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# STUDY OF ANTIOXIDANT ACTIVITY OF *PROPOLIS* FROM DIFFERENT AREAS OF PUNJAB, PAKISTAN

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**Abstract:**
This study investigates the antioxidant activity of *propolis* sourced from various regions of Punjab, Pakistan, specifically Jhang, Toba Tek Singh, and Sargodha. The findings indicate that *propolis* from these areas demonstrates significant antioxidant potential, suggesting its viability as a natural antioxidant for food supplements. However, further research is essential to isolate and quantify the key active compounds to better understand their specific biological activities. Expanding *propolis* collection to additional regions within Punjab will provide a more comprehensive evaluation of its antioxidant properties, enabling the identification of the most potent sources.

**Keywords:** Antioxidant, *propolis*, Natural products, Reactive oxygen species (ROS)

## 1. INTRODUCTION

Antioxidants are substances that, by attaching to free radicals and highly reactive molecules, can limit the autoxidation reaction. Because of their preventive activities in food and pharmaceutical goods against oxidative degradation and in the body, as well as against oxidative stress-mediated disease processes, antioxidants have gained popularity (Gulcin, 2020). They are required by the human body since oxidation occurs naturally in humans. If it occurs frequently, it can cause oxidative tension, which can lead to a variety of disorders (Calegari et al., 2019).

Natural products, such as *propolis*, can be used to acquire antioxidants (Christina et al., 2018). *Propolis*'s antibacterial and antioxidant qualities allow it to be used in the food sector, where it helps prevent lipid oxidation and lengthen food products' shelf lives (Tamfu et al., 2019; Almuhayawi, 2020). Biodiversity in ecosystems is directly dependent on bee pollination, which is a critical aspect; the world's agricultural supply is mostly sustained by these insects, who operate as "service providers" (Michener, 2007; Garófalo, 2004; Greenleaf and Kremen, 2006; Winfree et al., 2011). Bees offer people a variety of goods, the most essential and well-known of which are honey, *propolis*, royal jelly, wax, and bee venom (Apitoxin). In today's world, the usage of pesticides might

contribute to the extinction of bees; moreover, this extinction may be linked to ecosystem degradation, habitat fragmentation, plant species depletion, and global warming (Michener, 2007; Madras-Majewska et al., 2016; Calegari et al., 2019).

### 1.1 *Propolis*

*Propolis* is a naturally occurring substance obtained from plants that has a wide range of bioactivities due to its complex and changeable chemical makeup. It is made up of resins from buds, exudates, and other plant components that are combined with salivary enzymes and beeswax (Koh et al., 2013; Bankova et al., 2019; Tamfu et al., 2022). *Propolis*, which is derived from the Greek words pro, which means for or in defence, and polis, which means the city, meaning "defence of the hive." *Propolis* is a multipurpose substance that bees utilize to build and maintain their colonies. Bees utilize it to fill cracks in their honeycombs and smooth down interior walls (Salatino et al., 2005; Abu-Seida, 2015). *Propolis* is also used to keep intruders out and includes antibacterial compounds that are effective against several infections (Marco et al., 2017). The chemical composition and biological activities of *propolis* samples differ depending on geographical location, season of collection, and bee species. *Propolis*, also known as bee glue, is a sticky substance composed of waxes, resins, polysaccharides, volatile oils, polyphenols, and a variety of natural compounds that have antioxidant, antiulcer, antibacterial, anti-angiogenic, and antiviral properties (Tamfu et al., 2020; Tamfu et al., 2022).

There are three basic forms of *propolis* based on shape, behaviour, and biological geography: tropical region *propolis*, temperate region *propolis*, and Pacific region *propolis*. Flavonoids with no B-ring substituents, such as galangin, chrysin, pinocembrin, and pinobanksin, are among the distinctive components of the first kind of *propolis*. Regardless of geographical origin, the main component of temperate *propolis* with high biologic activity is caffeic acid phenethyl ester (Dezmirean et al., 2020; Alvear et al., 2021). Diterpenes and Prenylated phenyl propanoids are found in the second kind of *propolis*, which is found in tropical regions. The third kind of *propolis* includes geranyl flavanones and is usually found in the Pacific or African regions (Huang et al., 2014). *Propolis* chemical categories should be studied with regard to plant origin. The most frequent and widespread type of *propolis* is poplar, which comes from North America, New Zealand, Europe, non-tropical Asia, and even Africa. Populus species are the primary plant sources of *propolis* around the world, particularly in temperate climates. The poplar variety has a typical chemical composition with high levels of flavones and flavanones, low levels of phenolic compounds, and their esters (Dezmirean et al., 2020; Stanciauskaite et al., 2021; Alvear et al., 2021; Kurek-Górecka et al., 2022).

### 1.2. Composition of *propolis*

The primary components of *propolis* include resin (50%), beeswax (30%), aromatic compounds, essential oils (10%), pollen (5%), and the remaining 5% are different organic compounds such as polyphenols, flavonoids, amino acids, vitamins, and minerals (Ali and Kunugi 2021). Over 300 chemicals have been isolated from *propolis* from various locations of the world, although there is no obvious differentiation between *propolis* from different regions. The principal chemical compounds may be divided into two broad groups: those from tropical *propolis* and those from temperate *propolis*. Compounds identified from temperate *propolis* include flavonols, flavones, flavanonols, flavanones, lignans, pterocarpans, chalcones, aurones, phenolic acids, and their esters. Steroids, terpenoids, and xanthones are the most common compounds isolated from tropical and subtropical *propolis* (Tamfu et al., 2019). Although phenolic compounds are the most abundant in *propolis*, many other compounds have been identified in various species, including phenolic acids, flavonoids such as flavones, flavanones, flavonols and chalcones, terpenes, aromatic aldehydes, alcohols, fatty acids, stilbenes, steroids, amino acids, lignans, and sugars (Righi et al., 2011; Akyol et al., 2013; Andrade et al., 2017). *Propolis* includes vitamins B1, B2, B6, C, and E, as well as minerals including magnesium (Mg), calcium (Ca), potassium (K), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe). *Propolis* includes essential amino acids that are required for the

regeneration of cells, with proline and arginine being the most abundant, with a 45.8% concentration. The mineral composition of plants is also substantially impacted by their surroundings (Bankova, 2019; Mulyati et al., 2020).

### 1.3. Medicinal value of *propolis*

*Propolis* has been claimed to be utilized for medicinal purposes since antiquity (Ghisalberti, 1979). There is evidence that ancient Egyptians, Persians, and Romans used *propolis*. *Propolis*'s use dropped during the middle Ages, and it was not a popular drug in the medical sector. However, *propolis* knowledge has survived in traditional European medicine, which has been dubbed "Russian penicillin" (Kuropatnicki et al., 2013; Belmehdi et al., 2021). *Propolis* has been used empirically for centuries and has several biological applications, including the acceleration of regenerating processes in damaged cartilage and bones (Scheller et al., 1977; Stojko et al., 1978), immunomodulatory (Sforcin, 2007), antimicrobial (Cardoso et al., 2010), antioxidant (Ramadan et al., 2012), analgesic and anti-inflammatory agent (Ramos et al., 2012) and antitumoral property (Cinegaglia et al., 2013; Abu-Seida, 2015). *Propolis* has recently received recognition as a useful technique for addressing health issues. Because of its promising therapeutic properties, *propolis* was initially employed in pharmacology in 1985 (Salatino et al., 2005; ALaerjani et al., 2022). Over-the-counter preparations for cold syndrome (upper respiratory tract infections, common cold, flu-like infection), as well as dermatological preparations useful in wound healing, treatment of boils, acne, herpes simplex and genitalis, and neurodermatitis, among other ailments, are examples of current applications of *propolis* (Banskota et al., 2001; Freires et al., 2016; Galeotti et al., 2018). *Propolis*, which contains antioxidants, has been used to prevent and cure disorders associated with increased oxidative stress, such as cancer, aging, and cardiovascular disease (Araujo et al., 2016; Pratami et al., 2018). *Propolis* antioxidant compounds may extend food shelf life by slowing the peroxidation of lipids process, which is a major cause of food deterioration during storage (Silva et al., 2011; Al-Juhaimi et al., 2022). Aside from the beneficial characteristics of *propolis*, its toxicity must be considered before it is processed into natural products. A recent study found that *propolis* ethanolic extract (PEE) had no cytotoxicity against Caenorhabditis elegans after 24 hours of exposure (Abdullah et al., 2019; Shehata et al., 2020).

## 3. MATERIALS AND METHODS

### 3.1 Samples Collection

A total of 45 fresh *propolis* samples of *Apis mellifera* species were directly collected from apiaries in different areas of Punjab, Pakistan during the year of 2022-2023. Out of total 45 *propolis* samples each 20 *propolis* samples were collected from apiaries in different areas of District Jhang (31° 16′ 40.9656” N, 72°18′ 42.3360” E) and Toba Tek Singh (30° 53′ 41.5500” N, 72° 39′ 8.4924” E). Whereas 5 samples were collected from apiaries in different areas of District Sarghoda (32.0740° N, 72.6861° E). The samples were stored in plastic bags with proper labeling and date. All *propolis* samples were kept at room temperature till completion of analysis. These *propolis* samples were divided into three groups on the basis of their geographical areas.

