

DETERMINATION OF INHIBITION ACTIVITY OF α - AMYLASE ENZYME, ANTIOXIDANT ACTIVITY, ANTIBACTERIAL Activity AND PHENOLIC COMPOUNDS BY USING SOME MEDICAL PLANTS

S. I. Hussein A. F. Khalaf Y. Ahmed B. Ahmed A. Iyad
Assist. Prof. Assist. Lecturer Researcher Researcher Researcher

Dept. Biot. Col. Sci. University of Baghdad - Iraq

saharraheem2015@gmail.com

ABSTRACT

The inhibition of carbohydrate hydrolyzing enzymes such as α -amylase can be an important strategy in the postprandial blood glucose level in patients with type II diabetes, plants contains different chemical constituents with potential for inhibition of α -amylase and hence may be used as therapeutic. Nine types of plants include (Clove, Lemon, Chamomile, Radish, Ginger, Black seed, pomegranate, Beetroot and Garlic) were used in this study to select the optimum plant material that inhibited α - amylase enzyme. *Raphanus raphanistrum* was chosen, it had the highest inhibition activity of the enzyme (95.5%). Also sodium phosphate buffer (0.2 M, pH 7.5) was selected as a best extraction buffer of plants inhibitor with inhibition activity 83%. The optimum extraction ratio represented by 1:20 (w:v) after 90 min, it was given 96.8% enzyme inhibition activity. Antioxidant activities of plants were performed using DPPH free radical scavenging assay, and lemon had a highest by 94.4%. This confirms that the plant might protect cells from oxidative damage, resulting in certain diseases. The antimicrobial properties of lemon, garlic, clove have been proven the highest effective against selected human pathogens such as *Staphylococcus aureus*, *E. coli* and *Enterococcus* sp. Also total phenolic content (TPC) of lemon and garlic were estimated spectrophotometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE), lemon revealed the highest value compared with garlic.

Keywords: plant materials, diabetes mellitus, TPC, antimicrobial agents

حسين وآخرون

مجلة العلوم الزراعية العراقية - 2020: 51(1): 411-421

تحديد الفعالية التثبيطية لانزيم الاميليز ، الفعالية المضادة للاكسدة والفعالية المضادة للبكتريا باستخدام بعض النباتات الطبية

سحر ارحيم حسين أسيل فخري خلف ياسمين احمد بسمة احمد عبد الرحمن اياذ
استاذ مساعد مدرس مساعد باحث باحث باحث

المستخلص

يعتبر تثبيط الانزيمات المحللة للكربوهيدرات مثل انزيم الاميليز من الاستراتيجيات المهمة لتخفيض مستوى الجلوكوز في الدم بعد الاكل لمرضى النوع الثاني من مرض السكري. تحتوي النباتات على مكونات كيميائية مختلفة ذات فعالية تثبيطية لانزيم الاميليز. في هذه الدراسة تم اختيار تسعة أنواع نباتية مختلفة لغرض اختيار النبات الأمثل لتثبيط فعالية انزيم الاميليز شملت (القرنفل، الليمون، البابونج، الفجل، الزنجبيل، حبة البركة، الرمان، الشوندر والثوم). بينت النتائج ان نبات الفجل هو الأمثل من بينت النباتات المنتخبة، حيث امتلك اعلى فعالية تثبيطية لانزيم قدرت بـ 95.5%، كذلك استخدام بفر فوسفات الصوديوم بتركيز 0.2 مولاري وبدالة حموضة 7.5 كأفضل دارئ للاستخلاص بفعالية تثبيطية 83%. كما أظهرت النتائج ان نسبة 1:20 (وزن: حجم) هي أفضل نسبة لاستخلاص المثبط الانزيمي من نبات الفجل بعد 90 دقيقة اذ وصلت الفعالية التثبيطية الى 96.8%. درست أيضا الفعالية المضادة للأكسدة للنباتات المنتخبة باستخدام مادة DPPH (بطريقة حصاد الجذور الحرة) ، حيث امتلك الليمون اعلى فعالية مضادة للأكسدة اذا بلغت 94.4% وبهذا يلعب النبات دور في حمايه الخلايا من التدمير بالأكسدة الناجمة من امراض معينه. اختبرت كذلك الفعاليه المضاده للميكروبات باستخدام النباتات أعلاه وامتلك الليمون والثوم والقرنفل اعلى فعالية مضادة للمرضات البشرية مثل بكتريا *Staphylococcus aureus* و *E. coli* ، قدر المحتوى الفينولي الكلي لنبات الليمون والثوم باستخدام كاشف فولن Folin-Ciocalteu بوساطة جهاز المطياف الضوئي اعتمادا على المنحنى القياسي لحمض الكاليك حيث اعطى نبات الليمون محتوى فينولي اعلى من نبات الثوم.

كلمات مفتاحية: مواد نباتية، مرض السكري، المحتوى الفينولي الكلي، عوامل مضادة للميكروبات

