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# Study the effect of willow bark extracts on lipase activity

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**ABSTRACT**

The willow tree (genus Salix, family Salicaceae) is one of the fast-growing deciduous trees or shrubs. This tree is found all over the world and grows mostly in temperate regions. Willow bark contains many metabolites like phenolic glycosides, flavonoids, tannins, and phenolic acids. Beside that lipases can hydrolyze triglycerides and play a critical role in numerous industrial food processes and the activation or inhibition of this enzyme has many benefits in many of these industries. The effect of willow bark extracts of aqueous, ethanol, ethyl acetate, hexane on lipase activity was studied. The highest percentage of inhibition was observed with ethyl acetate and aqueous extracts, which found to be 85 and 61.43 % respectively. The lipase Inhibition by the presence of these extracts was investigated by drawing Lenweaver-Burk plot using different concentrations of para-nitro phenyl acetate as substrate. The results indicated that was noncompetitive inhibition mode using 2.5 mg/ ml of aqueous extract. The maximum velocity (Vmax) of lipase was reduced from 2.63 to 0.9 units /ml/ min. While, the results showed that was uncompetitive inhibition mode using 3 mg/ ml ethyl acetate extract. Vmax of lipase was which reduced from 2.63 to 1.03 units /ml/ min. On the other side, The effect of willow bark extracts by Soxhlet apparatus using the solvents petroleum ether, ethyl acetate, methanol on lipase activity was carried out. it was found the highest activation percentage was methanol extract, which founded to be 53.1%. However, the ethyl acetate and petroleum ether extracts had an activation percentage 51.6 and 23.3% respectively comparing with control.

## 1. Introduction

Willows (Salix L.) comprise approximately 450 species of deciduous trees and shrubs found primarily in moist Northern Hemisphere soils (Christenhusz et al., 2017). The major components of willow biomass are cellulose, hemicellulose, and lignin, with flavonoids and other polyphenols serving as minor constituents (Yan et al., 2021). Previous research has found that the bark of Salix L. species (Salicaceae family) is high in extractives such as bioactive phenolic compounds Tienaho1 Eet al., 2021), including flavan-3-ols, flavonols, flavanones, and chalcones (Tawfeek et al., 2021). Polyphenols from Salix tetrasperma Roxb. stem bark extract were also found to be effective in inhibiting Pseudomonas aeruginosa quorum sensing and virulence (Mostafa et al., 2020). The willow bark is used in phytopharmaceutical products to treat pain, headaches, fever, and inflammation (Tasneem et al., 2019; Ema, 2017). Salix cortex extracts have a high concentration of phenolic glycosides (Ostolski et al., 2021). In addition, willow bark extracts have been revealed to have strong antioxidant and radical scavenging properties (Bounaama et al., 2016; Ramos et al., 2019). Lipases (triacylglycerol hydrolases EC 3.1. 1.3) can hydrolyze triglycerides to obtain free fatty acids, monoacylglycerols, diacylglycerols, and glycerol (Casas et al., 2012; Sandoval, 2018). These enzymes play a critical role in numerous industrial food processes (Khan, et al., 2019 ; Szymczak et al 2021). The main source of lipases production can be some animals and plants as well as several microorganisms like fungi, bacteria (Lee and Park, 2019 ; Rivera et al., 2018). Because of the ability of lipase to catalyse various chemical reactions (Chowdary et al., 2000), they can be used in industries of food, chemical, pharmaceuticals, detergents etc. (Ananthi et al., 2014; Thakur et al., 2014]. Lipases in the food manufacturing and nutraceutical production be able to be used in aqueous extracts. Thay can be used to modify oils and and fats synthesize structured lipids or antioxidants with improved antioxidant power or modified lipophilicity, flavors, and aromas (Coelho et al., 2021; Li et al., 2020). Lipases contribute to the postmortem food spoilage of

seafood during handling, chilled frozen storage, and are broadly used for biotechnological applications in the dairy industry and oil processing (L´opez-Amaya et al., 2001).
The inhibition of this enzyme may be benefit in some of these industries, while its activation has benefits in other. The aim of the research was to study the effect of some extracts of willow bark on lipase in terms of its inhibition or activation because this bark contains important natural compounds and many secondary metabolites.