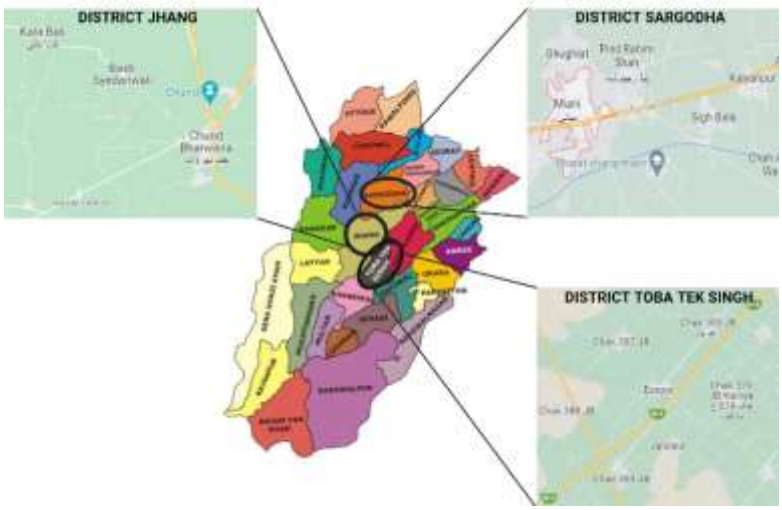



**Figure 3.1** Areas of *propolis* sampling from different Districts of Punjab

**Figure 3.2** Apairy located at Rivaz Bridge (J17)

District Jhang has an area of 8,809 square kilometers. The climate in the district is intense. Summer begins from April and lasts until October, with May and June being the hottest months. The winter season lasts from December to February. December and January are the coldest months. Over the course of the year, the temperature typically varies from 18.333°C to 41.111°C. The annual rainfall is approximately 348 millimeters. Winds and dust storms are prevalent in the summer and can inflict significant damage.

**Figure 3.3** Healthy worker Honey Bee

**Figure 3.4** *Propolis* on Inner cover

**Figure 3.5** Apiary located at Chak No. 383, Toba Tek Sing

District Toba Tek Singh has four Tehsils and an area of 4,364 square kilometers. The climate in this city is subtropical continental, with summers that are extremely hot, humid, and clear. This city's winters are pleasant, clear, and brief. The weather is usually dry throughout the year. Temperatures range from 16.667°C to 43.333°C. The yearly rainfall average is 376 millimeters. Toba Tek Singh is an agricultural city that helps to meet our country's nutritional demands by producing high-yield crops like wheat, sugarcane cotton, and maize. Citrus fruits are widely grown in this region.

**Figure 3.6** *propolis* on frames

**Figure 3.7** Apiary located at Sigh Bala, Sargodha

District Sargodha has an area of 5,864 square kilometers. In Sargodha, the average temperatures range between 15.5°C to 40.5°C. Precipitation in Sargodha averages 400 millimeters each year, making it rare. Sargodha and its surroundings are well-known for being one of the world's best citrus-producing regions.

**Figure 3.8** Healthy workers of *Apis mellifera*, pollens on the frame are visible

**Table 3.1** List of *propolis* samples from District Jhang (Js) and Toba Tek Singh (Ts)

| Location of Apairies in Jhang | Name of Sample | Location of Apairies in Toba Tek Singh | Name of Sample |
|---|---|---|---|
| Ali Pur | J1 | Esspur | T1 |
| Ghanva | J2 | Chinese Camp | T2 |
| Jand wala | J3 | Kashmir colony | T3 |
| Kari Wala | J4 | Chak No. 328 JB | T4 |
| Kilcha Pull | J5 | Chak No. 335 JB | T5 |
| Kuli Faqeer | J6 | Chak No. 379 JB | T6 |
| Mani Shareef | J7 | Chak No. 379 JB | T7 |
| Mouza Nankana | J8 | Chak No. 380 JB | T8 |
| Mouza Kuria Wala | J9 | Chak No. 381 JB | T9 |
| Mouza Billi Habib | J10 | Chak No. 382 JB | T10 |
| Mouza Maghyana | J11 | Chak No. 382 JB | T11 |
| Mouza Dadowana | J12 | Chak No. 383 JB | T12 |
| Mouza Gunyana | J13 | Chak No. 384 J.B | T13 |
| Mouza C. Baksha | J14 | Chak No. 385 JB | T14 |
| Mouza Salyana | J15 | Chak No. 386 JB | T15 |
| Mouza Khanwana | J16 | Chak No. 388 JB | T16 |
| Rivaz bridge | J17 | Chak No. 391J.B | T17 |
| Saho | J18 | Chak No. 392 J.B | T18 |
| Shay-ray ka Thatta | J19 | Chak No. 392 JB | T19 |
| Solakhna Wali | J20 | Chak No.479JB | T20 |

**Table 3.2** List of *propolis* samples from District Sargodha (Ss)

| Location of Apairies in Sargodha | Name of Sample |
|---|---|
| Miani | S1 |
| Mian ka banna | S2 |
| Sigh Bala | S3 |
| Pind Rahim Shah | S4 |
| Zainpur | S5 |

### 3.2 Methodology

#### 3.2.1 Sample solution

*Propolis* (1.33 g) was mixed with 100 mL of 100% ethanol to make the sample solution. This suspension was shaken at room temperature for 8 hours using a magnetic stirrer. The extract solution was then filtered using a Whatman no. 4 filter paper. The resultant solution was kept at -4°C until further use.

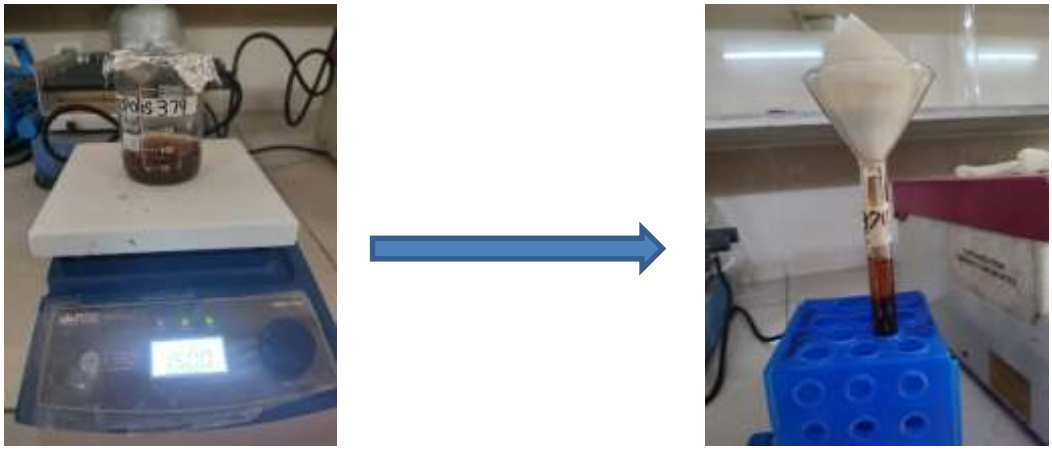


**Figure 3.9** Preparation of *Propolis* Sample Solution

#### 3.2.2 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The antioxidant activities in vitro of *propolis* ethanol extracts were determined using DPPH free radical based on the procedure reported by (Abdullah et al. 2019).

#### 3.2.3 Chemicals and Reagents

Name and quantity of chemicals and reagents used in the procedure for the determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of *propolis* extracts are mentioned in table 3.3.

**Table 3.3** List of chemicals and reagents for the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

| Sr. No. | Chemical/ Reagent | Quantity |
|---|---|---|
| **1** | *Propolis* extract | 2.3 mg |
| **2** | DPPH powder | 2.5 mg |
| **3** | Ethanol | 50 ml |
| **4** | Ethanolic DPPH solution | 52.5 ml |

#### 3.2.4 Procedure

In order to investigate DPPH radical scavenging activity of *propolis* samples, a dilution series were prepared for each *propolis* extract as follows: 400µg/mL; 200µg/mL; 100µg/mL; 50µg/mL; 25µg/mL. *Propolis* extract (0.5mL) from each diluted solution was then mixed with 3.5 mL of ethanol DPPH solution (50 mg $L^{-1}$). The mixtures were vigorously shaken and allowed to stand at room temperature for 30 min. The decrease of DPPH radical in the mixture, as indicated by the reduction of its purple color, was quantified by measuring the absorbance of the mixture at 517 nm using a single beam UV-vis spectrophotometer with ethanol acting as a blank. The assay was carried out in triplicate, and the radical scavenging activity (RSA) of the *propolis* extract was determined using the following equation:

$$RSA\ (\%) = (1 - A_S/A_0) \times 100$$

Where $A_S$ and $A_0$ is the absorbance of mixture with and without the *propolis* extract, respectively. The %age inhibition values were plotted against the *propolis* concentration to give a linear plot, and the IC50 (*propolis* concentration needed to scavenge 50% initial DPPH) was determined by using GraphPad prism version 10.0.0(153) software.