*Received:11/7/2019, Accepted:13/10/2019

INTRODUCTION

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level. Diabetes is a progressive disease and is one of the major killers in recent times. Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/type II). The treatment of type II diabetes is complicated by several factors inherent to the disease and elevated post prandial hyperglycemia (PPHG) is one of the risk factors (14). PPHG is elevated by the action of glucosidases, a class of enzymes that helps in the breakdown of complex carbohydrates into simple sugars such as maltose and glucose. Glucosidase inhibitors such as α -amylase inhibitors plays a major role in managing PPHG in diabetic patients. These α -amylase inhibitors inhibit the action of α -amylase enzyme leading to a reduction in starch hydrolysis which shows beneficial effects on glycemic index control in diabetic patients (2002). Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging (17,35). An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (34). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases (9). Herbal plants considered as good antioxidant since ancient times. Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged (24). Some *Staphylococcus* spp. and *Streptococcus* spp. involved in the pathogenesis of respiratory and skin infections, along with *Pseudomonads* and members of the Enterobacteriaceae causing gastrointestinal,

urogenital diseases and wound contamination are resistant to virtually all of the older antibiotics (19). Clinical isolates of *Staphylococcus aureus*, the leading cause of nosocomial infections, are increasingly resistant to an array of antimicrobial agents like penicillin, gentamicin, tobramycin, amikacin, ciprofloxacin, clindamycin, erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin (4). The problem of antibiotic resistance in humans and animals will continue for a long time (10). Against this backdrop, the development of alternative drug classes to treat such infectious diseases is urgently required (9). Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (10). These biomolecules are the source of plant-derived antimicrobial substances (PDAMs) (9). Some natural products are highly efficient in the treatment of bacterial infections (13). The aim of this project is trying to use some medical plants common in ways to reduce the effectiveness of amylase and reduce the proportion of glucose blood for diabetics. And to evaluate the antioxidant activity of several agents proposed for reversion of problems caused by bleaching procedures using the DPPH free radical assay, also to estimate the antibacterial activity and phenolic compounds of these plants extracts that might have support and help the research scientific community to study and encourage the use of medicinal plants for the treatment of diseases.

MATERIAL AND METHODS

Plants

Plants were used through this study were locally available in a market, include Clove (*Syzygium aromaticum*), Lemon (*Citrus lemon*), Chamomile (*Matricaria chamomilla*), Radish (*Raphanus raphanistrum*), Ginger (*Zingiber officinale*), Black seed (*Nigella stiva*), pomegranate (*Punica granatum*), Beet (*Beta vulgaris*) and Garlic (*Allium sativum*) were used as the source of material.

Determination Amylase assay

The activity of α -amylase enzyme was estimated according to the method describe by Bernfeld (6) which depend on standard curve

of maltose throughout decomposition reducing sugars liberated by the enzyme. A standard calibration curve was prepared for the maltose taking 1ml of 360-1800 µg/ml dilution of maltose, the percentage (w:v) of maltose in the reaction wells was calculated from the corrected absorbance of each test and using the equation of the calibration curve. Mixing 0.8 ml of starch solution with 0.2 ml of enzyme solution (saliva) for 20 min in water bath at 37 °C, then add 1 ml of 3,5-Dinitrosalicylic acid (DNSA) with boiling for 5 min then cooling and add 10 ml of D.W with vortex. Amylase activity was detected by measuring the absorbance increase at 540 nm. Enzymatic activity unit known as the amount of enzyme liberated 1 µmole from reducing sugars (glucose and maltose) in one minute under optimum conditions.

Estimation the standard curve of maltose

Put the following volume of standard maltose solution (2 mg/ml) that prepared previously in test tubes then add the appropriate volume of D.W (Table 1). After that add 1 ml of DNSA for each tube, then put the tubes in water bath at boiling temperature for 5 min. Thereafter the tubes were cooled by tap water and add 10 ml of distilled water for each tube. The solution was mixed by vortex for each tube and read the absorbance at 540 nm (Fig. 1).

Table 1. Estimate the standard curve of maltose

The number	Standard maltose solution (ml)	Distilled water (ml)	Maltose (mg/ml)
1	0	1	0
2	0.1	0.9	0.2
3	0.3	0.7	0.6
4	0.5	0.5	1
5	0.8	0.2	1.6
6	1	0	2

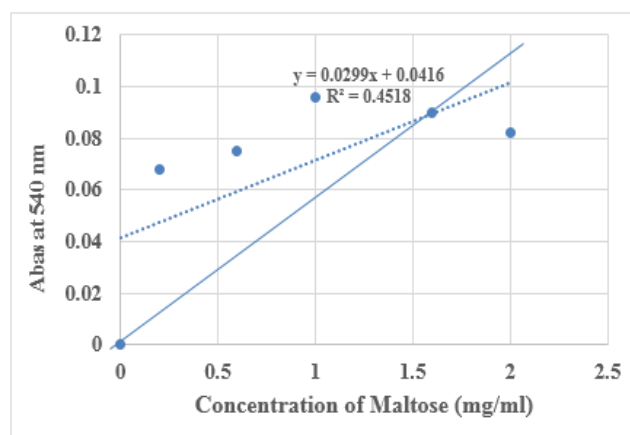


Figure 1. Maltose Standard Curve Performed by Bernfeld Method

Extraction and recovery of α-amylase inhibitor

One g of each plant was homogenizing with 10 ml of 0.2M of phosphate buffer, the mixing was crushed by mortar for 15 min at room temperature. The mixture was centrifuge at 10000 rpm for 15 min (17). The clear supernatant obtained represented the crude extract and used to assay the α- amylase inhibitor activity.

Determination of α-amylase inhibitor activity

A crude extract of each plant was incubated separately with a known volume of α-amylase enzyme (saliva) by 1:1 ratio for 30 min at room temperature, thereafter the α-amylase activity was estimated, the control representing 100% enzyme activity were conducted in the same manner replacing the plant extract with distilled water. Mixing 0.8 ml of starch solution with 0.2 ml of a mixture (enzyme-inhibitor) for 20 min in water bath at 37 °C, then add 1 ml of 3,5-Dinitrosalicylic acid (DNSA) with boiling for 5 min then cooling and add 10 ml of D.W with vortex. Amylase activity was detected by measuring the absorbance increase at 540 nm. The remaining activity is the present inhibitory activity of the enzyme with respect to the present enzyme activity without inhibitor according to the following equation:

$$\text{Remaining enzyme activity} = \frac{\text{Enzyme with inhibitor} - \text{enzyme without inhibitor}}{\text{Enzyme without inhibitor}} \times 100\%$$

Optimum conditions of α -amylase inhibitor Type of Extraction Buffer

Radish was homogenized with different types of buffers for 15 min at 30°C separately. These buffers were 0.2 M sodium acetate buffer (pH 5.5), 0.2M phosphate buffer (pH 7.5) and 0.2M of Tris-base buffer (pH 10). The remaining enzyme activity was determined in each experiment.

