

The Effect of Aqueous Extract of *Boswellia Papyrifera* on Salivary Peroxidase Activity

Maha Mohsin Khalaf, Suha Talal Abd and Eman Ali Salman

*Department of Basic Sciences, College of Dentistry, University of Baghdad, 10071 Baghdad, Iraq
Maha.Mohsin@codental.uobaghdad.edu.iq, Suhatalal_abd@codental.uobaghdad.edu.iq,
dr.eaman.alrubaiee@codental.uobaghdad.edu.iq*

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Abstract: This study investigated the effect of *Boswellia papyrifera* aqueous extract on human salivary peroxidase activity. Salivary peroxidase, a heme-containing enzyme secreted by acinar cells, plays a crucial role in maintaining oral health by generating antimicrobial hypothiocyanite ions (OSCN^-). Various concentrations of *Boswellia papyrifera* extract (2–15 g/100 mL) were tested to determine their influence on enzyme activity under different pH and temperature conditions. Results showed that enzyme activity increased significantly in a concentration-dependent manner, with maximal activation observed at 15 g/100 mL. The optimal enzymatic activity was recorded at 37° C and pH 6.5 under control conditions, while the presence of the extract enhanced activity across a broader temperature and pH range. Kinetic analysis revealed a marked increase in catalytic efficiency, as indicated by a higher V_{max} (260 U) and a lower K_m (0.02 mM) compared to control ($V_{\text{max}} = 204$ U; $K_m = 0.57$ mM). These findings suggest that bioactive components of *B. papyrifera*, such as boswellic acids and terpenoids, may stabilize or activate the enzyme's catalytic conformation, enhancing substrate affinity and turnover. Overall, the study highlights the potential of *B. papyrifera* as a natural modulator of salivary peroxidase.

1 INTRODUCTION

Human salivary peroxidase is an enzyme generated primarily by acinar cells located in the salivary glands. This enzyme contains in its structure a heme group; this heme group is essential for the function of the enzyme; it enables the enzyme to catalyze the oxidation of the substrate by hydrogen peroxide and other active substances [1]-[3]. The activity of this enzyme significantly impacts the maintenance of oral hygiene by exerting natural antimicrobial activity. Through its interaction with hydrogen peroxide and thiocyanate ions, salivary peroxidase generates hypothiocyanite ions (OSCN^-), which possess strong antimicrobial properties. These compounds inhibit the growth and metabolism of a wide range of oral pathogens, including *Streptococcus mutans*, a key contributor to dental caries [4]. Furthermore, salivary peroxidase maintains the normal microflora balance in the oral cavity, thus reduces the incidence of infections, such as gingivitis and periodontitis. The actions of this enzyme influence the overall health of oral as well as systemic through reducing bacterial growth as one of the first line defense mechanisms of

the body [5]-[7]. Accordingly, an increase in this enzyme activity can positively boost antimicrobial effect of the enzyme, as reported by researchers by using different types of substances, such as polyphenols [8]. Or other chemical substances such as using titanium dioxide nanoparticles to induce enzyme activity [9]. Natural plant extracts have long been studied for their therapeutic and biological properties, particularly their potential to modulate enzymatic activity and enhance host defense mechanisms. *Boswellia papyrifera*, a medicinal plant traditionally used for its anti-inflammatory, antioxidant, and neuroprotective effects, contains a variety of bioactive compounds, including boswellic acids and essential oils [10], [11]. Research indicates that boswellic acid's triterpenes reduce leukotriene production by blocking 5-LOX, a key enzyme in leukotriene synthesis, thereby curbing inflammatory responses [12], [13]. These constituents may interact with salivary enzymes and influence their activity, potentially boosting the oral cavity's natural antimicrobial defense [10], [14]. In this context, evaluating the effect of *Boswellia papyrifera* extract on salivary peroxidase activity provides a promising

avenue for exploring plant-based strategies to support oral hygiene and health [15]-[17].

