The E ffect of Aqueous *Albizia* Flowers Extract *Albizia lebbeck* on Production of Certain Enzymes from *Salmonella typhi*

تأثير المستخلص المائي لأزهار *Albizia lebbeck* على انتاج انزيمات معينة من *Salmonella typhi*

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Abstract

***Salmonella typhi* is Gram-negative and pathogenic bacteria, its produces variety of enzymes for growth. The present study aimed to study the effect of aqueous *albizia* flower extract 10 % on production of three enzymes from *Salmonella typhi*, and the effect of this extract on their activity. The production of amylase and protease enzymes from *Salmonella typhi*, were increased after addition of crude plant extract 10% into media with specific activity 141.3 and 72.6 U/mg respectively, while production of cellulase was decreased after addition, with specific activity 31 U/mg, compare with specific activity before addition of crude plant extract. Aqueous *Albizia* flowers extract 10% increases specific activity of amylase and protease, where they gave 16 and73.8 U/mg respectively after incubation with plant extract for 30 minutes, while specific activity 6.2 U/mg for cellulase was decreased after this incubation, compare with specific activity before incubation with plant extract. The phytochemicals screening of aqueous *Albizia* flower extract were positive for alkaloids, steroids, glycosides, flavonoids, tannins, phenols, and saponins, while were negative for terpens, resins, coumarines and anthraquinones.**

Key words: ***Salmonella typhi*, amylase, protease, cellulase and *Albizia lebbeck* flowers**

الملخص

***Salmonella typhi* بكتريا سالبة لصبغة غرام مرضية تنتج العديد من الانزيمات الضرورية للنمو. هدفت الدراسة الحالية الى دراسة تأثير المستخلص المائي لأزهار نبات الالبيزيا على انتاج ثلاث انزيمات من بكتريا *Salmonella typhi*، وتأثيره على فعالية تلك الانزيمات. بينت النتائج زيادة في انتاج انزيم الامليزوالبروتييز بفعالية نوعية 72.6 و 141.3 وحدة/ملغرام على التوالي عند اضافة مستخلص المائي الخام 10% لأزهار نبات الالبيزا بينما انخفض انتاج انزيم السلوليز مع فعالية نوعية31 وحدة/ملغرام بالمقارنة مع الفعالية النوعية للأنزيم قبل اضافة المستخلص. لوحظ عند حضن الانزيمات الثلاث مع المستخلص المائي الخام لأزهارالالبيزيا 10% لمدة 30 دقيقة زيادة في الفعالية النوعية لأنزيم الامليز و البروتييز بمقدار 16 و 73,8وحدة/ملغرام على التوالي في حين انخفضت الفعالية النوعية لأنزيم السلوليز بمقدار 6.2 وحدة/ملغرام. اظهرت نتائج الكشف عن وجود مركبات الايض الثانوي باستخدام الكشوفات الكيميائية في المستخلص المائي الخام للأزهار نتيجة موجبة مع كشف القلويدات, الستيرويدات, الكلايكوسيدات، الفلافونات، التانينات، الفينولات و الصابونيات, بينما اعطت نتيجة سالبة مع كشف التيربينس, الكومارين والانثروكواينون.**

الكلمات المفتاحية: بكتريا الـ *Salmonella typhi*، انزيم الامليز، انزيم البروتييز، انزيم السلوليز، ازهار نبات اللبخ

Introduction

Salmonella is an important pathogen that causes a variety of illnesses, ranging from localized gastroenteritis to severe and life-threatening typhoid fever [1]. *Salmonella typhi* was expressed a wide variety of enzymes which are necessary for growth, infection and the pathogenicity such as amylase, protease, L-4-aminoarabinose transferase, etc. [2]. Amylases are a class of enzymes that are capable of digesting glycosidic linkages in starch components and glycogen molecules, these enzymes refer to glycoside hydrolase enzymes group which have enzyme commission number (EC:3.2.1) [3].They are widely distributed in plant, microbial and animal kingdoms, which show varying in action patterns depending on the source. Proteases are degradative enzymes which catalyze the total hydrolysis of proteins. Proteases represent one of the three largest groups of industrial enzymes and find application in detergent, leather industry, food industry, pharmaceutical industry and bioremediation processes [4]. Proteases execute a large variety of functions and have important biotechnological applications [5]. Cellulases are the enzymes that hydrolyze ß-1,4 linkages in cellulose chains. They are produced by fungi, bacteria, protozoans, plants, and animals [6]. The catalytic modules of cellulases have been classified into numerous families based on their amino acid sequences and crystal structures [7,8].

