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Alpha-Amylase and Urease Inhibitory Activity of Leaf Extracts of *Prunus persica* (L.) Batsch

Ved Prakash[1]*, Anand Sagar[2]

Author's Affiliation
[1,2]Department of Biosciences, Himachal Pradesh University, Shimla-171005 India

***Corresponding Author:**
Ved Prakash
Department of Biosciences,
Himachal Pradesh University,
Shimla- 171005, India

E-mail:
vedp685@gmail.com

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Abstract

The use of herbal remedies for curing diseases is on the rise in developed as well as developing countries. The current study was undertaken to investigate the leaf extracts of *P*runus *persica* (L.) Batsch for their inhibitory activity against α-amylase and urease enzymes in three different solvents i.e. methanol, acetone and aqueous. The plant showed highest α- amylase enzyme inhibition (75.08±0.28%) at a concentration of 1 mg/mL in methanol extract whereas acetone and aqueous extracts showed moderate inhibition against α- amylase (59.95±1.25 and 50.00±1.00%respectively). In case of urease inhibition, methanol extract exhibited best inhibitory activity (55.08±0.28%) followed by acetone and aqueous extracts at a concentration of 1 mg/mL. The plant extracts showed concentration dependent inhibition of both the enzymes. The inhibitory activity altogether increased with increasing concentration of each plant extract in the range of 0.2-1.0 mg/mL. Moreover, the plant was found more effective against α-amylase than urease. The results further revealed that methanol leaf extracts displayed maximum inhibitory effects than other solvent extracts which tends to show that the active metabolites of the different plant parts are better extracted with methanol than other solvents. Thus the present study provides scientific evidence to the traditional uses of this plant in the treatment of diabetes, obesity, gastric intestinal infections, ulcers, kidney stones etc. Therefore, the leaf extracts of *P. persica* can be selected for further investigation to discover their ultimate therapeutic potential.

INTRODUCTION

Enzyme inhibition by plant-derived products has now become an important part of the modern drug discovery research as studies in this field have led to the discovery of wide variety of drugs useful in a number of physiological disorders[1]. Specific inhibitors mostly interact with enzymes and block their progression towards their corresponding natural/synthetic substrates[2]. α -Amylase and urease are two important enzymes that are often associated with a number of clinical disorders. α-Amylase (EC 3.2.1.1) is a classical calcium containing enzyme which catalyses hydrolysis of starch and related

carbohydrates by randomly cleaving internal α-D-glycosidic linkage[3]. Inhibition of α-amylase is considered a good strategy for the treatment of disorders related to carbohydrate uptake such as diabetes, obesity, dental caries, periodontal diseases etc.[4,5]. The drugs commonly used in clinic to treat or control diabetes are insulin, sulfonylureas, biguanide, glucosidase inhibitors, aldose reductase inhibitor, thiazolidinediones, carbamoylmethyl benzoic acid and insulin-like growth factor. The ultimate effect of these drugs is to decrease the blood glucose level[6,7].

Diabetes mellitus, a major global epidemic endocrine disorder is often characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Currently, in addition to insulin supplement, treatment of diabetes includes many oral hypoglycemic agents along with appropriate diet and exercise. One therapeutic approach for treating diabetes mellitus is to lower the post-prandial glucose levels and this can be done by lowering the absorption of glucose through the inhibition of carbohydrate hydrolysing enzyme i.e.α-amylase[8,9]. Inhibitors of α-amylase like acarbose, miglitol and voglibose generally delay carbohydrate digestion process and thus prolong overall carbohydrate digestion time causing reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. However, these drugs are known to be associated with various gastrointestinal side effects such as abdominal pain, flatulence and diarrhoea[10,11]. Therefore, it is the need of the hour to identify and explore α-amylase inhibitors from natural sources having fewer or no side effects.

On the other hand, Urease (E.C 3.5.1.5) is a ubiquitous nickel containing enzyme which is widespread in nature, being present in several forms of life ranging from bacteria to plants and animals[12]. It is considered as the most proficient protagonist in biochemistry carrying out the rapid catalysis and hydrolysis of urea to produce ammonia and carbon dioxide[13]. The product, ammonia (NH_3), of such decomposing reactions diffuses across the cytoplasmic membrane, increases the periplasmic space pH and as a result allows the bacterial growth in the presence of extra cellular gastric acid[14]. Additionally, urease contributes to arthritis, kidney stones formation, urolithiasis, pyelonephritis and gastric intestinal infections, and ultimately the urease imbalance leads to peptic ulcers[15,16].

