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Study the Effect of Some Methanolic and Aqueous Traditional Plants Extracts on Probiotic Bacteria

Sura A. Al-ani, Ayyad W. Al-Shahwany*

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Probiotics mean live microorganisms that have beneficial effects on their host's health. The purpose of this study was to compare aqueous, methanolic of some traditional plant extracts on the viability of dietary probiotic supplementation [a dietary probiotic (Protexin)]. Also, this study was conducted to evaluate the antibacterial effect of various extracts concentrations from 1.25 to 100 mg/ml of *Rosmarinus officinal*, *Glycyrrhiza glabra*, *Hibiscus sabdriffo*, *Curcuma longa*, *Citrus auratifolia* swingle, *Cinnamomum zeylancium*, *Urtica dioica*, *Thymus vulgaris*, *Punica grantum* and *Zingiber officinal* on activities of probiotic bacteria *Lactobacillus*, *Bifidobacterium*, *Streptococcus thermophilus* by determining the minimum inhibitory concentrations (MICs). Complete Randomized Design (CRD) was used as experimental design. Means were compared according to L.S.D. values at 5% significant level. The result appeared that methanolic extract gave high significant effect on bacteria compared with aquatic extracts which may be due to the highly acidity. Depending on phytochemical screening, the highest concentration of the total identified phenols and Flavonoids were (35.63 and 13.53 %) for *R. officinus* methanolic extracts. Based on the results of this study *R. officinus* and *C. auratifolia* methanolic extracts had the lowest MIC 6.25 mg/ml against probiotic bacteria than the other tested extracts. Taken together, evidence gathered in the present study indicates that *Z. Officinale*, *G. glabra*, *T. vulgaris* and *U. dioica* aquatics extracts can be more safely in treatment of digestive diseases. More study most conducted to evaluate the pH for the traditional plants extracts to guarantee their useful uses in treatment of digestive diseases.

Keywords: Methanolic, Traditional plants, Probiotic, pH.

دراسة تأثير المستخلصات الميثانولية والمائية لبعض الاعشاب الطبية على بكتريا المعززات الحياتية

سرى احمد معروف العاني، اياد وجيه رؤوف*

قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تعتبر بكتريا المعززات الحياتية Probiotics من الاحياء المجهرية الرئيسية المفيدة في جسم الانسان. هدفت هذه الدراسة الى للمقارنة بين تأثير المستخلصات المائية وميثانولية لبعض الاعشاب الطبية على حيوية بكتريا الـ Probiotics الموجودة في المكمل الغذائي Protexin. كما هدفت الدراسة الى قياس الفعالية التثبيطة للمستخلصات بتراكيز من 1.25 الى 100 ملغم /مل لنباتات الكليل الجبل *Rosmarinus officinal* و عرق السوس *Glycyrrhiza glabra* والكجرات *Hibiscus sabdriffo* والكرم *Curcuma longa* والمنومي بصرة *Citrus auratifolia* swingle و كمون *Cinnamomum zeylancium* و القريص *Urtica dioica* و الزعتر *Thymus vulgaris* و الرمان *Punica grantum* والزنجيل *Zingiber officinal* ضد بكتريا *Lactobacillus* و *Bifidobacterium* و *Streptococcus thermophilus* من