*Albizia lebbeck*is widely spread in the world, and its tree has large leaves and fragrant cluster of green-yellow flowers and long seed pods. Belonging to the family of Leguminosae, it is native to tropical Asia and widely

cultivated and naturalized in other tropical and subtropical regions including Malawi [9]. The flowers, bark, fruits, roots, and stems of *Albizia lebbeck* are all used for medicine [10]. A paste of leaves is used to treat skin problems [11]. *Albizia lebbeck*is also known for treating respiratory problems including allergies [12]. Furthermore, other parts of the plants are used to treat eye problem, purify blood, and promote health in teeth. Most importantly, ethanol extract from its pods is effective against some form of cancer [13].

The aim of this search was to study the effect of aqueous 9*6*Albisia* flowers extract on production of amylase, protease and cellulase from *Salmonella typhi* and effect of this extract on enzymes activity.

Materials and Methods

Media and chemicals

Nutrient agar, Muller-Hinton broth (MHA), brain heart infusion agar, and other media were obtained from Himedias (India), Coomassie brilliant blue, bovine serum albumin (BSA) and other chemicals were supplied by BDH Chemicals.

Collection and extraction of plant samples

Flowers of *A. lebbeck* were collected from the gardens of College of Science, Baghdad University. Plant was identified in Baghdad University Herbarium, College of Science. The Flowers of this plant were cleaned from dust, then left to dry at room temperature for three days. The samples were grounded into powder by electrical grinder, and the powder was kept at 4° C until use [14]. Coarse powders 25g were homogenized in 250 ml of tap water and boiling to 30 minutes. The crude extract was filtered from two layers and centrifuged at 6000 rpm for 15 min., and the suspension was used as crude extraction [15].

Preliminary phytochemical screening

The preliminary phytochemicals tests were carried out for all the extract as per standard methods.

-Detection of alkaloids

a- Mayer's test

Few drops of the freshly prepared Mayer's reagent were added to 5 ml of the sample, a white precipitate will appear if alkaloids were present [16].

b- Wagner's test

Few drops of freshly prepared Wagner's reagent were added to 1 ml of the sample, a brown precipitate will appear if alkaloids were present [17].

- Detection of glycosides

Ked's test

Few drops of Ked's reagent were added to 5 ml of the sample. The presence of glycosides was indicated by the formation of violet ring color [18].

- Detection of flavonoids

This test was accomplished by adding 4 ml of 95% ethanol to 1 ml of the plant extract sample and then placed in boiling water bath for 25-30 minute. Then few drops of potassium hydroxide 0.5N were added to 5 ml of the sample. The presence of flavonoids was indicated by the formation of dark yellow color [18].

- Detection of tannins

Two grams of crude plant extract was added to 50 ml of distilled water and boiled then left off to cool then few drops of lead acetate 1% were added to the 1 ml of the sample, appearance of white gel precipitate indicating tannins were present [18].

- Detection of saponins

Five milliliters of crude plant extract was added to 3 ml of mercuric chloride 1% solution, formation of white precipitate indicating presence of saponins [17].

- Detection of phenols

The phenol group in the molecule of phenol compounds can be determined by mixing equal volumes of 1% ferric chloride solution and crude plant extract. The appearances of blue-green color indicated the presence of phenols [19].

- Detection of terpens and Steroid

Four milliliters of acetic acid anhydride and 1 ml of concentrated sulfuric acid (H_2SO_4) then mixed with 1 ml of crude plant extract. The appearance of pink color indicated the presence of terpens while formation of blue color after leaving the sample for 1 minute indicated the presence of Steroids [18].

- Detection of resins

Ten milliliters of crude plant extract were added to 50 ml ethanol 95%, and after that the mixture was left in a water bath for two minutes then filtered and added to 100 ml of acidic distilled water with hydrochloric acid, formation of turbidity indicates to positive test [18].

- Detection of coumarines

One gram of dried plant extract was dissolved in some drops of alcohol in a test-tube then covered with filter paper, sprayed with NaOH and then placed in a water bath until boiling, then the filter paper was placed under UV light spectrum. The appearance of greenish-yellow color indicated the presence of coumarines [19].

-Detection of Anthraquinones

a-Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink color indicates the presence anthrax quinones [18].