## 2. Methodology

Plant collection: In this study, the bark of Willow tree was obtained from Al-Qasr village, Nineveh Governorate. The bark was dried by spreading it in a shade. After that, the bark was peeled and ground with a blender to be used in extraction processes.
Hexane extraction: Exactly 200 ml of hexane was added to 20 g of willow bark powder in a beaker, stirred for 3 hrs. by magnetic stirrer and left for 24 hrs. Filtration was performed, and the filtrate was taken to rotary evaporator vacuum to dry it which was dissolved in 5 ml of DMSO.
Ethyl acetate extraction: A resulting precipitate was taken from hexane extract and placed in a beaker, then 200 ml of ethyl acetate were added. Stirring was carried out for 3 hrs. left for 24 hrs. Filteration was done and the filtrate was taken to rotary evaporator to dry it which was dissolved in 5 ml of DMSO.
Ethanol extraction: A resulting precipitate was taken from ethyl acetate extract and placed in a beaker, then 200 ml of ethanol were added. Stirring was carried out for 3 hrs. left for 24 hrs. Filteration was done and the filtrate was taken to rotary evaporator to dry it which was dissolved in 20 ml of DMSO.
Aqueous extraction: A resulting precipitate was taken from ethanol extract and placed in a beaker, then 200 ml of water were added. Stirring was carried out for 3 hrs. left for 24 hrs. Filteration was done and the filtrate was taken to rotary evaporator to dry it which was dissolved in 20 ml of DMSO.
Soxhlet extraction (SE) : For extraction, the willow bark powder 5g/200 ml was subjected to soxhlet extraction and thoroughly extracted with petroleum ether 60-800C for 48 hours at for deffating. The extract was evaporated using rotary evaporator. The batch was macerated in 200 ml of ethyl acetate for 24 hrs, extracted by soxhlet for 6 hrs and evaporated. After that the batch was macerated again in 200 ml of methanol for 24 hrs, and extracted by soxhlet for 6 hrs and evaporated (Sharma and Janmed, 2014).

Lipase Assay: Lipase activity was assayed using the method of (Winkler and Stuckman, 1979). The method involves the hydrolysis of the substrate paranitrophenyl acetate (1.5 mM) catalyzed by lipase to paranitrophenol, whose absorbance is monitored at 410 nm.
Effect of extracts on lipase activity : The pure Aspergillus niger lipase activity was studied by incubating 50 microliters of each petroleum ether, ethyl acetate, methanol and 70% ethanol extracts with 0.1 microliters of enzyme for 5 minutes at 25 °C. The activity was measured at 410 nm using p-nitrophenyl acetate as substrate.

## 3. Results and Discussion

The effect of willow bark extracts on the lipase activity
The effect of willow bark extracts of aqueous, ethanol, ethyl acetate, hexane on the activity of the lipase was studied. Table (1) indicates that extracts revealed inhibitory effect on lipase activity compared to the control. The highest percentage of inhibition among other extracts was ethyl acetate and aqueous extracts, which amounted to be 85 and 61.43 % respectively while the lowest percentage of inhibition with hexane and ethanol extrac, which were excluded from the study because of its weak influence.

*Table 1 Effect of willow bark extracts on lipase enzyme activity*

| Extract | Activity at 410nm | Inhibitory effect % |
|---|---|---|
| Control | 1.4 | 0 |
| Hexane | 1.39 | 0.714 |
| EtOH | 1.35 | 3.57 |
| $H_2O$ | 0.54 | 61.43 |
| Ethyl acetate | 0.21 | 85 |

Assayed of enzyme inhibition at different concentrations of extracts

By using different concentrations of extract, table (2) shows the effect of the aqueous extract of willow bark on the lipase enzyme activity. It was found that the highest inhibition percentage at a concentration 2.5 mg / ml at 54.35%, while the lowest inhibition percentage was 1.47 % at a concentration of 4 mg / ml.

By studying the effect of different concentration of ethyl acetate extract on the lipase activity, it was found that the highest percentage of inhibition was at a concentration 9.09 mg / ml, and that was 80.797 %

*Table2 Effect of aqueous extract of willow bark on lipase activity*

| Extract conc. (mg/ ml) | Activity at 410nm | Inhibitory effect % |
|---|---|---|
| Control | 0.677 | 0 |
| 2.5 | 0.309 | 54.35 |
| 3 | 0.793 | 17.13 |
| 3.5 | 0.600 | 11.37 |
| 4 | 0.667 | 1.47 |
| 4.5 | 0.422 | 37.77 |