2 EXPERIMENTAL

Four different concentrations of *Boswellia papyrifera* (BP) were prepared and used (2, 5, 10, 15 g/100ml) according to references (5, 6). The mentioned concentrations were added to the saliva. The enzymatic activity was measured. With the help of the website EPITOOLS (https://epitools.ausvet.com.au/case_controls), the appropriate size of the sample was determined as 40 samples using a confidence interval of 95% and an error margin of 5%. Unstimulated whole saliva samples were collected from volunteers. The procedure ensured minimal discomfort and was easy to administer in a clinical setting. Proper labeling and storage of the samples were crucial to maintain their integrity for subsequent analysis. A colorimetric method was used to determine the activity of salivary peroxidase in the sample with and without aqueous extract of *Boswellia papyrifera* [8], [9]. Different pH values were tested to identify optimum pH condition. Salivary peroxidase activity was investigated in the range of pH 5.0-7.5 using phosphate buffer (0.2 M) on the pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5) with and without aqueous extract of *Boswellia papyrifera*. The effect of temperature was determined by measuring enzyme activity at different temperatures (4, 25, 37, 50° C) with and without aqueous extract of *Boswellia papyrifera* (9). Enzyme activity was measured by using five different concentrations of hydrogen peroxide (0.33, 0.5, 1.0, 1.7, 3.4 mM) for determining Km (Km is a substrate concentration and is the amount of substrate it takes for an enzyme to reach Vmax/2) and Vmax values (Vmax or maximum velocity of an enzymatic reaction can be defined as the rate of the reaction at which the enzyme shows highest turnover) of substrate with and without aqueous extract of *Boswellia papyrifera* [10].

3 RESULTS AND DISCUSSIONS

3.1 Effect of *Boswellia Papyrifera* Aqueous Extract on Salivary Peroxidase Activity

Under the same experimental conditions, the peroxidase enzyme activity was measured, first without the presence of *Boswellia* extract, as a

negative control, and then measured with 4 different concentrations of *Boswellia* extract. The results show a slight decline of the enzymatic activity at low doses of *Boswellia* (2g/100ml) from 22.857 ± 1.965 enzymatic activity in control to 14.265 ± 0.858 . followed by an increase in enzymatic activity when *Boswellia* was increased to 5 g/100ml to 24.964 ± 1.169 . A significant elevation of enzyme activity when the concentration of *Boswellia* increased to 10 /100 ml to reach 71.976 ± 6.832 . Moreover, at 15 g/100 ml the enzyme activity increased significantly and reached 112.178 ± 3.093 . This indicates that the extract has stimulatory effect on the enzyme activity.

These primitive experiments were to determine the optimal concentration that can influence and increase peroxidase activity. Enzyme activity peaked at a concentration of 15 g/100 ml as shown in Figure 1, suggesting this as the most effective dose under the experimental conditions.

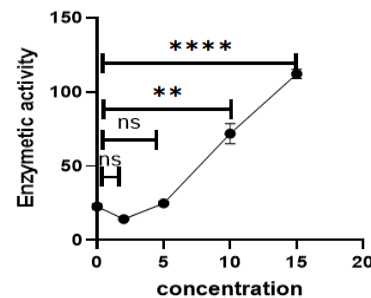


Figure 1: Salivary peroxidase enzymatic activity at different concentrations of *Boswellia papyrifera*.

A slight decline of enzymatic activity, followed by gradual increase when *Boswellia* increased. The data were significant ($p < 0.0001$) when analyzed with one way ANOVA test, followed by Sidak multiple comparison test as post hoc test.

3.2 Effect of Temperature on Salivary Peroxidase Activity

Different temperature settings were applied (four, twenty-four, thirty-seven, forty-five) on the experimental conditions to test the optimal temperature at which salivary peroxidase works the best, the experiment was conducted with and without the presence of *Boswellia* extract. *Boswellia* extract were used at 15 g/100 ml. Salivary peroxidase maximum activity was at 37°C without the presence of *Boswellia* extract; after that decreased with increasing temperature up to 50°C. Table 1 summarizes enzymatic activity at each temperature point, numbers expressed as average ± standard error of mean.

