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Comparative Study on Antioxidant Activity of Propolis of *Apis mellifera* from Different Regions of Nepal

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Abstract

Propolis is a waxy material obtained from honey bee hives. The physical and chemical property of this product is variable based on the source of hive, plant biodiversity where honeybee feed, season of collection, geographical origin etc. Propolis has several useful chemical compounds, and among them polyphenols are mainly contributing for their broad spectrum of medicinal quality such as antimicrobial, antifungal, antibacterial, and anti-inflammatory activities as well as antioxidant properties. The present study aims to analyze the ethanolic extract of propolis for their phenol and flavonoid content as well as its antioxidant characteristics. The samples (SPLs) were collected from farmers of six different locations of Nepal i.e. Jhapa, Lalitpur, Kathmandu, Banke and Chitwan districts. Total phenolic content (TPC) and total flavonoid content (TFC) were measured by Folin Ciocalteu method and the aluminum chloride method respectively expressed as the gallic acid (GAE) and quercetin (QE) and equivalent (GAE) per gram. The Diphenyl-Picrylhydrazyl (DPPH) assay method was used to evaluate the free radical scavenging activity. The antioxidant effect of propolis was reported in ascorbic acid equivalent antioxidant capacity per gram of propolis. The highest content of phenolic and flavonoid content was found in sample SPL 2. The range of these compounds' concentrations were from 127.36±5.50 mg GAE/gram to 242.7±4.50 mg GAE/gram. Similarly, total flavonoid content ranged from 1.3197±0.0261 QE mg/ grams to 5.3921±0.0261 QE mg/ grams. Whereas samples from SPL 2, and SPL 5 showed highest antioxidant properties. All samples were found to have strong antioxidant capacity which was greater than standard. It is concluded that there is no direct correlation on total antioxidant property of propolis with their total phenolic and total flavonoid content among collected samples. The phenolic characteristics of the samples were variable to the geographical location, and plant diversity of their origin.

Keywords: Nepalese Bee Propolis, Quality, Phenolics, Antioxidant, DPPH assay

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Introduction

Propolis is a resinous material, also known as bee glue, that bees collect from various plant sources for their hives. Bees create propolis, by combining saliva, beeswax, and exudate obtained from tree buds, sap flowers, or other botanical sources. It has a variety of biological activities and is an well-known natural remedy. Propolis is used to fill minor gaps, and burr comb is used to cover bigger gaps [1]. It possesses the antibacterial capabilities provide protection against infections [2, 3] . The caffeic acid phenethyl ester is considered as a main constituent to inhibit the nuclear factor κ-B, inhibition of cell proliferation, induction of cell cycle arrest and apoptosis.

The different climatic circumstances in which propolis is produced affect its chemical components and overall nature. Geographic origin is associated with variations in the chemical composition and consequently in the medicinal property of propolis. Propolis contains phenolic compounds, esters, flavonoids, aromatic aldehydes, resin, wax, and oil [4]. Numerous studies on propolis have revealed that it contains a variety of biologically active constituents as well as unique properties such as anti-inflammatory, antimicrobial, antioxidant, wound healing, and others [5]. The biological activity of several beneficial chemicals derived from propolis for antibacterial, antifungal, antiviral, antiinflammation, anticancer, antioxidant, and other properties can be applied in the pharmaceutical and health sectors [6–8]. Propolis can be employed as a free radical scavenger due to its significant oxidation inhibitory action [9]. The significant biological potency of propolis is demonstrated by its diverse composition [10]. Many researchers are working in the propolis because of its broad range of medicinal value and availability in different part of the world.

There is an escalating scientific concern in the impact of geographical origin of propolis on their chemical constituents, physical characteristics and biological

activities. Therefore the primary objective of this study was to calculate the flavonoids and phenolics, and evaluate the antioxidant activities of propolis collected from various locations of Nepal.

Materials and methods

Collection of propolis

Bee propolis were collected from Jhapa, Lalitpur, Kathmandu, Banke and Chitwan during June-July 2021. The propolis was collected as chunks in propolis traps every 15 days. The collected propolis chunks were stored in an air-tight container under refrigerated condition at 4^0C for subsequent research purposes.

Sample preparation

Total 10 grams of raw pulverized propolis was dissolved in 100 ml of 70% ethanol and warmed in a water bath. The mixture was transferred to conical flasks and set on a rotary shaker with suitable RPM for 72 hours. Thereafter, Whatman no 1 filter was used to obtain filtrate in conical flask. The extract was concentrated in rotavapor (Buchi R-215, Switzerland), 75-90 RPM, under 100 mbar pressure maintaining 40°C temperature of water bath (**Figure 1**).

Figure 1. Propolis sample preparation

Total Phenolic content

Total Phenolic Content (TPC) of developed propolis powder was estimated with slight modification in previously described method [11] . For each sample, 1 ml of Folin-Ciocalteu reagent was mixed with 0.2 ml of extract. After 3 minutes, 1 ml of 10% sodium carbonate was added, sodium carbonate helped speed up the oxidation reaction of phenol. The resulting mixture was incubated at room temperature for 30 minutes. Furthermore, the absorbance at 280 nm was measured in triplicates for each sample of propolis. The phenolic content of propolis was recorded in mg of gallic acid equivalent per gram. The standard curve was generated with concentration of 0.8mg/ml of gallic acid. All samples were calculated in triplicate.