The presence of urease has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health[17].The microorganism *Helicobacter pylori,* one of the main causes of gastrointestinal ulcers produces urease as a mechanism to survive in the acidic environment of stomach. It has been estimated that more than 50% of the population is affected with *H. pylori* infection worldwide[18]. It has already been established that the urease deficiency effectively risks the bacterial existence. Since inhibition of urease activity can terminate *H. pylori* infection, it can be considered to be a promising therapy for ulcer and kidney stones[19].However, the commercially available urease inhibitors are of low stability and toxic which prevents their clinical use[20]. Therefore, discovering of new active principles from plants or plant-based sources with possible urease inhibitory activity could help to cure ulcer, gastritis and other diseases caused by *H. pylori* infection[21].

The plant *P*runus *persica* (L.) Batsch (also named as *Amygdalus persica*) belonging to family Rosaceaeis a deciduous tree with a height of 5 to 10 m and commonly cultivated in West Asia, Europe, Himalayas and India up to an altitude of 1000 ft[22]. The leaves of this plant are found to be astringent, anthelmintic, insecticidal, sedative, diuretic, demulcent, expectorant and vermicidal. The flowers are considered as laxative and diuretic and are used to control constipation and oedema. Fruits being aphrodisiac and antipyretic, act as tonic to brain enhance the blood flow and remove bad smell from the mouth. Further, the plant exhibits antimicrobial, antioxidant, anti-tumour and anti-Oketsu syndrome effects[23]. In view of its above mentioned useful properties, we planned to investigate leaf extracts of *P. persica* for their possible α-amylase and urease inhibitory activities.

MATERIALS AND METHODS

Collection of plant material (study area)

Fresh Leaves of *Prunus persica* were plucked and collected from Rajgarh area of District Sirmaur, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further studies.

Processing of plant material

The Leaves of *P. persica* were washed thoroughly under tap water and then with 2% Mercuric chloride. Thereafter the leaves were cut into smaller pieces for quick drying. The plant material obtained after drying was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in air tight containers at room temperature.

Enzyme inhibitory activity assays

α- Amylase inhibition assay

α- Amylase inhibition activity of different plant extracts was determined by some modifications in the method reported by Giancarlo *et al*.[24]. The starch solution (1% w/v) was prepared by boiling and stirring 1 g of potato starch in 100 mL of sodium phosphate buffer for about 30 minutes. The porcine pancreatic α- amylase enzyme (EC 3.2.1.1; purchased from Sigma Aldrich-3176) was obtained by mixing 0.01 g of α- amylase in 10 mL of sodium phosphate buffer (pH 6.9) containing 0.0006 mM sodium chloride (NaCl). The leaf extracts were dissolved in DMSO to give concentrations ranging from 0.2 to 1.0 mg/mL (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). The colour reagent was used (a solution containing 0.1 g of 3,5-dinitrosalicylic acid plus 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and 10 mL phosphate buffer). 50µLof each plant extract plus150 µL of starch solution along with 10 µL of enzyme were mixed in a 96 well plate and incubated at 37^{o}C for 30 minutes. Then 20 µL of sodium hydroxide (NaOH) and 20 µL of colour reagent were added and the closed plate was placed into a 100°C water bath. After 20 minutes, the reaction mixture was removed from the water bath, allowed for cooling and α- amylase activity was determined by measuring the absorbance of the mixture at 540 nm using a UV-VIS spectrophotometer. Blank samples, where the enzyme was replaced with the buffer solution were used to correct the absorption of the mixture. Also, a control reaction was used, where the plant extract was replaced with 50 µL of DMSO and the maximum enzyme activity was determined. Acarbose solution (a positive control) was used as in the concentration range of 0.2-1.0 mg/mL. The complete experiment was performed in triplicate and the mean absorbance was used to calculate percentage of α- amylase inhibition. The inhibition percentage was assessed by the following formula:

$$\% \text{ α- Amylase inhibition} = \left(\frac{\Delta A\text{control} - \Delta A\text{sample}}{\Delta A\text{control}}\right) \times 100$$

Where, $\Delta A_{control} = A_{test} - A_{Blank}$

$\Delta A_{sample} = A_{test} - A_{Blank}$

The concentration of the plant extract (inhibitor) was determined from corresponding dose-response curves of inhibition percentage versus inhibitor concentration and compared to acarbose, a known inhibitor of α- amylase activity and a logarithmic regression curve was established to calculate the IC_{50}value (the concentration of the given sample required to inhibit the activity of urease enzyme by 50%) for each sample. Data were expressed as mean ± standard deviation (S.D.).