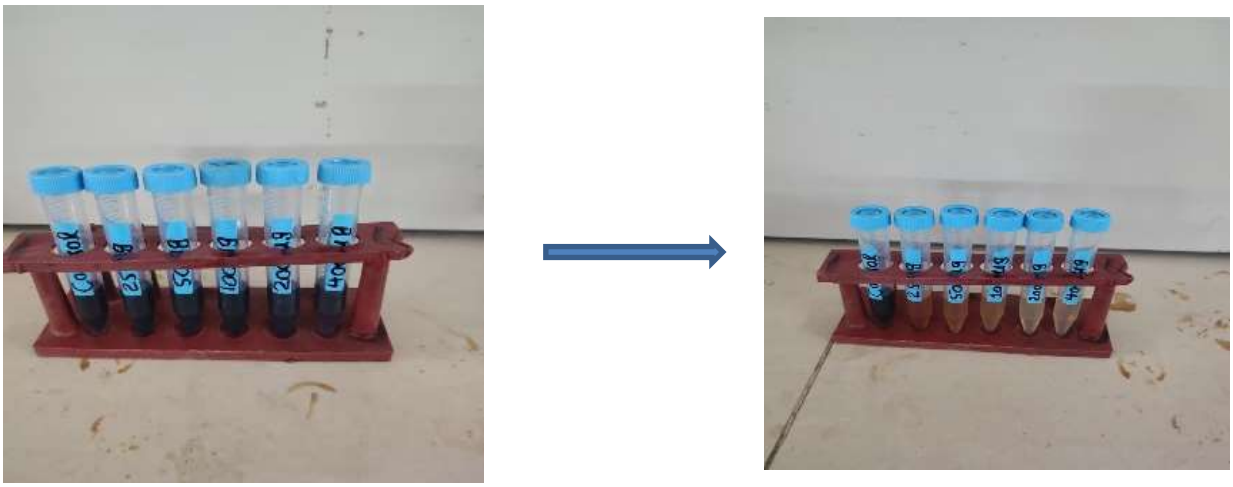


**Figure 3.10** Color change indicating reduction of DPPH radicals

### 3.2.5 Ferric reducing antioxidant power (FRAP) assay

The reducing ability of the *propolis* samples were investigated following a method reported by Temizer et al. (2017).

### 3.2.6 Chemicals and Reagents

Name and quantity of chemicals and reagents used in the procedure for the determination of Ferric reducing antioxidant power of *propolis* extracts are mentioned in table 3.4.

**Table 3.4** List of chemicals and reagents for the Ferric reducing antioxidant power (FRAP) assay

| Sr. No. | Chemical/ Reagent | Quantity |
|---|---|---|
| **1** | Ethanolic Extracts of *Propolis* | 6 ml |
| **2** | Distilled Water | 6 ml |
| **3** | Phosphate Buffered Saline | 6 ml |
| **4** | potassium ferricyanide | 6 ml |
| **5** | trichloroacetic acid | 6 ml |
| **6** | Iron Chloride | 1.5 ml |

### 3.2.7 Procedure

About 2.0 mL of the *propolis* sample solution was mixed with 2.0 mL phosphate-buffered saline (0.2 mol $L^{-1}$, pH 6.6) and 2.0 mL of 1.0% potassium ferricyanide. After incubating the mixture at 50°C for 20 minutes, trichloroacetic acid (2.0 mL, 10%) was added. Then, 2.0 mL of this solution was mixed with 2.0 mL distilled water and 0.5 mL of 0.1% $FeCl_3$. A control solution was prepared without *propolis* sample solution. The conversion of $Fe^{3+}$ to $Fe^{2+}$ was determined due to the presence of samples at 700 nm using a UV-vis spectrophotometer. The assay was carried out in triplicate, and the reducing ability of the *propolis* extract was determined using the following Equation:

$$FRAP\ (\%) = (A_s / A_c) \times 100$$

Where, $A_c$ is the absorbance of the control, and $A_s$ is the absorbance of the sample or standards.

**Figure 3.11** Blue color indicating Ferric reducing power of *propolis* samples

### 3.2.8 Determination of hydrogen peroxide scavenging activity (HPSA)

The HPSA was determined according to the method described by Dervisoglu et al. (2022).

### 3.2.9 Chemicals and Reagents

Name and quantity of chemicals and reagents used in the procedure for the determination of hydrogen peroxide scavenging activity of *propolis* extracts are mentioned in table 3.5.

**Table 3.5** List of chemicals and reagents for the determination of hydrogen peroxide scavenging activity (HPSA) of *propolis* extracts

| Sr. No. | Chemicals/ Reagents | Quantity |
|---|---|---|
| **1** | Phosphate Buffer | 7.2 ml |
| **2** | Distilled Water | 1000 ml |
| **3** | Hydrogen Peroxide | 1.8 ml |
| **4** | Ethanolic *Propolis* Extracts | 3 ml |

### 3.2.10 Preparation of Phosphate Buffer

For the preparation of 0.04M phosphate buffer with 7.4 pH firstly phosphate buffer of o.1M was prepared by using the following method. 15.6g of Sodium phosphate monobasic dehydrate ($H_6NaO_6P$) was dissolved in 500mL distilled water. 28.4g of Sodium phosphate dibasic anhydrous (Na2HPO4) was dissolved in 1L of distilled water. Then 57ml of $H_6NaO_6P$ solution and 243ml Na2HPO4 of were mixed to adjust the pH up to 7.4. Then 40ml of 0.1M phosphate buffer was mixed in 60ml of distilled water to make phosphate buffer of 0.04M with 7.4 pH.

### 3.2.11 Procedure

To determine this activity, a phosphate buffer of 0.04M with pH 7.4 and 40 mM hydrogen peroxide (prepared using the same buffer) were prepared fresh. Ethanolic extracts (1ml) of *propolis* (EEP) was mixed with 2.4 mL of buffer solution and finally 0.6 mL of hydrogen peroxide (40 mM) was added. The final volume of a tube was completed to 4 mL. The absorbance of the mixture was measured at 230 nm versus the blind sample after 10 min using a UV/VIS spectrophotometer. Phosphate buffer without hydrogen peroxide was used as blank. A decrease in the absorbance value indicated a high level of hydrogen peroxide scavenging activity.

A buffer solution containing hydrogen peroxide was used as control. The hydrogen peroxide removal was calculated as % of the following formula.

%Inhibition rate = $(A_C - A_S) / A_C) \times 100$

Where, $A_c$ is the absorbance of the control, and $A_s$ is the absorbance of the sample.

**Figure 3.12** HPSA assay performance

### 3.3 Statistical analysis

Data obtained was subjected for appropriate statistical analysis through computer by using Microsoft excel for Mean and standard error and graphic representations. One-way analysis of variance was performed by ANOVA procedures using SPSS (version 27 for Windows 2020, SPSS Inc.). Probability values of $p<0.0001$ were considered highly significant.

## RESULTS

The current research was designed to evaluate antioxidant activity of *propolis* collected from different locations of Jhang, Toba Tek Singh and Sargodha of province Punjab. Out of total 45 *propolis* samples each 20 *propolis* samples were collected from apiaries in different areas of District Jhang (31° 16′ 40.9656” N, 72°18′ 42.3360” E) and Toba Tek Singh (30° 53′ 41.5500” N, 72° 39′ 8.4924” E). Whereas 5 *propolis* samples were collected from apiaries in different areas of District Sarghoda (32.0740° N, 72.6861° E).

### 4.1 Hydrogen peroxide ($H_2O_2$) scavenging activity (HPSA)

Hydroxyl radical scavenging activities of *propolis* samples collected from District Jhang and Toba tek singh have been shown in table 4.1. The %age inhibition of hydroxyl radical by *propolis* samples collected from District Jhang ranges from 64.34% to 74.91%.

**Table 4.1** Hydrogen peroxide scavenging activity of *propolis* from District Jhang and Toba Tek Singh

| Sample Name | % inhibition | Sample Name | % inhibition |
|---|---|---|---|
| J1 | 71.68459 | T1 | 67.02509 |
| J2 | 72.22222 | T2 | 65.05376 |
| J3 | 74.91039 | T3 | 70.78853 |
| J4 | 65.23297 | T4 | 72.40143 |
| J5 | 64.33692 | T5 | 71.14695 |
| J6 | 67.02509 | T6 | 69.35484 |
| J7 | 64.51613 | T7 | 72.04301 |
| J8 | 66.30824 | T8 | 74.19355 |
| J9 | 68.99642 | T9 | 65.77061 |
| J10 | 68.63799 | T10 | 73.11828 |
| J11 | 70.60932 | T11 | 74.01434 |
| J12 | 66.84588 | T12 | 75.44803 |
| J13 | 64.87455 | T13 | 74.91039 |

| | | | |
|---|---|---|---|
| J14 | 67.02509 | T14 | 70.96774 |
| J15 | 67.74194 | T15 | 76.34409 |
| J16 | 72.58065 | T16 | 66.30824 |
| J17 | 65.23297 | T17 | 65.41219 |
| J18 | 69.35484 | T18 | 75.98566 |
| J19 | 70.2509 | T19 | 74.73118 |
| J20 | 65.05376 | T20 | 76.16487 |
| **Mean ± SD** | **68.1720±3.07910** | **Mean ± SD** | **71.5591±3.86738** |

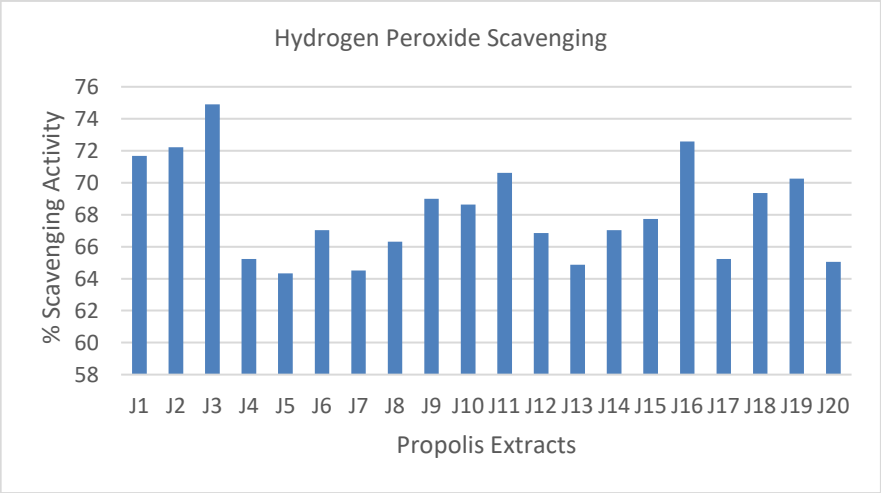



**Figure 4.1** Hydroxyl radical scavenging activities of *propolis* sample collected from Jhang (J1-J20) The minimum (64.34%) hydrogen peroxide scavenging activity was found in sample J5, while, the maximum (74.91%) activity was shown by sample J3. The %age inhibition of hydrogen peroxide ($H_2O_2$) by *propolis* samples collected from District Toba Tek Singh were found to be between 65.05%- 76.34%. The minimum (65.05%) and maximum (76.34%) scavenging activity was shown by T2 and T15 respectively.

