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Phytochemical Screening and Anti-Obesity Activity of *Salvia Officinalis L.*

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Abstract:
Phytochemical screening of leaves of Salvia Officinalis L., have been determined, and the results showed that the leaves of Salvia Officinalis L. contained the following compounds:phenols,steroids,terpenoids, carbohydrates, flavonoids, , tannins, and glycosides. While it has shown the absence of alkaloids and Cardinols, , the total antioxidants, phenols ,and flavonoids were determined by using different solvents (MeOH; MeOH70%;EtOH; EtOH70%) by spectrophotometer. The results show that methanolic 70% extract has the highest content of total antioxidants and total flavonoids ,whereas the methanolic extract has the highest content of total phenols.The anti-obesity activity effect of Salvia OfficinalisL.were screened by studied the inhibition lipase and α –amylase activity by used different extracts[MeOH; MeOH 70% EtOH ; EtOH70%].The results showed that the highest effect on lipase activity was in the MeOH extracts but the highest effect onα –amylase activity was in the MeOH 70% extracts.

Keywords: *antioxidants, phenols ,flavonoids,lipase, α-Amylase, inhibition , obesity, Salvia Officinalis L.*

INTRODUCTION

The results from the WHO global survey on traditional, complementary/alternative, and herbal medicines showed that the market for these kinds of medicines is growing steadily worldwide[1].

In fact, the usage of herbs and nutraceuticals is rapidly and continuously expanding. Recently, many people have been using these formulations in the treatment or prevention of various diseases and disorders in different national healthcare systems. Moreover, many patients often use herbal medicines to complement treatment with conventional medicines [2],[3]. Herbals for the treatment of excess weight and obesity were among the most used remedies, especially in developing and developed countries, since these metabolic disorders became very prominent [4],[5].

In fact, obesity poses a worldwide concern, not only for the harm which excess weight alone may cause, but also due to associated health problems such as endocrine, metabolic, and cardiovascular disorders [6],[7],[8].

Inhibition of enzymes involved in the metabolism of carbohydrates such as α-amylase is an important therapeutic approach for reducing postprandial hyperglycemia[9]. Moreover, one of strategies used in the discovery of anti-obesity drugs is to search for potent lipase inhibitors from plant extracts. In fact, several synthetic drugs such as acarbose and orlistat are widely used as inhibitors of these enzymes in patients with obesity[10],[11].

Orlistat is a commercial drug that commonly used as anti-obesity medications by inhibiting pancreatic lipase activity. It has adverse effects including abdominal pain, bloating, flatulence, oily stools, diarrhea, and decreasing in fat soluble vitamins absorption.[12] Acarbose have been potent reversible inhibitor of α-amylase and α-glucosidase. However, undesirable side effects limit its use.[13],[14]

In this study, the leaves of *Salvia Officinalis L* were evaluated for their anti-lipase and anti-α-amylase activity by using a simple, fast, efficient, and reliable spectrophotometric method, in an attempt to investigate these new agents for their ability to impair the digestion and assimilation of dietary fats and carbohydrate. In addition, they were compared with Orlistat and Acarbose in order to assess their potential use as an alternative to this chemical agent.

MATERIALS AND METHODS

2.1.Chemicals and Equipment:

Chemicals: Aluminum Chloride, Ferric Chloride, Sulphuric Acid, Ammonia 25%, Hydrochloric Acid 32%, Lead Acetate,α Naphthol, MeOH, EtOH, Chloroform, Folin-Ciocalteau reagent, sodium carbonate, potassium acetate, ammonium molybdate, sodium phosphate, acetic anhydride acid, Dimethyle sulfoxide (DMSO), were purchased from Merk, Germany Orlistat,Acarbose , *p*-nitrophenylpalmitate (NPP),Lipase type II and α- AmylaseEC 3.2.2.1 from porcine pancreas,Strach,3,5 dinitrosalysic acid were purchased from Sigma Aldrich.
Equipments: Ultrasonic Bath (Hwashin،Power Sonic 405), Rotary evaporator (HeidolphLaborata 4000, Germany), UV-Vis spectrophotometer (Jasco, V-650, Japan).