Total Flavonoid content

Total flavonoid content was measured with slight modification in the procedure of previously described method [12]. For each sample, approximately 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 mol/L potassium acetate and 2.8 ml of water was added to 0.5 ml of extract. For about 30 minutes, the mixture was incubated at room temperature. After that, the absorbance at 415 nm was measured in UV-Visible spectrophotometer. Each sample of propolis was measured in triplicates expressing the flavonoid content in mg of quercetin equivalent per gram of propolis. The standard curve was generated with concentration of 0.8 mg/ml of quercetin.

DPPH radical scavenging activity assay

DPPH (2, 2′- diphenyl-1-picrylhydrazyl) assay was used to measure the free radical scavenging activity of the fractions as per the method described earlier [13]. The stock solution in 100 ml Methanol was prepared by dissolving 3.94 mg DPPH. Ethanolic extracts of the propolis samples, and Ascorbic acid of different concentrations (i.e., 60, 120, 180, 240 and 300 ppm) were prepared and reacted with aliquot solution of DPPH in the ratio 1:3. The resulting mixture was incubated at room temperature for 30 minutes. It was kept in a dark place to protect from light. After that, the absorbance at 517 nm was measured in triplicate. Based on the percentage of DPPH radical scavenged, the scavenging activity was calculated using the following equation:

$$Inhibition\ \% = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100\%$$

Where $A_{Control}$ is the mean Absorbance reading of 0 ppm solution against Methanol as Blank

A_{Sample} is the mean Absorbance reading of different concentrations of the solutions

Results and discussions

Propolis from various locations of Nepal were subjected for their quality analysis with the prospective of phenolics and antioxidant property. The electron donating capacity of natural products can be estimated by using 2, 2'-diphenyl-1- picrylhydrazyl radical (DPPH) reagent. This method is based on scavenging of DPPH radical through the addition of antioxidant which reduces the free radical of DPPH leading to its decolorization. According to the study's findings, propolis extract contains phytoconstituents which can donate hydrogen to a free radical to scavenge possible damage.

Total Phenolic content

Gallic acid was used to perform the total phenolic content assay, and the standard curve equation was y = 0.001x + 0.5883, where R^2 = 0.9894. The absorbances were put in the equation obtained from the Gallic acid standard, and TPC were determined (**Figure 2**). The range of these compounds' concentrations was 127.36±5.50 mg GAE/gram to 242.7±4.50 mg GAE/gram. SPL 2 had the highest concentration of phenols, followed by SPL 6. The lowest concentration was found in SPL 3 and SPL 4. Others had average total phenolic content. In propolis sample, phenolic acids such as benzoic acid, cinnamic acid, and their derivatives are present. P-hydroxybenzoic acid, anisic acid, and gallic acid are some of the most prominent benzoic acid derivatives. Additionally, there is vanillic acid, salicylic acid, gentisic acid, 3,4-dimethoxybenzoic acid, protocatechuic acid, and 2-amino-3-methoxybenzoic acid present. [14].

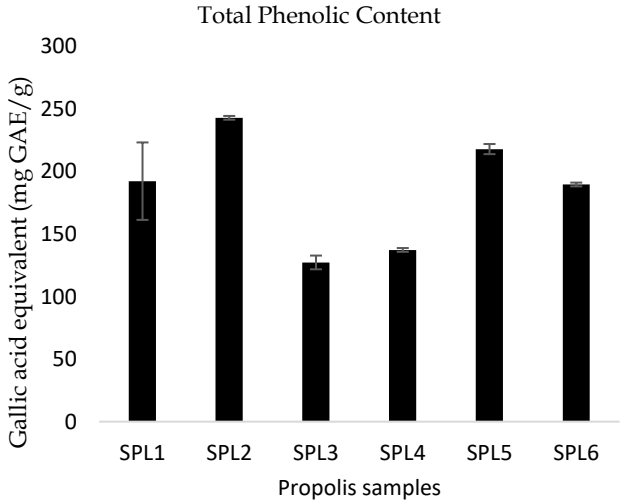

Figure 2: Total phenolic content of propolis collected from different area of Nepal**.**

Total Flavonoid Content

Quercetin used as the standard to estimate TFC in the samples comparing the standard curve equation. Quercetin equivalent was calculated in milligram quercetin equivalent (QE) per gram. The samples were evaluated based on the quercetin equivalent. Total flavonoid content ranged from 1.3197±0.0261 QE mg/ grams to 5.3921±0.0261 QE mg/ grams. SPL 2 was found to have the highest content of flavonoids 5.3921±0.0261 mg QE/gram (**Figure 3**). Phenolic compounds such as; Odoratin, 7,3',4'-Trihydroxy-5'-methoxyisoflavonoid, 6,7,3'-Trihydroxy-4'-methoxyisoflavonoid, 7,3'-Dihydroxy-6,5'- methoxyisoflavonoid, Neoflavonoid 1 to Neoflavonoid 10, (S)-3'-hydroxy-4-methoxydalbergione, (S)-3',4'-dihydroxy-4-methoxydalbergione were also reported from Nepalese propolis [15-16].

