Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.4025

# *IN-VITRO* AMYLASE AND UREASE INHIBITORY ACTIVITY OF *MORINGA OLEIFERA* LEAVES AND *CHENOPODIUM QUINOA* SEEDS

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**Abstract**

Amylase and urease are two important enzymes and drug targets in crosstalk, with oxidative stress playing a vital role in developing and originating diseases, including diabetes mellitus. In diabetic patients, alpha-amylase inhibition is a vital therapeutic target in regulating increased postprandial blood glucose levels. Increased urea production increases the local pH and produces a favorable environment for bacteria like *helicobacter pylori, leading* to peptic ulcer and stone formation. Despite the latest innovations in modern medicine, medicinal plants and natural treatments are getting more attention. Herbs have been used to cure numerous diseases for a long. However, scientists have shown more interest in extracting herbal bioactive compounds and their pharmacological properties because of their side effects and consumption of synthesized medicines. This study investigated the amylase and urease inhibitory activity of *Moringa oleifera* leaves and *Chenopodium quinoa* seeds by inhibitory enzyme assay, which shows excellent results. The methanolic and aqueous extracts of *M. oleifera* leaves and *C. quinoa* seeds were prepared to check their enzyme inhibitory activity. It showed significant enzyme inhibitory action against urease and

alpha-amylase, respectively. Consumption of these edible medicinal plants illustrates therapeutic efficacy against gastrointestinal ulcers and postprandial hyperglycemia. The isolation of valuable amylase and urease inhibitors might achieve through assay-guided phytochemical analysis.

**Keywords**: Moringa oleifera leaves, chenopodium quinoa seeds, amylase inhibition, urease inhibition

## INTRODUCTION

Inhibition of enzymes is a vital pharmaceutical research area that led to the innovation of a wide variety of valuable drugs in treating various diseases. Enzymes and specific inhibitors interact and block their action concerning analogous natural substrates. Enzyme inhibitors have treated numerous physiological conditions because of their vast drug importance [1]. Essential bioactive chemicals found in medicinal plants have long been recognized for their therapeutic usefulness. Medicinal plants include a variety of bioactive compounds, each of which can exert a physiological effect on the human body [2]. Until chemically produced medications were invented, people sought natural drugs to treat or cure diseases [3]. The rising adverse effects of synthetic pharmaceuticals have reintroduced the importance of medicinal herbs and natural medicines.

Urease is a metalloenzyme that occurs when urea is hydrolyzed into carbon dioxide and ammonia [4]. Pathologically, ammonia produced by urease can neutralize abdominal acid, allowing harmful bacteria such as Helicobacter pylori to survive [5]. This can result in gastric cancer, gastrointestinal disorders, and duodenal or peptic ulcer formation [6].

The urease enzyme inhibitor of ammonia is critical for protecting plants from the toxic effects of ammonia, reducing urea loss, and eliminating urea-related illnesses such as peptic ulcers [7]. Naphthoquinones, phosphorodiamidates, and polyhalogenated benzo are some synthetic urease inhibitors. Only a few studies demonstrate their efficacy, while most cause bacterial resistance [8]. Due to the toxicity of synthetic Urease inhibitors, researchers have concentrated their efforts on identifying natural ureas inhibitors with little toxicity for pharmaceutical development [9,10].

Amylase is a digestive enzyme present in saliva and pancreatic juice. It breaks down complex carbohydrates and glycogen into single glucose molecules or smaller polysaccharides that the body can absorb [11]. In diabetic patients, alpha-amylase inhibitors are critical for treating PPHG and lowering starch hydrolysis [12]. Miglitol and acarbose are well-known glucosidase inhibitors used to treat diabetes [13,14]. Miglitol and acarbose are well-known glucosidase inhibitors used to treat diabetes by blocking intestinal and pancreatic -glucosidase enzymes.

Moringa oleifera is found worldwide and is cultivated in subtropical and tropical climates. When preserved as a dried powder, the leaves retain their nutritional value for a more extended time. The leaves and Chenopodium quinoa seeds exhibit a range of pharmacological activities, including anti-inflammatory and antioxidant activity [15,16]. When preserved as a dried powder, the leaves of M. oleifera retain their nutritional value for a longer time.

*Chenopodium quinoa* is a seed crop cultivated for more than 5,000 years in the Andean region. Because of its genetic diversity, quinoa adapts to various challenging environments like drought, highlands, frost, and salinity. Polyphenols, such as flavonoids, tannins and phenolic acids, are the bioactive secondary metabolites of plants with anti-inflammatory, antimicrobial, antitumor and antioxidant effects. Various countries like the USA, Bolivia, Japan and Peru reveal that cultivated quinoa seeds have antioxidant activity due to the antioxidant components like polyphenols. Pakistan is one of those countries where traditional Unani medicine is popularly practiced among its large population.

## Materials and Methods

### Plant material

Medicinal plants were taken from the University of Agriculture Faisalabad-Pakistan and taxonomically recognized from the Department of Botany, Government College University Faisalabad-Pakistan. Selected medicinal plants, including *Moringa oleifera* and *Chenopodium quinoa*, were used to prepare the extract. The *M. oleifera* leaves were shaded dried for three days, and then by using a blender, the leaves were ground to powder form and the seeds of *C. quinoa*. Then this fine powder is used to make the extracts of *M. oleifera* and *C.quinoa* to determine its amylase and urease enzyme inhibition. Four samples of finely powdered M. oleifera (MO) leaves and three samples of C. quinoa seeds were prepared at varying RPMs and for varying lengths of time to optimize yield for desired activity.

**Figure 1.** a) Chenopodium quinoa seeds, b) Moringa oleifera leaves, c) Chenopodium quinoa powder, d) Moringa oleifera leaves powder.