Urease inhibition assay

The urease enzyme inhibition was determined through catalytic effects of urease on urea by measuring change in absorbance in the absence and in the presence of inhibitor at 640 nm, using UV-VIS spectrophotometer. The leaf extracts that exerted significant inhibition were tested in a particular concentration range: 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. Just after addition of 10 mL of phosphate buffer to accurate weight of enzyme, sonication was performed for about 60 seconds, followed by centrifugation and absorbance of upper solution was measured at 280 nm. One can calculate the concentration of initial urease solution by using equation A= εbc, where c is concentration of solution (mol/L), b is length of the UV cell and ε represents molar absorptivity. The concentration of enzyme

solution was adjusted to 2 mg/mL following proper dilution. The reaction mixture containing 1.2 mL of phosphate buffer solution (10mM potassium phosphate, 10 mM lithium chloride and 1 mM EDTA, pH 8.2 at 37°C), 0.2 mL of urease enzyme solution and 0.1 mL of test compound was subjected to incubation for about 5 minutes. After pre-incubation, 0.5 mL (66 mM) of urea was added to the reaction mixture and then incubated for 20 minutes. Eventually, urease activity was determined by measuring the ammonia released during the reaction by modified spectrophotometric method as described by Weatherburn[25]. Briefly, 1 mL of phenol reagent (1% w/v sodium nitroprusside) and 1 mL of an alkaline reagent (1% w/v NaOH and 0.075% active chloride NaOCl) were added to each test tube. The control contained all the reagents except the test sample. Thiourea (standard inhibitor) was used as a positive control. The increase in absorbance at 640 nm was measured after 30 minutes using a UV-VIS spectrophotometer. The inhibition percentage was determined using the formula:

$$\% \text{ Urease Inhibition} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Where,
A_s= absorbance of the sample under study
A_c= absorbance of the control

Each experiment was performed thrice and average was calculated. Data were expressed as mean ± standard deviation (S.D.). IC_{50} value for each sample was determined from the dose-response curves.

RESULTS

In the present investigation, the leaf extracts (methanol, acetone and aqueous) of *P. persica* were tested for their enzyme inhibitory activity against α-amylase and urease enzymes and it was observed that all the extracts showed strong inhibition at a concentration of 1 mg/mL. The inhibitory activity increased with increasing the concentration of each plant extract in the range of 0.2-1.0 mg/mL. At a concentration of 1 mg/mL, the inhibitory activity of α-amylase was 75.08±0.28 (IC_{50}: 0.55 mg/mL), 59.95±1.25 (IC_{50}: 0.84 mg/mL) and 50.00 ± 1.00% (IC_{50}: 0.97 mg/mL) for methanol, acetone and aqueous extract respectively as shown in Table 1. The results were compared with standard acarbose which showed greater inhibition (78.56±0.45%) than plant extracts with lowest IC_{50} value of 0.53 mg/mL. The results further indicated that methanol extracts exhibited maximum inhibitory effects than other solvent extracts.

Furthermore, the urease inhibitory activity of *P. persica* leaf extracts was studied against jack bean urease by using phenol hypochlorite method as compiled in Table 2. All the three extracts (methanol, acetone and aqueous) were reported to exert inhibitory effects on jack bean urease enzyme. Among these, methanol extract showed maximum inhibition of 55.08±0.28%followed by acetone (38.15±1.25) and aqueous extract (38.00±1.41) with IC_{50} value of 0.89, 1.44 and 1.32 mg/mL respectively. Further, Thiourea (standard) showed better urease inhibition (81.26±1.25) as compared to plant extracts.

