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Evaluation of Antioxidants, Antibacterial and Antidiabetic Activities of Aqua-alcoholic Marjoram Extract

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Abstract

Plant extracts possess special significance towards human health and vital activities for a wide range of diseases. The current study aims at verifying the presence of active ingredients in *marjoram* leaves extract, and determining antioxidants, antibacterial and antifungal activities, LD50 and its antidiabetic activity. The active ingredients were qualitatively analyzed by specific test methods. Antioxidant, LD50, antibacterial and antifungal activities were measured by their standard method. The antidiabetic activity was determined by assessed hyperglycemia, hyperlipidemia and liver functions by spectrophotometric methods. The results confirmed the presence of several active components including flavonoids, alkaloids, terpenoids, tannins, and saponins. Also, the results of antioxidants activity showed that marjoram leaves extract possesses an antioxidant activity similar to ascorbic acid. LD50 results revealed that the allowed dose is 12.5 mg/ml. Biological activity results indicated that *marjoram* leaves extract possesses antibacterial and antifungal activities against wide range of pathogenic bacteria and fungi, but there is no effect of extract on E. coli and Staphylococcus aureus even at high concentrations. *Marjoram* extract revealed high antidiabetic activity when treated for two weeks 4mg of the extract daily. Also, lipid profile and liver functions, and testosterone levels were improved after treatment in the diabetic group related to the control group. It can be concluded that *marjoram* leaves extract has high antioxidant activity with high biological activity and high antidiabetic activity with a significant enhancement of fertility affected in diabetes and a high level of tolerated doses according to the LD50 value.

Keywords: Antibacterial activity, Antidiabetic activity, Antifungal activity, Antioxidant activity, Marjoram extract.

Introduction

Recently, several studies about plant extracts were conducted that included their properties in nano size, antioxidant [1-4] , their phenolic content , and their cytotoxicity due their bio-activities and safety [5-7], in addition to other studies that concerned with diabetes complications [8-11]. *Marjoram* is an aromatic herbaceous plant and it is from the famous mint group and contains many vitamins and minerals, as well as volatile oils, flavonoids, hydroquinone glycosides, sugars, and triterpenes. These ingredients give *Marjoram* important health properties such as antimicrobial, anti-inflammatory, analgesic, antidepressant, and antiviral. *Marjoram* has been employed for the treatment of a wide range of diseases as a traditional medicine including disorders of the heart, lungs, gastric and nerves [12].

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Plant extracts represent the major source of the antioxidants compounds due to the presence of the active compounds like phenolic acids and flavonoids [13]. These types of phytochemical have the ability to scavenger free radicals and protect cells from damage by reducing oxidative stress [14]. More recently, natural products are choosing for synthetic additives. Generally, plant extracts are used as an important source of phytochemicals that possess multi-biological activities including antioxidants, antiviral and antimicrobial activities [15]. Oxidative stress considers one of the main reasons for the initiation and progress of diabetes mellitus, heart and cancer diseases due to generate reactive oxygen and nitrogen species, which represent unstable molecules occurred endogenously through aerobic metabolic processes [16].

Carotenoids and tocopherol consider natural antioxidants that obtained from plant extracts, in addition to other important secondary metabolites like kaempferol and quercetin that were found to be as excellent antioxidants [17]. More recently, medicinal plant extracts showed a high rank of antioxidants activity toward degenerative disease that reached to 75% in terms of the inhibition percentage of oxidants. Furthermore, it was found that no significant effect of the extraction solvents on the obtained plant extract yield [18.] In another recent study, it was reported that that the extracts of medicinal plants act as vital antioxidants in treatment of wide range of degenerative diseases and inhibition oxidants to avoid the oxidative stress [19].

One of the major public health problems is the acquisition of bacterial resistance to traditional antibiotics, which is required a continuous search for new antibiotics that work properly and safety as effective antibiotics [20]. Due to this problem, plant extracts have received great attention because they contain biologically active and non-toxic components to study the extent of their effect on bacterial and fungal cells [21].

