

**Effect of selected doses of ethanol extract of *Seidlitzia rosmarinus* leaves on serum total cholesterol (TC) concentration in male rats.**

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**Abstract**

This study was carried out on the chemical extract of leaves of the plant of Al-Shinan and was aimed at the most important chemical components of plant leaves, and quantitative determination major phytochemicals then tested on laboratory animals to know the most effective dose in reducing the concentration of total cholesterol in the serum blood of experimental animals. The Chemical tests showed that the leaves contain alkaloids, clicosides, phenols, flavinoids, soap and tannins. On the other hand, the total value of the yield of the ethanolic extract was 18.92%. and the results also showed the extract contained 1.112% phenol, 0.393% flavonoids and 1.5225% soap.

In order to extract the most effective dose of the extract of this study, thirty mature male rats were selected and divided into five equal groups. The first was considered as a control group and only the distilled water was administered and the other groups were given 100,200,300 and 400 mg of crude ethanol / kg body weight. For four weeks. After two and four weeks of treatment, the results of the total cholesterol concentration measurements of the animals' forbidden blood samples for more than eight hours showed that the 200 mg / kg B.W. dose caused the highest reduction in total cholesterol concentration.

**Key wards:** *Seidlitzia rosmarinus*, cholesterol , flavonoids

### الخلاصة

اجريت هذه الدراسة على المستخلص الكحولي لاوراق نبات الشنان وكانت تهدف لمعرفة المكونات الكيميائية النباتية لاوراق النبات والتقدير الكمي لاهم المكونات الكيميائية في اوراق نبات الشنان,ومن ثم اجريت تجربة على الحيوانات المختبرية لمعرفة الجرعة الاكثر تأثيرا في تخفيض تركيز الكوليسترول في مصل الدم في حيوانات التجربة . وقد استخلصت الدراسة ان الكشوفات الكيميائية بينت احتواء اوراق النبات على القلويدات والكلايكوسيدات والفينولات والفلافونويدات والصابونين والتانين وان القيمة الاجمالية للعائد من المستخلص الخام الإيثانولي من أوراق الشنان (*Seidlitzia rosmarinus*) هي 18.92 % . و نتائج التقدير الكمي اظهرت احتواء المستخلص على 1.112% فينول و 0.393% فلافونويدات و 1.5225% صابونين. ولأجل استخراج الجرعة الاكثر تأثيرا لمستخلص هذه الدراسة تم اختيار ثلاثين من ذكور جردان الالبينو الناضجة وقسمت الى خمس مجاميع متساوية. اعتبرت الاولى كمجموعة ضابطة جرعت فقط ماء مقطر وأعطيت المجاميع الأخرى 100،200،300 و 400 ملجم من المستخلص الإيثانولي الخام / كجم من وزن الجسم. لمدة أربعة أسابيع. بعد أسبوعين وأربعة أسابيع من العلاجات ، أوضحت نتائج قياسات تركيز الكوليسترول الكلي لعينات دم الحيوانات الممنوعة من الاكل لمدة اكثر من ثمان ساعات أن جرعة 200 مغ / كغ من وزن الجسم ، تسببت في أعلى انخفاض في تركيز الكوليسترول الكلي.

### Introduction

A survey that carried out by World health organization (WHO) indicated that coronary heart disease alone accounts for more than half of the total mortalities associated with cardiovascular diseases. Atherosclerosis is the focal point of pathogenesis of these diseases. The American Heart Association identified the primary risk factors

associated with atherosclerosis as elevated levels of cholesterol and triglycerides in the blood<sup>[1]</sup>.

Oxidative stress induced by reactive Oxygen species (ROS) is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery disease<sup>[39]</sup>. Oxidative stress (OS) plays a major role in

hypercholesterolemia that is an essential risk factor for coronary Clinical studies indicated that hypercholesterolemia is an essential risk factor for CAD, where low-density lipoprotein (LDL) cholesterol plays a major role in the atherosclerosis and pathogenesis of CAD and other vascular diseases [3]. Furthermore, several studies showed that hyperlipidemia induces oxidative stress and the oxidative modification of lipoproteins in vessel walls might play a key role in atherogenesis [4,5].

The harmful action of the free radicals can, however, be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism [6].