### Extraction of M. oleifera leaves

### Aqueous extract

The aqueous extract of moringa oleifera leaves was prepared by mixing 20g dried moringa powder with 200ml distilled water. 30 minutes at low heat, then 24 hours at room temperature. Then filtered with filter paper and evaporated (Fig 1). This method resulted in a yield of 18.5 percent.

### Methanolic extracts:

Methanolic extracts of Moringa oleifera leaves were prepared by adding 10g moringa leaf powder and 20g moringa leaf powder to the other beaker. 100ml methanol is contained in a beaker containing 10g moringa powder, while 200ml methanol contains 20g moringa powder. Following that, the beaker was shaken for 2 and 4 hours at 200 RPM. The extracts were then placed in a water bath to evaporate the methanol at 40C. A yield of 15% was recorded after 2 hours and 23.05 percent after 4 hours (Table 1).

### Extraction of *chenopodium quinoa* seeds

### Methanolic extraction #1

In a 1000ml flask with 600g C. quinoa crushed seeds, 500ml methanol was added with 100ml water. Then it was covered with foil and left for 5-6 days. After filtering with filter paper, the extract was rotary evaporated to remove the methanol with a yield of 2.98 percent. (Fig. 2).

**Methanolic extraction #2**

200ml methanol was added to 20g powdered C. quinoa seeds. The flask was then shaken for 2 hours at 200 RPM. Water bathed the extract to evaporate the methanol. After filtration, the extract is transferred to a petri dish to dry. The percentage yield obtained using this method was 1.18 percent.

***Moringa oleifera* leaves extract**

**Table # 1**

| Experiment # | Dry Wt. (g) | Solvent (ml) | RPM | Time (hr) | Yield |
|---|---|---|---|---|---|
| 1 | 20g | Methanol (200 ml) | 200 | 2 | 15% |
| 2 | 20g | Methanol (200 ml) | 200 | 4 | 23.05% |
| 3 | 20g | Water<br>(200 ml)<br>Decoction method | | | 18.5% |

***Chenopodium quinoa* seeds extract**

**Table # 2**

| Experiment # | Dry wt. (g) | Solvent (ml) | RPM | Time (hr) | Yield |
|---|---|---|---|---|---|
| 1 | 10g<br>(Fine powder) | Methanol<br>(200ml) | 200 | 2 | 1.9% |
| 2 | 20g | Methanol<br>(200ml) | 200 | 2 | 1.18% |
| 3 | 150g<br>(crushed seeds) | Methanol<br>(500ml +<br>water 100ml)<br>placed for 5-6 days | | | 2.98% |

**Phytochemical analysis**

**Total phenolic content (TPC)**

The Folin-Ciocaletu reagent method was used to determine the TPC content of plant extracts [17]. 0.1 mL, 0.5 mL, and 5.0 ml of the extract, Folin Ciocalteau reagent, and sodium carbonate, respectively, were added. After 30 minutes at 760nm, the absorbance of this reaction mixture was determined using a spectrophotometer.

**Total Flavonoid content (TFC)**

The TFC concentration was determined using a colorimetric assay to determine the absorbance at 540nm using a spectrophotometer [18]. In brief 1 mL extract 0.3 mL (5 percent ) NaNO2 and 4mL H2O were added. The final volume was 10 mL by mixing water. After mixing, at 510 nm, the absorbance was calculated, and Quercetin was used as the standard.

**Enzyme inhibition assay**

**Amylase inhibition assay**

Alpha-amylase inhibition was studied using the Giancarlo et al. method with minor modifications. Each plant extract (50l), starch solution (150l), and enzyme (10l) were mixed in 96 well plates. To determine the highest activity of the enzyme, DMSO (50 l) solution was used as a control reaction in place of the plant extract. The inhibition % of alpha-amylase was evaluated by the following formula:

$$I\alpha\text{–Amylase}\% = 100\times (\Delta A\text{control} - \Delta A\text{sample})/\Delta A\text{control}$$
$$\Delta A\text{control} = A\text{test} - A\text{Blank}$$
$$\Delta A\text{sample} = A\text{test} - A\text{Blank}$$

**Urease inhibition assay**

The inhibitory activity of the urease enzyme was determined by the catalytic effects of urease on urea using a spectrophotometer set to 625nm absorbance [17]. This assay used 96 well Elisa plate reader. 100mm urea, 0.01M Lithium Chloride, 0.01M Dipotassium Phosphate, and 1mM EDTA

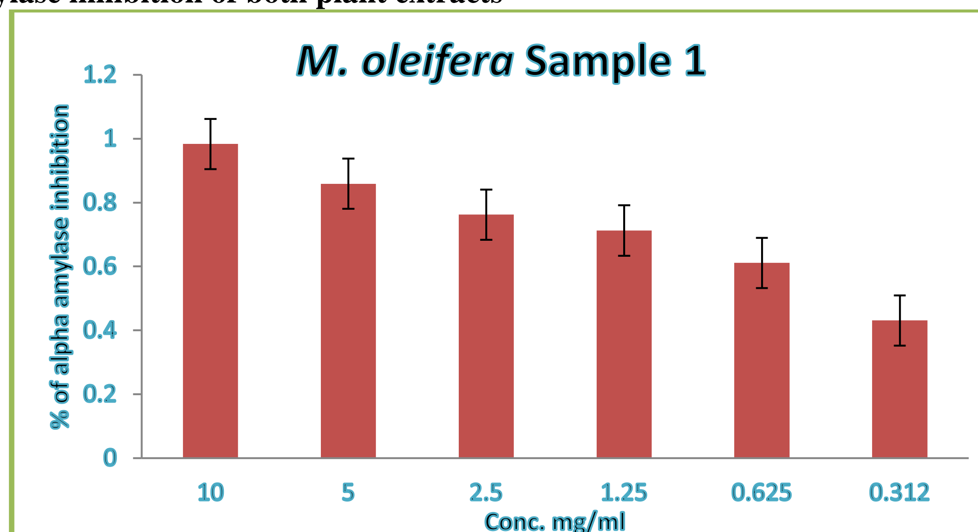
make up this 8.2 pH buffer. Then 10ul of test compounds were put to each well. Then 10ul of urease enzyme (5u/ml) is added to the wells. This plate was incubated at 37°C for 30 minutes. The phenol reagent contained 0.005% sodium nitroprusside and 1% phenol. Then, it was mixed with an alkali reagent (0.1 percent sodium hypochlorite, 0.5 percent sodium hydroxide). The plate was then incubated at 37°C for 30 minutes. After incubation, the absorbance at 625nm was measured using a microplate reader.