Microorganism and culture conditions

Salmonella typhi isolate from University of Baghdad/ College of Science/ Biotechnology Department (isolated from infected person), was cultured on nutrient agar and incubated at 37 C° for 24 hours. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB), then the bacteria was incubated for 24 hrs at 37C° . The cultures were diluted with fresh Muller-Hinton broth to achieve 10^6 cells/ml.

Production of enzymes

Salmonella typhi isolate was cultured in 250 ml flask containing Muller-Hinton broth (pH 7.0) supported with cellulose, casein and starch separately, and then incubated for 24 hrs at 37C° . The broth culture was centrifuged at 6000 rpm for 30 minutes. The activity, protein concentration and specific activity were estimated for amylase, cellulose and protease respectively.

Determination of amylase and cellulose activity

Determination of enzyme activity of crude enzyme is using miller method [20] by DNSA reagent (substrate for amylase activity was starch and cellulose for cellulose activity). One enzyme activity unit was defined as the amount of enzyme releasing 1 µg of reducing sugar as glucose per gram of dry substrate per minute, under standard assay conditions [21].

Determination of protease activity

Protease activity was determined according to the methods described by [22]. Proteases activity is expressed as µmol of free amino acids equivalent per min/ml of the culture filtrate.

Determination of protein concentration

Determination of proteins concentration of all enzymes were determined according to the methods described by Bradford method [23] using Coomassie Brilliant Blue G-250, and Bovine serum albumin as standard. Determination of specific activity is calculated by dividing U/ml (activity) by the protein concentration in mg/ml to get U/mg.

Effect of *Albizia* flowers extract on enzymes production

Salmonella typhi isolate was cultured in 250 ml flask containing Muller-Hinton broth (pH 7.0) supported with cellulose, casein and starch separately, and 10% from crude plant extract was added to each flask, and then incubated for 24 hrs. at 37C° . The broth culture was centrifuged at 6000 rpm for 30 minutes. The activity, proteins concentration and specific activity were estimated for amylase, cellulose and protease respectively.

Effect of *Albizia* flowers extract on enzymes activity

Salmonella typhi isolate was cultured in 250 ml flask containing Muller-Hinton broth (pH 7.0) supported with cellulose, casein and starch separately, and then incubated for 24 hrs. at 37C° . The broth culture was centrifuged at 6000 rpm for 30 minutes, and supernatant was used as crud enzyme. The specific activity was estimated for each enzyme. Two ml of each crud enzyme separately, was mixed with 2ml of *Albizia* flowers extract previously prepared, in test tubes and incubated in water bath at 50 C° for 30 min, then cooled directly in cold water bath and the specific activity was estimated for each treatment.

Results and discussions

Preliminary phytochemical screening

The crude plant extract was subjected to preliminary phytochemical test to determine the presence of functional groups present. The results of these experiments are shown in Table (1).

Table (1): Qualitative phytochemical analysis flower extracts of *Albizia lebbeck*.

Phytochemicals	Result
Alkaloids: mayer's test, wagner's test	+, +
Glycosides	+
Flavonoids	+
Tannins	+
Saponins	+
Phenols	+
Terpens	_
Steroids	+
Resins	_
Coumarines	_
Anthraquinones	_

(+) = Positive result, (-) = Negative result

The results of the phytochemical screening of the aqueous extract of *Albizia lebbeck* flower revealed the presence of alkaloids, steroids, glycosides, flavonoids, tannins, phenols, and saponins while the terpens**,** resins, coumarines and anthraquinones, gave negative result. Padamanabhan *et.al* [24], found that hydroalcoholic extract of *Albizia lebbeck* flower revealed the presence of steroids, Terpenoids, and Saponins while the hydroalcoholic extract of *Albizia lebbeck* pod revealed the presence of alkaloids, flavonoids, phenols, and saponins.

Effect of *Albizia* flowers extract on enzymes production

The effect of the plant extract on production of certain enzymes from *Salmonella typhi*, were shown in Table (2). The results in this table shows that production of amylase and protease enzymes from *Salmonella typhi*, gave specific activity 141.3 and 72.6 U/mg respectively. The addition of crude plant extract into media increased the specific activity of these enzymes, compare with specific activity before agitation agent *Albizia* flowers extract.While production of cellulase from this bacterium decreased to give specific activity 31 U/mg.

Table (2): Effect of *Albizia* flowers extract on amylase, protease and cellulase production from *Salmonella typhi*.