It was reported that vegetal sources are used in development in many drugs, because plant extracts possess important properties like anti-inflammatory, antibacterial and antioxidant effects. These properties can be employed in development of new drugs with no side effects [22]. More recently, several plant extract tests showed high action against selected pathogenic bacteria. Antibacterial activities can be attributed to presence bioactive compounds that support the antibacterial activity [23]. Antibacterial activities were evaluating in plant extracts. It was found that these activities are associated with certain phytochemical components. These components include tannins, saponins, flavonoids, alkaloids. They are support the activities against bacteria, diabetes, oxidants and cancer [24, 25].

Diabetes is considered one of the diseases that is a major challenge to public health, and it represents a group of metabolic disorders that are characterized by hyperglycemia, beta-cell impairment in insulin secretion, insulin resistance, or both. Moreover, diabetic complications could be associated with variation in the body's antioxidant defense system, oxidative stress, and dyslipidemia [26]. Studies reported that plant extracts that contain the active components like isoflavones possess hypoglycemic activity. Furthermore, it was found that the presence of alkaloids, phenolic compounds, flavonoids, and terpenoids leads to lowering the glucose level in diabetic patients [27].

More recently, antidiabetic activity of plant water crude extracts has been investigated. The study referred to that glucose level was decreased regularly with increase treatment time and the rate of glucose decreasing was increased with increasing of the applied dose [28]. Antidiabetic activity was detected in another plant extracts with different extraction solvents. The study confirmed that the plant extract of honeybush tea displayed antidiabetic and antioxidant activities. Furthermore, the study reported that this type of plant extracts considers a promising therapeutic agent in avoidance and controlling on diabetes and its complications [29]. The extract of *Chromolaena odorata* was used to treat diabetes and several infection diseases. The results of this study support the ability of this type of plant extract to be as promising antidiabetic agent and some infection disease [30].

The aim of this study is to determine the bio activities of marjoram extracts toward diabetes in terms of certain relevant biochemical factors including glucose, insulin, liver functions, lipid profile and testosterone via treatment diabetic mice group related to control group and its activities toward pathogenic bacteria and oxidants.

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Materials and Methods

Extraction of Marjoram Leaves

Powder of dried leaves of marjoram (10 gm) was extracted by 70% methanol to obtain the aqua methanolic extract depending on the ratio 1:10 (weight: volume) at 60-80 Cº for 6-8 hrs. The process was conducted by soxhlet. The result of the extraction process was filtered by a filter paper type Whatman No. 1, then the filtrate dried at 40 Cº and stored at -21 Cº, then qualitative tests for alkaloids, terpenes, and flavonoids were conducted depending on Mujeeb F, et al [31]. Also, the antioxidant activity of the studied extract was conducted depending on the DPPH method which was reported elsewhere [32]. The antibacterial activity test was achieved by a plate agar method [33]. The toxicity of marjoram extract was studied by applying the LD50 procedure [34]. The antidiabetic activity was assessed in terms of many biochemical factors including glucose, insulin, liver functions, and lipid profile in alloxan-diabetic mice. The mentioned biochemical parameters were evaluated by their suitable kits by spectrophotometric methods. Fertility improvement in the treatment mice group was evaluated in terms of testosterone level in both diabetic and control groups.

Antidiabetic Activity

The antidiabetic activity was estimated depending on certain animal model design. The model includes diabetes induction by alloxan in a group of mice and then the diabetic mice were treated with marjoram extract with a certain dose and period related to positive and negative control groups, as reported below.

Animals Care

Thirty-two healthy mice were employed in the current study. The animals were purchased from the Center of Biotechnology, University of Al-Nahrain. The age of animals is three months and the weight of each mouse is about 25 grams. Cages of mice were cleaned with soap and tap water together with 70% ethanol for sterilization. Furthermore, mice have been exposed to light at a room temperature, 25°C for 12 hrs.

