis contains bioactive compounds capable of scavenging free radicals while maintaining acceptable biological safety within the tested concentration range. These properties support its potential application as a natural antioxidant and a promising adjunct in oral healthcare and dental caries prevention. Further studies are recommended to identify the active constituents and investigate their antimicrobial efficacy against cariogenic microorganisms.

Keywords: Propolis, antioxidant activity, cytotoxicity, zebrafish embryo, DPPH assay, hydrogen peroxide scavenging, dental caries.

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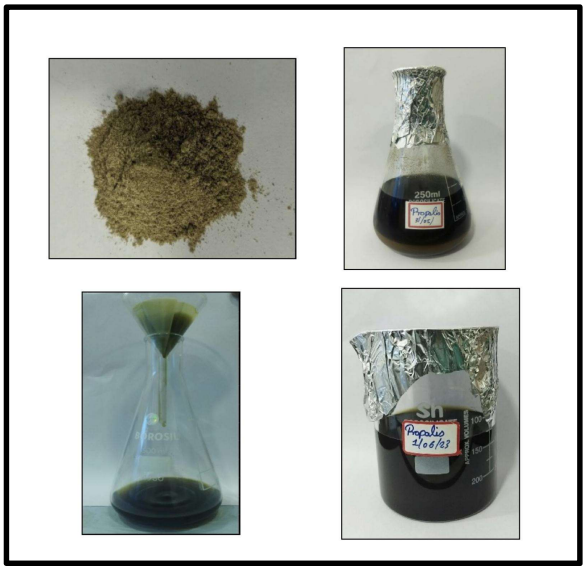
INTRODUCTION:

Natural products have long been recognized as valuable sources of therapeutic agents due to their diverse biological activities and relatively low toxicity. Among these natural products, propolis has gained considerable scientific interest because of its broad spectrum of pharmacological properties. Propolis is a resinous material produced by honeybees (*Apis mellifera*) through the collection of resins from buds, leaves, and bark of plants, which are then mixed with beeswax and salivary enzymes. Bees use propolis to protect and sterilize their hives against microbial invasion, and this protective function has inspired extensive research into its medicinal applications.The chemical composition of propolis varies depending on geographical location, plant source, season, and bee species. However, it is generally rich in biologically active compounds such as flavonoids, phenolic acids, aromatic esters, terpenoids, and other polyphenolic

substances. These compounds are largely responsible for the antimicrobial, anti-inflammatory, antioxidant, antiviral, anticancer, and immunomodulatory properties associated with propolis. Due to these beneficial effects, propolis has been incorporated into various pharmaceutical, nutraceutical, and oral healthcare products.Oxidative stress is a major contributing factor in the pathogenesis of numerous chronic diseases and oral disorders. It occurs when the production of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defense systems, leading to cellular and tissue damage. Excessive ROS can damage lipids, proteins, and nucleic acids, thereby contributing to inflammation, aging, and disease progression. In the oral cavity, oxidative stress has been implicated in dental caries, periodontal disease, oral mucosal lesions, and delayed wound healing. Therefore, the identification of natural antioxidants capable of neutralizing free radicals has become an important area of biomedical research.Propolis is considered a promising natural antioxidant due to its high content of phenolic compounds and flavonoids, which can donate electrons or hydrogen atoms to stabilize free radicals. Several studies have reported that propolis exhibits strong free radical scavenging activity in various antioxidant assays, including DPPH and hydrogen peroxide scavenging methods. These antioxidant properties may contribute to its protective effects against oxidative damage and support its potential use in preventive and therapeutic healthcare applications.In addition to efficacy, the safety of natural products must be carefully evaluated before their clinical application. Although propolis is generally regarded as safe, its biological effects may vary depending on concentration and chemical composition. Cytotoxicity studies are therefore essential to determine the potential adverse effects of propolis and establish safe dosage ranges. The zebrafish (*Danio rerio*) embryo model has become a widely accepted tool for toxicity assessment because of its rapid embryonic development, transparency, genetic similarity to humans, and sensitivity to toxic substances. Zebrafish-based assays provide reliable preliminary information regarding the safety and biocompatibility of natural products.Furthermore, the growing prevalence of dental caries worldwide has encouraged the search for natural agents that can contribute to oral health promotion. Dental caries is a multifactorial disease characterized by the demineralization of tooth structures resulting from acid production by cariogenic microorganisms. Oxidative stress and inflammation are known to exacerbate oral tissue damage associated with caries progression. The antioxidant potential of propolis, combined with its reported antimicrobial activity against cariogenic bacteria such as *Streptococcus mutans*, suggests that it may serve as a beneficial natural agent for the prevention and management of dental caries.Therefore, the present study aimed to evaluate the antioxidant activity of propolis using DPPH radical scavenging and hydrogen peroxide scavenging assays and to assess its cytotoxic effects using zebrafish embryos. The findings of this study may provide valuable insights into the therapeutic potential, safety profile, and possible application of propolis as a natural antioxidant and oral healthcare agent.