2.2.Plant material:

The leaves of *Salvia Officinalis L.* collected from Latakia Kasab in Syria. Identification of leaves of *Salvia Officinalis L.* was done by the dipartites in the Agriculture faculty- Aleppo University. leaves of *Salvia Officinalis L.* were washed , and dried in shade at ambient temperature (25-30°C) for 15-20 days , then they were ground into fine powder**.**

Figure (1): leaves of *Salvia Officinalis L.*

2.3. Extracts Preparation:

3g from leaves of *Salvia Officinalis L.* were extracted with 100ml of [MeOH, MeOH 70%, EtOH, EtOH 70%,], three times, then the extracts were collected and evaporated by using a rotary evaporator under decreased pressure, at 40°C until obtain the crude extract.

For the assay of pancreatic lipase inhibition and pancreatic α -amylase inhibition crude extracts were dissolved in DMSO that did not affect enzyme activity[15].

2.4.Chemical Content Study:

Tannins: About 0.02 g of each crude extract was dissolved in 10 ml of distilled water in a test tube at range of pH=6-8, few drops of lead acetate solution 9.5% was added, formation of a white or brown precipitate indicates the presence of tannins[16].
Steroids:2 ml of acetic anhydride acid. The color changes from violet to blue or green in samples indicate the presence of steroids[17].
Terpenoids:0.2 g of the extracts was mixed with 2 ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish- brown coloration of the interface will form to indicate positive results from the presence of terpenoids[17].
Phenols: 2ml of each extract was mixed with 1ml of the ferric chloride solution, occurring of blue or green color indicates the presence of phenols [18].
Flavonoids: 2ml of each extract was mixed with 1ml of the 5% ethanolic aluminum chloride, occuring of yellow colour indicates the presence of Flavonoids [19],[20].
Carbohydrates: 1ml of each extract was mixed with 1ml of the molish reagent, then few drops of sulfuric acid was added into the sides of the test tube, formation a violet ring indicates the presence of carbohydrates[21].
Alkaloids: About 0.1 g of each crude extract was soaked in Hydrochloric acid solution 1% for 24 hours, then filtered and added the ammonia solution to the filtrate until pH=9, then it was extracted several times using chloroform, the chloroform layer was separated and evaporated , after that 2ml of Hydrochloric acid solution 1% was added and few drops of mayer reagent, formation of turbidity indicates the presence of alkaloids[22].
Glycosides: In a test tube 1ml of Hydrochloric acid (1%), was added to 2ml of each extract ,after two hours, filtered and 3ml of chloroform was added to the filtrate ,shaken vigorously, then 1m of ammonia solution 10% was added , occuring of pink colour indicates the presence of glycosides[18].
Cardinols: 1ml of each extract was mixed with 2ml of benzene occuring of a brown color indicates the presence of cardinols[23].
Saponins: About 0.02g of each crude extract was dissolved in 7 ml of distilled boiling water, and shaken vigorously a stable persistent froth indicates the presence of saponins [24].

2.5.Total Antioxidant content assay:

The antioxidant potential of the extracts was assessed by the phosphomolybdenum reduction assay .The reagent solution contained ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (600 mM) mixed with the extracts, the samples were incubated for 60 min at 90°C and the absorbance of the green phosphomolybdenum complex was measured at 695 nm. For reference, the appropriate

solution of ascorbic acid was used and the reducing capacity of the extracts was expressed as the ascorbic acid equivalents. Calibration equation for Ascorbic acid[25].

$y = 3.646x+0.0711$; (R^2=0.9985).

2.6.Total Phenols content Assay:

Total phenol content of the extracts was determined using Folin-Ciocalteau method [15]. This test is based on the oxidation of phenolics groups. The aliquot (400µL) of each extract was mixed with 2 ml of Folin- Ciocalteau reagent and 1.6 ml of 4% sodium carbonate. The mixture was allowed to stand for 2 h with intermittent shaking for reaction. After oxidation, the green-blue complex formed was measured at 750 nm The phenols content was expressed as gallic acid equivalent in % w/w of the extracts[26]. Calibration equation for gallic acid

$y = 2.5731x-0.0074$; (R^2=0.9941).

2.7.Total Flavonoids content Assay:

Aluminum chloride colorimetric technique was used for total flavonoid estimation. Flavonoids are capable of forming complexes with metal ions and act as antioxidants. A known volume (1ml) of the extract was mixed with 3ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415 nm with the help of Perkin Elmer UV Visible Spectrophotometer. The flavonoid content was expressed as Rutin equivalent in % w/w of the extracts[27].Calibration equation for Rutin

$y = 1.13x-0.019$; (R^2=0.9924).