Extraction Ratio

One gram of radish was homogenized in different volume of (0.2M phosphate buffer pH 7.5) at 1:10, 1:20 and 1:30 (w:v) ratio. The mixing was crushed by mortar for 15 min at room temperature. Remaining enzyme activity was determined in each experiment.

Extraction Time

One gram of radish was homogenized with 0.2M of phosphate buffer pH 7.5 at ratio 1:20 in different time, include (15, 30, 60 and 90) min at 30°C. Remaining enzyme activity was determined in each experiment.

Antioxidant Assay: Free Radical Scavenging Activity

The free radical scavenging activity of the nine medical plants was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by (32). Briefly, 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3 ml of the solution of all extracts separately. The mixtures were shaken vigorously and allowed to stand in dark at room temperature for 30 minutes, then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The ability of sample extract to scavenge DPPH radical was calculated by the following equation:

$$\text{Antioxidant activity} = \frac{\text{Ab of control} - \text{Ab of sample}}{\text{Ab of control}} \times 100\%$$

Where:

Ab of control = is the absorbance of DPPH radical only, **Ab of sample** = is the absorbance of DPPH radical with sample extract.

Determination of Antimicrobial Activity

Nutrient agar prepared and poured in the sterile petri dishes and allowed for solidify. Twenty-four hour overnight bacterial cultures of *Staphylococcus aureus*, *E. coli* and *Enterococcus* sp. were swabbed separately on the media using sterile cotton buds, then two wells (8 mm diameter) were made by using a sterile cork borer. One hundred microliter of each extract from chosen medical plants were loaded in the wells. The plates then incubated at 37°C for 24h. After incubation, the diameter of inhibition zone was measured. Tetracyclin, Pipracillin, Gentamycin, Sulfametazol-trimethoprim, Azthereonam and Ciprofloxacin were used as positive control.

Determination of the Total Phenolic Content (TPC)

The total phenolic contents of the lemon and garlic extracts were estimated using the Folin Ciocalteu reagent as described by Singleton and Rossi (33) with some modifications. The calibration curve fig. (2) was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350 and 400 mg/ml of gallic acid solutions with 0.5 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 10 min at 765 nm. For both plants extracts, 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 10 min, the absorbance was measured to determine the total phenolic contents in both extracts separately using the formula,

$$C = C1 \times V/m$$

Where:

C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), **C1**= concentration of Gallic acid established from the calibration curve in mg/ml, **V**= volume of extract in ml, and **m**= the weight of the plant extract in g.

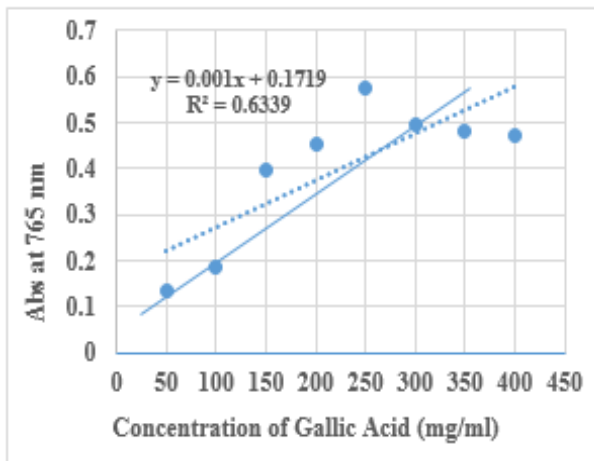


Figure 2: Standard Curve of Gallic Acid

Result and Discussion

Optimum Conditions of α - Amylase Inhibitor

Different bioprocess conditions that effect on α - amylase inhibitor were optimized for maximum inhibition activity, a large number of factors affect the extraction of inhibitor such as types of plants, types of buffer, extraction ratio and extraction time etc. Hence, optimization of these conditions helps to reduce extraction cost and help to obtain a high yield of α - amylase inhibitor.

Types of Plants

Nine types of plants, commonly used in this study, namely Clove (*Syzigium aromaticum*), Lemon (*Citrus lemon*), Chamomile (*Matricaria chamomilla*), Radish (*Raphanus raphanistrum*), Ginger (*Zingiber officinale*), Black seed (*Nigella staiva*), pomegranate (*Punica granatum*), Beetroot (*Beta vulgaris*) and Garlic (*Allium sativum*) were obtained and screened for their enzymatic inhibition activity. As can see from fig. (3) radish was gave the highest inhibition activity of 95.5%, followed by lemon 87.3% whereas the inhibition activity of Matricaria, Clove, Ginger, Black seed, Garlic, Pomegranate and Beetroot were reached to (83.5, 66.6, 60.6, 9, 72, 81 and 59.4) % respectively. Plants α -amylase inhibitors are drug-design targets for treatment of diabetes and digestion disorders (5). Radish has been identified as having anti-diabetic effects, making it favorable for those with diabetic conditions. This may be due to its ability to enhance the antioxidant defense mechanism and reduce the accumulation of free radicals, affect hormonal-induced glucose

hemostasis, promote glucose uptake and energy metabolism, and reduce glucose absorption in the intestine. Furthermore, the aqueous extract of radish inhibited both α -amylase and α -glucosidase enzymes *in vitro*, it is well-known that these enzymes are required for the degradation of poly- and oligosaccharides in the intestine before absorption. Therefore, such enzymatic inhibition may reduce the amount of glucose absorbed, which could be effective for the management and prevention of diabetes (5). Bibek with coworkers (7) found that radish seed and rapeseed have antioxidant activity, alpha-amylase inhibitory activity and antibacterial activity.