*Email: a61_bio@yahoo.com

خلال قياس اقل تركيز مثبتي تثبيطي (MIC). استعمل تصميم القطاعات الكاملة المعشاة كتجربة عامليه بثلاثة مكررات وتم اختبار أقل فرق معنوي LSD للمقارنة بين المتوسطات عند مستوى معنوية 5%. اظهرت النتائج وجود تاثيرات تثبيطية كبيرة للمستخلصات ميثانولية بمقارنة بالمستخلصات المائية للنباتات اعلاه ، وربما يعود السبب للحموضة المرتفعة (اكثر حامضية) للمستخلصات الكحولية قياسا بمستخلصات المائية. وبينت نتائج التحليل الكيميائية للمستخلصات تفوق تراكيز الفينولات والفلافونيات في مستخلصات الكحولي لنبات اكليل الجبل والتي بلغت 35.63 و 13.53% ، على التوالي. كما واطهرت نتائج هذه الدراسة ان اقل تركيز تثبيطي كان لمستخلصات الكحوليه لنبات اكليل الجبل ونبات النومي بصرة، اذ بلغ 6.25 ملغم /مل لكل منها. وبالاعتماد على نتائج هذه الدراسة والتي اوضحت مقاومة بكتريا المعزيزات الحيوية لتاثير المستخلصات المائية لنبات عرق السوس والزنجبيل والزعر والقرص مما يمكن اعتمادها في علاجات امراض الجهاز الهضمي . واخيرا توصي هذه الدراسة الى اجراء المزيد من البحوث لدراسة تاثيرات مستخلصات النباتات الطبية على فعالية المعزيزات الحيوية قبل استخدامها في علاج امراض الجهاز الهضمي.

INTRODUCTION

The ‘probiotics’ was first used by Lilly and Stillwell [1] to elect unidentified growth promoting substances formed by a ciliate protozoan that stimulated the growth of another ciliate. The term today covers a much broader group of organisms. Fuller [2] was dangerous of the presence of the word ‘substances’ and redefined probiotics as “a live microbial feed supplement which helpfully affects the host by refining its intestinal microbial balance”. The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Working Group definite probiotics as “live micro-organisms, which when administered in adequate amounts converse a health benefit on the host” [3].

This description is widely accepted and adopted by the International Scientific Association for Probiotics [4]. Probiotics may increase the inhabitants of useful micro-organisms as well as *lactobacilli* and *bifidobacteria* which then inhibit growth of damaging micro-organisms by creating inhibiting substances (bacteriocins and/or organic acids) and by viable exclusion [5].

Balance-Protexin a dietary probiotic supplementation [a dietary probiotic (Protexin)], a probiotic capsule produced by Probiotics International(Ltd., Lopen Head, UK). Each probiotic included several kinds of probiotic bacteria include: *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Streptococcus thermophiles*, by 2×10^8 CFU. The Protexin was used in this study as some sores for probiotic bacteria [6].

Traditional plants as a basis of medicinal mixes have sustained to play a main role in the care of human health since early times. Affording to the World Health Organization, Traditional plants extracts are used as folk medicine in therapies of 80% of the world’s inhabitants. Over 50% of all current clinical drugs are of natural product source [7]. Traditional plants refer to the plants which are used for medicinal purposes. As herb prates includes seeds, roots, leaves, bark, flowers and stem used for treatment purpose in early times. In this study evaluated effect of aqueous and methanolic extracts of (*Rosmarinus officinal*, *Glycyrrhiza glabra*, *Hibiscus sabdriffo*, *Curcuma longa*, *Citrus auratifolia swingle*, *Punica granatum*, *Urtica dioica*, *Thymus vulgaris*, *Zingiber officinales* and *Cinnamomum zeylancium* on probiotic bacteria. These plants extract have biological activity rang as from antibiotic to anticancer. This because of the natural products, which are using in treating and preventing medicinal problem [8].

Many results were obtained the effects of plant extracts ironic in polyphenolic composites on the growth of probiotic bacteria and other microorganisms. It was evidenced that plant extracts can stop the growth of food related pathogens and microorganisms esponsible for food treating, as well as intestinal microflora, both pathogenic and physiological [9,10]. Some researchers indicate that polyphenolics compounds from different plant extracts may have, also, negative effect on bacteria which are desirable for human health. The aims of this study were to evaluated the aqueous and methanolic of some traditional plants extracts on the viability of probiotic bacterium.

Material and Method

Collection of plant samples

Ten plants used in this study were collected from local herbarium market in Baghdad city, Iraq. The plants identified in herbarium, Department of Biology - College of Science - University of Baghdad. The plants parts dried and grinded in to powder form by mechanical grinder, it saved at 4°C until extra investigations.