MATERIALS AND METHODS :

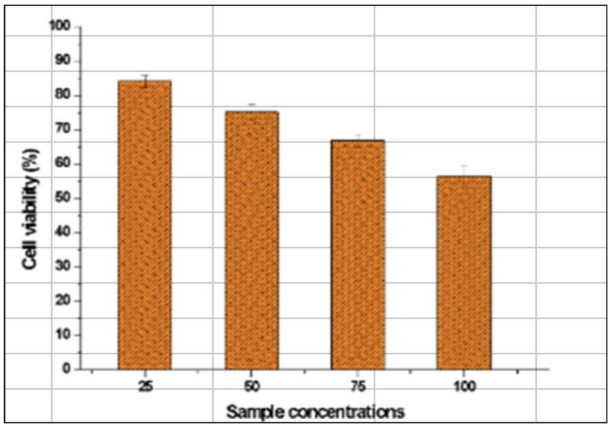
The study was conducted in Saveetha Dental College and Hospital. Here the embryos of Zebra fish and the propolis extract was taken. Embryos of zebra fish was used to measure the cytotoxicity effect of biosynthesised propolis extract . In three different test tubes around 10 larvae were added along with 10 ml sea water and 1 ml of propolis extract were added to the test tube.A control test tube was prepared by omitting propolis extract.After 24 hrs the number of dead and alive larvae were counted and percentage were calculated.Antioxidant assay was performed by DPPH assay and Hydrogen peroxide assay.



RESULTS :

Cytotoxicity Activity- Embryos of Zebrafish

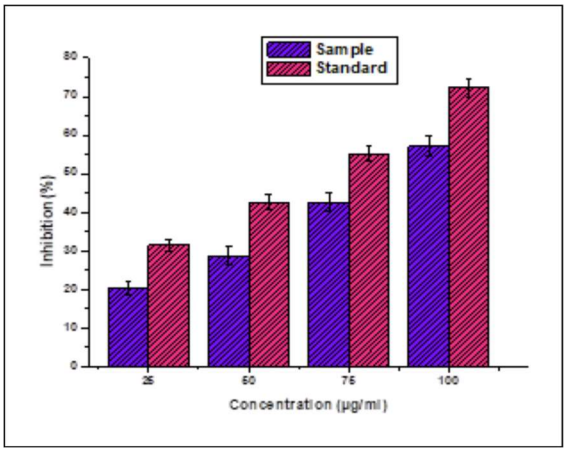
µg/ml	% of viability	SE
25	84.2	1.8
50	75.2	2.2
75	66.8	1.8
100	56.4	2.8



The cytotoxic effect of the sample was evaluated using zebrafish embryos at concentrations ranging from 25 to 100 ug/mL.The percentage viability of embryos decreased in a concentration-dependent manner, with viability values of 84.2%, 75.2%, 66.8%, and 56.4% at 25, 50, 75, and 100 kg/mL, respectively. The results indicate that increasing sample concentration resulted in reduced embryo survival, suggesting dose-dependent toxicity.