Antioxidants can be defined as substances able to inhibit or delay the oxidative damage of protein, nucleic acid and lipid caused by dramatic increase of ROS [7] by inhibiting the

artery disease (CAD), induced atherosclerosis [2].

initiation or propagation of oxidizing chain reactions [8].

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [9]. Phenolics or polyphenols, including flavonoids have received considerable attention because of their physiological functions such as antioxidant, antimutagenic and antitumor activities [10,11].

Plants have been used for several years as a source of traditional medicine to treat various diseases and conditions [11]. A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can provide therapeutic effect [12].

The knowledge on the use of medicinal plants was acquired by trial and error and handing on from generation to generation [13].

Traditional medicine is widespread and plants still

presents a large source of natural antioxidants that might serve as leads for the development of novel drugs <sup>[14]</sup>.

*Seidlitzia rosmarinus* belong to the family Chenopodiaceae it is a perennial woody plant well adapted to grow along the banks of salt marshes and also in saline

soils <sup>[15,16]</sup>. It used in soap and detergent industries. The ash has also antiseptic and antibacterial properties<sup>[17]</sup>.this study was aimed to determine the antioxidant activity(invevo and invetro) of *Seidlitzia rosmarinus* leaves.

## Materials and Methods

### Preparation of plant material

The fresh leaves of shnan were collected during October and November from Alshehaymia area in Wasit governorate, Iraq. The vouchers specimen of the plant were deposited to be

identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib, The leaves were washed in fresh water to remove adhering dust and then dried under shade.

### Preparation of ethanol extract of *Seidlitzia rosmarinus* leaves

The air dried powdered leaves were extracted by continuous hot extraction continuously with 70% ethanol for 24 hrs using double-thickness cellulose extraction thimbles. The extracted material

were evaporated or concentrated by rotary evaporator at tempreteur below 45°C .The weight of the crude extract was measured and kept at -20°C till use.

### Calculation of yield matter

The amount of crude extract from 125gm of *Seidlitzia rosmarinus* powder (table 2), was

found to be equal to 23.7gm (approximately equal to 18.92%) of yield.

### Phytochemical screening

Table (1) showed a qualitative chemical tests of the essential compounds of the aqueous and ethanol extract of powdered leaves of *Seidlitzia rosmarinus* were carried out as follows:

#### Quantitative chemical constituents of crude ethanol extract of *Seidlitzia rosmarinus* leaves.

#### Preparation of the extract:

Fifty grams of powdered *Seidlitzia rosmarinus* leaves were extracted in 500ml of absolute methanol by maceration process (48hrs), the solvent was

Alkaloids<sup>[18]</sup>, Glycosides,<sup>[19]</sup> Phenols<sup>[20]</sup>, Flavonoids<sup>[21]</sup>, Saponin<sup>[18,22]</sup>, Tannins<sup>[22]</sup>, Resins<sup>[23]</sup>, and Terpene<sup>[24]</sup>.

removed under vacuum at a temperature below 50°C and the extract was stored at 4-8°C<sup>[25]</sup>, determination of flavonoids according to Chang *et al.*,<sup>[26]</sup> Total phenols were determined according to the method that used by McDonald *et al.*<sup>[27]</sup>, and Determination of Saponin according to the method used by Obadoni and Ochuko<sup>[28]</sup>.

#### Experimental design :

Different doses of crude ethanol extract *Seidlitzia*

*rosmarinus* leaves were given daily to male rats orally by using gavages' needle.

Thirty adult male rats were randomly divided into five equal groups and treated daily as follows for four weeks.

**GI:** Animals in this group received 4ml/kg B.W of 10% Dimethyl sulphoxide (DMSO) orally using gavages needle and served as a control group.

**GII:** Animals in this group received 100 mg/kg B.W. of crude ethanol extract of *Seidlitzia rosmarinus* leaves dissolved in 10% DMSO for four weeks.

### Blood collection

Fasting blood samples were collected at 0 time , 3<sup>rd</sup> , 6<sup>th</sup> and 9<sup>th</sup> weeks during the experiment

**GIII:** Animals in this group received 200 mg/kg B.W. of crude ethanol extract of *Seidlitzia rosmarinus* leaves dissolved in 10% DMSO for four weeks.

**GIV:** Animals in this group received 300 mg/kg B.W. of crude ethanol extract of *Seidlitzia rosmarinus* leaves dissolved in 10% DMSO for four weeks.