Extract preparation

a. Aqueous extraction

From ten powdered plant 10 grams were taken and infused in 250 ml of distilled water. The samples transferred to water bath in 70°C for 4-5 hour until complete exhaustion. Then cooled and filtered many times through muslin cloths, then it sterilized by Millipore filter 0.22mm.

b. Methanol extraction

Alcoholic extract was made by taken 20 grams of each ten powdered plants and extracted with 200 ml of methanol, using a Soxhlet extractor for 6 hours [11]. In order to evaporate its methanol that dried in 40°C in oven for 3-4 days until it tainted in to powder and saved in refrigerator. The extract yields were weighted, stored in a small bottle in fridge at 5°C and the yield percentages were designed using the following formula:

Extract yield percentages = $R / S \times 100$

(R; weight of extracted plants residues and S; weight of plant raw sample) [12].

Preparation of different concentrations of plant extracts:

Stock solutions were prepared by mixing 2 g from the dried extract with 20 ml ethylene glycol, and then it was sterilized with Millipore membrane filter (0.22 μm). Then many concentrations of (10, 5, 1) mg. ml^{-1} were set by mixing known volume from the stock solution with Ethylene glycol using the following equation: $C_1V_1 = C_2V_2$.

C_1 = Concentration of stock solution .

V_1 = Volume that attained from stock solution.

C_2 = Ending concentration.

V_2 = Final volume .

Ethylene glycol was used as diluent solution.

The minimum inhibitory concentration (MIC)

MIC of plant extracts was minimized by microdilution method in sterile 96 - wells microtiter plates according to the protocol described previously [13]. Many plant extracts concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.625 $\mu\text{g/ml}$) (W/V) were set containing bacterial cells similar to McFarland standard No. 0.5 in an ending volume of 200 μl . Sterile distilled water, broth and plant extracts were using as a negative control, while broth and bacteria used as positive control. After 24 h at 37° C, the MIC of each sample was resolute. The MIC measured the lowest concentration of an antimicrobial that will inhibit the observable growth of a microorganism after 24 h incubation [14].

Compound detection

Determination of total phenols by spectrophotometric [15] method:

The fat free sample was boiled with 50 ml of ether for the extraction of phenol constituent for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, after that 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. This was minimized at 505 nm.

Alkaloid determination using Harborne [16] method:

The 5 g of the sample was balanced into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was extra and covered and allowed to stand for 4 h. This was filtered and the extract was concerted on a water bath to one-quarter of the novel volume. Concerted ammonium hydroxide was supplementary drop wise to the extract until the precipitation was broad. The complete solution was allowable to settle and the precipitated was composed and washed with dilute ammonium hydroxide and then filtered. The rest is the alkaloid, which was dried and weighed.

Tannin determination by Van-Burden and Robinson [17] method:

The 500 mg of the sample was balanced into a 50 ml plastic flask. 50 ml of distilled water was added and shaken for 1 h in a shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M

FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was minimized at 120 nm within 10 min.

Saponin determination

This method was according to Obadoni and Ochuko [18]. The samples were ground and 20 g of each were put into a conical hipflask and 100 cm³ of 20% aqueous ethanol were extra. The samples were heated over a hot water bath for 4 h with continuous moving at 55°C. The mixture was filtered and the rest re-extracted by added 200 ml 20% ethanol. The joint extracts were concerted to 40 ml over water bath at 90°C. The concentrate was moved into a 250 ml separatory funnel and 20 ml of diethyl ether was supplementary and shaken dynamically. The aqueous layer was recovered while the ether layer was wasted. The purification method was repeated. The 60 ml of n-butanol was extra. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The residual solution was boiling in a water bath. After evaporation the samples were dry in the oven to a continual mass and the saponin content was considered as percentage.

Flavonoid determination according to Bohm and Kocipai- Abyazan [19] method.

The 10 g of the plant sample was extracted frequently with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman paper No42 (125mm). The filtrate was later moved into a pot and evaporated into aridness over a water bath and balanced to a constant weight.