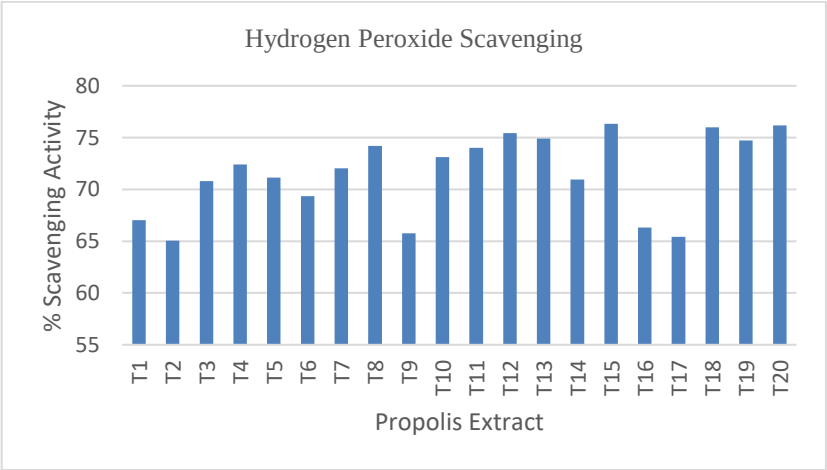



**Figure 4.2** Hydroxyl radical scavenging activities of *propolis* sample collected from Toba Tek Singh (T1-T20)

**Table 4.2** $H_2O_2$ scavenging activity of *Propolis* from Sargodha

| Sample Name | %age inhibition |
|---|---|
| S1 | 71.12 |
| S2 | 68.58 |

| | |
|---|---|
| S3 | 70.89 |
| S4 | 67.38 |
| S5 | 72.22 |
| **Mean±SD** | **70.03±1.98** |

The %age inhibition of hydroxyl radical by *propolis* samples collected from District Sargodha have been represented in table 4.2. The results indicated that the %age inhibition values varies between 67.38% and 72.22 %. The minimum scavenging activity, shown by S4, was 67.38%, while the highest scavenging activity, shown by S5, was 72.22%.

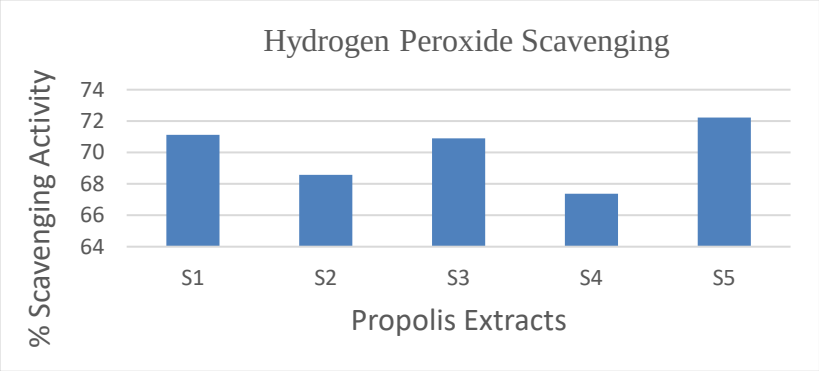



**Figure 4.3** $H_2O_2$ scavenging activity of *propolis* from Sargodha

**Table 4.3** Comparison of %age inhibition (Mean±SD) of *propolis* from Jhang, Toba Tek Singh and Sargodha

| Sample Name | Mean ± SD of % inhibition |
|---|---|
| Jhang | 68.1720±3.07910 |
| Toba Tek Singh | 71.5591±3.86738 |
| Sargodha | 70.0394±1.98986 |

A comparison between means of %age inhibition values for hydroxyl radical scavenging by *propolis* samples from three different areas (Jhang, Toba Tek Singh and Sargodha) has been represented in table 4.3. The overall scavenging activity of *propolis* determined was more (71.5591%) in Toba Tek Singh (T1-T20) samples followed by Sargodha (S1-S5) (70.0394%) and Jhang (J1-J20) (68.1720%).

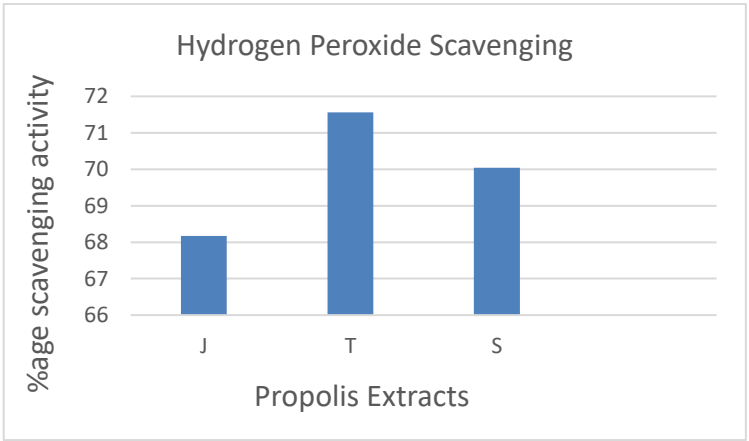



**Figure 4.4** Comparison between mean %age Inhibition of samples (Js, Ts, and Ss)

### 4.2 Ferric reducing antioxidant power (FRAP) assay

The principle behind the reducing power test technique is that compounds with reduction potential combine with potassium ferricyanide ($Fe^{3+}$) to generate potassium ferrocyanide ($Fe^{2+}$) which then interacts with ferric chloride to form ferric-ferrous complex with an absorbance maximum at 700 nm. A compound's reducing capacity is an important indication of its potential antioxidant ability. The reducing ability of *propolis* samples collected from District Jhang and Toba Tek Singh have been shown in table 4.4.

**Table 4.4** Percentage reduction of *propolis* extracts from District Jhang and Toba Tek Singh in ferric reducing power determination model

| Sample Name | % Reduction | Sample Name | % Reduction |
|---|---|---|---|
| J1 | 64.08333 | T1 | 66.75 |
| J2 | 62 | T2 | 64.5 |
| J3 | 60.66667 | T3 | 61.75 |
| J4 | 59.25 | T4 | 64.25 |
| J5 | 64.75 | T5 | 63.58333 |
| J6 | 61.83333 | T6 | 66.08333 |
| J7 | 60.5 | T7 | 62.33333 |
| J8 | 61.08333 | T8 | 63.66667 |
| J9 | 64.25 | T9 | 66.58333 |
| J10 | 59.5 | T10 | 61.41667 |
| J11 | 61.16667 | T11 | 65 |
| J12 | 64 | T12 | 65.66667 |
| J13 | 64.91667 | T13 | 61.41667 |
| J14 | 63.66667 | T14 | 66 |
| J15 | 61.58333 | T15 | 62.25 |
| J16 | 64.08333 | T16 | 64.16667 |
| J17 | 61.33333 | T17 | 63.41667 |
| J18 | 60.33333 | T18 | 66.33333 |
| J19 | 64.16667 | T19 | 63.83333 |
| J20 | 63.91667 | T20 | 61.33333 |
| **Mean ± SD** | **62.2400 ± .01000** | **Mean ± SD** | **64.0203 ± .00950** |

The percentage of the reductive ability of *propolis* extracts from District Jhang was found to vary between 59.25% and 64.916%. The minimum ferric ion reducing activity (59.25%) was found in sample J4, while sample J13 exhibited the maximum value (64.916%) for the ferric reducing antioxidant power assay. In Jhang, 10% samples exhibited low (59%) scavenging activity, 25% of the samples showed moderate (61%) scavenging activity, while 35% samples demonstrated high (64%) scavenging activity.