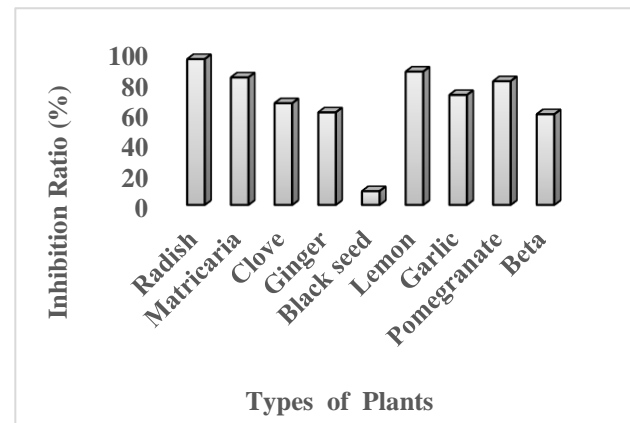


Figure 3. Effect the Types of Plants on α -Amylase inhibition using Phosphate Buffer pH 7.5 at 30°C and for 15 min.

Type of Extraction Buffer

The inhibition activity of α -amylase was estimated after extraction, using different buffer, and the results were illustrated in (Fig 4). These results show that sodium phosphate buffer (0.2 M, pH 7.5) was a best extraction buffer with inhibition activity 83%. While other buffer with different pH were given low inhibition activity. α -amylase inhibitors was extracted from *Pouteria sapota* by using phosphate buffer in pH 7.5 (30). In addition, Hind with coworker (16) were extracted α -amylase inhibitor from *Ononis angustissima* by using distilled water in pH 7. Wang with coworkers (34) were extracted α -amylase inhibitors from seeds of common bean mutants by water pH was adjusted to 6.5.

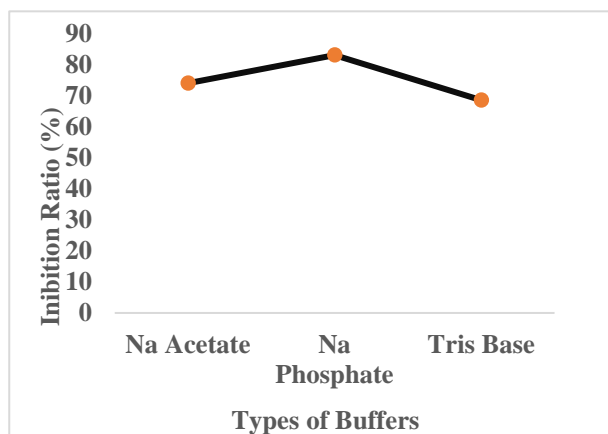


Figure 4. Effect of Types of Buffer on Extraction α - amylase Inhibitor from Radish at 30°C for 15 min.

Extraction ratio

Three extraction ratio were chosen 1:10, 1:20 and 1:30 (w:v) to determine the best extraction ratio on extraction the inhibitor material from radish by using 0.2M phosphate buffer pH 7.5. The best result was obtained at extract ratio 1:20 in rate of 75.45%, While the other gave 66.48% and 30.39% for 1:10 and 1:30 respectively (Fig.5). Kondo and Ida (19) found that an α -Amylase inhibitors from wheat (*Triticum aestivum*) was extracted with water in a 1:10 (w:v). Also Wang with coworkers (1) were extracted α -amylase inhibitors from seeds of common bean mutants by using tert-butanol. A preliminary trial for optimization of ratio of crude extract to tert-butanol volume required for Three-phase partitioning (TPP) was determined by comparing the 1:0.5, 1:1, and 1:1.5 ratios of crude extract totert-butanol. Salivary α - amylase inhibitor was extracted from barley by using water at a ratio (1:11) (19).

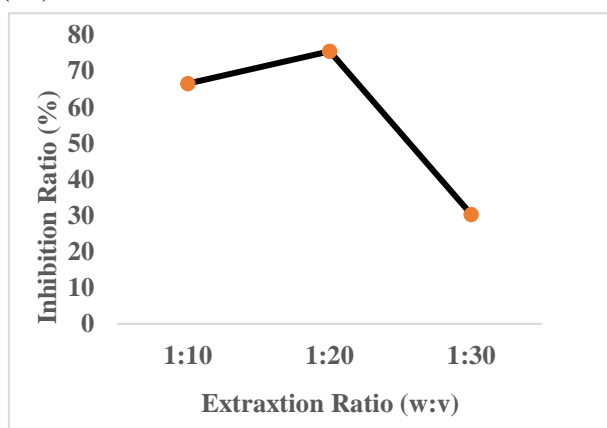


Figure 5. Effect of Extraction Ratio on Extraction α - amylase Inhibitor from Radish at 30°C for 15 min.