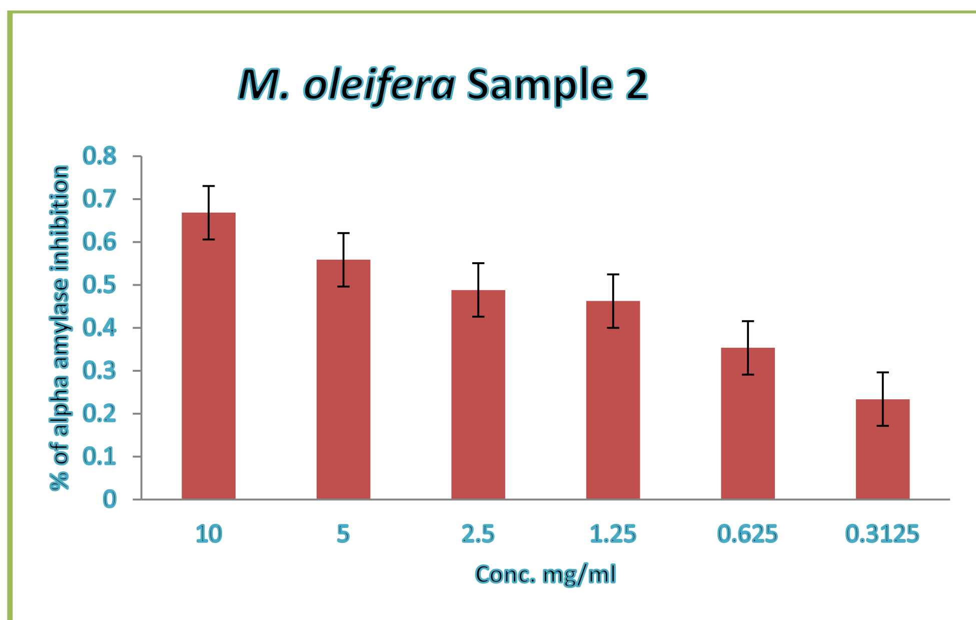
## Results

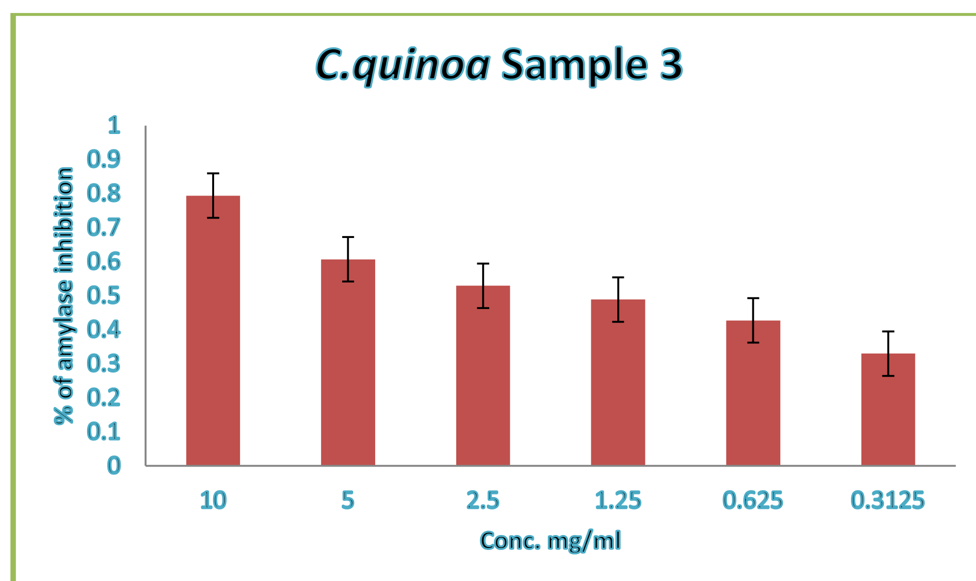
Fig. shows inhibition of alpha-amylase of both plants at different concentrations. The three samples of *M. oleifera* leaves extracts showed the inhibition rate of 26.09% (sample 1), 15.15% (sample 2) and 29.32% (sample 4). In contrast, the *C.quinoa* extracts showed 9.70% (sample 3), 16.08% (sample 5) and 10.69% (sample 6, Table. 1 & 2) against alpha-amylase enzyme. Moreover, sample 7 is the mixture of both extracts, which showed the highest inhibitory activity, i-e 51.97%. The results indicate that the sample with both plant extracts showed higher alpha-amylase inhibitory activity because of phenolic components in both plants [18].