Table 1: Salivary Enzymatic activity at 4 different temperature settings values, expressed as mean of 3 independent experiments ± standard error of mean.

Temperature	Enzyme activity without Boswellia	Enzyme activity with Boswellia
Four	90.8 ± 2.7	187.01 ± 3.1
Twenty-five	109.6 ± 9.3	254.5 ± 10.7
Thirty-seven	165.8 ± 0.2	239.3 ± 4.2
Forty-five	17.02 ± 0.5	51.1 ± 1.8

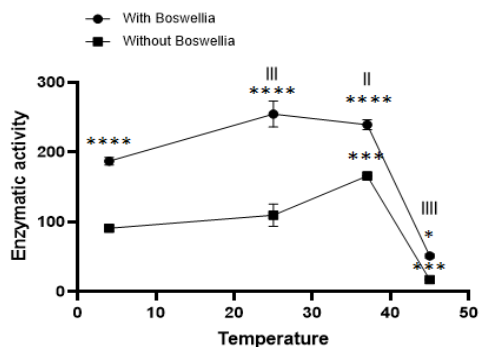


Figure 2: Effect of temperature on enzymatic activity.

There was a significant difference in enzymatic activity between with and without the presence of Boswellia at the same temperature at all four temperature points (*p < 0.05, **** p < 0.0001). In the presence of Boswellia extract, there was a significant increase between four and twenty-four degrees, four and thirty-seven (II p<001, III p< 0.001). Between four and forty-five, there was a significant reduction in enzymatic activity (IIII p< 0.0001). As well as, without the presence of Boswellia, there was a significant increase of enzymatic activity between five and thirty- seven degrees (***p< 0.001).

3.3 Effect of pH and Ionic Strength on Salivary Peroxidase Activity

The effect of variable values of pH were applied to test the effect on peroxidase activity with and without the presence of Boswellia extract (15 mg/100ml) (Fig. 3), to determine the influence of changes of pH on the enzymatic activity, and whether the changes of the pH can influence the enzyme or even the effect of the extract on the enzymatic activity. Among the tested pH, 6.5 yields the highest activity of peroxidase enzyme with and without the presence of Boswellia extract. The presence of *Boswellia papyrifera* aqueous extract caused a higher enzyme activity compared to the control group (Fig. 3). Numbers are expressed as mean ± standard error of mean included in Table 2.

Table 2: Values of peroxidase enzyme activity in different pH settings.

pH value	Enzymatic activity without Boswellia	Enzymatic activity with Boswellia
5.0	89.16 ± 2.48	423.10 ± 11.35
5.5	47.01 ± 0.94	434.46 ± 6.55
6.0	294.23 ± 0.81	544.84 ± 13.36
6.5	348.53 ± 13.79	647.02 ± 6.50
7.0	288.55 ± 3.38	637.08 ± 14.59
7.5	272.34 ± 4.84	505.78 ± 3.24

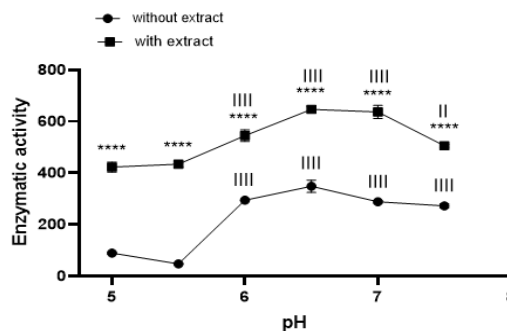


Figure 3: Effect of pH and Boswellia papyrifera on peroxidase enzymatic activity.

A significant increase in activity at the same pH point between with and without the presence of extract (**** p < 0.0001), as well as there was a significant increase of enzyme activity when pH was increased, the significant differences was between five pH and the followed numbers (IIII p< 0.0001. II p< 0.01). the data were analyzed with one way ANOVA, followed by Tukey’s multiple comparison test as post hoc analyses.