**GV: Animals** in this group received 400mg/kg B.W. Of crude ethanol extract of *Seidlitzia rosmarinus* leaves dissolved in 10% DMSO for four weeks.

period (4weeks) for measuring serum total cholesterol. The effective dose of the crude extract was chosen .

### Statistical Analysis

Data were analyzed to investigate the effects of treatment, period and the interaction between treatment and period by using SAS program (SAS.,2000). According to the following model:

$$Y_{ijK} = M + A_i + P_j + AP_{ij} + e_{ijK}$$

Where:  $A_i$ = the effect of treatment ,  $P_j$ = the effect of period ,

$AP_{ij}$ = the interaction between treatment and period ,

$P_{ijK}$ = the rondum error.

Differences between means were tested by using F-test at 0.05 levels.

## Results and Discussion

### Phytochemical screening

Table (1) illustrated qualitative phytochemical screening of the crude ethanol extract of *Seidlitzia rosmarinus* leaves. The results showed that alkaloids, glycosides, phenols, flavonoids, saponin, as well as tannins were the major leaves extract

constituents, while Resins and terpenes were not present.

Total yield value of the crude ethanol extract of *Seidlitzia rosmarinus* leaves Out of 100g of powdered *Seidlitzia rosmarinus* leaves was approximately (18.92)gm

**Table (1): Qualitative phytochemical screening of the crude ethanol extract of *Seidlitzia rosmarinus* leaves.**

Constituents	Alkaloids	Glycosides	Phenols	Flavonoids	Saponin	Tannins	resins	Terpene
Results	+	+	+	+	+	+	–	–

**Quantitative chemical constituents of crude ethanol extract of *Seidlitzia rosmarinus* leaves.**

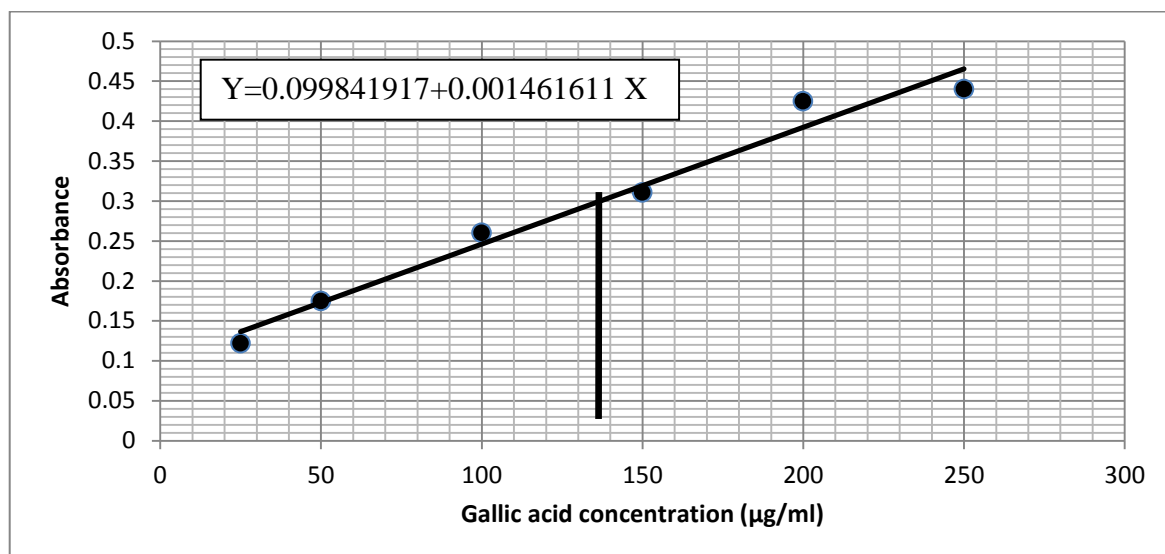
Table(5) showed total yield value of the crude ethanol extract of *Seidlitzia rosmarinus* leaves Out of 100g of powdered *Seidlitzia rosmarinus* leaves was approximately (18.92)gm.

Also, the recorded percentage yield values of phenols, flavonoids and saponin were found to be 1.112, 0.393 and 1.5225 % respectively.