Identification of Protexin probiotic bacterial

Test microorganisms

In this trial, bacterial strain was obtaining from pharmaceutical products Protexin-Balance (a mixture of advantageous bacteria). Protexin-Balance provides a complex blend of 7 strains of friendly bacteria and prebiotic. The text was made to be sure that Protexin consist at least three types of probiotic bacteria (*Lactobacillus sp.*, *Bifidobacterium sp.* and *Streptococcus thermophiles*).

Culture media preparation

All the media were prepared according to the manufacturing company; the constituents were dissolved in distilled water (D.W.). sterilized by autoclaving at 121 °C for 15 min at 15 b, there after distributed into sterile tubes or Petri dishes.

Laboratory prepared culture media

A. M17 media

It was made according to Tabasco *et al.*, [20] by adding the following ingredients to 900 ml of distilled water: 5 gm peptone, 5 gm casein, 2.5 gm yeast extract, 5 gm beef extract, 5 gm lactose, 0.5 gm ascorbic acid, 19 gm disodium-β-glycerophosphate and 0.25 gm MgSO₄.7H₂O, pH was adjusted to 6.2 and the volume was completed to 1 liter with distilled water. The medium was dispensed into tubes (10 ml) and autoclaved at 121°C for 15 minutes and used as a culture medium.

B. MRS agar L-cysteine HCl

Used for *Bifidobacterium sp.* isolation, 0.05% L-cysteine. HCl was extra to MRS medium as a reducing agent to provide sterner anaerobic situations needed to stimulate *Bifidobacterial* growth, and then incubated at 35-37°C for 48 hours [21].

Activation of bacteria

A. *Lactobacillus sp.*

Bacterial cultures were stimulated in tubes 5 mL of MRS broth and then incubated at 35-37°C for 24 hours below an aerobic situation until the inoculum turbidity equivalent to 0.5 McFarland standard.

B. *Bifidobacterium sp.*

Bacterial cultures were activated in test tubes containing 5 mL of MRS broth L-cysteine HCL and then incubated at 37°C for 24-48 hours below some aerobic situations until the inoculum turbidity equivalent to 0.5 McFarland standard.

C. *Streptococcus thermophiles*

Bacterial cultures were stimulated in test tubes 5 mL of M17 broth and then incubated at 37 °C for 24 hours below aerobics conditions until the inoculum turbidity equivalent to 0.5 McFarland standard

Morphological identification

A. Microscopical characteristics

Probiotic bacteria were Gram stained and examined under high magnification (100X lens) by light microscope.

B. Colonial characteristics

The colonies of *Lactobacillus* on MRS agar media. Bifidobacterium on L-cysteine agar media and *streptococcus thermophiles* on M17 agar media were tested by color, appearance and size of colony [22]

Antibiotic susceptibility test

The antibiotic susceptibility test of isolates was assessed using antibiotic discs diffusion method. the broth cultures of LAB were prepared using MRS and adjacent to 0.5 McFarland standard. A sterile cotton swab was dipped into the bacterial suspension. The surface of an MRS agar plate was inoculated by bacterial separate as follows: the whole surface of the plate was streaked with the swab, then the plate was rotated through a 45° angle and streaked the whole surface again; finally, the plate was rotated another 90° and streaked once more. Ten minutes later, by some sterile forceps the antimicrobial disc was picked up and placed on the surface of inoculated plate. The disc was pressed gently in to full contact with the agar. The plates were incubated anaerobically at 37 °C for 24-48hours. After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) around the antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc [23].

Statistical Analysis

The Statistical Analysis System- SSPS (20) was used to study the effect of different factors in the study parameters. The significant differences between the means in this study were determined by using Least Significant Difference (LSD) test. Results were considered significant when p values were less than 0.05.

Result and Discussion

Antibiotic Susceptibility of probiotic bacteria

Susceptibility tests were summarized in Table-1 using 8 different antibiotics by disc diffusion method recommended by CLSI (2013) guidelines. These antibiotics included Ampicillin, Cefotaxime, Azithromycine, Ciprofloxacin, Vancomycine, Erythromycin, Gentamycine and Nitrofurantoin. These antibiotics included Ampicillin, Cefotaxime, Ciprofloxacin, Erythromycin, and Nitrofurantoin were used because of their mode of act inhibiting cell wall synthesis which basis the release of the bacterial cell DNA into the surrounds, While Azithromycin, Gentamicin and Vancomycin were used due to their action in the inhibition of protein synthesis in bacteria [24].