3.4 Kinetic Studies

Results in this study show changes in peroxidase enzyme activity when H₂O₂ substrate concentration changes, in the presence and absence of Boswellia extract (Fig. 4). Enzyme activity increased significantly with increasing substrate concentration in both groups. As well as there was a significant increase in the enzyme activity when tested at the presence of Boswellia extracted in compare to the other group without the extract. The data are presented in Table 3 as a mean of three independent experiments ± standard error of mean.

The obtained reaction velocities were plotted against substrate concentration to construct Michaelis- Menten curves, showing the effect of Boswellia papyrifera on the enzyme’s kinetic parameters (Km and Vmax) (Fig. 5).

Table 3: Values of peroxidase enzyme in the presence and absence of Boswellia with different concentration of H₂O₂ substrate.

H ₂ O ₂ concentration in mM	Enzyme activity without Boswellia	Enzyme activity with Boswellia
0.33	35.99 ± 0.32	197.45 ± 2.27
0.50	42.31 ± 0.81	289.85 ± 0.00
1.00	247.13 ± 1.00	344.80 ± 5.03
1.70	152.22 ± 6.97	235.38 ± 7.13
3.40	132.44 ± 3.70	197.93 ± 11.83

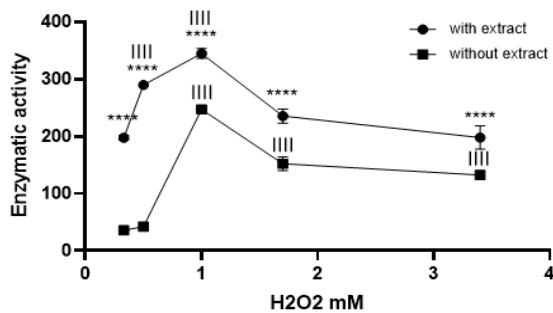


Figure 4: The effect of substrate concentration on enzyme activity was assessed in the presence and absence of Boswellia.

Peroxidase activity increased significantly when substrate concentration increased (IIII p < 0.0001). Adding Boswellia extract increased the enzyme activity in comparison to the other group with no Boswellia at the same substrate concentration points (**** p < 0.0001).

Effect of changes in substrate (H₂O₂) on enzyme activity in the presence and absence of Boswellia Extract.

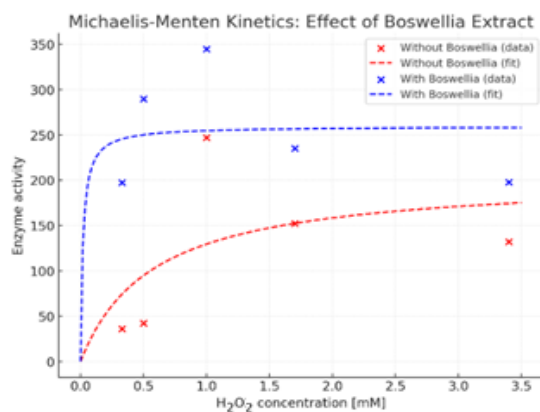


Figure 5: Michaelis- Menton Kinetic: Red (without Boswellia): lower V_{max} (~204) and higher K_m (~0.57 mM), showing weaker catalytic efficiency and substrate affinity.

Blue (with Boswellia): higher V_{max} (~260) and much lower K_m (~0.02 mM), showing stronger catalytic capacity and much tighter substrate binding.

In this study, we investigated how the addition of Boswellia papyrifera affects salivary peroxidase activity. The main findings indicate that Boswellia extract significantly increased enzymatic activity in a concentration-dependent manner. Under control conditions, the enzyme showed maximum activity at 37° C and pH 6.5, whereas in the presence of Boswellia (15 g/100 mL), the apparent optimum temperature shifted to 25° C, while the optimum pH remained at 6.5. Enzyme kinetic analysis revealed that the K_m decreased from 0.57 mM (control) to 0.02 mM (treated), and V_{max} increased from 204 to 260 units. Together, these results suggest that B. papyrifera enhances enzymatic efficiency and stability, potentially through activation or stabilization of the enzyme’s active conformation, or via antioxidant components that preserve the active site from oxidative inhibition (17).