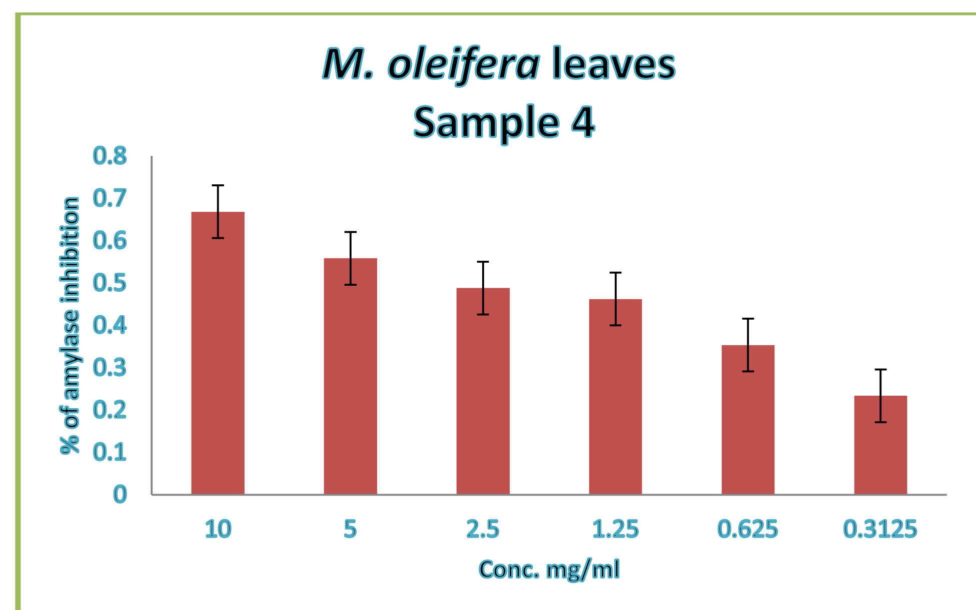
*H. pylori* is the major cause of gastrointestinal disorders like peptic ulcer, duodenal ulcer, gastric cancer and gastritis [19]. This bacterium disturbs the pathogenic state of humans and becomes the cause of diseases such as peptic ulcer, hepatic coma, urinary stone formation and pyelonephritis [20]. The acidic stomach medium is favorable for *H.pylori* and is significantly dependant on urease enzyme activity. An exclusive characteristic of *H.pylori* has been seen due to urease enzyme buffering activity. The urease enzyme alters the stomach medium when it changes into the urea through hydrolysis to form carbon dioxide and ammonia and neutralizes the gastric acid, which is supportable for bacteria. For this reason, it is the need to evaluate urease inhibitory activity from different medicinal plants [21]. The rate of urease inhibitory activity of methanolic extracts of M. oleifera leaves (MO) were 60%, 56.12% and 71%, which showed that MO act as a potent inhibitor of *H. pylori* while C.quinoa seeds also showed valuable results, which might be due to the presence of the rich amount of saponins and Quercetin which has anti-helicobacter pylori effect [22]. The results showed that the amylase and urease activity of Moringa oleifera have potent enzyme inhibitory activity compared to chenopodium quinoa because of the presence of numerous vital bioactive components having anti-diabetic and anti-ulcer properties. It is also suggested by this study that the methanolic extract showed higher enzyme inhibition as compared to aqueous extract. Both medicinal plants are vital and provide notable results on combining the extracts that showed their significant enzyme inhibitory bioactive compounds. The results demonstrated that the increased or decreased alpha-amylase and urease enzyme inhibitory activity depends on the plant extracts with different concentrations at different RPM [23].

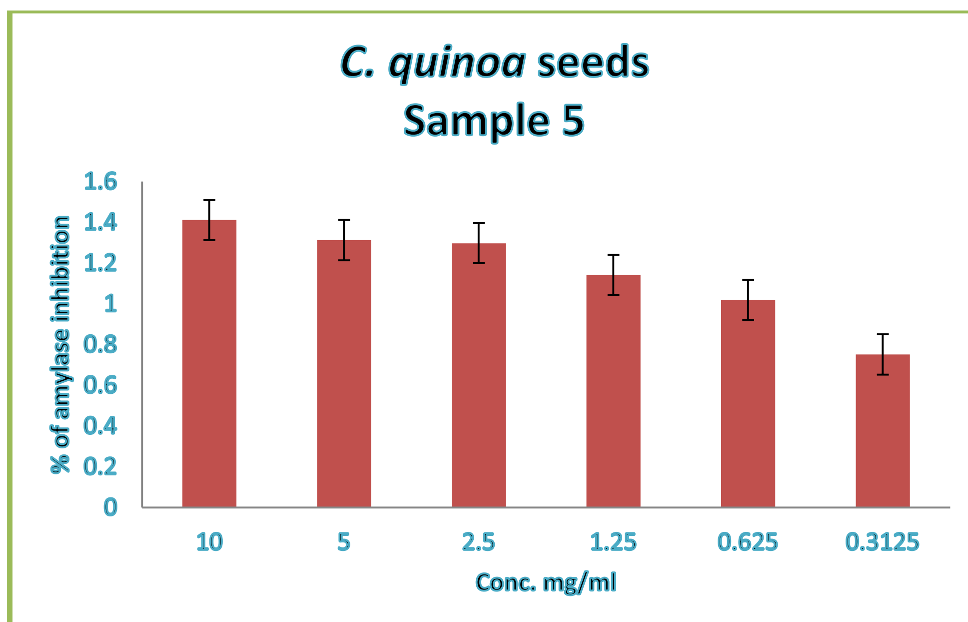
### Alpha-amylase inhibition of both plant extracts