Diabetes Induction

Diabetes was induced in mice by injecting (IP) with alloxan monohydrate after mixed with saline in a ratio 5% w/v and volume 0.1 ml and at dose (150mg/kg BW). The diabetic condition was formed after 48 hrs., of injection with alloxan and was confirmed by weight loss and hyperglycemia.

Mice groups

In the case of the anti-diabetic effect by *Marjoram* extract in the current study, thirty-two mice were employed to achieve this part of this study. The animals were divided into four groups which are classified as follows.
Group 1: normal mice (normal control group).
Group 2: normal mice injected with alloxan (diabetic group, positive control group).
Group 3: diabetic mice treated with 0.5 ml of marjoram extract solution containing 4mg of extract (treated group).
Group 4: normal mice treated with the same dose of extract used in group 3 (negative control group).

Blood Samples Collection

Samples of blood were collected by heart puncture occasionally at the period of the treatment by *Marjoram* extract. The mice were killed by cervical dislocation after they fasted overnight.

Measurements of Biochemical Parameters

Agappe diagnostic kits were used to estimate all the studied biochemical parameters in the current study. Level of fasting blood glucose, lipid profile, testosterone and enzymes of AST and ALT were determined according to kits procedures and spectrophotometric methods.

Statistical Analysis

Data for all the studied parameters in the current study were stated by mean SD. Statistics data were estimated statistically by analysis of variance [ANOVA] using SPSS program version 17. The significant differences were taken when P 0.05.

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Results

Qualitative Tests

The brownish-red precipitate was the result after adding 1%HCl and few drops of Dragendorff reagent to the filtered boiled methanolic extract. This colored precipitate indicates the presence of an alkaloid. The presence e of saponins was confirmed after a vigorous shake for a few minutes of aqua extract to form a stable foam. A blue–black precipitate referred to the presence of tannins in this extract. The precipitate formed from the addition of $FeCl_3$ reagent (few drops) to the aqueous extract. Flavonoid was indicated by a yellow coloration formed from the addition of concentrated H_2SO_4 to the mixture of aqua extract and ammonia solution. A reddish brown color confirmed the existence of the active component terpenoids in the studied extract when a chloroform extract was added to the concentration of sulphuric acid carefully, as shown in Table 1.

Table 1. Detection results of the active ingredients in *marjoram* extract

Active ingredients	Detection results
Alkaloids	Brownish-red precipitate. (positive test)
Saponins	Foam formation. (positive test)
Terpenoids	Reddish brown color formation. (positive test)
Tannins	Blue–black precipitate. (positive test)
Flavonoids	Yellow color formation. (positive test)

Determination of the Half Lethal Dose (LD50)

The results of LD50 have been recorded in Table 2. Fig. 1 shows that the value of LD50 was found to be 80.47 mg/kg BW, also the allowed dose was found to be 12.5 mg/kg that means for a person with 70 kg body weight the allowed dose of this extract is equal to 875 mg/70 kg BW, (0.875 gm/70 kg BW).

Table 2. LD50 results

	Number of mice			
Dose mg/Kg	**Lived**	**Died**	**Total**	**Percentage of death %**
200	0	7	7	100.00
100	2	5	7	71.43
50	4	3	7	42.86
25	5	2	7	28.57
12.5	7	0	7	0.00

	LD50	mg/Kg
	50	**80.47005**

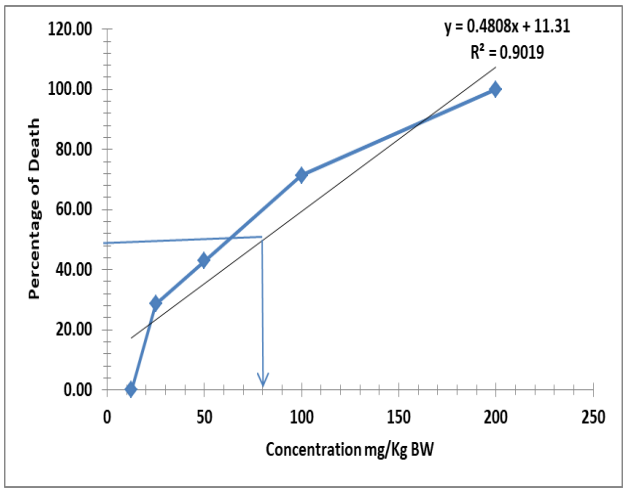

Figure 1. Shows the relation between mice death percentage and extract concentration for LD50 determination