Table 1-Diameter interpretive standards of inhibition zones for probiotic bacteria according to CLSI (2013)

Bacterial	Azithromycin	Ciprofloxacin	Vancomycin	Erythromycin	Gentamicin	Nitrofurantoin	Ampicillin	cefotaxime
Protexin Probiotic bacteria	S	S	R	S	R	S	S	S

R=Resistant, S=Sensitive

The antibiotic resistance associated with probiotics for Vancomycin and Gentamicin may be due to resistance genes which are usually present in plasmids, transposons and integrons of bacteria and can handover from one bacterium to another (intra- or inter-species) by machines of horizontal gene transmission [25,26]. Although *Lactobacillus* spp. are measured one of the harmless bacteria used as probiotics, many species of these bacteria harbour one or more antibiotic resistance genes [27,28] However, approximately of the foodborne species of *Lactobacillus* have antibiotic resistance genes, that are capable of actuality moved horizontally to pathogenic bacteria and linked with moveable elements [29].

Examining and isolation Protexin probiotic bacteria

For the purpose of ascertaining the presence of these bacteria in Protexin capsules, the tests were carried out to detect and isolate three main types of bacteria strains (*Lactobacillus* spp., *Bifidobacterium* sp. and *Streptococcus thermophiles*). The preliminary results indicated their presence in the preparation as follows:

A. *Lactobacillus* spp.

MRS medium is the most selective medium for *Lactobacillus* spp., thereafter; plates were incubated at 37°C in anaerobic jar for 24-48 hour. After incubation period, *Lactobacillus* colonies were observed as smooth convex, Creamy gray, mucoid colonies Figure-1. In addition, *Lactobacillus* colonies appeared to be just below the surface of the medium. Such results coincide with those mentioned by Garg *et al.* [30].



Figure 1- characteristics of *lactobacillus* sp. on MRS agar

Microscopic examination Figure-2 observed that cells were Gram positive, non-motile, non-spore formers bacilli [31,32].



Figure 2- Microscopical characteristics of the *Lactobacillus* sp.

B. *Bifidobacterium* sp.

colony with milky white color or slightly creamy, rounded form [32] Figure-3.



Figure 3- characteristics of *Bifidobacterium* sp. on MRS L-cysteine agar

Microscopic examination observed that cells were non-motile and Gram-positive Figure-4. The cells appeared in Pleomorphic forms. These appeared in the form of long curved bronchial bacilli or short ovoid bacilli. The cells were clustered in irregular shapes, chains, and the characteristic bifurcated form of these bacteria was not observed in any of the microscopic swabs preparation [33].



Figure 4-Microscopical characteristics of the *Bifidobacterium* sp.

C. *Streptococcus thermophilus*

Streptococcus thermophilus colonies on M17 agar platter looked to be creamy white, circular, entire, low convex Figure-5. The cells were circular in form and usually taking place in chains Figure- 6. They were Gram positive, non-motile, and non-spore former [34].



Figure 5-characteristics of *S. thermophilus* on M17 agar



Figure 6-Microscopical characteristics of the *S. thermophiles*

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) value is important to determine efficacy of antibacterial agent. Low MIC value may be due to an indication of high efficiency or that microorganism has no possible to develop resistance to the bioactive composite. Table 2 showed the effects of MIC values of aqueous and methanol plant extracts with different concentrations on probiotic bacteria of potexin capsules. Obviously, minimum and maximum values were in concentrations between 100 to 6.25 mg/ml for probiotic bacteria in Protexin capsules. The results indicated that *Rosmarinus officinal* and *Citrus auratifolia swingle* methanolic extracts where the minimum MIC value 6.12 mg/ml, while probiotic bacteria showed a highly resistant to *Glycyrrhiza glabra*, *Zingiber officinal*, *Thymus vulgaris* and *Urtica dioica* aqueous extracts (Table-2).