3.5 Concentration-Response (Dose Dependence)

Addition of Boswellia caused a clear concentration-dependent increase in salivary peroxidase activity. At 15 g/100 mL, activity increased by approximately 390.7%, from 22.86 ± 1.97 to 112.18 ± 3.09 (n = 3, p < 0.0001). This dose-response pattern suggests that compounds within the extract may directly interact with the enzyme to enhance its catalytic activity [18].

3.6 Temperature Effects

The enzyme exhibited maximum activity at 37 °C under control conditions. In the presence of Boswellia, the apparent optimum shifted to a lower temperature (25° C), although activity remained relatively high at 37° C. In both conditions, activity declined sharply at 45 °C, indicating thermal denaturation at higher temperatures. The shift toward lower optimum temperature may reflect conformational stabilization by extract constituents at moderate temperatures, allowing the enzyme to maintain high activity without requiring its normal thermal optimum [19].

3.7 pH Effects

The pH–activity profile showed a maximum activity at pH 6.5 in both control and treated samples, indicating that the extract did not change the enzyme’s optimum pH. However, Boswellia

markedly enhanced activity across all tested pH values, particularly in the acidic and neutral ranges. Additionally, the enzyme retained relatively high activity between pH 6.0 and 7.0 when treated with *Boswellia*, suggesting that the extract broadened the effective pH range and enhanced enzyme stability. This effect may result from interactions between bioactive components of the extract and the enzyme surface, potentially minimizing conformational changes or protecting ionizable residues critical for catalysis.

3.8 Enzyme /Kinetics (V_{max} / K_m)

Kinetic analysis showed that treated samples displayed a lower K_m and higher V_{max} relative to control, indicating increased substrate affinity and catalytic efficiency. These changes are consistent with activation or stabilization of the enzyme's active conformation by extract constituents. Possible mechanisms underlying these effects include:

- 1) Direct binding of small molecules to the active or allosteric sites, enhancing catalysis.
- 2) Metal chelation, if the enzyme requires cofactors, altering substrate binding or turnover.
- 3) Antioxidant effects, which may protect the enzyme from oxidative damage and maintain structural integrity.
- 4) Indirect effects, such as changes in assay conditions (e.g., surfactants or pigments in the extract) affecting apparent activity.

Distinguishing among these mechanisms will require targeted follow-up experiments.

3.9 Comparison with Literature

Similar enzyme-activating effects have been reported for plant extracts rich in flavonoids, tannins, or terpenoids, which can modulate enzyme activity through direct binding or stabilization of the active conformation [20], [21]. Our findings align with these reports, supporting the notion that bioactive phytochemicals in *Boswellia* can enhance peroxidase activity and stability under varying environmental conditions.

3.10 Limitations and Future Work

Limitations of this study include the use of a crude extract (active constituents unknown), potential assay interference from pigments or other extract components, and in vitro conditions that may not fully

reflect physiological environments. Future work should aim to: isolate and identify the active compounds, determine K_i values and inhibition/activation types at multiple extract concentrations, conduct structural studies (CD, fluorescence, or X-ray) to map binding sites, and test activity in physiologically relevant or cell-based assays.

4 CONCLUSIONS

This study demonstrates that the aqueous extract of *Boswellia papyrifera* significantly enhances salivary peroxidase activity in a concentration-dependent manner, with maximum activation observed at 15 g/100 mL. The extract also improved enzyme stability across a wider range of temperature and pH conditions without altering the optimal pH (6.5).

Kinetic analysis revealed a decrease in K_m and an increase in V_{max} , indicating enhanced substrate affinity and catalytic efficiency. These effects suggest that bioactive compounds in *Boswellia papyrifera* may stabilize or activate the enzyme's functional conformation.

Overall, the findings highlight the potential of *Boswellia papyrifera* as a natural modulator of salivary peroxidase, with possible applications in improving oral antimicrobial defense. Further studies are required to identify the active components and confirm these effects in vivo.

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