Antioxidant activity by DPPH assay

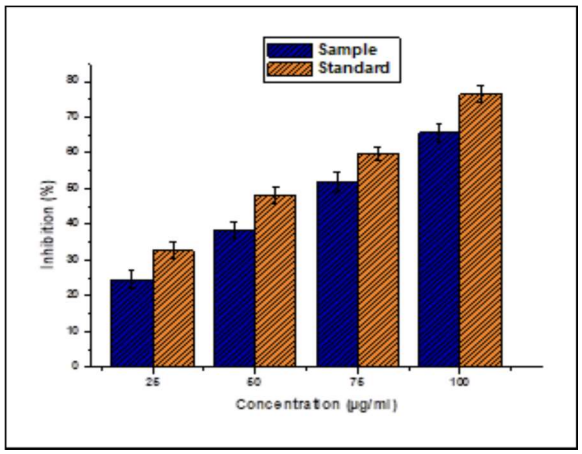
DPPH assay			STD Ascorbic acid	
Concentration (µg)	sample	S. Er	std	S. Er
25	20.5	1.8	31.5	1.5
50	28.8	2.2	42.6	2
75	42.5	2.4	55.2	1.8
100	57.2	2.7	72.2	2.2



The DPPH radical scavenging assay demonstrated concentration-dependent antioxidant activity. The sample exhibited inhibition values of 20.5%, 28.8%, 42.5%, and 57.2% at concentrations of 25, 50, 75, and 100 Mg/mL, respectively.In comparison, the standard antioxidant ascorbic acid showed significantly higher scavenging activities of 31.5%, 42.6%, 55.2%, and 72.2% at corresponding concentrations. Although the sample was less potent than ascorbic acid, it displayed substantial free radical scavenging activity, particularly at higher concentrations.

Antioxidant activity by hydrogen peroxide assay

Hydrogen peroxide assay			STD Ascorbic acid	
concentration(µg	sample	S. Er	Std	S. Er
25	24.5	2.6	32.6	2.3
50	38.3	2.4	48.1	2.2
75	51.7	2.8	59.8	1.8
100	65.6	2.6	76.5	2.4



The hydrogen peroxide scavenging assay also showed a concentration-dependent increase in antioxidant activity. The sample achieved scavenging percentages of 24.5%, 38.3%, 51.7%, and 65.6% at 25, 50, 75, and 100 Mg/ mL, respectively.The standard ascorbic acid exhibited stronger activity, with values of 32.6%, 48.1%, 59.8%, and 76.5% at the same concentrations.Nevertheless, the sample demonstrated notable hydrogen peroxide scavenging potential, reaching over 65% inhibition at the highest concentration tested.

DISCUSSION :

Zebrafish embryos are widely recognized as an effective in vivo model for toxicity assessment due to their genetic similarity to humans, rapid development, and transparent embryos. The present findings revealed a concentration-dependent reduction in embryo viability, indicating that the bioactive constituents of the sample exert toxic effects at elevated concentrations.