Table 2-Minimum inhibitory concentration (MIC) of aqueous and methanol plant extracts were determined by using microtiter plate.

Plant extracts	Protexin probiotic bacteria	
	Aquatic extracts mg/ml	Methanol extracts mg/ml
<i>Cinnamomum zeylancium</i>	50	25
<i>Citrus auratifolia swingle</i>	50	6.25
<i>Curcuma longa</i>	100	25
<i>Glycyrrhiza glabra</i>	R	50
<i>Hibiscus sabdriffo</i>	25	25
<i>Punica grantum</i>	25	12.5
<i>Rosmarinus officinal</i>	50	6.25
<i>Zingiber officinal</i>	R	R
<i>Thymus vulgaris</i>	R	50
<i>Urtica dioica</i>	R	50
Least significance of difference 0.05	2.70	

R= Resistant

Also, the result in Table-3 summarized the plants methanolic extracts MIC against three probiotic bacteria isolates. These bacteria were isolated from Protexin capsule. Based on the results of this study, the highest MIC was at 50 mg/ml, while the lowest was 6.25mg/ml. According to the result, all kinds of probiotic isolation were sensitive to the plants methanolic extracts, except for *Zingiber officinal* extract which probiotic was resistant to their effect Table-3.

Table 3-Minimum inhibitory effect (MIC) of aqueous and methanol plant extracts were determined by using microtiter plate.

Plant methanolic extracts	MIC for Protexin isolates probiotic bacteria (mg/ml)		
	<i>Strep.</i>	<i>Bifi.</i>	<i>Lac.</i>
<i>Rosmarinus officinal</i>	6.25	6.25	6.25
<i>Glycyrrhiza glabra</i>	50	50	50
<i>Urtica dioica</i>	50	50	50
<i>Hibiscus sabdriffo</i>	50	25	25
<i>Curcuma longa</i>	50	25	25
<i>Thymus vulgaris</i>	25	50	50
<i>Punica grantum</i>	12.5	12.5	12.5
<i>Citrus auratifolia swingle</i>	6.25	6.25	6.25
<i>Zingiber officinal</i>	R	R	R
<i>Cinnamomum zeylancium</i>	25	25	25
Least significance of difference 0.05	2.85		

R= Resistant to all tested concentrations

pH values for methanolic and aqueous extracts:

The result in Figure-7 demonstrated the aqueous and methanolic extracts pH values for ten plants under study. The result indicated that the highest pH value was 8.1 for *H. sabdariffa* aqueous extract, while the lowest pH value was 1.7 for the *C. zeylanicum* methanol extract. Besides, all aqueous extracts were more alkaline (higher pH value) than the methanolic extracts.