Extraction time

Four extraction periods were chosen (15, 30, 60 and 90) min to determine the best extraction time on extraction the α -amylase inhibitor by using sodium phosphate buffer (0.2 M, pH 7.5). The highest inhibition activity of radish was measured in 90 min; it was reached to 96.8 %, compared to the lower of inhibition activity in 15, 30 and 60 min (87, 89.3 and 91.5) % respectively (Fig. 6). Abood and Hakeem (1) were extracted salivary α -amylase inhibitor from barley by using water at a ratio (1:11) at 60 min. Alpha-amylase inhibitor in white kidney beans (*Phaseolus vulgaris*) was extracted by stirred 1, 2, 3 hour with 0.05M, 0.10M, 0.15M (1:6 w/v) of solvent at room temperature (25°C). The homogenate was centrifuged at 10,000 g for 30, 45, 60 min at 4 °C (21).

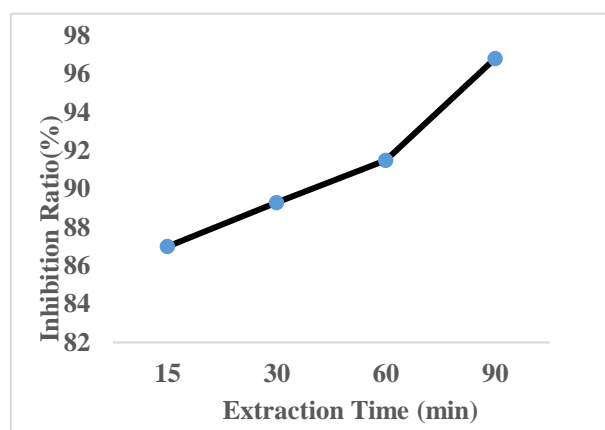


Figure 6. Effect of the Extraction Time on α - amylase Inhibitor Extraction from Radish at

Antioxidant Assay: Free Radical Scavenging Activity

The nine types of medical plants were used to examine α - amylase inhibition, also selected to measure the antioxidant activity by using DPPH. The results shown that lemon has a highest activity of radical scavenger (94.4%) between the other plants (Fig. 7). The DPPH radical scavenging assay is a commonly used tool for accessing the antioxidant capacity of plant materials because of the relative cheap cost and speed of completion. The model of scavenging the stable of DPPH radical was commonly used to evaluate the free radical scavenging ability of various samples. DPPH is a commercial oxidizing radical that can be

reduced by antioxidants. In this assay, the violet color of DPPH changed to pale yellow fig (8), due to the abstraction of hydrogen atoms from the antioxidant compound. When there are more antioxidants in an extract, more DPPH was reduced (11). Natalie (24) was found that citrus fruits like lemons are high in vitamin C, a primary antioxidant that helps protect cells from damaging free radicals. Lemon fruit extract act as an antioxidant agent against histo-pathological changes induced by cyclophosphamide in the testes of albino mice (29).

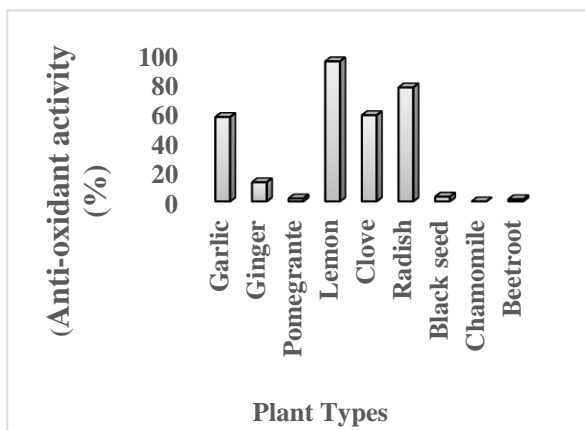


Figure 7. Antioxidant Activity of Some Medical Plants

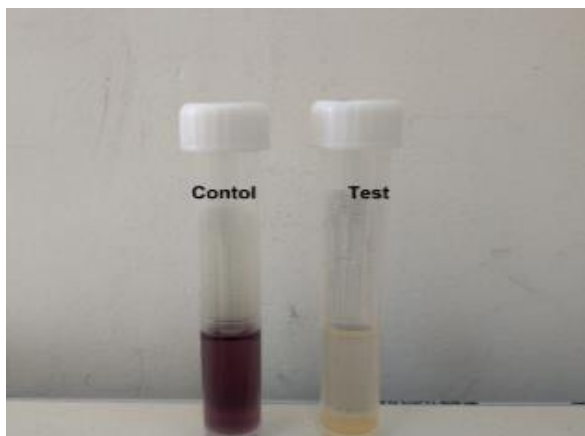


Figure 8. Antioxidant Activity of Lemon Plant by DPPH

Antibacterial Activity

The selected medical plants that used to determined α - amylase inhibition and antioxidant activity were used to measure the antibacterial activity by selected three different pathogenic bacteria included *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus* sp. after growing on nutrient agar at 37°C for 24 h. The results shown in table (2) that lemon has a highest antibacterial activity against *E.coli* comparison with other plants fig. (9), whereas *Staph aureus* was inhibited by lemon and Garlic separately fig (10), also garlic and ginger were used to inhibit the growth of *Enterococcus* sp. (Fig.11). Lemon is an important medicinal plant of the family Rutaceae. It is a rich source of vitamin C and it is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root and flower) of lemon against clinically significant bacterial strains has been reported also its consists of about 5% citric acid that gives a sour (tarty) taste to the lemon (31, 26). (11) found that lemon juice has the antibacterial susceptibility against some gram-positive and -negative bacterial strains. Also garlic is a potential inhibitor for food pathogens. Foods contaminated with pathogens pose a potential danger to the consumer's health. The use of garlic can increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods. Garlic aqueous extract has antibacterial properties against *S. aureus*. In addition, garlic has antibacterial properties against other Gram-positive and Gram-negative bacteria, which must be investigated in further studies (3).