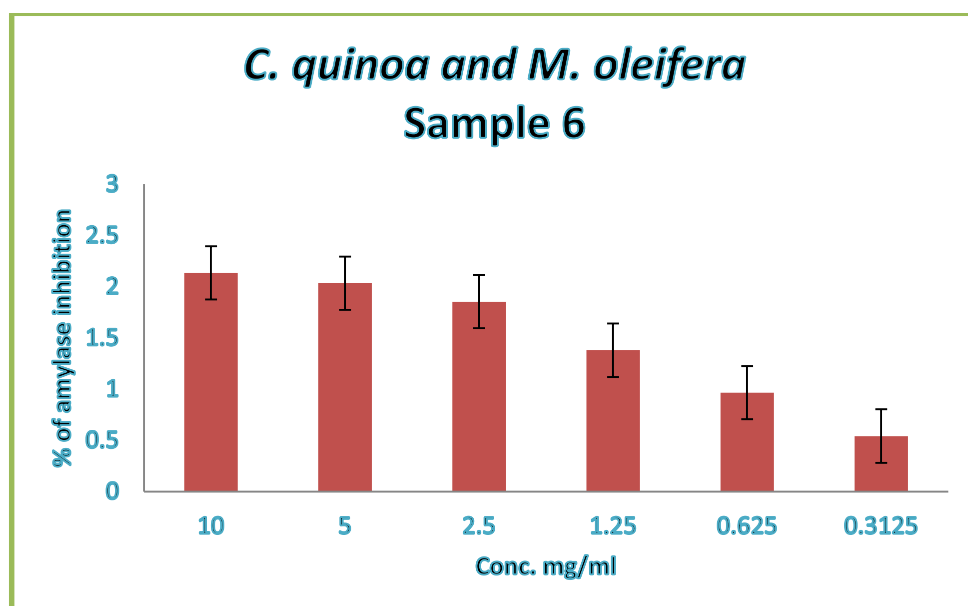


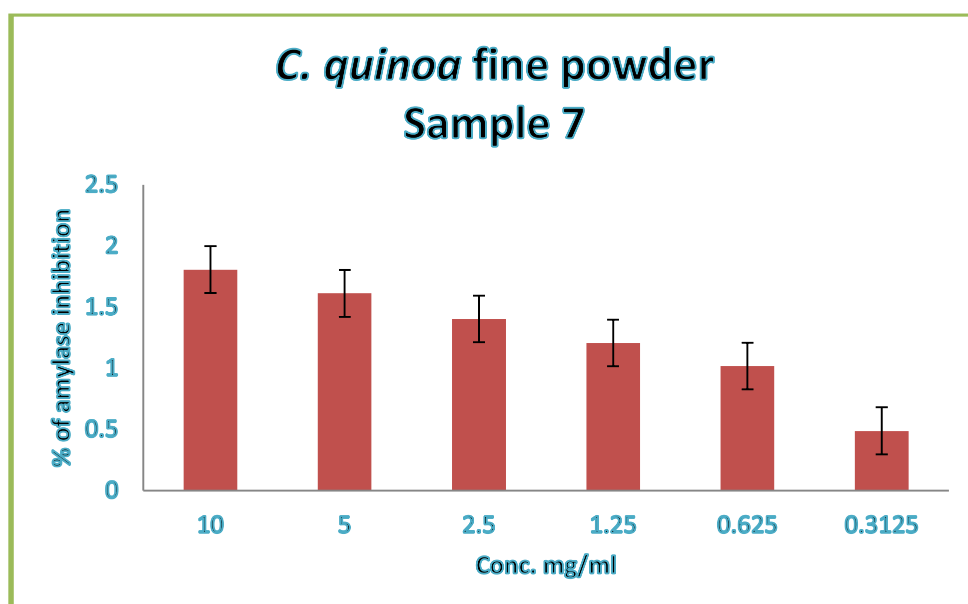

M. oleifera Sample 2
% of alpha amylase inhibition
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml


C.quinoa Sample 3
% of amylase inhibition
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml


M. oleifera leaves
Sample 4
% of amylase inhibition
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml


C. quinoa seeds
Sample 5
% of amylase inhibition
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml

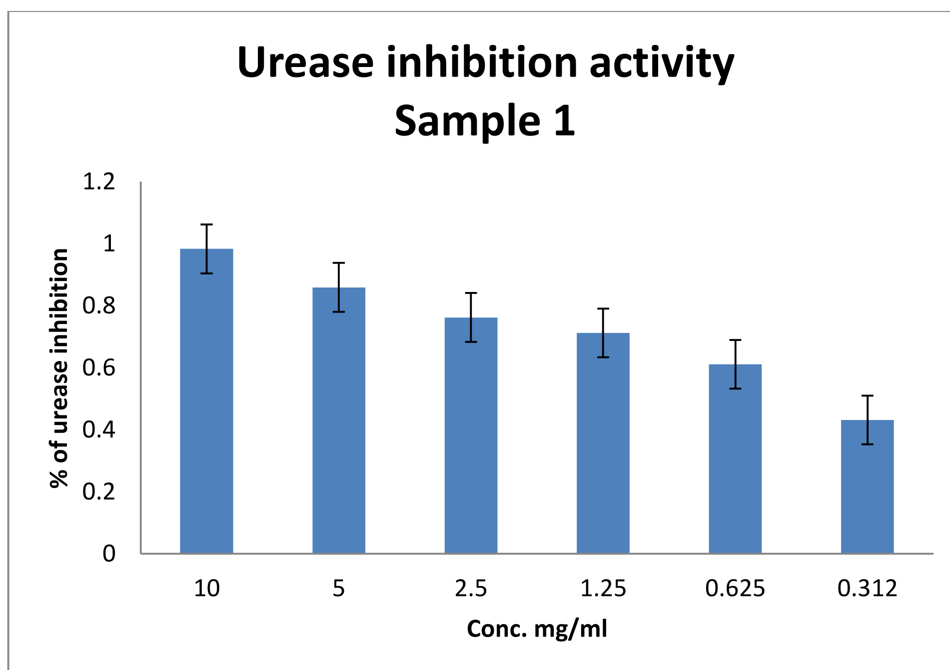

C. quinoa and M. oleifera
Sample 6
% of amylase inhibition
3
2.5
2
1.5
1
0.5
0
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml

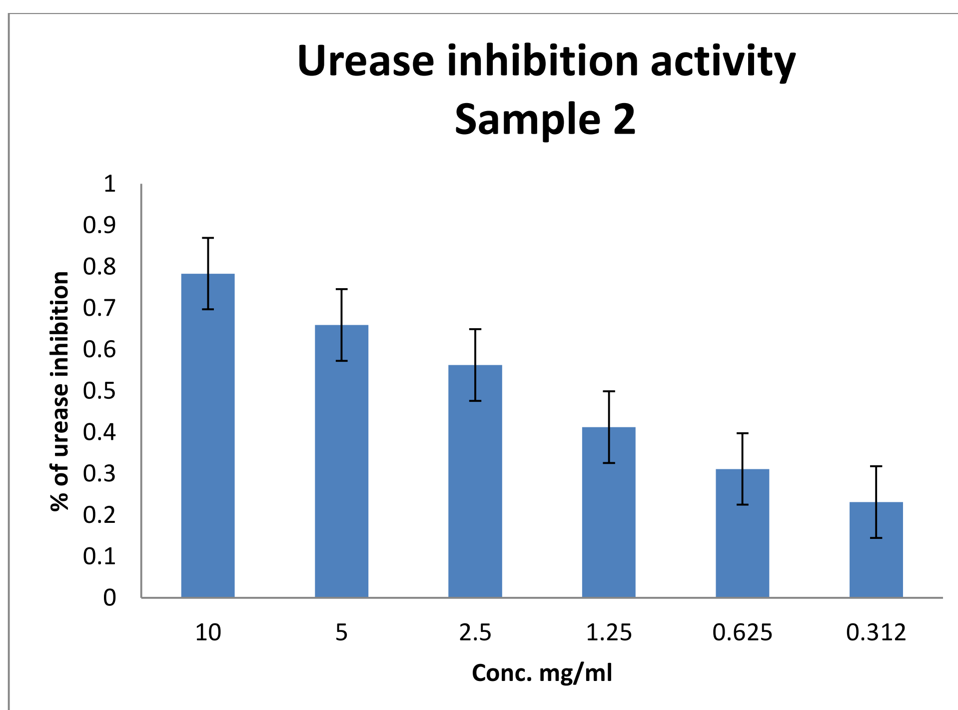

C. quinoa fine powder
Sample 7
% of amylase inhibition
2.5
2
1.5
1
0.5
0
10
5
2.5
1.25
0.625
0.3125
Conc. mg/ml

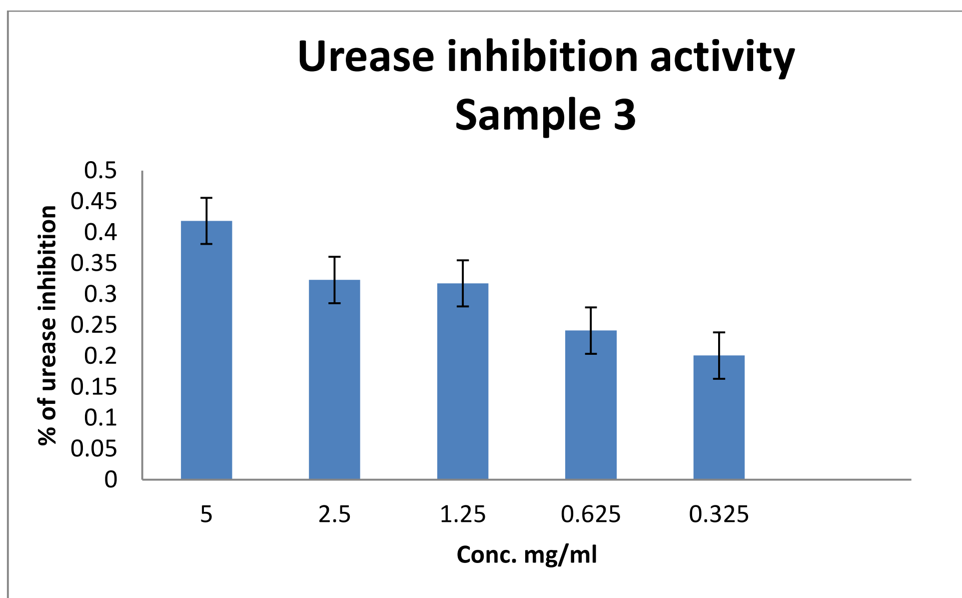
| Sample no | Alpha Enzyme inhibition (10) | D1 (5) | D2 (2.5) | D3 (1.25) | D4 (0.625) | D5 (0.325) |
|---|---|---|---|---|---|---|
| 1 | 0.6855 | 0.5408 | 0.4028 | 0.3298 | 0.3131 | 0.2887 |
| 2 | 0.7935 | 0.6440 | 0.5356 | 0.4618 | 0.3020 | 0.2383 |
| 3 | 0.7940 | 0.6068 | 0.5293 | 0.4889 | 0.4272 | 0.3302 |
| 4 | 0.3684 | 0.2586 | 0.2083 | 0.1623 | 0.1236 | 0.1138 |
| 5 | 1.2692 | 1.2013 | 0.1944 | 0.1541 | 0.1198 | 0.1021 |
| 6 | 2.1348 | 2.1358 | 1.8532 | 1.7803 | 0.6082 | 0.8413 |
| 7 | 1.8050 | 1.7112 | 1.6017 | 0..4069 | 0.3720 | 0.2875 |