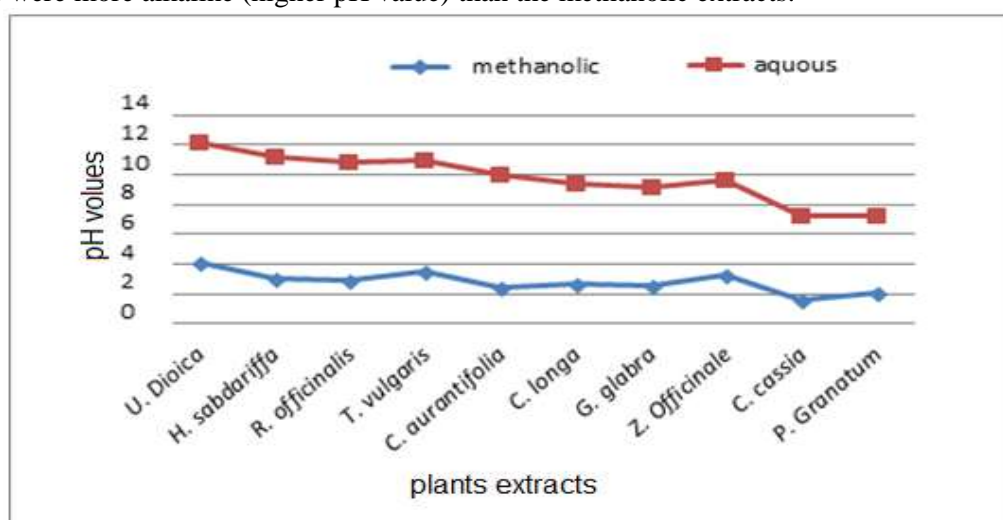


Figure 7-pH values for methanolic and aqueous extracts

Microorganisms vary in their optimum pH requirements for growth. Most bacteria favor conditions with a near neutral pH. The certain bacteria are acid tolerance and will survive at reducing pH value.

Notable acid tolerant bacteria consist of the *Lactobacillus* and *Streptobacillus* species which play a role in fermentation of dairy and vegetable products. The optimum pH range for the growth of *Lactobacillus* sp. is 5.5 to 6.0. Nevertheless, the growth of *L. acidophilus* will stop at pH < 3.6 [35]. Indeed, the optimum pH ranges of *Bifidobacteria* are 6.0 to 7.0. *Bifidobacteria* cannot grow at pH < 4.5 and > 8.5 [35].

According to pH values in Figure-7, the probiotic bacteria were more sensitive to the methanolic extracts at lowest acidity. Usually, all probiotic strains favored higher pH with upmost viability of *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus thermophilus* observed at pH 8.1 [36]. Thus, it is important that more study most conducted to evaluate the pH for the medicinal plants extracts to guarantee their useful uses in treatment of digestive diseases.

Phytochemical screening

Chemical analysis tests for the aqueous and methanol extracts were to identify the medicinally active constituents as described in Table (4, 5). Alkaloids, tannins, saponin, flavonoids and glycosides were detected in all solutions. The results inducted that quantitative estimation of the percentage crude chemical constituents in methanolic extracts solution. The methanolic extracts were contained the highest percentage of the phytochemicals compounds compared with other aqueous extracts solution. The result showed significant different between phenolic, Flavonoids, tannins, saponins and alkaloids means percentage, which were 14.9, 9.97, 20.06, 1.58 and 6.94% for methanolic extracts, and 12.90, 7.06, 14.75, 0.83 and 3.62% for aquatics extracts, respectively. This may be the resin behaves the strong effect of the methanolic extracts on probiotic bacteria. Depending on this result, the highest phytochemicals compounds means of *R. officinus* and *C. cassia* were 22.44 and 20.88 %, caused the highest MIC of probiotic bacteria, especially with methanolic extracts Table (2, 3). A better understanding of highest MIC for *R. officinus* and *C. cassia* methanolic extracts maybe due to the highest phenolic concentration which were 32.61 and 48.22%, respectably. Similarly, according to low concentration in Table-4, probiotic bacteria showed resisting to some aquatic extracts such as *Z. Officinale*, *G. glabra*, *T.vulgaris* and *U. dioica*.

Many studies showed that plants include specific metabolites that are acknowledged to perform a range of purposeful activities. These studies induct that Phenolic and Flavonoid composites were biologically active components, which were the chief managers that can give hydrogen to free radicals and therefore break down the chain reaction of lipid oxidation at the first start step. This high possible of these mixtures were to scavenge radicals may be explained by their phenolic hydroxyl groups [37].

The results gathered agreed with a study conducted by Alasalvar *et al.*[38], whom study the effects of secondary compound (as antioxidants; both pure herbal extracts and antioxidants) was evaluated on cultures of *Lactobacillus casei*, as a sample of probiotic microorganisms.