*Table3 Effect of aqueous extract of willow bark on lipase activity*

| Extract conc. (mg/ml) | Activity at 410nm | Inhibitory effect % |
|---|---|---|
| Control | 0.677 | 0 |
| 3.03 | 0.33 | 51.255 |
| 9.09 | 0.130 | 80.797 |
| 15.15 | 0.267 | 60.561 |
| 21.21 | 0.480 | 29.098 |
| 30.3 | 0.424 | 37.37 |

The presence of phytoconstituents may have contributed to the inhibitory activity of the extract in this study. Lipase activity was found to be affected when incubated with Ethyl Acetate extract of Terminalia bellerica Roxb seeds. The inhibitory effect was discovered to be dose dependent, i.e., higher inhibition of enzyme was observed as extract concentration increased (Kumar et al., 2010). A nother studies have been carried out by 95% ethanol extract of Taraxacum officinale which inhibited lipase activity by 86.3% at a concentration of 250µg/ml (Zhang et al., 2008). At a concentration of 1 mg/ml, a seed extract of Vitis vinifera inhibited pancreatic lipase by 80% (Moreno et al., 2003 ). An extract of Nomame herba was found to have dose-dependent inhibitory activity against pancreatic lipase (Yamamoto et al., 2000). In a previous study, it was found that extracts of Quercus infectoria, Eucalyptus galbie, Rosa damascene, and Levisticum officinale, which are rich in alkaloids, had an inhibitory effect on lipase activity by 85, 64, 57, and 55%, respectively (Gholamhoseinian et al., 2010).

Inhibition mode: The inhibition of lipase activity was examined in the presence of extracts as inhibitors by drawing Lenweaver-Burk plot using different concentrations of para-nitro phenyl acetate as substrate ranging 1-5 mM. The results indicate that the mode of inhibition was noncompetitive by using 2.5 mg/ ml of aqueous extract (Figure 1). The value of Vmax was calculated which reduced from 2.63 to 0.9 units /ml/ min), while the value of Km was stable at 5 mM.

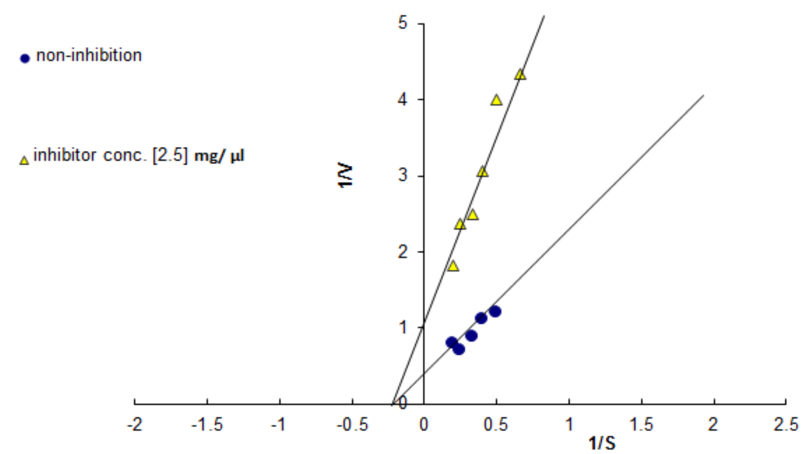


**Figure1:** The inhibition mode of lipase by aqueous extract

On the other hand, The results indicate that the mode of inhibition was uoncompetitive by using 3 mg/ ml of ethyl acetate extract (Figure 2). The Vmax value was calculated which reduced from 2.63 to 1.03 units /ml/ min), while the value of Km decreased from 5 mM to 2 mM.