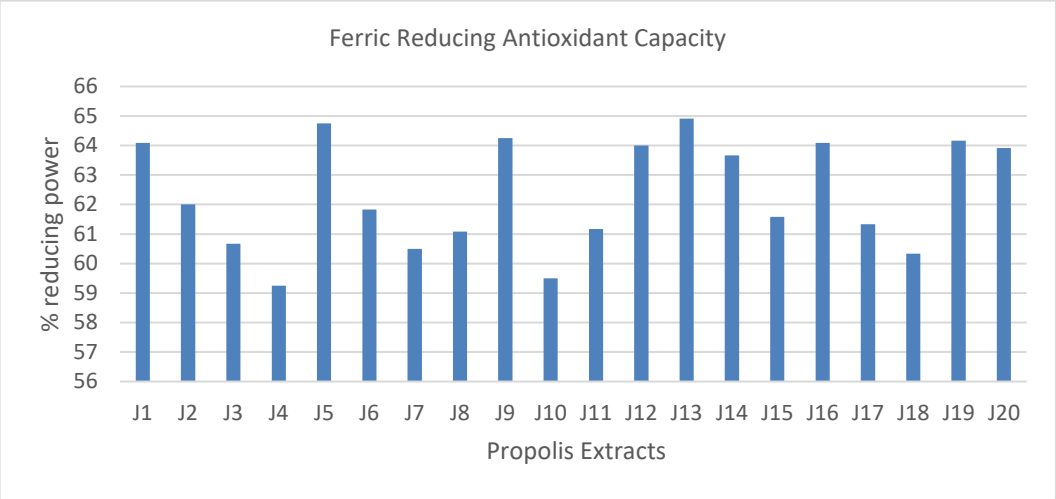



**Figure 4.5** Ferric reducing antioxidant capacity of *propolis* samples from District Jhang (J1-J20)

Similarly, the reduction potential of *propolis* samples from District Toba Tek Singh ranges between 61.33% and 66.75%. Among the samples, the minimum ferric reducing ability (61.33%) was shown by sample T20, while the maximum (66.75%) was found in sample T1.

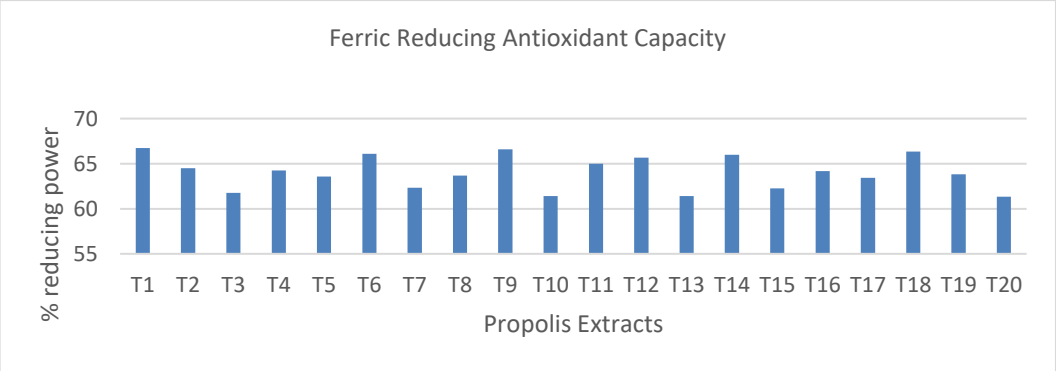



**Figure 4.6** Ferric reducing antioxidant capacity of *propolis* samples from District Toba Tek Singh (T1-T20)

In Toba Tek Singh, 20% samples had a maximum (66%) reductive potential, 20% showed moderate (63%) reductive potential, and another 20% exhibited low (61%) reductive potential. The remaining 40% of the samples displayed reducing abilities ranging from 62% to 65%, falling within the range of 61.33% (minimum) to 66.75% (maximum).

**Table 4.5** Percentage reduction of ferric ion by *propolis* of Sargodha

| Sample Name | % Reduction |
|---|---|
| S1 | 64.33333 |
| S2 | 61.58333 |
| S3 | 65.66667 |
| S4 | 62.08333 |
| S5 | 64.16667 |
| **Mean ± SD** | **63.2768 ± .80171** |

Table 4.5 presents the ferric reducing capacity of *propolis* samples from District Sargodha, showing

values ranging from 61.58% to 65.67%. Among the samples, S2 exhibited the minimum (61.58%) reducing capacity while S3 demonstrated the maximum value (65.67%) for ferric reducing potential. Among the samples tested from Sargodha 40% showed moderate (64%) reducing ability.

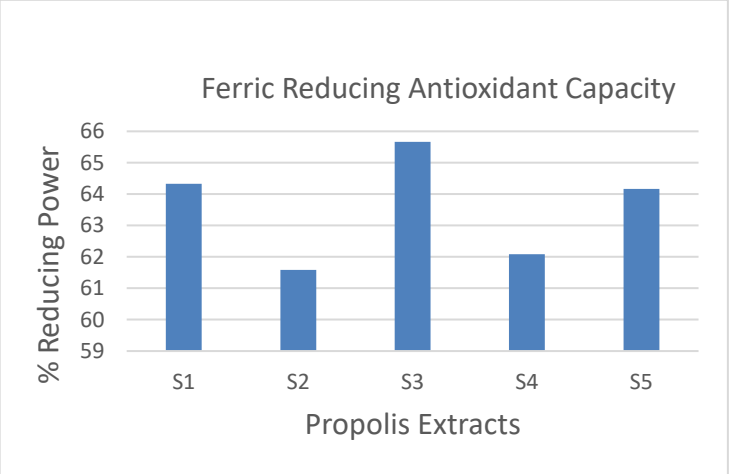



**Figure 4.7** Ferric reducing antioxidant capacity of *propolis* samples from Sargodha

**Table 4.6** Comparison of %age reduction (Mean ± SD) of extracts from Jhang, Toba Tek Singh and Sargodha

| Sample Name | Mean ± SD of % Reduction |
|---|---|
| Jhang | 62.2400 ± .01000 |
| Toba Tek Singh | 64.0203 ± .00950 |
| Sargodha | 63.2768 ± .80171 |

The data in table 4.6 represents mean values of the percentage reduction along with the standard deviation (SD) for samples from three different locations: Jhang, Toba Tek Singh, and Sargodha. Potential for reducing ferric ion followed the trend: Toba Tek Singh (64.0203%) > Sargodha (63.2768%) > Jhang (62.2400%). Overall 15% in Toba Tek Singh, 35% in Jhang and 40% *propolis* extracts from Sargodha had shown 64% reducing ability.

**Table 4.7** Percentage inhibition and IC-50 Value of *Propolis* from District Toba Tek Singh at various concentrations (µg/ml) in DPPH radical scavenging model

| Sample Name | % inhibition of DPPH radicals at different concentrations | | | | | IC-50 ± SD |
|---|---|---|---|---|---|---|
| | 25 ug/ml | 50 ug/ml | 100 ug/ml | 200 ug/ml | 400 ug/ml | |
| T1 | 16.1233 | 28.0867 | 51.68 | 69.29 | 84.66 | 100.6 ±0.233 |
| T2 | 18.21 | 30.07 | 53.0767 | 67.55 | 81.29 | 95.2 ± 0.011 |
| T3 | 19.43 | 27.07 | 50.77 | 70.77 | 89.77 | 101.3 ±0.012 |
| T4 | 17.1 | 30.9867 | 51.77 | 69.55 | 84.76 | 94.6 ± 0.029 |
| T5 | 16.07 | 27.4467 | 53.77 | 68.44 | 81.33 | 96 ± 0.027 |
| T6 | 20.1 | 31.44 | 57.29 | 70.78 | 86.06 | 98.3 ± 0.018 |
| T7 | 20.98 | 32.88 | 59.87 | 73.67 | 82.88 | 84.8 ± 0.513 |
| T8 | 16.77 | 28.5467 | 50.78 | 70.55 | 87.55 | 78.7 ± 0.029 |

| | | | | | | |
|---|---|---|---|---|---|---|
| T9 | 17.44 | 27.98 | 53.65 | 69.45 | 81.33 | 100.7 ±0.016 |
| T10 | 17.88 | 33.9767 | 54.9 | 67.88 | 88.05 | 96.6 ± 0.033 |
| T11 | 21.42 | 31.74 | 50.53 | 71.3267 | 82.77 | 86.9 ± 0.013 |
| T12 | 22.77 | 34.07 | 51.22 | 73.66 | 85.33 | 93.8 ± 0.031 |
| T13 | 17.55 | 27.54 | 56.77 | 68.33 | 89.66 | 88.3 ± 0.013 |
| T14 | 19.32 | 29.77 | 58.44 | 67.08 | 81.32 | 93.1 ± 0.034 |
| T15 | 16.87 | 34.88 | 56.88 | 73.66 | 88.87 | 86.2 ± 0.020 |
| T16 | 23.66 | 27.91 | 54.98 | 71.98 | 81.54 | 82.51 ±0.037 |
| T17 | 18.55 | 31.55 | 57.88 | 67.44 | 86.65 | 90.4 ± 0.022 |
| T18 | 16.43 | 32.56 | 53.77 | 72.1667 | 89.44 | 85.2 ± 0.037 |
| T19 | 19.0767 | 29.0567 | 54.08 | 67.88 | 87.08 | 90.4 ± 0.004 |
| T20 | 23.07 | 34.68 | 52.55 | 70.12 | 82.22 | 85.9 ± 0.037 |
| **Mean ± SD** | **18.9 ± 2.37** | **30.6 ± 2.61** | **54.2 ± 2.81** | **70 ± 2.15** | **91.6 ± 6.32** | **91.47 ± 3.13** |

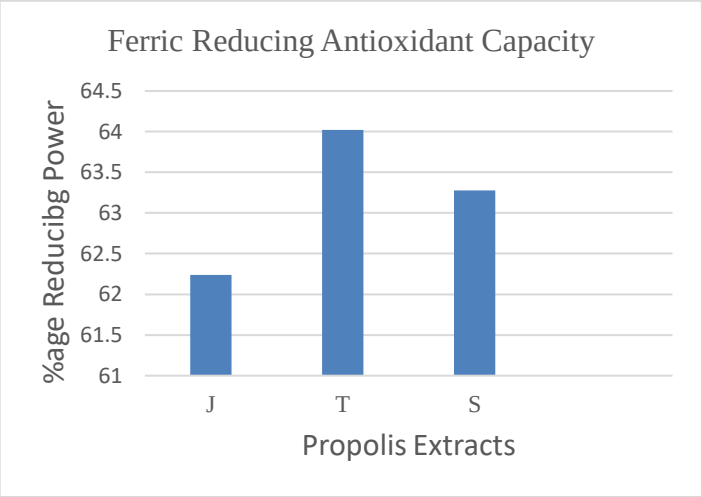



**Figure 4.8** Mean %age reducing power of samples from Jhang, Toba Tek Singh, and Sargodha

### 4.3 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH is a stable purple radical that becomes pale yellow as it absorbs free radicals.