Table 4-Qualitative analysis of the phytochemicals medicinal plants aquatic extracts

No.	Plant methanol extracts	Phenolic %	Flavonoids %	Tannins %	Saponins %	Alkaloids %	Mean
1	<i>Punica Granatum</i>	16.22	12.41	37.3	0.88	5.8	12.66
2	<i>Zingiber Officinale</i>	7.51	5.82	0.89	0.17	2.8	4.22
3	<i>Hibiscus sabdariffa</i>	1.59	4.76	28	2.8	17.9	5.57
4	<i>Glycyrrhiza glabra</i>	0.72	0.22	4.8	0.6	1.8	1.16
5	<i>Cinnamomum cassia</i>	49.25	25.32	0.81	0.83	2.7	23.94
6	<i>Rosmarinus officinus</i>	33.63	13.53	2.5	3.5	1.5	26.21
7	<i>Cinnamomum cassia</i>	0.92	1.25	41.6	0.001	0.001	1.21
8	<i>Citrus aurantifolia</i>	4.19	3.27	78.9	3.4	25.6	7.67
9	<i>T. vulgaris</i>	23.5	5.9	3.9	1.8	9.3	12.11
10	<i>U. dioica</i>	11.5	27.3	1.9	1.9	2	8.54
	Mean	14.903	9.978	20.06	1.58	6.94	
	L.S.D 0.05	1.62	7.14	0.76	0.29	1.29	

Table 5-Qualitative analysis of the phytochemicals medicinal plants methanolic extracts

No.	Plant aquatics extracts	Phenolic %	Flavonoids %	Tannins %	Saponins %	Alkaloids %	Mean
1	<i>Punica Granatum</i>	15.12	11.2	25.5	0.77	4.9	9.99
2	<i>Zingiber Officinale</i>	6.5	4.81	0.79	0.8	0.6	2.68
3	<i>Hibiscus sabdariffa</i>	0.49	3.36	18	2.8	7	2.87
4	<i>Glycyrrhiza glabra</i>	0.5	2.2	0.7	0.6	0.7	1.3
5	<i>Cinnamomum cassia</i>	48.22	24.3	0.7	0.71	0.6	20.88
6	<i>Rosmarinus officinus</i>	32.61	12.51	2.5	0.8	0.4	22.44
7	<i>Cinnamomum cassia</i>	0.92	0.35	30.6	0.001	0.001	0.67
8	<i>Citrus aurantifolia</i>	3.18	2.29	65.9	0.4	15	4.33
9	<i>T. vulgaris</i>	10.2	3.7	2.1	0.8	5.2	6.93
10	<i>U. dioica</i>	11.3	5.9	0.8	0.7	1.8	3.94
	Mean	12.90	7.06	14.75	0.83	3.62	
	L.S.D 0.05	1.30	6.71	0.54	0.12	0.64	

Medicinal plants play a major role as antimicrobial agents. The drug which derivative from plants have enhanced in recent years which are rich in a varied kinds of secondary metabolites compounds, such as tannins, terpenoids, alkaloids, and flavonoids. These compounds have an antimicrobial property [39]. their results induct that natural secondary compound such as Phenolic and Flavonoids had an inhibitors effect on the growth of *Lactobacillus* [40]. The biologically active components of Phenolic and Flavonoid compounds, are the mean agents that can donate hydrogen to free radicals and so break the chain reaction of lipid oxidation at the first start step. This high effective of these mixtures to scavenge radicals might be clarified by their phenolic hydroxyl groups [37].

The result in this study was agree with Al-Bayati [41], who study the *Mentha longifolia* biological activity and antimicrobial action against many selected pathogenic and non-pathogenic microorganisms; *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa* and the yeast *Candida albicans*. The result showed that menthol (the major secondary compounds in *Mentha longifolia*) concentrations were effective against all tried bacteria except for *P. aeruginosa*.

Taken together, evidence gathered in the present study indicates that *Z. Officinale*, *G. glabra*, *Thymus vulgaris* and *Urtica dioica* aquatics extracts can be more safely in treatment of digestive diseases, scientifically maintained by the results found in this work.

Conclusion

Thus, it can be said that, on the one hand plant additives can enhance the health and nutritional value (Vitamins, Antioxidants), on the other hand can decrease the number of probiotic bacteria significantly. Hence, further studies should be conducted the effect of traditional plant material on probiotic bacteria in probiotic products containing plant material.

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