

## Aqueous and Alcoholic Bee Pollen Extract effects on Peroxidase in the Sera of healthy individuals

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### Abstract

**Background:** Many studies have concentrated on the pro-health and therapeutic pictures of phytochemical's material products, coalesce bee products for it. Bee pollen have been highly containing on important huge compounds such as flavonoids, terpenoids and phenolic acids which had been an inhibiting effects on large enzymes types. Peroxidase was an important metabolics enzymes products.

**Objectives:** To investigation the effect of alcoholic and aqueous extracts of bee pollen on peroxidase activity in the sera of healthy human.

**Materials & Methods:** Extracts were prepared using two solvents, were 0.6 g of collected bee pollen was extracted with 50ml water, and with 50 ml absolute ethanol; then, stirred for 24 hours at room temperature; after that, filtered in two steps: filter paper and medicinal lint

**Results:** The results of the current study show the obvious incidence of these extracts in which have been an inhibitory effect in the present study on peroxidase activity. different extracts and volumes had been used in this study which gave the inhibitory percentage at maximum volume 75  $\mu$ l extract equal to 65.74 & 23.51% in peroxidase in ethanol and aqueous extracts with respectively.

**Conclusions:** The recorded Michaelis-Menten Kinetics ( $K_m$ ) values in ethanolic extract was higher than  $K_m$  in the aqueous extract, where as the recorded Velocity maximum ( $V_{max}$ ) values in aqueous extract were higher than  $V_{max}$  in ethanolic extract.

**Keywords:** Bee pollen, Peroxidase.

## **Introduction**

In recent studies, there are large studies have been focused on the healthy and therapeutic properties of phytochemical material products; including bee products <sup>1</sup>. Bee pollen is used as an immune stimulator antimicrobial, anti-viruses, wounds burns healing, anti-tumor, and local analgesic. Some studies have been shown that bee pollen and honey reducing the symptomatic of menopause in women and breast cancer patients, as it relieves or reduced the symptoms of menopause in patients who use aromatase inhibitors/inactivators and tamoxifen <sup>2,3</sup>.

Bee pollen is collected by the worker individual bees and pressed into grains from trees and flowers, then transported to the hive. It is dampened with a fragmented and saliva via flightless bee, packed in honeycomb cell, then covered with a thin layer of honey and waxes. The color of bee pollen grain varies from yellow to black <sup>4,5</sup>. There are many factors which contribute to the formation of bee pollen, including climates, plant source and the actions of beekeepers <sup>6</sup>. Pollen is an important source of saccharids, proteins, cure fiber, lipids, phenolic compounds, vitamins, miscellaneous, and minerals <sup>7-9</sup>.

Experiments were conducted on the laboratory mouse's that poisoned with ammonium fluoride, carbon tetrachloride, trichloroethylene and ethionine have confirmed that the bee pollen act to remove the toxicity or poisoning of these organic solvents which destroy the liver cell organ as well as cause liver cirrhosis <sup>10</sup>. Other articles have confirmed that bee pollen is an anti-inflammatory that effectively inhibition of the activity of lipoxygenase also cyclooxygenase, These enzymes are responsible for converting arachdonic acids into a toxic materials such as leukotrienes and prostaglandin, where it is based on the formation of inflammation in the tissues. Because of that it was recommended in the treatment of inflammations, liver diseases and any symptoms of poisoning in this organ <sup>11</sup>.

**Peroxidase [E. C . 1. 1 1.1.7]** is a heme-including enzyme, which catalyzes oxidation of many types of organic and inorganic substrates by uses hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as the electron acceptor <sup>12, 13</sup>, as well as an aromatic substrates which oxidized by one-electron oxidation of (AH<sub>2</sub>) proved by the Chance–George mechanism <sup>14</sup>. The native this pure enzyme is oxidized by a substrate (H<sub>2</sub>O<sub>2</sub>) and subsequent oxidized some compounds. Else, it oxidized a two molecule as an organic compound to free radicals; then terminally enzyme return to its native state <sup>15</sup>.