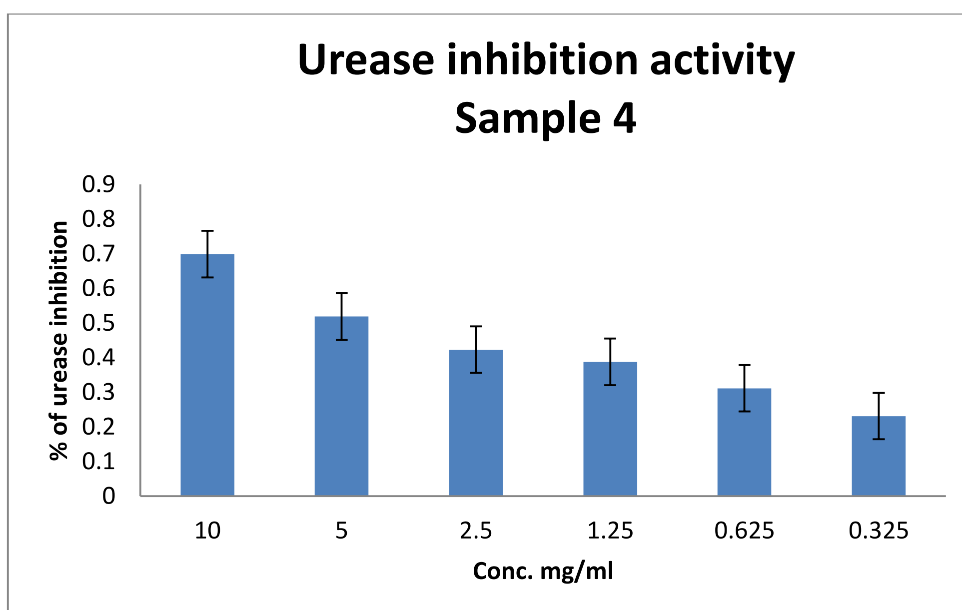
**Amylase inhibition of M.oleifera leaves and C. quinoa seeds at different concentrations**

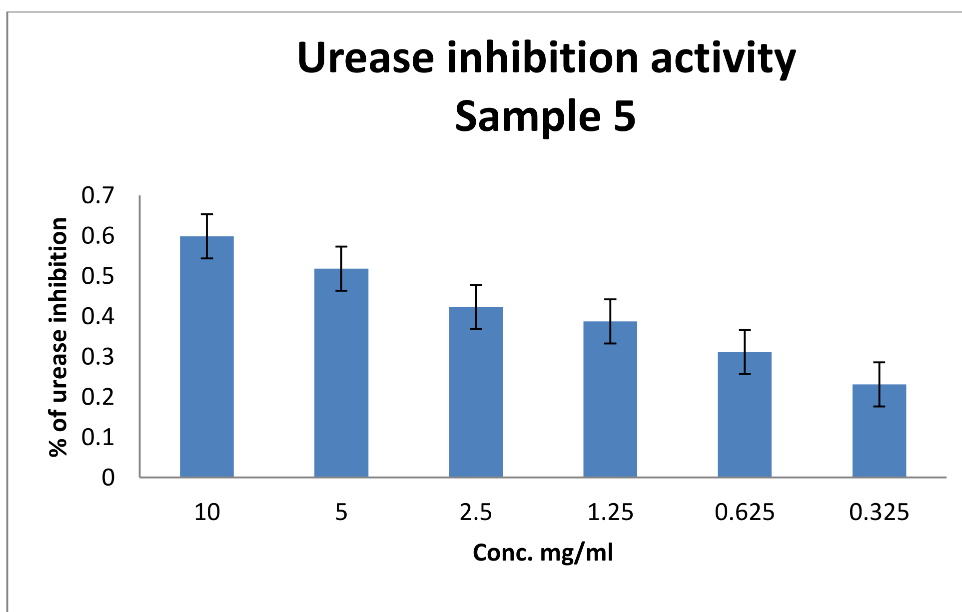
**Urease inhibitory activity of both plant extracts:**