DISCUSSION

The biological activity of plant/plant-derived substances may be considered as a source of new anti-enzyme drugs. Therefore, traditional Indian plants which are commonly used as remedies to control various diseases have been screened to discover possible plant-derived α- amylase and urease inhibitors[20]. The medicinal plants have been widely used for their therapeutic potential in controlling various disorders caused by these enzymes. Scientists are unifying traditional knowledge with experimental methodology for evaluating the efficacy and safety of herbal preparations[26]. Rural population in India, like most developing countries, heavily relies on valuable heritage of medicinal plants. It is therefore of high interest to find out the possible reasons for efficacy of indigenous medicinal plants which are commonly used by local population and traditional practitioners. Therefore, the present study on α- amylase and urease enzyme inhibition of *P. persica* collected from Himachal Pradesh was undertaken. Jack bean urease enzyme has been used in the study because it shares more than 50% similarity with the bacterial urease. Additionally, it has been found that the

mechanism of action and the kinetics of inhibition for bacterial urease and jack bean urease are almost similar[27].

Current study presents an investigation of different leaf extracts of *P. persica* for their enzyme inhibitory activity (against α- amylase and urease). From the results it was found that the plant was more effective against α- amylase than urease as all the three leaf extracts displayed better inhibition of α- amylaseas compared to urease. In case of α- amylase, methanol leaf extract showed maximum inhibition of 75.08% whereas for methanol extract of *P. persica* against urease,maximum inhibition of 55.08% was reported which was less than α- amylase. The plant extracts showed concentration dependent inhibition of enzymes as shown in Fig. 1 and 2.Against both the enzymes, aqueous extract proved to be least effective which indicated that the water soluble constituents of *P. persica* have little ability to inhibit these enzymes.The results further indicated that methanol extracts exhibited maximum inhibitory effects than other solvent extracts against both the enzymes. This tends to show that the active constituents or metabolites of the different plant parts are better extracted with methanol than other solvents. A large number of phytochemicals i.e. alkaloids, terpenoids and flavonoids have been isolated from *P. persica*[28]. The chemical constituents of the plant include cyanogenic glycosides, amygdalin and prunasin as major components along with glycerides, sterols and emulsion which could be attributed to its medicinal properties[29].

Our findings are in accordance with the results of Gilani*et al*.[30] and Chatragadda *et al*.[31]who conducted various experiments to explore inhibitory potential of different leaf extracts of *P. persica* against α- amylase. In similar studies, various extracts of *P. persica* were found to possess significant inhibitory effects on starch break-down *in vitro*. At a concentration of 0.5 mg/mL, the activity of α-amylase was 31.28% whereas it was 62.67% at 2.5 mg/mL. Results showed dose-dependent increase in α-amylase inhibitory activity[32]. The fruit extracts of *P. persica* at concentration range of 1-10 mg/mL were tested for urease inhibition but low inhibition (9.47%) was reported against urease in *H. pyroli*[33]. As per literature survey, there is no previous report found on urease inhibitory activity of leaf extracts of this plant.

Table 1: α-Amylase inhibitory activity (%) of *P. persica* leaf extracts at different concentrations

Concentration (mg/mL)	Methanol extract	Acetone extract	Aqueous extract	Acarbose
0.2	25.78±1.10	14.75±1.05	12.10±0.30	29.50±0.70
0.4	41.82±0.34	26.90±0.15	28.30±0.86	40.85±2.15
0.6	54.40±0.52	32.70±0.24	33.88±0.25	56.45±1.25
0.8	66.30±2.20	48.00±0.60	42.30±1.55	66.22±0.52
1.0	75.08±0.28	59.95±1.25	50.00±1.00	78.56±0.45
IC_{50} (mg/mL)	0.55	0.84	0.97	0.53

Values are given as mean ± S.E.

Table 2: Urease inhibitory activity (%) of *P. persica* leaf extracts at different concentrations

Concentration (mg/mL)	Methanol extract	Acetone extract	Aqueous extract	Thiourea
0.2	13.12±1.10	10.75±1.00	10.10±0.50	28.38±0.78
0.4	23.87±1.30	16.90±0.15	18.00±0.80	41.58±0.55
0.6	34.40±0.55	20.00±0.20	24.88±0.66	56.30±1.20
0.8	46.30±2.24	27.00±0.60	32.30±1.90	69.20±0.50
1.0	55.08±0.28	38.15±1.25	38.00±1.41	81.26±1.25
IC_{50} (mg/mL)	0.89	1.44	1.32	0.51

Values are given as mean ± S.E.