The observed decrease in viability from 84.2% to 56.4% suggests that the sample may contain secondary metabolites capable of affecting embryonic development when administered at higher doses. Similar concentration-dependent toxicity patterns have been reported for numerous plant extracts, nanoparticles, and natural products tested in zebrafish embryos. Previous studies have shown that toxicity often increases with concentration due to oxidative stress induction, membrane damage, disruption of cellular metabolism, or interference with developmental pathways.Despite the reduction in viability, survival remained above 50% at the highest concentration, suggesting that the sample possesses relatively low to moderate toxicity within the tested range. This finding may indicate a favorable safety profile for potential therapeutic applications, although further studies involving LC50 determination, developmental endpoints, and histopathological analyses are necessary.The DPPH assay is commonly employed to evaluate the ability of compounds to donate hydrogen atoms or electrons and neutralize free radicals. In the present study, the sample demonstrated significant DPPH radical scavenging activity that increased with concentration.The lower activity compared with ascorbic acid is expected because pure antioxidant standards generally exhibit stronger radical scavenging capacities than crude extracts or partially purified fractions. The antioxidant effect observed may be attributed to the presence of phenolic compounds, flavonoids, tannins, alkaloids, or other phytochemicals capable of donating electrons to stabilize DPPH radicals.Previous studies have consistently reported a positive correlation between total phenolic content and DPPH scavenging activity. The concentration-dependent increase observed in the current study is therefore consistent with findings reported for medicinal plant extracts and natural antioxidant sources. The inhibition value of 57.2% at 100 ug/mL suggests that the sample contains bioactive compounds with considerable free radical scavenging potential.Hydrogen peroxide is a reactive oxygen species that can penetrate biological membranes and generate highly reactive hydroxyl radicals. Therefore, compounds capable of scavenging hydrogen peroxide may contribute to protection against oxidative stress-related cellular damage.The present study demonstrated that hydrogen peroxide scavenging activity increased progressively with concentration, reaching 65.6% inhibition at 100 mg/mL.Although lower than the standard ascorbic acid, the observed activity indicates an effective antioxidant mechanism.These findings agree with previous reports demonstrating that plant-derived antioxidants and natural bioactive compounds can neutralize hydrogen peroxide through electron donation and free radical stabilization. The strong concentration-dependent response suggests that the antioxidant constituents of the sample are sufficiently abundant to exert protective effects against reactive oxygen species.Interestingly, the sample showed slightly higher scavenging activity in the hydrogen peroxide assay than in the DPPH assay at corresponding concentrations. This may indicate that the active compounds possess a stronger affinity toward reactive oxygen species such as hydrogen peroxide than toward the stable DPPH radical.The present study demonstrated concentration-dependent

antioxidant activity and cytotoxicity of the sample. In the DPPH assay, radical scavenging activity increased from 20.5% to 57.2%, while hydrogen peroxide scavenging activity increased from 24.5% to 65.6% with increasing concentration. Although the activities were lower than those of ascorbic acid, the results indicate the presence of bioactive compounds capable of neutralizing free radicals.These findings are consistent with previous studies reporting that plant extracts and natural products rich in phenolics and flavonoids exhibit dose-dependent antioxidant activity in both DPPH and hydrogen peroxide assays. Similar trends have been observed by Blois (1958) for DPPH radical scavenging and by Ruch et al. (1989) for hydrogen peroxide scavenging, where antioxidant activity increased with concentration due to enhanced electron or hydrogen-donating capacity.Overall, the results suggest that the sample possesses promising antioxidant properties with acceptable toxicity levels, supporting previous reports that natural products can provide beneficial antioxidant effects while maintaining reasonable biological safety at appropriate concentrations.

CONCLUSION :

The sample exhibited significant antioxidant activity and moderate cytotoxicity in zebrafish embryos in a concentration-dependent manner. Antioxidant activity increased steadily with concentration in both DPPH and hydrogen peroxide scavenging assays, although it remained lower than that of the standard antioxidant ascorbic acid. The zebrafish embryo assay revealed decreasing viability with increasing concentration, indicating some toxic effects at higher doses.

Collectively, these findings suggest that the sample contains bioactive compounds with promising antioxidant potential while maintaining acceptable toxicity levels within the tested concentration range. Further phytochemical characterization and in vivo studies are recommended to identify the active constituents and establish their therapeutic safety and efficacy.Since oxidative stress plays an important role in the progression of dental caries by promoting inflammation and tissue damage within the oral cavity, the observed antioxidant activity may contribute to oral health benefits. Therefore, the sample may have potential as a natural agent for the prevention or management of dental caries, either alone or as an adjunct in oral healthcare formulations. However, further studies evaluating its antimicrobial activity against cariogenic bacteria such as Streptococcus mutans, biofilm inhibition, and clinical efficacy are required to confirm its suitability for dental applications.

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CONFLICT OF INTEREST:

The authors declare that there was no conflict of interest in the present study.

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