**Table (2) : Total phenols determination Calculated**

X	Y
25	0.136382191
50	0.172922465
100	0.246003013
150	0.319083561
200	0.392164109

250	0.465244657
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**Figure 1** Calibration curve of standard gallic acid for determination of total Phenol contents

\*Note :values in the curve was multiplied by 8(time of dilution)and by10(concentration of extract) to detect the total phenols (µg) in one gram of dry matter.

**Table (3): Total Flavonoids determination Calculated**

X	Y
12.5	0.053878048
25	0.112603658
50	0.230054877
75	0.347506095
100	0.464957314



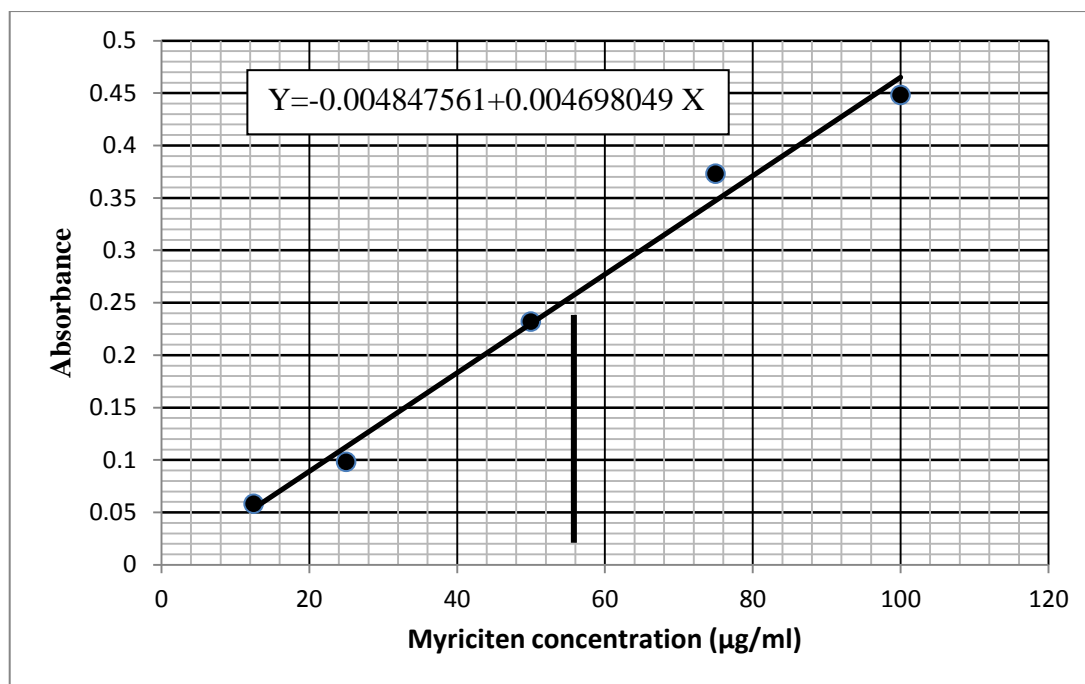


Figure 2. Calibration curve of standard Myrecetin for determination of total flavonoid content

\*Note: the value in curve was multiplied by 8(time of dilution)and by 10(concentration of extract), to detect the total flavonoids (µg) in one gram of dry matter.

Table (4): Quantitative total crude extract, phenols, Flavonoids, and Saponin of crude ethanol extract of *Seidlitzia rosmarinus* leaves.

chemical	Percentage
Crude extract	18.92
Phenols	1.112
Flavonoids	0.393
Saponin	1.5225

**The effect of selected doses of ethanol extract of *Seidlitzia rosmarinus* Leaves on serum total cholesterol**

The effect of four successive increasing doses of ethanol crude extract of *Seidlitzia rosmarinus* leaves on mean values of serum total cholesterol concentration

(mmol) of male rats is shown in table (5). The results showed that after two weeks of treatment no significant ( $P>0.05$ ) differences in serum TC concentration were recorded between the treated groups GII, GIV and GV comparing with the control. Moreover, with the exception of GIII group, serum TC in all treated groups showed no significant ( $P>0.05$ ) differences

comparing with the control, and the significant ( $P<0.05$ ) reduced values after 4weeks of treatment in GIII group were ( $2.23\pm0.04$ ) comparing with the control ( $2.36\pm0.04$ ). With time, with the exception of GIII no significant ( $P>0.05$ ) differences in treated groups were detected, while the recorded significant mean values for GIII after 2 and 4 weeks of treatments were documented.