The %age inhibition and IC-50 Values of *propolis* samples from Toba Tek Singh for scavenging DPPH free radical at different concentrations have been presented in table 4.7. The scavenging activity showed a concentration-dependent increase, with the activity increasing as the extract dose was increased. The %age scavenging activity rise from 18.9% to 91.6% with the increase of concentration from 25ug/ml to 400ug/ml.

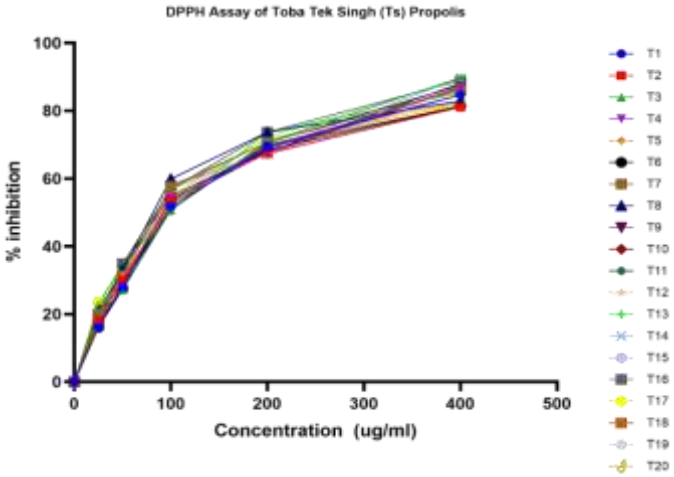



**Figure 4.9** %age inhibition of DPPH free radical scavenging activity of Toba Tek Singh *Propolis* *Propolis* samples showed a stronger scavenging activity against DPPH radical with IC-50 between 101.3 ± 0.012 and 78.7 ± 0.029 ug/ml. The IC-50 (half-maximal inhibitory concentration) is a measure of the concentration of *propolis* required to scavenge 50% of the DPPH radicals. A lower IC-50 value indicates a more potent antioxidant activity of the *propolis* sample. T8 with IC-50 value of 78.7 ± 0.029 ug/ml indicated highest antioxidant activity among samples of Toba Tek Singh while the lowest activity was showed by T3 with IC-50 value of 101.3 ± 0.012.

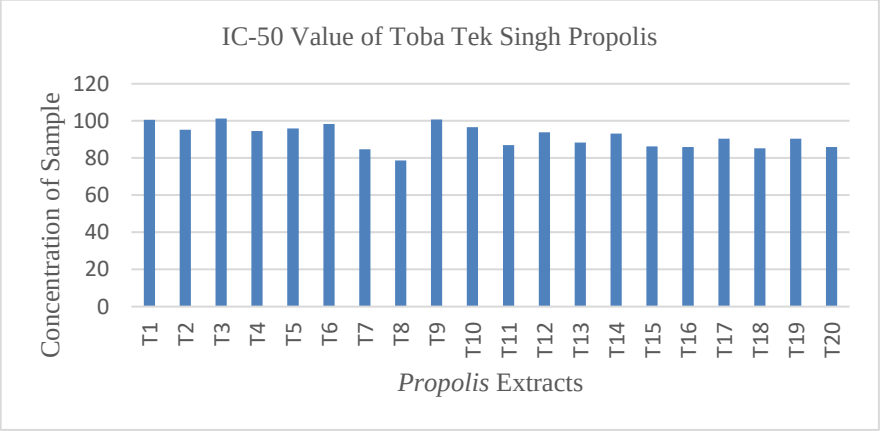



**Figure 4.10** IC-50 Value of *Propolis* Extracts of Toba Tek Singh

The percentage inhibition and IC-50 values for the scavenging ability of *propolis* samples from District Jhang at different concentrations have been presented in table 4.8. The IC-50 values ranges between 115.2 ± 0.024 ug/ml to 86.1 ± 0.028 ug/ml. The maximum IC-50 value (115.2 ± 0.024 ug/ml) was found in J1, indicating a lower level of scavenging activity. Conversely, the minimum IC-50 value (86.1 ± 0.028 ug/ml) observed in J20 showcased high DPPH scavenging.

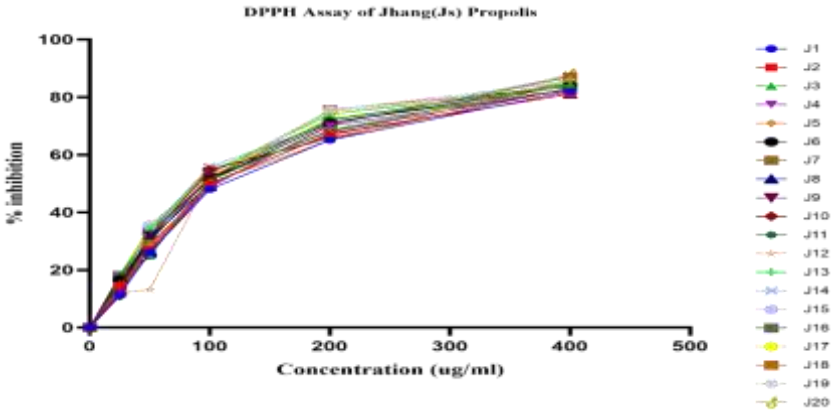
**Table 4.8.** Percentage inhibition and IC-50 Value of *Propolis* Samples from District Jhang at various concentrations (µg/ml) in DPPH radical scavenging model

| Sample name | % inhibition of DPPH radicals at different concentrations | | | | | IC-50 ± SD |
|---|---|---|---|---|---|---|
| | 25 ug/ml | 50 ug/ml | 100 ug/ml | 200 ug/ml | 400 ug/ml | |
| J1 | 11.5 | 26.07 | 48.07 | 65.11 | 82.07 | 115.2 ± 0.024 |
| J2 | 14.58 | 27.1133 | 50.07 | 67.09 | 81.12 | 106.7 ± 0.025 |
| J3 | 15.1167 | 25.0267 | 51.07 | 68.54 | 84.33 | 106.5 ± 0.009 |
| J4 | 12.0767 | 26.1133 | 49.1133 | 70.04 | 82.44 | 108.1 ± 0.007 |

| | | | | | | |
|---|---|---|---|---|---|---|
| J5 | 11.77 | 28.0833 | 50.11 | 65.0767 | 86.07 | 109.3 ± 0.027 |
| J6 | 16.66 | 25.1133 | 51.1267 | 71.1 | 84.11 | 103.7 ± 0.014 |
| J7 | 13.22 | 29.08 | 48.76 | 70.43 | 87.02 | 104.9 ± 0.023 |
| J8 | 13.6533 | 31.1133 | 49.78 | 67.32 | 81.08 | 102.1 ± 0.010 |
| J9 | 14.6633 | 32.0567 | 52.02 | 68.1133 | 82.88 | 96 ± 0.016 |
| J10 | 11.0233 | 31.07 | 54.7667 | 65.77 | 83.11 | 95.9 ± 0.038 |
| J11 | 17.07 | 30.1133 | 51.98 | 72.12 | 84.76 | 95.6 ± 0.008 |
| J12 | 12.12 | 25.7933 | 52.11 | 75.05 | 82.44 | 100 ± 10.4 |
| J13 | 16.0333 | 34.08 | 53.56 | 74.05 | 83.66 | 88.3 ± 0.028 |
| J14 | 15.0367 | 34.12 | 55.77 | 71.05 | 87.44 | 86.3 ± 0.02 |
| J15 | 15.15 | 35.0867 | 53.0867 | 75.43 | 85.32 | 87.9 ± 0.012 |
| J16 | 18.02 | 33.22 | 54.33 | 71.33 | 82.67 | 87.8 ± 0.019 |
| J17 | 18.1133 | 35.1133 | 52.55 | 72.88 | 81.28 | 87.5 ± 0.007 |
| J18 | 17.1133 | 33.08 | 50.9867 | 75.65 | 83.5367 | 92.1 ± 0.015 |
| J19 | 14.0267 | 35.88 | 52.4467 | 68.08 | 84.77 | 90.3 ± 0.02 |
| J20 | 18.0867 | 34.56 | 55.02 | 66.24 | 87.98 | 86.1 ± 0.028 |
| **Mean ± SD** | **14.7 ± 2.3** | **30.5 ± 3.7** | **51.8 ± 2.16** | **70 ± 3.44** | **97.5 ± 9.08** | **83.9 ± 2.06** |

**Figure 4.11** %age inhibition of DPPH free radical scavenging activity of Jhang *Propolis*

Five different concentrations (25ug/ml, 50ug/ml, 100ug/ml, 200ug/ml, 400 ug/ml) were evaluated for the scavenging activity and the activity increased with the increase in concentration of *propolis* extract. There was a dose dependent increase in the percentage antioxidant activity for all concentrations tested as shown in figure 4.11.