Antibacterial Antifungal Activities

Table 3 shows the activity of *Marjoram* extract toward selective pathogenic bacteria and fungi. Four different doses including 4mg, 2mg, 1mg, and 0.5mg were applied as allowed doses according to LD50 results. Pathogenic bacteria include *Eshreshia, coli*, *Pseudomonas aeruginosa*, *Klebsiella*, Acinetobacter baumannii, as gram negative bacteria species, and *Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus hominis, Staphylococcus epidermidis, Enterococcus Spp., as* gram positive bacteria species together with *Candid albicans* and *Candida tropicalis as pathogenic fungi.* The results revealed that marjoram extract possesses high activity toward each *Pseudomonas aeruginosa*, *Streptococcus pyogenes, Staphylococcus epidermidis, Enterococcus Spp., Acinetobacter baumannii, Candid albicans and Candida tropicalis* while both *Klebsiella* and *Staphylococcus hominis* were affected by extract at the first upper concentration and first and second upper concentrations, respectively, but there is no effect of the extract on *E. coli* and *Staphylococcus aureus* even at high concentrations (doses), as shown in Table 3.

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Table 3. *Marjoram* extract activity against pathogenic bacteria and fungi species

Species	Dose 1	Dose 2	Dose 3	Dose 4	Control
Eshreshia coli	-	-	-	-	-
Pseudomonas aeruginosa	40 mm	27 mm	23 mm	17 mm	-
Staphylococcus aureus	-	-	-	-	-
Streptococcus pyogenes	25 mm	22 mm	15 mm	10 mm	-
Klebsiella	15 mm	-	-	-	-
Staphylococcus hominis	10 mm	02 mm	-	-	-
Staphylococcus epidermidis	20 mm	10 mm	05 mm	2.5 mm	-
Enterococcus Spp.	35 mm	20 mm	10 mm	07 mm	-
Acinetobacter baumannii	15 mm	10 mm	03 mm	02 mm	-
Candid albicans	35 mm	28 mm	18 mm	15 mm	-
Candida tropicalis	10 mm	07 mm	05 mm	02 mm	-

Dose1= 4mg/mL; Dose2 = 2mg/mL; Dose3=1mg/mL; Dose4=0.5 mg/mL

Antioxidant Activity

Four different concentrations of *Marjoram* extract were applied to evaluate the extract's antioxidant activity, they are including 25µg/ml, 50µg/ml, 100µg/ml, and 200µg/ml. ascorbic acid was used as reference antioxidant to determine the extract's antioxidant activity. The results of the *Marjoram* extract were recorded in Table 4. They showed high antioxidant activity in the concentrations 200,100 and 50 µg/ml, but no 25 µg/ml, there is a significant difference between extract antioxidant activity and antioxidant activity of ascorbic acid at this concentration (25µg/ml), whereas, no significant differences between ascorbic acid antioxidant activities and extract antioxidant activities at the concentrations 200, 100 and 50 µg/ml. Thus, the antioxidant activity of extract is similar to ascorbic acid activity at 200,100 and 50 µg/ml concentrations but no 25 µg/ml concentration, it is significantly decreased than ascorbic acid activity at this concentration (25 µg/ml), as recorded in Table 4.