2.8.Pancreatic Lipase Inhibition Assay:

The inhibition of Porcine pancreatic lipase (PPL) activity was measured by using *p*-nitrophenylpalmitate (*p*-NPP) as a substrate the released *p*-nitrophenol, was monitored at 410nm. 1ml of one of of crude extract (0.1 ,to 0.5 mg/ml) or (Orlistat as a positive control)at the concentrations (0.08, to 0.16mg/ml) was mixed with 0.5ml lipase solution (20 mg/ml of PPL type II in 0.05 M Tris-HCL pH 8.5) [28]. It was incubated for 30min at 37°C. then 1ml substrate solution (8mM *p*-NPP in dimethyl formamide)[29], was added into it. After incubating the mixture for 2h at 37 °C, its absorbance was recorded at 410nm against a blank [30].

The inhibitory activity was calculated according to the following formula:

$$\% \text{ of lipase inhibition} = [E-T/E] \times 100$$

Where: E the absorbance of the reaction without crude extract.
T the absorbance of the reaction with crude extract.

2.9.Pancreatic α -amylase Inhibition Assay:

In the test tube was taken 1ml of plant extracts (0.1,to 0.5 mg/ml) (or Acarbose as a positive control) at the concentrations (0.04, to 0.12 mg/ml), and added 1ml of enzyme solution(0.5 unit/ml) was

added (enzyme solution: 0.001g of α- amylase EC 3.2.2.1 in 100ml of 20mM sodium phosphast pH8.5 containing 6.7mM sodium chloride) [31].The mixture was incubated for 30min at 25 °C. 1ml of this mixture was taken and added to 1ml of starch solution(0.5%w/v) as a substrate, the mixture further incubated for 5min at 25 °C, to 1ml of this mixture, 1ml of color reagent was added (reagent solution:1g of 3.5 dinitrosalysic acid DNSA,30g of sodium potassium tartarate added to 20ml of 2N sodium hydroxide and made up to a final volume 100ml with distilled water) [32], after that the mixture placed in a water bath at 85 °C for 15min, then the mixture was removed from a bath water and cooled, 9ml of distilled water was added to the mixture, and the absorbance value determined at 540nm against a blank by spectrophotometer[31].
The inhibitory activity was calculated according to the following formula [33]:

Inhibition (%) = [Abs 540 (control) – Abs 540 (extract)]/ Abs 540(control)×100

2.10.Statistical analysis:

All measurementsofpancreatic lipase inhibition andpancreatic α -amylase inhibitionwere carried out in triplicates. Mean ± SD was used for multivariate analysis (ANOVA) with Tukey's Post hoc test. Difference at $p<0.05$ was considered statistically significant..

RESULTS AND DISCUSSION

3.1.Phytochemical Study:

Results of phytochemical study are shown in Table (1)

Table (1):The chemical content of leaves of *Salvia Officinalis L* .

The extract	MeOH	MeOH70%	EtOH	EtOH70%
Tannins	+++	+	+	+
Steroids	+	++	+	+
Terpenoids	+	+	+	+
Phenols	+++	++	+	+
Flavonoids	++	+++	+	++
Carbohydrates	++	+++	++	+++
Alkaloids	-	-	-	-
Glycosides	++	+++	+	++
Cardinols	-	-	-	-
Saponins	+	++	+	+

+Indicates trace, ++ Indicates the presence, +++ Indicates presence abundantly**.**

The results of phytochemical study of different extracts of leaves of ***Salvia Officinalis L.***showed,that contained: carbohydrates, , flavonoids, , saponins, phenols, steroids, terpenoids, tannins, and glycosides. While the alkaloids and cardinols were absent, the quantity of saponins, flavonoids, glycosides, and carbohydrates were more in the MeOH70% extract in compare with other extracts, while phenols, tannins, were abundant in MeOH extract, also there was a similarity in the chemical composition among the different extracts.

3.2.Total Antioxidant content:

Results of total antioxidant of leaves of *Salvia Officinalis L* .are shown in table(2)

Table (2): The total antioxidant content of different extracts of leaves of *Salvia Officinalis L* .