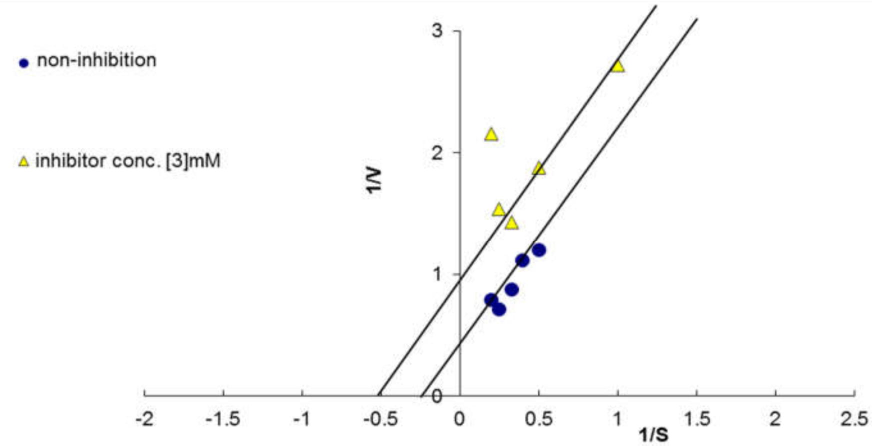


**Figure 2:** The inhibition mode of lipase by ethyl acetate extract

It was revealed in previous studies that Levisticum officinale extract showed mixed inhibition of the lipase enzyme, while the inhibition was non-competitive when using Quercus infectoria, Eucalyptus galbie and Rosa damascene extracts. The values of Vmax in the presence of extracts of Quercus infectoria, Eucalyptus galbie and Rosa damascene were 0.051, 0.056, 0.049 units /ml/ min) respectively

The effect of Soxhlet extraction of willow bark extracts on lipase activity

The effect of willow bark extracts was studied by using the solvents (petroleum ether, ethyl acetate, methanol) on lipase activity. Table 4 shows the activation effect of all extracts on enzyme. it was found the highest activation ratio among the other extracts was the methanol extract, which amounted to 53.1%. The ethyl acetate and petroleum ether extracts had an activation ratio 51.6 and 23.3% respectively comparing with control.

*Table4 The effect of Soxhlet extracts of willow bark on activity lipase*

| Extract | Enzyme activity at 410nm | Activation effect % |
|---|---|---|
| Control | 0.774 | 0 |
| Petroleum ether | 0.954 | 23.3 |
| Ethyl acetate | 1.173 | 51.6 |
| Methanol | 1.185 | 53.1 |

Assayed of enzyme activity at different concentrations of extracts Soxhlet extracts
Table (5) shows the effect of different concentrations of willow bark petroleum ether extract by Soxhlet on lipase activity. We found that the best activation was 160% at higher conc. of 3.65 mg/ml, while the lowest activation rate reached 7%, at conc. of 0.365 mg/ml .

*Table5 Effect of petroleum ether extract conc. of willow bark on lipase activity*

| Extract conc. (mg/ml) | Activity at 410nm | Activation effect % |
|---|---|---|
| Control | 0.774 | % |
| 0.365 | 0.72 | 7 |
| 1.095 | 0.99 | 28 |
| 1.825 | 1.548 | 100 |
| 2.555 | 1.65 | 113.18 |
| 3.65 | 2.01 | 160 |

By studying the effect of ethyl acetate extract on the activity, the results indicated that the lowest activation was 18% at conc. of 3.03 mg/ ml, but the highest activation was 392.25% at conc. 30.3 mg/ml, with Table (6) .

*Table6 Effect of ethyl acetate extract of willow bark on lipase activity*

| Extract conc. (mg/ml) | Activity at 410nm | Activation effect % |
|---|---|---|
| Control | 0.677 | 0% |
| 3.03 | 0.912 | 18 |
| 9.09 | 1.146 | 48.1 |
| 15.15 | 1.224 | 58.14 |
| 21.21 | 1.374 | 77.52 |
| 30.3 | 3.81 | 392.25 |

Table (7) shows the effect of methanol extract of willow bark on the activity of lipase, It was found that the highest activation was 107% at a conc. of 4.5 mg/mL. The lowest activation was 13.2% at conc. of 2.5 mg/mL.

*Table 7 Effect of methanol extract of willow bark on lipase activity.*

| Extract conc. (mg/ ml) | Activity at 410nm | Activation effect % |
|---|---|---|
| Control | 0.677 | 0% |
| 1.65 | 0.876 | 13.2 |
| 4.96 | 0.906 | 18 |
| 8.26 | 1.185 | 54 |
| 11.56 | 1.572 | 103.1 |
| 16.52 | 1.602 | 107 |

Activation of the lipase enzyme is important in many food industries because it has a role in the decomposition of fats and thus converting them from one form to another to be useful in them. Pomegranate peel extracts with ethyl acetate and chloroform revealed an activation effect on lipase ( Alabbas et al., 2021).

## 4. Conclusion

To our information, this is the first study in which the effects of willow bark extracts and some of its constituents on lipase activitt are directly compared. In this study, we have investigated inhibitory activity of aqueous and ethyl acetate extract of willow bark against lipase. The mode of inhibition was noncompetitive and uncompetitive respectively. On the other hand, the willow bark was powdered and extracted with Petroleum ether, ethyl acetate and methanol in soxhlet apparatus. The extract was tested on lipase activity and found the activation effect for all extracts at different concentration

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