## **Materials with Methods**

### **Bee pollen collection with its extracts preparation**

The pollen granule (bee pollen) was collected from Iraqi beekeepers, during the primary summer season of 2020. Extracts were prepared using two solvents, where (0.6g) of collected bee pollen was extracted with (50ml) water, and with (50 ml) absolute ethanol; then, stirred for 24 h at room temperature; after that, filtered in two steps: filter paper and medicinal lint. The percentage of inhibition enzyme could calculated by depending on the following equation:

$$(\%Inhibition = 100 - \frac{activity\ with\ extract}{activity\ without\ extract} \times 100)..... [16].$$

### **Peroxidase Activity Assay**

Peroxidase activity was determined in 20 ml sera of a healthy individual by using manual colorimetric method. Then added in four test tubes (1.4 ml) of phenol, and (1.5 ml) of four different concentrations of hydrogen peroxide substrate (0.0018, 0.01, 0.015, 0.017 mM) for each test tubes was added to estimate Michaelis-Menten Kinetics and Velocity maximum ( $K_m$  &  $V_{max}$ ) with types of inhibitions by Lineweaver-Burk plot, with incubation 3 minutes, thus (100  $\mu$ l) of serum and (20 $\mu$ L) distilled water was add with mixing and read at 515 nm at zero time and 5 minutes to calculation the difference in absorbance ( $\Delta A$ ) [17]. The former steps were re-conducted with replace the volume of distilled water by bee pollen extracts for two set of four test tubes alone to calculate the effect of extract on this enzymatic activity and calculate the inhibition percentage. Also tested the different volume extracts for each alone that equal to 10, 25, 50, 75  $\mu$ l at constant the substrate concentration that equal to 0.017 mM. Peroxidase activity and inhibition percentage was calculated according to the following equations:

$$\text{Activity (U/L)} = \frac{\Delta A/\text{min}}{\epsilon V_s} \times V_t \times 10^6. \text{ (} V_t\text{=total volume 3.1 ml), (} V_s\text{=sample volume (100 } \mu\text{l), (}\epsilon\text{= 6580).}$$

### **Results and Discussion**

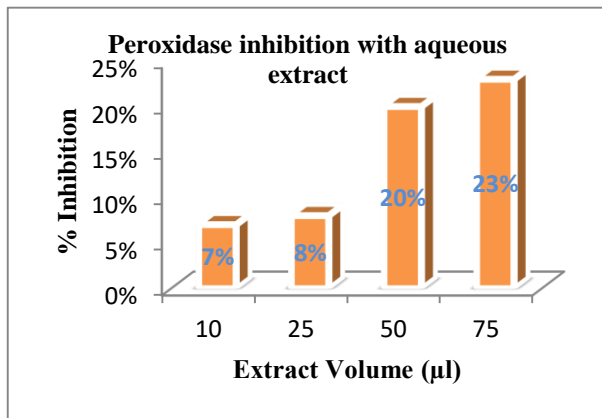
Recently all studies on Bee pollen recorded information on its ingredients, that contained on materials might be interact and reacted with many chemical compounds and acting to be anti oxidants, therefore the experimented their effects in the present study on some enzymes that participate in oxidation reduction reactions. The results in the present study demonstrated the different volume effects on peroxidase activity, which the effects were clear and significant as inhibitors for two extracts (ethanolic and aqueous) on this enzyme. The maximum volume were used from two extracts from stock solution which equal to 75 $\mu$ l, which gave maximum inhibition percentage and shown in figure 1, 2 and table 1 and 2, that equal to 23.5% when using aqueous pollen extraction, and 65.74% when using ethanolic extract with activity 117.33 and 52.55 IU/ml respectively. The kinetic parameters also were calculated according to the Lineweaver-Burk plot as shown in figure 3 and 4, and the results in the presence of inhibitors that recorded in the present study; that demonstrated in table 3 equal to  $K_m=0.0013M$  ( $v_{max}=101$ ) by using aqueous extracts, and  $km=0.0017M$  ( $v_{max}=38.3$ ) by using ethanolic extract.

**Table 1: Effect of different volumes of the alcoholic Bee pollen extract on the activity of peroxidase in in sera of a healthy individual.**

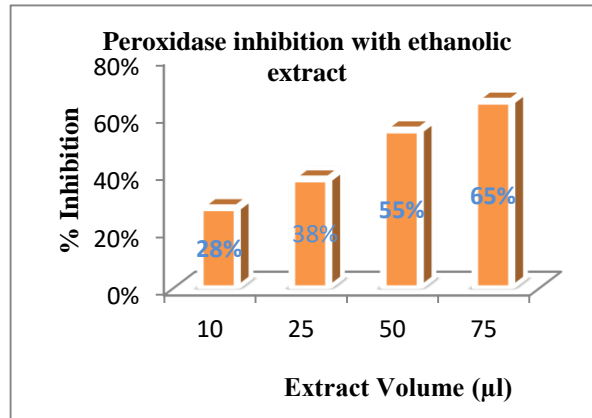
Alcoholic con. ( $\mu$ l)	Peroxidase activity (IU/ml)	Inhibition %
0	153.40	0.00
10	110.16	28.18
25	93.73	38.89
50	68.45	55.37
75	52.55	65.74