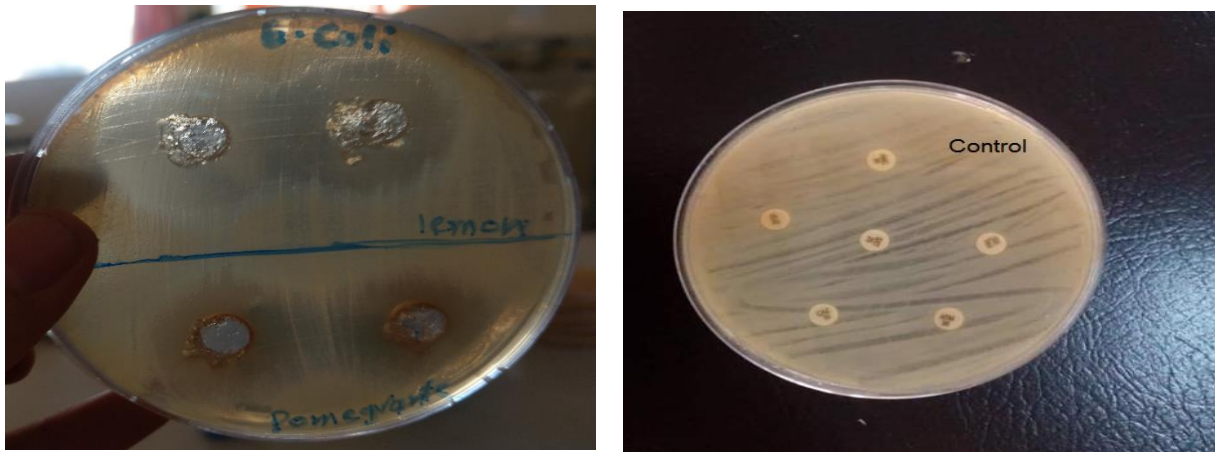


Figure 9. Antibacterial Activity of Lemon against *E.coli*

Control: Tetracyclin, Pipracillin, Gentamycin, Sulfametazol-trimethoprim, Azthereonam, Ciprofloxacin

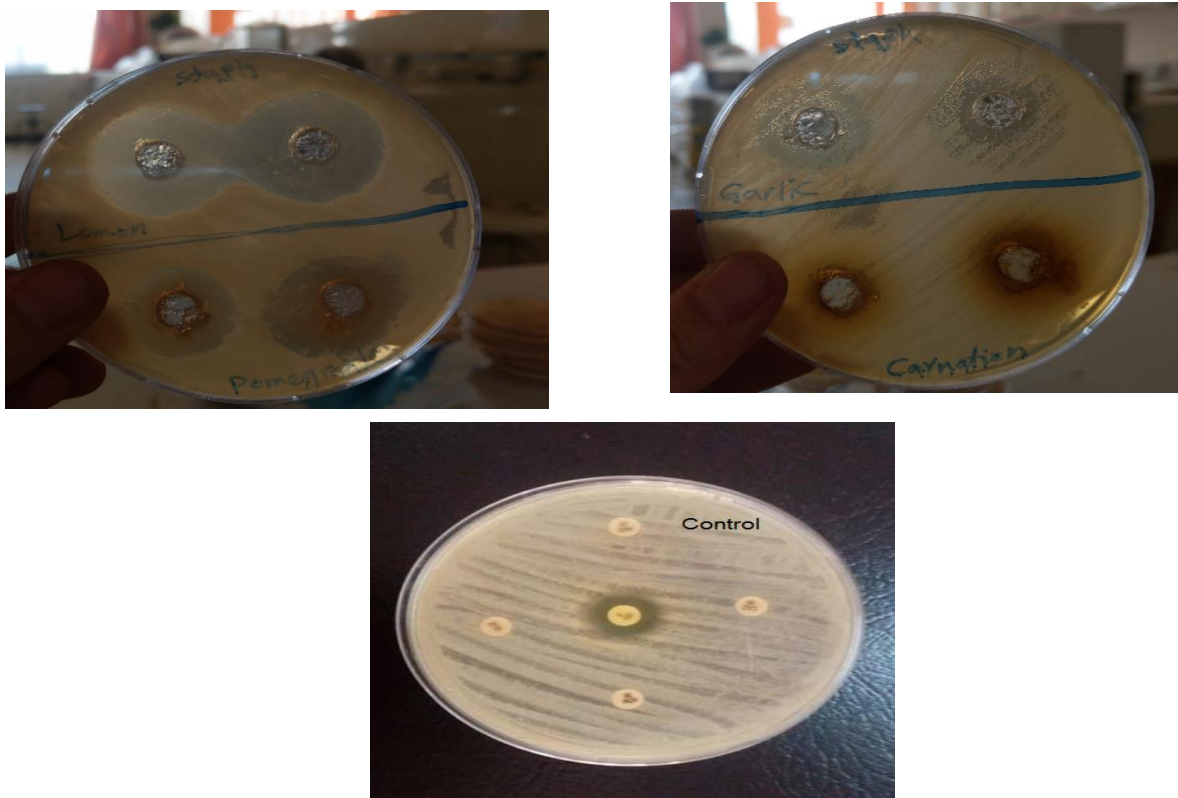


Figure 10. Antibacterial Activity of Lemon and Garlic Against *Staph. aureus*

Control: Tetracyclin, Pipracillin, Gentamycin, Sulfametazol-trimethoprim, Azthereonam, Ciprofloxacin