The extract	The total antioxidant of leaves of *Salvia Officinalis L* . e.q. Ascorbic acid mg/10g
MeOH	102.48±0.30
MeOH70%	133.93±0.36
EtOH	41.96±0.17
EtOH70%	110.53±0.43

The results showed that the different extracts of ***Salvia Officinalis****L* .have a high content of total antioxidant, and the MeOH70% extract has the highest content then the EtOH70% extract comparative with the MeOH and EtOH extracts, while the EtOH extract has the lowest content of the total antioxidant, with a convergence of total content of antioxidant among the MeOH and EtOH70% extracts.

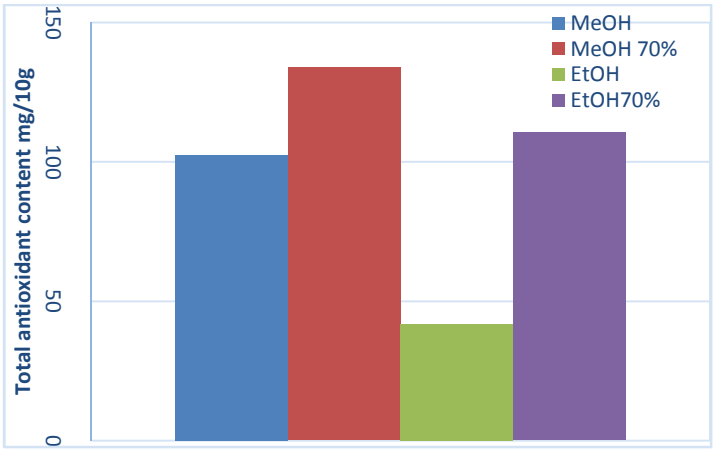


Figure (2): the total antioxidant content of different extracts of leaves of *Salvia Officinalis L.*

3.3. Total Phenols content:

Results of total phenols of leaves of *Salvia Officinalis L* . are shown in table (3).

Table (3): The total phenols content of different extracts of leaves of *Salvia OfficinalisL* .

The extract	The total phenols of leaves of *Salvia OfficinalisL* . e.q. gallic acid mg/10g
MeOH	93.41±0.53
MeOH70%	79.68±0.27
EtOH	29.19±0.12
EtOH70%	72.89±0.75

As showed in the table (3) the different extracts of ***Salvia Officinalis*** *L* .have a good content of total phenols, and the MeOH extract has the highest content then the MeOH70% extract, while the EtOH extract has the lowest content of the total phenols.

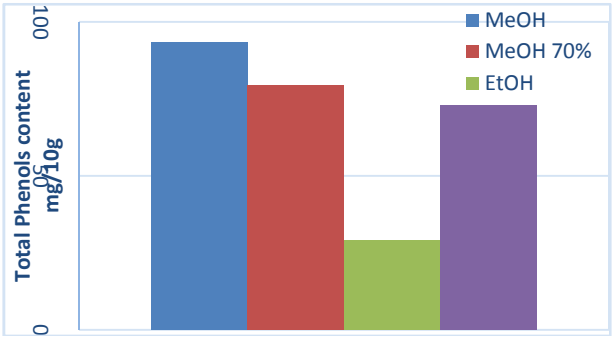


Figure (3): the total phenols content of different extracts of leaves of *Salvia Officinalis L.*

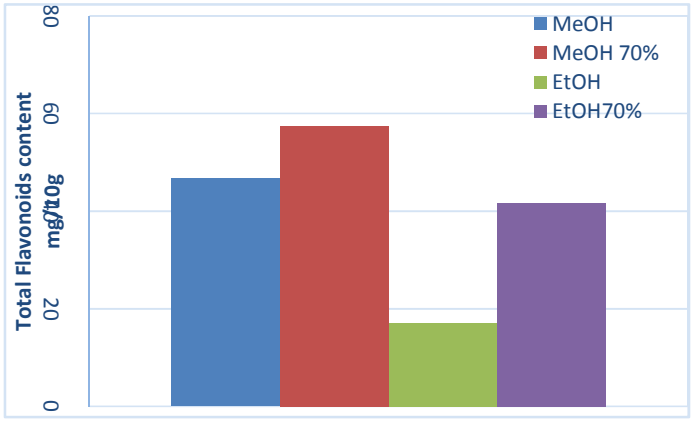
3.4. Total Flavonoids content:

Results of total flavonoids of leaves of *Salvia Officinalis L* . are shown in table(4)

Table (4):The total flavonoids content of different extracts of leaves of *Salvia OfficinalisL* .