Table 4. Antioxidant activities of *marjoram* extract and ascorbic acid

Marjoram extract	Antioxidant activity		
Concentrations	Marjoram acid	Ascorbic acid	P value
200 µg/ml	71.95 ±2.101	75.82± 2.102	0.891 NS
100 µg/ml	63.46 ±4.070	65.64±3.025	0.973 NS
50 µg/ml	50.00± 4.036	56.12±3.028	0.531 NS
25 µg/ml	32.83 ±7.342	43.56±4.163	0.032 S

Antidiabetic Activity

Twenty mice were divided into four groups (5 mice for each group) to study the antidiabetic activity of *Marjoram* extract. Group one represents the control group, the second group was injected with alloxan to convert to induced diabetes without treatment. Group three represents diabetic group with alloxan and treated with 0.5ml of DMSO containing 4 mg of extract daily for two weeks. Group four represents normal mice that injected with *Marjoram* extract. Table 5 shows the results of glucose levels in all the studied groups. The results revealed that glucose level was increased in diabetic group (G2) (174.982 ± 6.950). While the level of glucose in treated diabetics with *marjoram* extract dose (G3) is decreased toward normal case (108.172 ± 3.780). At the same time, the same dose of the extract was injected to the normal mice group (G4) but the glucose level was not affected by extract dose in comparison with control group.

Table 5. Glucose level in all the studied groups

Groups	Glucose mean ± SD mg/dl
Control group (G1)	84.516 ± 5.453^{c}
Diabetic group (G2)	174.982 ± 6.950^{a}
Diabetic group treated with extract dose (G3)	108.172 ± 3.780^{b}
Normal group treated with extract dose (G4)	87.455 ± 3.443^{c}

Different small letters referred to significant differences between groups at p value less than 0.05

The results of insulin in all the studied groups are reported in Table 6. The results showed that insulin level was decreased in diabetic group G2 (14.858 ± 1.482^{b}). at the same time, glucose

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level was improved in diabetic group that treated with extract dose G3 (19.649 ± 1.966[a]) in comparison with control group G1 (21.177 ± 1.477[a]). also, the results showed that insulin level is not affected by extract in normal mice group that treated with extract dose G4 (21.802 ± 1.305[a]).

Table 6. Insulin level in all the studied groups

Groups	insulin mean ± SD mg/dl
Control group (G1)	21.177 ± 1.477[a]
Diabetic group (G2)	14.858 ± 1.482[b]
Diabetic group treated with extract dose (G3)	19.649 ± 1.966[a]
Normal group treated with extract dose (G4)	21.802 ± 1.305[a]

Different small letters referred to significant differences between groups at p value less than 0.05

Table 7 shows the results of lipid profile of all the studied groups. The results of TGs revealed that the level of TGs in diabetic group G2 (138.230 ±6.65[a]) was increase in comparison with control group G1 (74.338 ±3.452[c]), whereas, TGs level in diabetic group with extract dose G3 was decreased toward normal level (97.552 ±2.625[b]). Also, TGs level in normal group with extract dose G4 (75.80 ±6.505[c]) does not affected by extract dose related to control group. The results of total cholesterol level (TC) showed significant increasing (109.629 ±6.58[a]) in diabetic group (G2) related to control group (G1) (66.138 ±3.452[c]). At the same time, TC level in treated diabetic group (G3) significantly decreased (76. 482 ±2.850[b)] in comparison with diabetic group (G2). In contrast, TC level in treated normal group (G4) was not affected with marjoram extract dose (68.252 ±2.721[c]), it was found to be similar to its level in control group (G1).

The results of HDL level showed significant decrease (18.367 ±3.095[c]) in diabetic group (G2) in comparison with control group (G1) (38.650 ±1.715[a]). The treatment with *Marjoram* extract in treated diabetic group (G3) led to increase of HDL level significantly. Also, HDL level in normal group that treated with *Marjoram* extract (G4) was not affected by treatment and its level (37.487 ±3.380[a]) was similar to control group (G1) (38.650 ±1.715[a]), as noticed in Table 7. In similar manner, both LDL and VLDL levels significantly increased in diabetic group (G2) (63.619 ±5.872a), (27.646 ±1.330[a]), respectively, in comparison with control group (G1) for both LDL and VLDL (12.611±379[c]), (14.878 ±0.690[c]), respectively. Also, levels of LDL and VLDL in treated diabetic group (G3) were significantly decreased in comparison with diabetic group (G2). Whereas, levels of LDL and VLDL in normal group (G4) that treated with *marjoram* extract were not affected by treatment (15.605 ±4.876[c]), (15.160 ±1.301[c]) respectively, as noticed in Table 7.