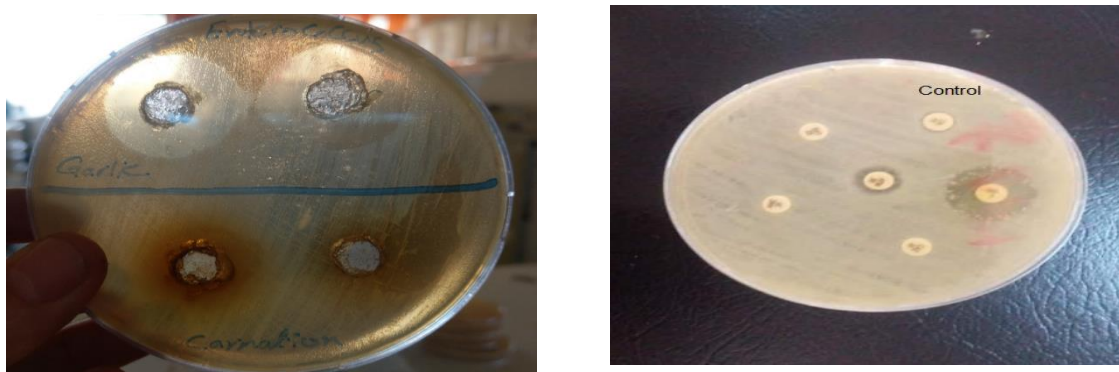


Figure 11. Antibacterial Activity of Garlic against *Enterococcus* sp.

Control: Tetracyclin, Pipracillin, Gentamycin, Sulfametazol-trimethoprim, Azthereonam, Ciprofloxacin

Table 2. Antimicrobial Activity of Some Medical Plants

Plant	Inhibition Zone (cm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>Enterococcus</i>
Garlic	2.5	3	3
Clove	1.8	1.6	2
Ginger	2	2.5	2.8
Lemon	4	3	2.5
Pomegranate	2.8	2.5	2.5

Determination of the Total Phenolic Content

Two types of medical plants (Lemon and garlic) that gave the highest antibacterial activity against the selected pathogens were used to screening for TPC in aqueous extracts, and both of extracts showed the presence of phenolics. The total phenolic contents were determined using the Folin Ciocalteu method in terms of the Gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in fig (2), and the standard curve equation was $y = 0.001x + 0.1719$ where $R^2 = 0.6339$. The total phenolic contents (Gallic acid equivalents, mg/g) in the extracts were calculated to be 47.5 and 35 mg/g, respectively (Fig 12). Phenolic compounds are commonly reported in plants and they are known to exert various biological activities, including antioxidant activity (2, 27) as well as possess antibacterial properties (23, 28). Phenolic compounds are also known to possess α -amylase inhibitory potential (22). Thus, the antioxidant, α -amylase inhibition and antibacterial activities of medical plants

extracts of this study may be attributed to their high phenolic contents. Elkhatim with coworkers (15) were estimated the TPC of whole fruit, peel, and pulp containing seeds citrus (orange, lemon, and grapefruit) and founds that the peels of grapefruit had the highest total phenolic content followed by lemon and orange, which was reached to 77.3, 49.8, and 35.6 mg of gallic acid equivalent/g of peels, respectively. While (8) mentioned that garlic had a various levels of phenolics (0.05–0.98 mg gallic acid equivalents/g of dry extract).