**Table 2: Effects of different volumes of aqueous Bee pollen extraction on activity of peroxidase in in sera of a healthy individual.**

Aqueous extract volume (µl)	Peroxidase activity (IU/ml)	Inhibition %
0	153.40	0.00
10	142.21	7.29
25	140.05	8.70
50	121.72	20.65
75	117.33	23.51



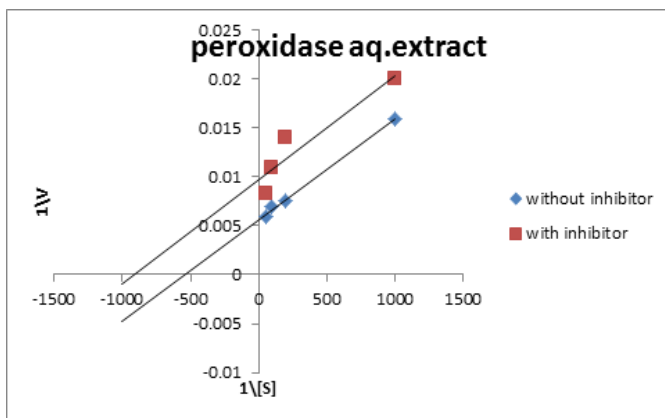
**Figure 1: Peroxidase inhibition percentages with different volume of aqueous extract in sera of a healthy individual.**



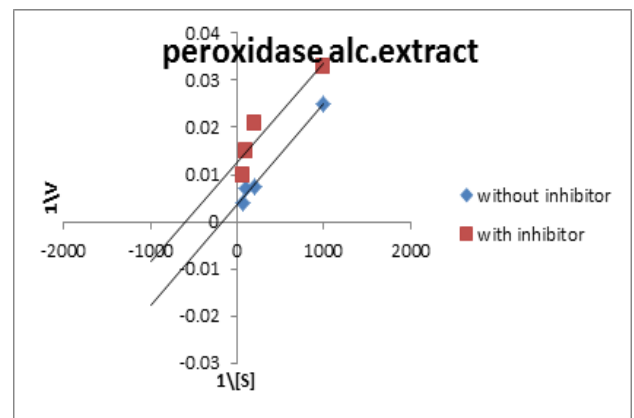
**Figure 2: Peroxidase inhibition percentages with different volume of ethanolic extract in sera of a healthy individual.**

**Table 3: Kinetic parameter; Km , V max, and type of inhibition for alcoholic with aqueous bee pollen extracts on peroxidase activity in sera of a healthy individual.**

Enzyme	Km (M)	Vmax mol/ml/min)	Type of inhibition
Peroxidase aq.	0.0013	101.0	Competitive
Peroxidase alc.	0.0017	38.3	Competitive



**Figure 3: Lineweaver-Burk plot with the presence and absence of bee pollen aqueous extract for peroxidase in sera of a healthy individual.**



**Figure 4: Lineweavers-Burk plot with the presence and absence of bee pollen alcoholic extract for peroxidase in sera of a healthy individual.**

### **Discussions:**

The present study showing that alcoholic extract have a higher effect than an aqueous extract because alcoholic extract as an organic solvent have been might and able to extract more ingredients compounds that may be cause the inhibition of enzymes. This study supports the use of the bee pollen extracts which inhibited peroxidases and exhibited significant free radical scavenging and antioxidation activities which is related to inflammation treatment. People administration antidepressants drugs may suffer from crisis called hypertensive, and it may be fatal <sup>18</sup>. Because of bee pollen have many of phenolic compounds, it have anti-Parkinson effects <sup>19</sup>. Else, bee pollen is not only useful as a dietary supplements but as functional one, therefore we recommend to carry out more studies about bee pollen extracts and using it to treatment some illness such as inflammation, cancer and depression because its inhibition of peroxidase and other enzymes regulates serotonin and catecholamin in nervous system <sup>20</sup>.

Bee pollen is rich in polyphenols materials <sup>21</sup>; which may be responsible inhibitory effect for these extracts. Due to the Peroxidase exhibited to different compounds that extracted from bee pollen, through carried out of enzymatic reaction. The important basic role of polyphenol in chemical reaction properties is its ability to form a complexes with metal ion <sup>22</sup>. Interaction of these polyphenols with iron ion that incorporated with chemical structure of peroxidase, therefore these polyphenols prevent oxidation-reduction of enzymatic reaction and finally inhibition of enzyme <sup>23</sup>.

In conclusion, bee pollen extract can be used to treat depression because it has an effect on inhibiting of peroxidase, inflammation and cancer because it is act as aromatase inhibitors.

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