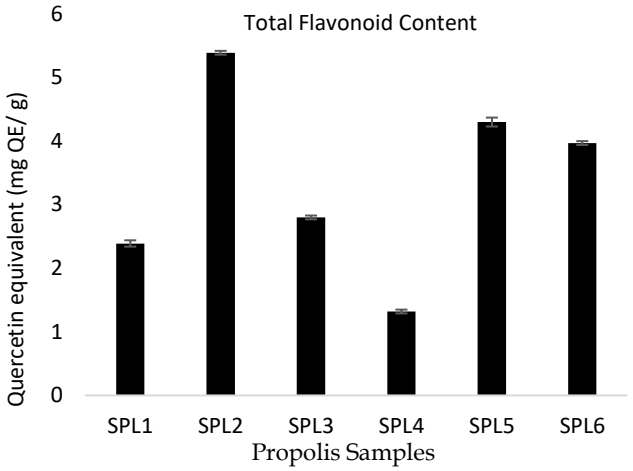

Figure 3. Bar graph comparing total flavonoid content in given samples

Antioxidant activity

The results from our experimental studies shows that the antioxidant capacity of the Nepalese originated propolis samples are very strong (**Figure 4**). The IC_{50} range of the sample were from 35 to 218 as compared with ascorbic acid standard. The sample's antioxidant property has an inverse relationship with the IC_{50} value. In other words, if the IC_{50} value is lower, the sample will need less to scavenge the free radical, and vice versa. Free radical scavenging activity in the sample is caused by the presence of bioactive components known as antioxidants. Additionally, based on the above total phenolic content and total flavonoid content, SPL 2 contains the highest TFC and TPC, resulting in the strongest IC_{50} value. DPPH• is a stable purple radical that turns pale yellow when it absorbs free radicals. The antioxidant effect of propolis was reported in ascorbic acid equivalent antioxidant capacity (AEAC) per gram of propolis. All propolis showed an antioxidant capacity greater than 35 AEAC; 5 samples exceeded 100 AEAC, and sample number 6 exceeded 200 AEAC. SPL2 has a lower IC50 value, indicating higher antioxidant activity, as less extract is required to inhibit 50% of the DPPH radical. Propolis comprises components from several different chemical families, including flavonoids, four aromatic carboxylic acids, and eleven phenolic acid esters, which are responsible for its antioxidant properties, according to a prior study. Numerous studies show that the overall number of phenolic compounds is related to antioxidant activity [17]. The current investigation has also reinforced the antioxidant property of Nepalese originated propolis samples. The results also supported the assumption that an increase in the flavonoid concentration can lead to higher antioxidant activity.

It was observed that bee propolis chemistry and antioxidant properties need to be compared together with their geographical factors and plant species found around the beehive and bee feeding area having prominent Nepalese antioxidant herbs [18-20]. The future research on the propolis, can be focused on feeding experiments, together with local biodiversity to identify the species and behavior on propolis. The chemical composition of the propolis and its biological activities can be correlated with their geographical origin of the samples.

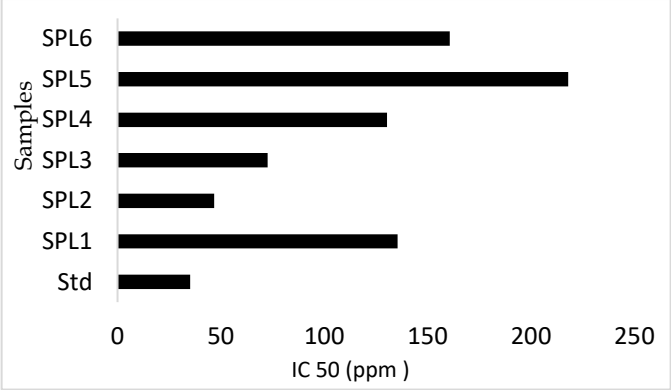

Figure 4. Comparison of IC50 value of samples**.**

Conclusion

It is concluded that the propolis of *Apis mellifera* from different regions of Nepal possesses various strengths of antioxidant property. SPL 2 exhibited the highest levels of TFC and TPC, but antioxidant property was stronger in another sample, SPL 5. The concentrations of phenolic compounds in sample might be influenced by the vegetation of bee farming area.

Author's contribution

Project supervisors are Dr. Rajendra Gyawali, Dr. Rajan Shrestha. Lab, fieldwork and writing manuscript were done by Aparna Paudel, Albina Maharjan, Babita Lamsal, Nina Khaitu and Pranisha Bhatta. Review and final editing were done by Rajendra Gyawali, Aparna Paudel and Babita Lamsal. All authors read and approved the final manuscript.

Competing interest

No competing interests were disclosed.

Ethical approval and consent

Not applicable.

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