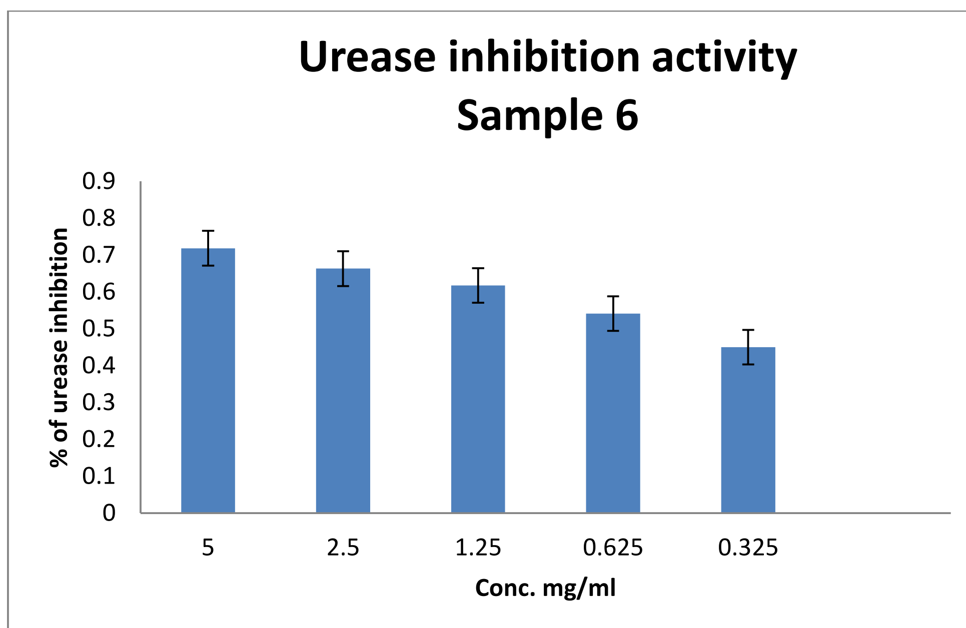


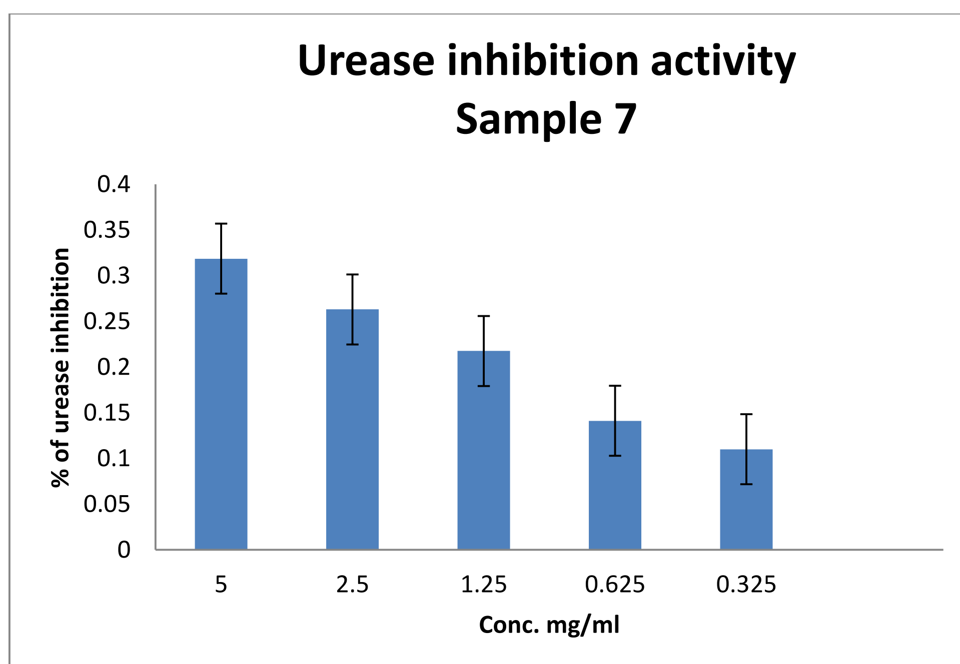




Urease inhibition activity
Sample 3
% of urease inhibition
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
5
2.5
1.25
0.625
0.325
Conc. mg/ml


Urease inhibition activity
Sample 4
% of urease inhibition
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
5
2.5
1.25
0.625
0.325
Conc. mg/ml


Urease inhibition activity
Sample 5
% of urease inhibition
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
5
2.5
1.25
0.625
0.325
Conc. mg/ml




| Sample no | Enzyme inhibition (10) | D1 (5) | D2 (2.5) | D3 (1.25) | D4 (0.625) | D5 (0.325) |
|---|---|---|---|---|---|---|
| **1** | 0.9855 | 0.8408 | 0.7028 | 0.6298 | 0.5331 | 0.4887 |
| **2** | 0.8935 | 0.7440 | 0.6356 | 0.5618 | 0.5020 | 0.4383 |
| **3** | 0.5940 | 0.4068 | 0.3993 | 0.3289 | 0.2272 | 0.2302 |
| **4** | 0.7684 | 0.6586 | 0.5883 | 0.5223 | 0.4536 | 0.4138 |
| **5** | 1.2692 | 1.3113 | 0.5744 | 0.4441 | 0.4198 | 0.3521 |
| **6** | 2.1348 | 1.1358 | 0.8532 | 0.6803 | 0.6082 | 0.5413 |
| **7** | 0.4050 | 0.3112 | 0.2617 | 0.2069 | 0.1720 | 0.1075 |

**Urease inhibitory activity of M. oleifera leaves and C. quinoa seeds at different concentrations.**

## Discussion

This study demonstrates the inhibitory activity of various extracts of M. oleifera leaves and C. quinoa seeds against alpha-amylase. The alpha-amylase inhibition assay is significant because hyperglycemia is a common risk factor for diabetes and its complications.

Alpha-amylase and alpha-glucosidase are two enteric enzymes located at the intestinal border critical for starch hydrolysis and carbohydrate absorption following food intake. Therefore, studies suggest that inhibiting these enzymes prevents their activity for starch hydrolysis, thereby controlling blood glucose levels and diabetes [24].
As a result, blood glucose control is critical for the early detection and prevention of micro and macrovascular complications associated with diabetes mellitus. This study aimed to determine how M. oleifera leaves and C. oleifera leaves Quinoa seeds inhibited alpha-amylase activity.
The figure depicts the inhibition of alpha-amylase in both plants at various concentrations. The three samples of M. oleifera leaf extracts demonstrated a 26.09 % inhibition rate (sample 1), a 15.15 % inhibition rate (sample 2), and a 29.32 % inhibition rate (sample 3). (sample 4). In comparison, the C.quinoa extracts inhibited alpha-amylase enzyme by 9.70% (sample 3), 16.08 % (sample 5), and 10.69 % (sample 6; Tables 1 & 2). Additionally, sample 7 is a mixture of the two extracts that exhibited the highest inhibitory activity, 51.97 percent. The results indicate that the sample containing both plant extracts exhibited significantly higher alpha-amylase inhibitory activity due to the phenolic compounds found in both plants [25].

## Conclusion

The results indicated that *Moringa oleifera* has significantly higher amylase and urease inhibitory activity than *Chenopodium quinoa* due to the presence of numerous vital bioactive components with anti-diabetic and anti-ulcer properties. Additionally, this study indicates that the methanolic extract inhibited enzymes more effectively than the aqueous extract. Both medicinal plants are critical, and their combined extracts demonstrated significant enzyme inhibitory bioactive compounds. The results indicated that the increased or decreased inhibitory activity of alpha-amylase and urease enzymes is dependent on the concentration of plant extracts at various RPMs.

## Conflict of Interest

The authors have no conflict of interest.

## References

extract (in the range of 0 to 100 μL) and phosphate buffer (100 mM, pH 7.4) to reach the total value of 985 μL. The enzymatic reactions started with the addition of 15 μL of to have signi□cant inhibitory characteristics for concentration of 1 mg/mL

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