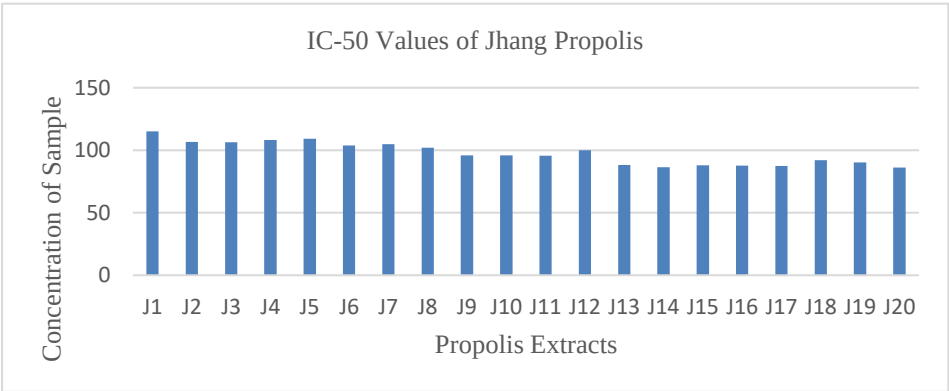




**Figure 4.12** IC-50 Value of *Propolis* Extracts of Jhang

**Table 4.9** Percentage inhibition and IC-50 Value of *Propolis* Samples from District Sargodha at various concentrations (µg/ml) in DPPH radical scavenging model

| Sample name | % inhibition of DPPH radicals at different concentrations | | | | | IC-50 ± SD |
|---|---|---|---|---|---|---|
| | 25 ug/ml | 50 ug/ml | 100 ug/ml | 200 ug/ml | 400 ug/ml | |
| S1 | 16.54 | 28.55 | 48.08 | 74.88 | 83.32 | 101.9 ± 0.021 |
| S2 | 20.02 | 30.02 | 50.19 | 69.08 | 86.55 | 98.9 ± 0.487 |
| S3 | 14.52 | 27.55 | 55.51 | 71.38 | 84.03 | 95 ± 0.018 |
| S4 | 19.08 | 35.52 | 49.08 | 75.5567 | 84.44 | 93.9 ± 0.024 |
| S5 | 17.33 | 32.22 | 51.15 | 73.32 | 88.66 | 91 ± 0.03 |
| **Mean ± SD** | **17.4 ± 2.16** | **30.7 ± 3.18** | **50.80 ± 2.87** | **72.84 ± 2.64** | **96.14 ± 4.28** | **85.40 ± 2.18** |

Percentage inhibition and IC-50 Value of *propolis* samples from District Sargodha for scavenging DPPH radical have been presented in table 4.9. The minimum and maximum IC-50 value represented by S5 and S1 were 91 ± 0.03 and 101.9 ±0.021 respectively. S5 depicted the lowest value of IC-50 which means it has the highest scavenging activity among Sargodha samples whereas highest IC-50 with lowest scavenging activity was found in S1. Figure 4.13 depicted that with an increase in concentration, the antioxidant capacity of *propolis* extracts demonstrated a linear increase.

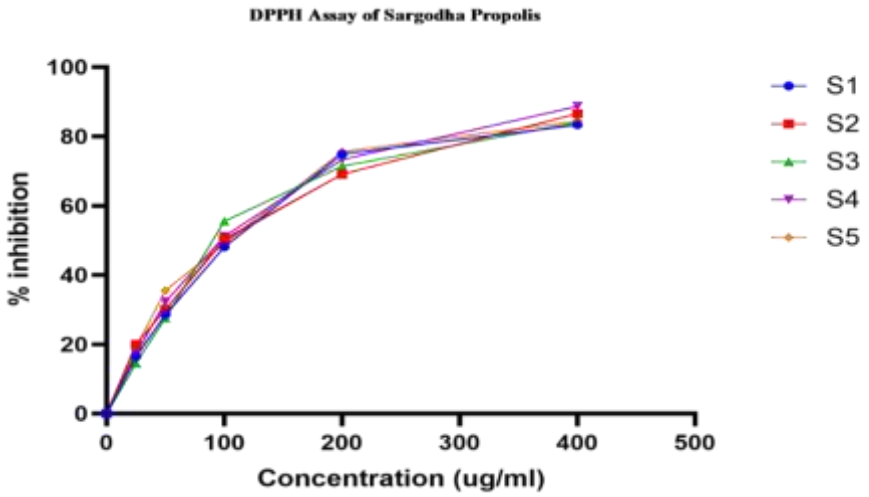



**Figure 4.13** %age inhibition of DPPH free radical scavenging activity of Sargodha *Propolis*

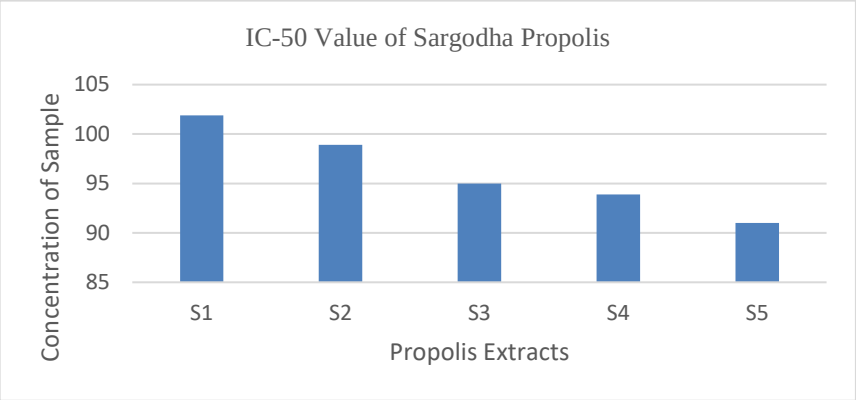



**Figure 4.14** IC-50 Value of *Propolis* Extracts of Sargodha

The table 4.10 presented the IC-50 values (half-maximal inhibitory concentration) for the antioxidant capacity of *propolis* extracts from three different regions: Jhang, Toba Tek Singh, and Sargodha. The IC-50 values are measured in micrograms per milliliter (ug/ml).

**Table 4.10** Comparison of the Percentage inhibition and IC-50 Value of *Propolis* from District Jhang, Toba Tek Singh, and Sargodha in DPPH radical scavenging model

| Sample name | % inhibition of DPPH radicals at different concentrations | | | | | $IC_{50}$ ± SD |
|---|---|---|---|---|---|---|
| | 25ug/ml | 50ug/ml | 100 ug/ml | 200 ug/ml | 400 ug/ml | |
| Jhang | 14.7 ± 2.3 | 30.5 ± 3.7 | 51.8 ± 2.16 | 70 ± 3.44 | 97.5 ± 9.08 | 83.9 ± 2.06 |
| Toba Tek Singh | 18.9 ± 2.37 | 30.6 ± 2.61 | 54.2 ± 2.81 | 70 ± 2.15 | 91.6 ± 6.32 | 91.47 ± 3.13 |
| Sargodha | 17.49 ± 2.16 | 30.7 ± 3.18 | 50.80 ± 2.87 | 72.84 ± 2.64 | 96.14 ± 4.28 | 85.40 ± 2.18 |

The *propolis* extracts from Jhang demonstrated the strongest antioxidant activity with the lowest mean IC-50 value of 83.9 ug/ml. This indicated that the *propolis* extracts from Jhang were most effective in scavenging DPPH free radicals among the three regions. The *propolis* extract from Sargodha showed a slightly lower mean IC-50 value of 85.40 ug/ml compared to Toba Tek Singh (91.47 ug/ml). This suggested that the *propolis* from Sargodha had a slightly stronger antioxidant capacity than Toba Tek Singh. The antioxidant activity of the three regions followed the order of Jhang > Sargodha > Toba Tek Singh.

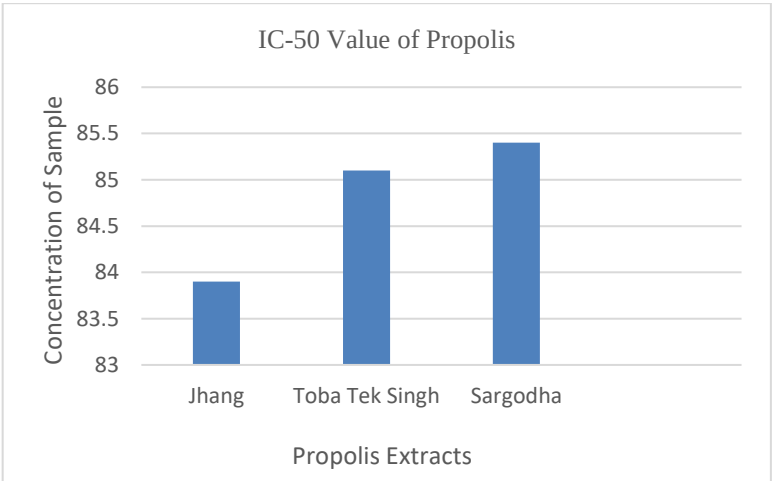



**Figure 4.15** Mean IC-50 Value of *Propolis* Extracts of Jhang, Toba Tek Singh and Sargodha

**Table 4.11** The analysis of variance (ANOVA) of HPSA assay of Toba Tek Singh *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 853.226 | 19 | 44.907 | 201148.4 | .000 |
| Within Groups | .009 | 40 | .000 | | |
| Total | 853.235 | 59 | | | |