The extract	The total flavonoids of leaves of *Salvia OfficinalisL* . e.q. rutin mg/10g
MeOH	46.70±0.13
MeOH70%	57.37±0.79
EtOH	17.08±0.11
EtOH70%	41.69±0.44

The results showed that the different extracts of ***Salvia Officinalis****L* .have a good content of total flavonoids, and the MeOH70% extract has the highest content then the MeOH extract, while the EtOH extract has the lowest content of the total phenols, also we noticed that there is a convergence of total content of flavonoids among MeOH and EtOH70% extracts.



Figure(4): the total flavonoids content of different extracts of leaves of *Salvia Officinalis L.*

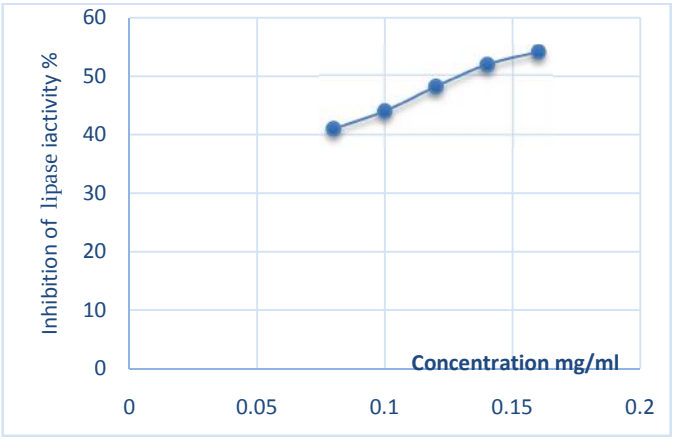
These phytochemical compounds (phenols, flavonoids) are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities in this plant extract used for this study.

3.5.Pancreatic Lipase Inhibition:

The inhibitory activity of positive control- Orlistat on lipase was determined and the IC_{50} value was 0.133 mg/ml, results are shown in Table 5.

Table (5): lipase inhibitory of Orlistat

Concentration mg/ml	Inhibition of lipase activity%
0.08	41.00±0.26
0.1	43.96±0.0.46
0.12	48.07±0.33
0.14	51.75±0.11
0.16	53.87±0.56



Figure(5): lipase inhibitory of Orlistat

Percent of lipase inhibition of the different extracts of the leaves **of *Salvia OfficinalisL.*** shown in table (6)

Table (6): lipase inhibitory of different extracts of leaves of *Salvia Officinalis L.*

Extracts	Concentrations (mg/ml)	Inhibition of lipase activity%
MeOH	0.1	17.44±0.11*
	0.2	29.54±0.47*
	0.3	46.37±011
	0.4	58.37±0.63
	0.5	80.47±0.33*
MeOH 70%	0.1	13.87±0.32*
	0.2	21.76±0.39*
	0.3	39.55±0.75*
	0.4	51.43±0.29
	0.5	73.43±0.66*
EtOH	0.1	8.63±0.05*
	0.2	14.38±0.12*
	0.3	31.47±0.53*
	0.4	46.19±0.78*
	0.5	61.99±0.73*
EtOH 70%	0.1	11.36±0.04*
	0.2	18.49±0.48*
	0.3	35.15±0.27
	0.4	48.12±0.15
	0.5	66.38±0.30*

⋆p<0.05, compared the concentrations of the extracts to the concentrations of Orlistatdata were presented as mean ± SD (n=3).

The results showed that the different extract of leaves of *Salvia Officinalis L.* have a good inhibition lipase activity, and the highest effect on lipase activity was in MeOH extract(IC_{50}0.32 mg/ml) , then MeOH 70% extract (IC_{50}0.36 mg/ml), follow them EtOH 70%extract(IC_{50}0.39 mg/ml) , then EtOH extract(IC_{50}0.42 mg/ml), also the inhibition lipase activity was increased as concentration of crude extracts increased.