Table 7. Lipid profile levels in all the studied groups

Groups	TGs mg/dl	TC	HDL	LDL	VLDL
Control group **G1**	74.338 ±3.452[c]	66.138 ±3.452[c]	38.650 ±1.715[a]	12.611±379 [c]	14.878±0.690[c]
Diabetic group **G2**	138.230 ±6.65[a]	109.629 ±6.58[a]	18.367 ±3.095[c]	63.619 ±5.872[a]	27.646±1.330[a]
Treated diabetic group **G3**	97.552 ±2.625[b]	76. 482 ±2.850[b]	32.800 ±2.823[b]	24.171±5.708[b]	19.510±0.525[b]
Treated normal group **G4**	75.80 ±6.505[c]	68.252 ±2.721[c]	37.487 ±3.380[a]	15.605±4.876b[c]	15.160±1.301[c]

Different small letters referred to significant differences between groups at p value less than 0.05

The levels of AST and ALT were recorded in Table 8. The results showed that AST level increased in diabetic group G2 (153.370 ± 7.750[a]) compared to the control group G1(139. 600 ± 13.10 [b]). While the treated group with marjoram extract dose G3 decreased to normal level (145.420 ± 5.330 [ab]). Also, the level of AST in normal group G4 that treated with extract group was not affected by *Marjoram* extract as shown in Table 4. In another hand, ALT level in diabetic group G2 (132.040 ± 13.30[a]) increased related to the control group G1(69.22 ± 4.650[c]), whereas, ALT level in the treated group with extract dose decreased toward normal case (112.260 ± 11.88[b]). It was noticed that the level of ALT in normal group does not affect by the extract dose and maintained its normal level as in the control group (66.70 ± 2.93c), as shown in Table.8.

Table 8. AST and ALT levels in all the studied groups

Groups	AST IU/ml	ALT IU/ml
Control group (G1)	139. 600 ± 13.10[b]	69.22 ± 4.650[c]
Diabetic group (G2)	153.370 ± 7.750[a]	132.040 ± 13.30[a]

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Treated diabetic group (G3)	145.420 ± 5.330[ab]	112.260 ± 11.88[b]
Treated normal group (G4)	137.080 ± 6.050[b]	66.70 ± 2.93c

Different small letters referred to significant differences between groups at the same column at p value less than 0.05

Table 9 shows the testosterone levels in all the studied groups. The diabetic group G2 revealed decreasing in testosterone level (3.194 ± 0.188[c]) in comparison with control group G1(.11.652 ± 1.219[a]). In contrast, the level of testosterone in the treated group with extract dose G3 increased toward the normal state (8.369 ± 0.458[b]), while its level in normal group that treated with extract dose is not affected with extract (11. 613 ± 0.670[a]), as shown in Table 9.

Table 9. Testosterone levels in all the studied groups

Groups	Testosterone ng/ml
Control group (G1)	11.652 ± 1.219[a]
Diabetic group (G2)	3.194 ± 0.188[c]
Treated diabetic group (G3)	8.369 ± 0.458[b]
Treated normal group (G4)	11. 613 ± 0.670[a]

Different small letters referred to significant differences between groups at the same column at p value less than 0.05

Discussion

The study of the antioxidant activities of plant compounds showed the important role of these compounds in the treatment of oxidants present in the biological system. Phenols represent the main compounds in plant extracts that possess antioxidant activity due to their structure containing aromatic rings that enable them to be stable and capable of donating hydrogen atoms to neutralize free radicals without being affected [35]. Accordingly, the qualitative tests of *marjoram* extract confirmed the presence of flavonoids and terpenoids which represented the major source of the antioxidant components. So, the results of qualitative tests of *marjoram* extract support the results of the antioxidant activity of the extract [34].