**Table 4.12** The analysis of variance (ANOVA) of HPSA assay of Jhang *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 585.201 | 19 | 30.800 | 206.378 | .000 |
| Within Groups | 5.970 | 40 | .149 | | |
| Total | 591.171 | 59 | | | |

**Table 4.13** The analysis of variance (ANOVA) of HPSA assay of Sargodha *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 47.776 | 4 | 11.944 | 137814.4 | .000 |
| Within Groups | .001 | 10 | .000 | | |
| Total | 47.777 | 14 | | | |

**Table 4.14** The analysis of variance (ANOVA) of FRAP assay of Toba Tek Singh *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 193.862 | 19 | 10.203 | 7929.331 | .000 |
| Within Groups | .051 | 40 | .001 | | |
| Total | 193.913 | 59 | | | |

**Table 4.15** The analysis of variance (ANOVA) of FRAP assay of Jhang *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 160.031 | 19 | 8.423 | 42.086 | .000 |
| Within Groups | 8.005 | 40 | .200 | | |
| Total | 168.036 | 59 | | | |

**Table 4.16** The analysis of variance (ANOVA) of FRAP assay of Sargodha *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 34.474 | 4 | 8.619 | 9.11 | .000 |
| Within Groups | .000 | 10 | .000 | | |
| Total | 34.474 | 14 | | | |

**Table 4.17** The analysis of variance (ANOVA) of DPPH assay of Toba Tek Singh *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2437 | 19 | 128.3 | 7777 | .000 |
| Within Groups | 0.6597 | 40 | 0.01649 | | |
| Total | 2438 | 59 | | | |

**Table 4.18** The analysis of variance (ANOVA) of DPPH assay of Jhang *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 4706 | 19 | 247.7 | 45.39 | .000 |
| Within Groups | 218.3 | 40 | 5.457 | | |
| Total | 4924 | 59 | | | |

**Table 4.19** The analysis of variance (ANOVA) of DPPH assay of Sargodha *Propolis* Extracts

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 220.6 | 4 | 55.15 | 1147 | .000 |
| Within Groups | 0.4806 | 10 | 0.04806 | | |
| Total | 221.1 | 14 | | | |

## DISCUSSION

Reactive oxygen species (ROS) and their counterparts, reactive nitrogen species (RNS), play essential roles in various biological processes. However, their presence also poses a significant threat to the integrity of biological material and cellular physiology (Sies et al. 2017). The body employs protective mechanisms, such as antioxidants and antioxidative enzymes, to regulate the levels of reactive oxygen species (ROS) within cells, ensuring they remain at appropriate physiological concentrations. Both internal and external factors can influence the amount of ROS by modulating the activity of enzymes responsible for ROS production and degradation (Schuermann and Mevissen 2021). The danger of oxidative stress in the body, which may cause several diseases, can rise if there is an imbalance between reactive oxygen species (ROS) and endogenous antioxidant mechanisms (Guzelmeric et al. 2021). On a cellular level, oxidative stress impairs the function of tissues and organs, and eventually the entire body (Dossena and Marino 2021).

Habryka et al. (2020) stated that *propolis* and other bee products are rich in physiologically active chemicals. *Propolis*, more than any other bee product, demands special attention. It is a viscous and sticky resin-like material created by bees from a combination of insect secretions and plant resin. The chemical composition of *propolis* is strongly influenced by the surrounding vegetation near the beehive. In recent years, *propolis* has gathered significant attention as a valuable natural product, mainly because of its remarkable ability to scavenge radicals and act as an effective antioxidant (Guzelmeric et al. 2021).

For direct application, *propolis* requires purification through extraction methods. These techniques are essential to obtain *propolis* enriched with bioactive compounds while eliminating excess wax content (Zainal et al. 2022). In fact, extraction is an essential step in utilizing *propolis*' bioactive ingredients. The extraction of bioactive elements from bee glue attempts to dissolve the essential plant-derived chemicals while removing the wax, which is always present in *propolis* up to 20% (Bankova et al. 2021). *Propolis* extracts are more typically prepared using traditional methods such as aqueous or ethanolic extraction or Soxhlet extraction (Machado et al. 2016; Galeotti et al. 2017; Galeotti et al. 2018). Suran et al. (2021) declared ethanol as the most important solvent for the medicinal and chemical analysis of crude *propolis*. Considering the detrimental effects of free radicals on the human body and the potential of *propolis* in mitigating these harmful effects, the purpose of this study was to evaluate the antioxidant capacity of *propolis* from Jhang, Toba Tek Singh, and Sargodha districts in Punjab, Pakistan. In this current study raw *propolis* samples were extracted using 100% ethanol. Antioxidant potential of the *propolis* extracts was assessed by using invitro analysis. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay, Ferric reducing antioxidant power (FRAP) assay, and Hydrogen peroxide ($H_2O_2$) scavenging activity (HPSA) assay were used for determining the antioxidant potential.

The DPPH radical scavenging activity has been frequently utilized to assess the free radical-scavenging capacity of various matrices (Pereira et al. 2006, Oliveira et al. 2007, Sousa et al.

2008, Oliveira et al. 2008). DPPH is a stable free radical that dissolves in ethanol and has a distinct absorbance at 517 nm due to its purple color. By donating hydrogen, antioxidant molecules neutralize the free radical, and the color of the DPPH test solution changes to light yellow, leading to a decrease in absorbance. One of the recognized processes by which antioxidants reduce lipid oxidation is free radical scavenging (Moreira et al. 2008). In present study, the decrease of DPPH radicals displayed a concentration-dependent trend for all *propolis* samples tested. *Propolis* samples from Jhang region presented a very high scavenging activity at very low extract concentration: at 25µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, and 400µg/ml the %age inhibition for DPPH radicals was 14.7%, 30.5%, 51.8%, 70%, and 97.5% respectively and the IC-50 value was 83.9µg/ml. In a different study conducted by Dervisoglu et al. (2022) on Bingol *propolis* reported a %age inhibition of 17.06% at 200µg/ml (extract/solvent) concentration. Comparing current study results with the findings of Dervisoglu et al. (2022) *propolis* from Jhang region showed a significantly higher %age inhibition (70%) at the same concentration (200µg/ml). *Propolis* extracts from Toba Tek Singh region also showed similar % age of antioxidant activity (70%) for scavenging DPPH radicals at the same concentration. Sargodha *propolis* samples showed even higher antioxidant capacity of 72.84% at 200µg/ml. Aboulghazi et al. (2022) conducted a study on Moroccan *Propolis* and found IC-50 value for DPPH radical scavenging activity to be 21 to 39.15 µg/mL by using methanol as the solvent. Jug et al. (2014) and Bayram et al. (2020) reported that ethanol is frequently employed in the extraction procedure since most of the components in *propolis*' chemical structure are lipophilic. When methanol is employed, the production of flavanones and flavonols, which are among the most significant bioactive components of *propolis*, increases; however, methanol is a harmful solvent for human health.

When the antioxidant activity was evaluated through the capacity for scavenging $H_2O_2$, beyond those samples with the highest activity for scavenging DPPH free radicals (T8, J20, S5), other ones were equally good scavengers of $H_2O_2$, such as T15 (76.34%), J3 (74.91%), S5 (72.22%) had the best capacity for scavenging hydrogen peroxide among all the samples tested. Malaysian *propolis* tested by Nna et al. (2018) for the assessment of its antioxidant activity revealed $H_2O_2$ radical scavenging activity to be $53.94 \pm 1.88\%$ (soft *propolis itama*), $43.34 \pm 0.51\%$ (*propolis itama*), and $35.08 \pm 0.84\%$ (*propolis apicalis*). Comparing present work results with the findings of Nna et al. (2018) all *propolis* samples from Jhang (68.1720±3.07%), Toba Tek Singh (71.5591±3.86%), and Sargodha (70.0394±1.98%) showed higher hydrogen peroxide scavenging activity. Guendouz et al. (2018) conducted a research on Moroccan *Propolis* and found hydrogen peroxide scavenging activity to be significantly higher for samples 1 ($IC_{50}$ = 0.086 mg/mL) and 23 ($IC_{50}$ = 0.048 mg/mL). Once the unities are different, it is not possible to compare the results obtained by Guendouz et al. (2018) with the values obtained in the present study. In terms of capacity for reducing Fe3+ to Fe2+ evaluated through FRAP assay *propolis* samples J13 (64.916%), T1 (66.75%), and S3 (65.67%) depicted the highest ferric reducing activity. In FRAP assay, Toba Tek Singh samples and Sargodha samples exhibited higher activities than samples from Jhang, and their relative activities were Toba Tek Singh > Sargodha > Jhang. A study conducted by Temizer et al. (2017) on *propolis* of Irano-Turanian region documented the ferric reducing power to be 90.73±0.24%. Comparing present study results with Temizer et al. (2017) *propolis* extracts from the 3 studied areas showed less ferric reducing activity than *propolis* extracts of Irano-Turanian region. Huang et al. (2014) and Kekecoglu et al. (2020) documented that the presence of such variations between samples can be explained by the composition being influenced by elements such as local flora, collection region, bee breed, production period, and hive material.

## CONCLUSION

The present work findings concluded that *propolis* from the studied areas (Jhang, Toba Tek Singh, and Sargodha) can be a good source of antioxidants for food supplements because *propolis* collected from these areas had presented different antioxidant potential. However, it is critical to identify and quantify the key active chemicals independently in order to assess their biological activity. *Propolis* collection can be done from more areas of Punjab so that we can have a bigger clear picture about

their antioxidant potential and select the best out of them.

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