Enzymes	Specific activity (U/mg) before addition of crude plant extract	ecific activity (U/mg) after additi of crude plant extract
Amylase	14.4	141.3
Protease	55.8	72.6
Cellulase	58.9	31

The amount of carbon and protein source in culture mediais important for the growth and production of extracellular amylase and protease in bacteria. The higher yields of amylase and protease can be obtained in media with complex raw material containing starch and protein from *Albizia lebbeck* flower extract, because its carbohydrate-protein-rich foods. Also there are significant positive changes in the biochemical and physiological parameter related to protein, carbohydrates and lipid metabolism for bacteria with *albizia* flower extract [25]. The medicinal properties of the plants could be attributed to the presence of one or more of the detected plant secondary metabolism. Aqueous extract of *Albizia lebbeck* flower contains alkaloids, glycosides, flavonoids, steroids, tannins, phenols, and saponins which has antioxidant properties [26]. Ander and Eriksson [27], found that phenolic compounds may greatly affect the synthesis of celluloses and xylanases in mycelial fungi and bacteria. In fungi and bacteria the production of cellulases may also be regulated by other factors than induction and repression by sugars. Several lignin-related phenolic compounds, which are found in association with cellulose in nature, stimulate or inhibit cellulose production. The capacity of fungi and bacteria to produce high levels of hydrolases is of importance in supplying the growing cultures with a carbon and protein source essential for their biosynthetic activity [28].

Effect of *Albizia* flowers extract on enzymes activity:

Incubation of crud enzymes with *Albizia* flowers extract for 30 minutes gave the following results73.8 U/mg for protease and 16 U/mg for amylase, while before incubation gave 14.4 U/mg for amylase and 55.8 U/mg for protease Figure (3). The specific activity 58.9 U/mg for cellulose was decreased after this incubation to give 6.2 U/mg.

Table (3): Effect of *Albizia* flowers extract on activity of amylase, protease and cellulose production from *Salmonella typhi*.

Enzymes	Specific activity (U/mg) before incubation with crude plant extract	Specific activity (U/mg) after incubation with crude plant extract
Amylase	14.4	16
Protease	55.8	73.8
Cellulase	58.9	6.2

Malla, *et. al.* [29], found that *Albizia lebbeck* recorded the high mineral content, such as sodium, calcium, magnesium, iron, zinc and copper. Essential trace minerals such as Zinc (Zn), Copper (Cu) and Iron (Fe) are known to play important role in the maintenance of redox homeostasis. Amylases are mostly metalloenzymes and require calcium and manganese ions for activity, structural integrity and stability [30]. Calcium enhances amylase activity by its interaction with negatively charged amino acid residues such as aspartic and glutamic acids [31, 35]. Regulation of protease activity depends on the nature of ion. Protease enzyme activated by Ca^{+2} and Fe^{+2}. The Ca^{+2} ions stimulated the protease activity indicating calcium ion involvement in stabilization of the molecular structure of enzyme. In fact, calcium ions are known to be inducer and stabilizer of many enzymes and protect them from conformational changes. Such type of metal dependent variation in proteases activity has also been reported with serine proteases [32].Varadi [33], found that phenolic compounds may greatly affect the activity of celluloses in fungi and bacteria. The inhibition of cellulolytic enzymes is broad-based. Causes include substrate and product inhibition, mass transfer resistance, particle size effects, and non-productive enzyme binding with lignin. In addition phenolic molecules produced by plants also inhibit of some enzymes hydrolysis. Inhibitors from plants protect themselves against pathogens that utilize cellulose to gain entry into plant cells. Inhibitors for cellulase are phenolics and oligosaccharides which released during hydrolysis of lignocelluloses [34].

Conclusions

1- The phytochemicals screening of aqueous *albizia* flower extract were positive for alkaloids, steroids, glycosides, flavonoids, tannins, phenols, and saponins, while were negative for terpens, resins, coumarines and anthraquinones.

2- The production of amylase and protease enzymes from *Salmonella typhi*, were increased after addition of aqueous *Albizia* flowers extract10% into media.

3- The production of cellulase from *Salmonella typhi* was decreased after addition of aqueous *Albizia* flowers extract 10% into media.

4- Aqueous *Albizia* flowers extract 10 % increases specific activity of amylase and protease, where they gave 73.8 and 16 U/mg respectively after incubation with plant extract for 30 minutes, while specific activity 6.2 U/mg for cellulose was decreased after this incubation, compare with specific activity before incubation with plant extract.

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