**Table (5): The effect of selected doses of ethanol extract of *Seidlitzia rosmarinus* leaves on serum total cholesterol (TC) concentration (mmol) in male rats.**

<b>T \ G</b>	<b>GI</b>	<b>II</b>	<b>GIII</b>	<b>GIV</b>	<b>GV</b>
Zero	2.35 $\pm$ 0.06 A a	2.36 $\pm$ 0.05 A a	2.41 $\pm$ 0.03 A a	2.41 $\pm$ 0.05 A a	2.39 $\pm$ 0.04 A a
Two Week	2.38 $\pm$ 0.03 AB a	2.45 $\pm$ 0.08 AB a	2.27 $\pm$ 0.04 B b	2.32 $\pm$ 0.04 B a	2.46 $\pm$ 0.02 A a
Four Weeks	2.36 $\pm$ 0.04 A a	2.45 $\pm$ 0.02 A a	2.23 $\pm$ 0.04 B b	2.41 $\pm$ 0.03 A a	2.38 $\pm$ .03 A a

LSD=0.13 \* Capital letters denote differences between groups,  $P<0.05$  vs. control.

\* Small letters denote differences within group,  $P<0.05$  vs. control.

Values are expressed as mean  $\pm$ SE, n =6 each group

**G I:** control group drenched of 10% DMSO (4ml/kg B.W) by gavages' needle daily for four weeks.

**G II:** drenched of 100mg /kg B.W crude extract of *Seidlitzia rosmarinus* leaves by gavages' needle daily for four weeks.

**G III:** drenched of 200mg /kg B.W crude extract of *Seidlitzia rosmarinus* leaves by gavages' needle daily for four weeks.

**G IV:** drenched of 300mg /kg B.W crude extract of *Seidlitzia rosmarinus* leaves by gavages' needle daily for four weeks.

**G V:** drenched of 400mg /kg B.W crude extract of *Seidlitzia rosmarinus* leaves by gavages' needle daily for four weeks.

## Discussion

Chemical analysis revealed that the crude extract of *Seidlitzia rosmarinus* leaves contained glycosides, phenols, flavonoids, and saponin, that have an antioxidant activity and a significant hypocholesterimic effect <sup>[29-31]</sup>. It has been hypothesized that cells respond to phytochemicals through direct interactions with receptors or enzymes involved in signal transduction, or through modifying gene expressions that may result in alteration of the redox status of the cell that may trigger a series of redox-dependent reactions <sup>[32]</sup>.

For instance, recent studies suggested that flavonoids decrease plasma lipids and atherosclerosis and it is denoted that antihypercholesterolemic effect of flavonoids is related to a decrease of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) through enhancing phosphorylation of HMG-CoA

reductase indirectly thus diminish endogenous cholesterol production, and decrease in apo B secretion in hepatocytes <sup>[33,34]</sup>. Flavonoids, probably exert their influence on steroid metabolism of other pivotal points. That is, flavonoids bind to cytoplasmic steroid receptor due to hydrophobicity of their aglycones portion and this complex is likely to interact with steroid regulatory elements. Alternatively, the flavonoids may intercalate it between the bases of DNA segments, leading to transcription of gene involved in lowering blood cholesterol level <sup>[35]</sup>.

Moreover, saponin, one of the major constituent of ethanol extract of *Seidlitzia rosmarinus* leaves (1.5225%), may lower cholesterol level in treated rats in this study since a number of studies shown that saponin from different sources lowered serum cholesterol levels in a variety of animals and human subjects <sup>[36]</sup>.

Large mixed micelles formed by the interaction of saponin with bile acid account for their increased excretion when saponin

rich foods such soya bean consumed <sup>[37]</sup>. Decreased intestinal cholesterol absorption induced by some saponin <sup>[38]</sup>.

## Conclusions

1. The yield of crude ethanolic extract of *Seidlitzia rosmarinus* leaves was approximately 18.92g/100g of dry matter.
2. The potent foaming property of Al-shinan (*Seidlitzia rosmarinus*) may be

attributed to high saponin content (1.5225%) of the crude extract .

3. Administration of ethanolic crude extract (200mg/kg B.W), caused significant reduction in serum total cholesterol.

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