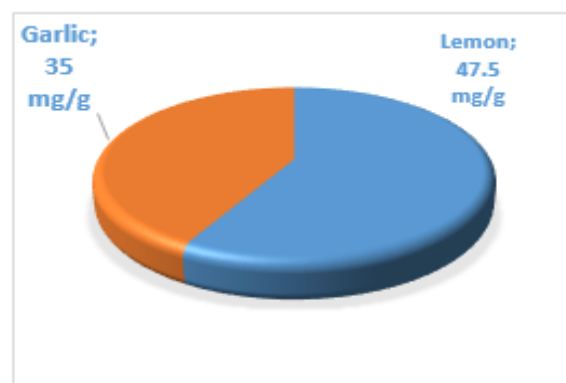


Figure 12. TPC of Lemon and Garlic

REFERENCES

1. Abood, S. C. and I. M. Hakeem. 2016. Purification and Characterization of Salivary Amylase Inhibitor Extracted from Barley. *The Iraqi Journal of Agricultural Sciences*. 47(4):1039-1048.
2. Almoulah N.F., Voynikov, Y., Gevrenova, R. and H. Schohn, 2017. Antibacterial, antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species. *South African Journal of Botany*. 112:368-374.
3. Amir, S. M. N. Shahrokh, S., Mansour, B. and H. Ebrahim 2014. Antibacterial Effect of Garlic Aqueous Extract on *Staphylococcus aureus* in Hamburger. *Journal of Microbiology*. 7 (11):1-5.
4. Andersson, D.I. and D. Hughes. 2011. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiology Reviews*. 35:901–911.
5. Baenas, N., Piegholdt, S., Schloesser, A., Moreno, D.A., García-Viguera, C., Rimbach, G. and A.E. Wagner. 2016. Metabolic Activity of Radish Sprouts Derived Isothiocyanates in *Drosophila melanogaster*. *International Journal of Molecular Sciences*.18:17(2):251.
6. Bernfeld P. Amylases, alpha and beta. In: Colowick SP, Kaplan NO. (Eds.), *Methods in Enzymology*, vol. 1. Academic Press, New York. 1955; 149–158.
7. Bibek, K., Sobita, K., Ashmita, D., Ansu, J., Abdul Rehman, M. and S. Pradeep. 2018. Total phenolic content, antioxidant activity, alpha-amylase inhibitory activity and antibacterial activity of radish seed and rapeseed. *European Journal of Biotechnology and Bioscience*. 6 (2): 21-25.
8. Biljana, B., Neda, M.-D., Ruzica, and A. G., Isidora. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chemistry Journal*. 111: 925–929
9. Chiavaroli, V., Giannini, C., De Marco, S., Chiarelli, F. and A. Mohn. 2011. Unbalanced oxidant-antioxidant status and its effects in pediatric diseases. *Redox Report Journal*. 16:101–107.
10. Das, K., Tiwari, R.K.S. and D.K. Shrivastava. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *Journal of Medicinal Plants Research*.4:104-111.
11. Ehigbai, I. O., Ehimwenma, S. O., Faith, E. O. and O. Kelly. 2016. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food Science & Nutrition Journal*. 4(1): 103–109.
12. Elkhatim, K. A. S., Randa, A. A. E. and B. H. Amro. 2018. Content of phenolic compounds, vitamin C, and antioxidant activity in wasted parts of Sudanese citrus fruits. *Food Science & Nutrition Journal*. 6(5): 1214–1219.
13. Fernebro, J. 2011. Fighting bacterial infections - Future treatment options. *Journal of Drug Resist Updat*.14:125–139.
14. Gin, H. and V. Rigalleau. 2000. Post-prandial hyperglycemia. Post-prandial hyperglycemia and diabetes. *Diabetes and Metabolism Journal*. 26 (4): 265–272.
15. Heidari, R. and M. Heidarizadeh. 2005. Extraction, Purification, and Inhibitory Effect of Alpha - Amylase Inhibitor from Wheat (*Triticum aestivum* Var. Zarrin). *February Pakistan Journal of Nutrition*. 4(2).101-105.
16. Hind, L., Nabila, B., Sara, A. and D. Rabah. 2017. In vitro α -amylase and α -glucosidase inhibitory activity of *Ononis angustissima* extracts. *Journal of Applied Pharmaceutical Science*. 7 (02): 191-198.
17. Hussein, S. I. 2013. Extraction and purification of protease inhibitor from seeds of some plants and its antimicrobial activity. *International Journal of Science and Technology*. 8 (2).
18. Kanwar, J.R., Kanwar, R.K., Burrow, H. and S. Baratchi. 2009. Recent advances on the roles of NO in cancer and chronic inflammatory disorders. *Current Medicinal Chemistry Journal*. 16:2373–2394.
19. Kondo, N. K. and E.I. Ida. 1995. Extraction, purification and some partial characterization of alpha-amylase inhibitors from wheat Iapar 28-Igapó. *Archivos Latinoamericanos de Nutrición Journal*. 45(4):310-6.
20. Lowy, F. 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*.

- Journal of Clinical Investigation. 111:1265–1273.
21. Manatwiyangkool, J. 2014. α - amylase inhibition activity of white kidney bean (*Phaseolus vulgaris*) .A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Technology Suranaree University of Technology, Academic Year 2014.
 22. McCue, P.P. and K. Shetty. 2004. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition*, 13(1).
 23. Nascimento, G.G., Locatelli, J., Freitas, P.C. and G.L. Silva. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*. 31(4):247-256.
 24. Natalie, O. 2018. Seven ways your body benefits from lemon water. Medically reviewed by Natalie Olsen, RD, LD, ACSM EP-C on December 10, 2018 — Written by Annette McDermott.
 25. Neu, H.C. 1992. The crisis in antibiotic resistance. *Science Journal*. 257:1064–1073.
 26. NPCS (2012) Handbook on Agro Based Industries (2nd eds), Amazon. Published by Niir Project Consultancy Services, India, pp. 75-78.6.
 27. Qasim, M., Abideen, Z., Adnan, M.Y., Gulzar, S., Gul, B., Rasheed, M. and M.A. Khan. 2017. Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. *South African Journal of Botany*. 110:240-250.
 28. Rauha, J.P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H. and P. Vuorela. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International journal of food microbiology*. 56(1):3-12.
 29. Salwa, M., Q. 2016. Evaluation of lemon fruit extract as an antioxidant agent against histopathological changes induced by cyclophosphamide in the testes of albino mice. *Electron Physician Journal*. 8(1): 1824–1831.
 30. Sathishkumar, T., Abarna, S., Malini, M., Nithya, S., Prathishta P. and J. Lavavya. 2011. Optimal extraction process and identification of A-amylase inhibitors from *Pouteria sapota*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 3(4).
 31. Satoru, K., Yasuhiko, T., Eriko, K., Kazunori, O. and Y. Masamichi. 2000. Quantitative study of flavonoids in leaves of Citrus plants. *Journal of Agricultural and Food Chemistry* .48(9): 3865-3871.
 32. Shen, Q., Zhang, B., Xu, R., Wang, Y., Ding, X. and P. Li. 2010. Antioxidant activity in vitro of selenium-contained protein from the se-enriched. *Bifodobacterium animalis*. *Journal of Anaerobe*. 16: 380-386.
 33. Singleton, V.L. and J.A. Rossi. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*.16: 144-158.
 34. Wanga, H.H., Chena, C.L., Jengb, T.L. and J.M. Sungc. 2011. Comparisons of α -amylase inhibitors from seeds of common bean mutants extracted through three phase partitioning. *Food Chemistry Journal*. 128 (4): 1066-1071.
 35. Wu, Y.Y., Li, W., Xu, Y., Jin, E.H. and Y.Y. Tu. 2011. Evaluation of the antioxidant effects of four main the aflavin derivatives through chemiluminescence and DNA damage analyses. *Journal of Zhejiang University Science B*. 12:744–751.
 36. Yamagishi, S. and T. Matsui. 2011. Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy-Friend or foe? *Pharmacological Research Journal*. 64:187–194.