Bacterial resistance to common antibiotics has led to a search for new antibiotics. Plants contain many and varied chemical compounds that have various properties and activities against infections, microbes and various diseases. The biologically active plant ingredients are safe and have a proven ability to treat various diseases. Furthermore, the effect of plant extracts on bacteria species differs due to the structure of bacterial cell membranes and the nature of the effective components of the extract. Generally, the mechanism of plant extract effect on bacteria includes the effect on the bacterial membrane to penetrate it, caused inhibit the DNA and protein synthesis, in addition to inhibiting a number of metabolic enzymes such as succinate dehydrogenase and malate dehydrogenase, increasing the osmotic pressure inside the cell, and organic acids can also lead to increase in the pH inside the cell. However, cell membrane damage is considered one of the main pathways affecting and inhibiting bacterial cells [36].

The acute toxicity of plant extract should be determined by LD50 method. So, evaluating the LD50 value guides to determine the permissible doses to indicate their effect on the disease or ailments to be treated. LD50 value of *marjoram* extract is found to be 50.84 mg/kg BW, which is considered a high value that can give permissible limits equal to 12.5 mg / kg body weight. However, the dose of 4 mg/ml was used as a value within the permissible limits for the treatment of diabetes and against pathogenic bacteria as upper doses with other lower doses include 2,1 and 0.5 mg/ml, which are all be under permissible dose limits [37].

It was reported that plant extracts have high activity as antidiabetic factors. The activity includes glucose transport and metabolism regulation. Health advantages of plant extracts occurred due to presence of active bio components called the phytochemical compounds which are including flavonoids, terpenoids, alkaloids and phenolic acids like ellagic acid and ferulic acid. So, the presence of such compounds support the antidiabetic activity of the plant extract [38]. Furthermore, plant extracts lead to regulate blood glucose, in addition, the antioxidant and anti-inflammatory features suppressed lipid oxidation that can be associated with insulin resistance [39]. It was found that plant extract mechanisms of their actions to be as anti-hyperglycemic factors, occurred through utilized their properties in suppression of glucosidases, rise

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in glucose uptake and elevation of insulin secretion [40].

Studies referred to that plant extracts possess beneficial roles in the reduction of TC, LDL-C, and TGs levels and elevation of HDL-C levels, due to its function in reverse cholesterol transport in addition to its connection with antioxidant enzymes like paraoxonase 1that can reduce pro-atherogenic components that can may occur during lipid oxidation. Subsequently, *marjoram* extract regulates the lipid profile in the diabetic group in comparison with the control group and has no effect on the normal group that was treated with extract under the same condition [41]. Plant extract also found to be enhanced kidney and hepatic functions by decreasing the abnormal levels of GOT, GPT during diabetes period to the normal levels during the treatment period. Plant extracts activate SOD, GSPx and GSH, so they protect the cells from damage that occurred by free radicals. Moreover, it was found that certain plant extracts decreased the liver fibrosis in terms of decreasing collagen 1 and connective tissue [42].

In diabetic rats, plant extracts like *Alpinia officinarum* extract improved sperm damage and its morphology in addition to repairing the histological defects in the testis [43]. In another study, methanolic extract of *equisetum arvense* treats the diabetic adverse effects on fertilization and sperm quality, so, this action may be linked with its bioactivities like antioxidant and anti-hyperglycemia activities [44].

In recent study, it was reported that antidiabetic activity was increased with increasing of treatment time and the applied dose of plant extract [28]. This study is agreeing with current study in reducing the level of glucose and lipid levels. In another recent study, the activity of medicinal plant extracts attributed the active components represented by the secondary metabolites like flavonoids, phenolic acids, tannins, saponins and glycosides. So, the activity of this raw materials encourages to convert they into pharmaceutical agents [45]. Antidiabetic activity of the plant extract of *Euphorbia neriifolia* was investigated. The results showed increasing in beta cells numbers and insulin secretion. This result is consistent with the obtained results in current study [46].