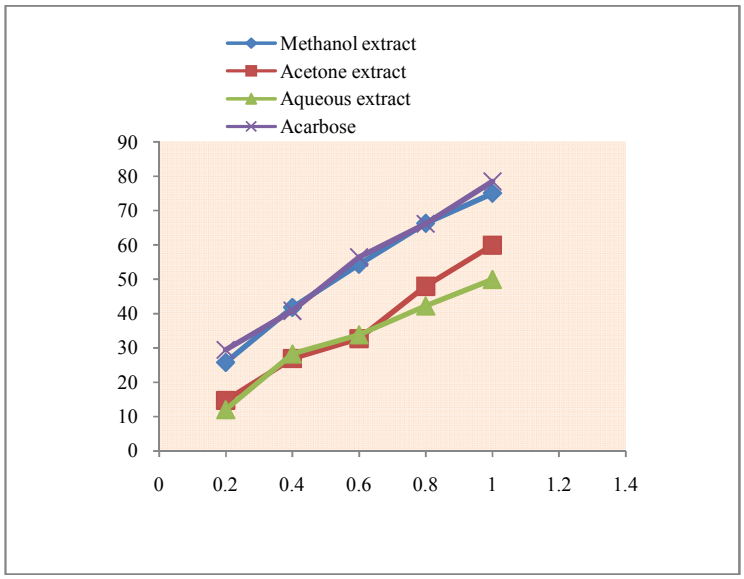

Figure 1: α-amylase inhibition profile of different extracts of *P. persica* against porcine α-amylase. The extracts were tested at a concentration range of 0.2-1.0 mg/mL. The activity altogether increased with increase in concentration. Vertical bars indicate mean ± standard error.

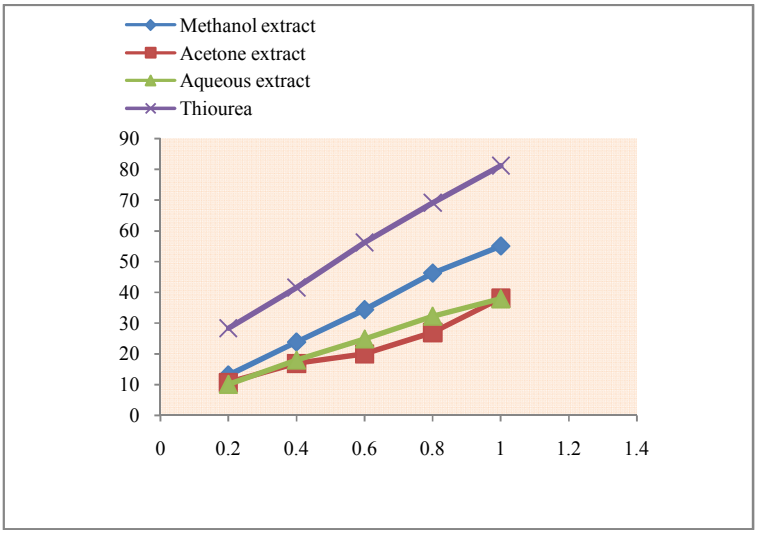

Figure 2: Urease inhibition profile of different extracts of *P. persica* against porcine α-amylase. The extracts were tested at a concentration range of 0.2-1.0 mg/mL. The activity altogether increased with increase in concentration. Vertical bars indicate mean ± standard error.

CONCLUSION

As a conclusion, it could be speculated that the results of α- amylase and urease inhibitory studies are encouraging as all the tested leaf extracts (methanol, acetone and aqueous) of *Prunus persica* showed significant inhibition. α-amylase inhibition ranged from 12.10-75.08%. Similarly, urease inhibitory activity ranged from 10.10 ± 0.50 to 55.08 ± 0.28% suggesting a strong α- amylase and urease inhibitory effects of this plant. Besides this, methanol leaf extracts were found to be more effective against both the enzymes used compared to acetone and aqueous extracts. Hence it is clear from the

results that leaf extracts under study displayed variable enzyme (α- amylase and urease) inhibitory activities thereby confirming their roles in the treatment of various diseases caused by the malfunctioning of these enzymes. Further research is needed to find the exact mechanism of action and the chemical constituents responsible for its anti-enzyme activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the manuscript and experimentation done.

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