Conclusion

On the basis of the obtained results in the antioxidant activity of this extract, it can be concluded that the methanolic leaves extract of marjoram has high antioxidant capacity compared to ascorbic acid. This activity attributed to the presence of phytochemicals in extract like polyphenol and terpenoids. Also, the results revealed the powerful antimicrobial activity of the marjoram leaves extract that it can be stopping or inhibit the growth of the studied species of pathogenic bacteria and fungi. So, using this extract as an antidiabetic agent support the immunological system of diabetic patients. Moreover, the study of antidiabetic activity showed that marjoram extract has the ability to reduce the level of blood sugar in diabetic mice and improve lipid profile, liver function and fertility disorders. Further studies should be conducted to isolate and characterize the pure active phytochemicals for improvement and supporting these bioactivities are recommended.

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Author's Contribution

K.K.G. conducted acquisition of data, did laboratory analytics, interpretation, as well as the interpretation, revision and proofreading the manuscript. F.M.K. did the conception and design the idea of MS, interpretation, revision, and proofreading the manuscript.

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تقييم الفعاليات المضادة للاكسدة والبكتريا والسكري لمستخلص البردقوش الكحولي - المائي

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الخلاصة

تمتلك المستخلصات النباتية أهمية خاصة تجاه صحة الإنسان والأنشطة الحيوية لمجموعة واسعة من الأمراض. هدفت الدراسة الحالية إلى التحقق من وجود مكونات فعالة في مستخلص أوراق البردقوش ، وتحديد مضادات الأكسدة ، والأنشطة المضادة للبكتيريا والفطريات ، و LD50 ونشاطها المضاد لمرض السكر. تم تحليل المكونات النشطة نوعيا من خلال طرائق اختبار محددة. وتم قياس الأنشطة المضادة للأكسدة ، LD50 ، مضادات البكتريا والفطريات بالطرائق القياسية. وحددت الفعالية المضادة للسكري عن طريق تقييم ارتفاع السكر وفرط شحميات الدم ووظائف الكبد بالطرائق الطيفية. اكدت النتائج على وجود العديد من المكونات الفعالة تتضمن مركبات الفلافونويد والقلويدات والتربينويدات والعفص والصابونين. كما أظهرت نتائج فعالية مضادات الأكسدة أن مستخلص أوراق البردقوش يمتلك نشاطًا مضادًا للأكسدة مشابهًا لحمض الأسكوربيك. وأظهرت نتائج LD50 أن الجرعة المسموح بها هي 12.5 ملغم / مل. أشارت نتائج الفعالية الحيوية إلى أن مستخلص أوراق البردقوش يمتلك فعالية مضادة لمدى واسع من البكتريا والفطريات المرضية ، ولكن لا يوجد تأثير للمستخلص على E. coli و Staphylococcus aureus حتى عند التراكيز العالية. أظهر مستخلص البردقوش فعالية عاليَة مضادًا للسكري عند المعالجته لمدة أسبوعين بـ 4 ملغم من المستخلص يوميا. مع تحسن مستوى الدهون ووظائف الكبد ومستويات هرمون التستوستيرون بعد العلاج في مجموعة مرضى السكري مقارنة مع مجموعة التحكم. يمكن الاستنتاج أن مستخلص أوراق البردقوش يحتوي على فعالية عالية مضادة للأكسدة مع فعالية حيوية عالية ضد البكتريا والفطريات المرضية ونشاط عال مضاد للسكري مع تعزيز كبير للخصوبة ومستوى عالٍ من الجرعات المسموح بها وفقًا لقيمة LD50.

الكلمات المفتاحية: الفعالية المضادة للبكتريا، الفعالية المضادة للسكري، الفعالية المضادة للفطريات،الفعالية المضادة للاكسدة،مستخلص البردقوش