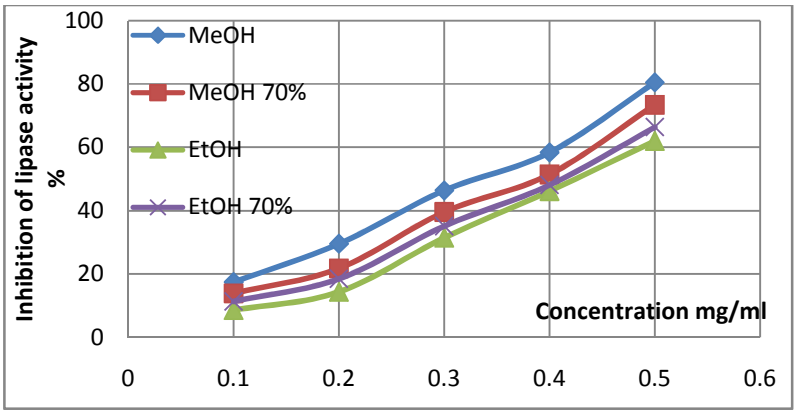


Figure (6): lipase inhibitory of different extracts of leaves of *Salvia Officinalis L.*

The results in table (6) indicate that the extracts [MeOH,MeOH70%,EtOH,EtOH70%] of *Salvia OfficinalisL.* contain a good percentage of compounds that can have inhibitory effect of lipase activity as the phenols, flavonoids, polyphenols, tannis and saponins which have shown many studies that these compounds have a good inhibitory lipase activity [34],[35]. The results in table(3) showed that the MeOH extract rich in phenols, therefore these compounds have a inhibitory effect on lipase activity more than other compounds such as flavonoids.

3.6.Pancreatic α -amylase Inhibition:

The inhibiton activity of positive control- Acarbose on α -amylase was determined and the IC50 value was 0.099 mg/ml ,the results are shown in Table 7.

Table (7):inhibitory of α -amylase Acarbose

Concentration mg/ml	Inhibition of α-amylase activity%
0.04	27.03±0.40
0.06	32.94±0.46
0.08	40.76±0.29
0.10	51.17±0.43
0.12	58.37±0.63

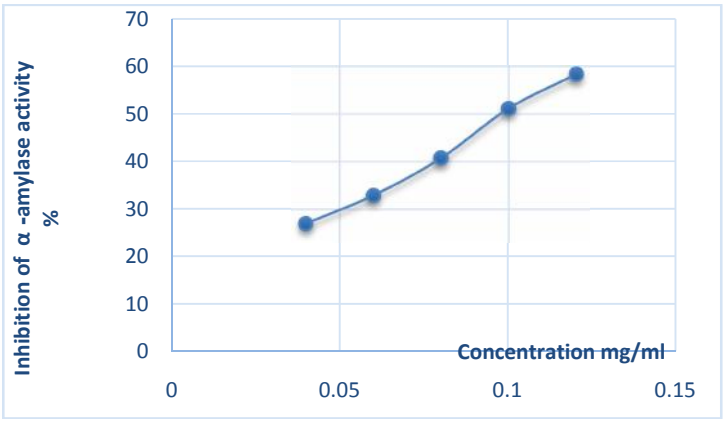


Figure (7): α -amylase. inhibitory of Acarbose

Percent ofα -amylase inhibition of the different extracts of the leaves of *Salvia OfficinalisL.* are shown in the table [8].

Table (8): α- amylase inhibitory of different extracts of leaves of *Salvia Officinalis L.*

Extracts	Concentrations mg/ml	Inhibition of α-amylase activity% leaves
MeOH	0.1	15.65±0.10*
	0.2	33.41±0.54
	0.3	47.28±0.75*
	0.4	68.39±0.49*
	0.5	80.21±0.56*
MeOH 70%	0.1	21.66±0.40*
	0.2	38.16±0.29*
	0.3	53.65±0.55*
	0.4	71.42±0.11*
	0.5	86.10±0.33*
EtOH	0.1	10.33±0.07*⋆
	0.2	18.50±0.43*
	0.3	35.31±0.11*
	0.4	50.79±0.83
	0.5	71.21±0.21*
EtOH70%	0.1	13.50±0.04*
	0.2	27.66±0.65*
	0.3	41.49±0.17
	0.4	60.37±0.44*
	0.5	76.85±0.30*

⋆p<0.05, compared the concentrations of the extracts to the concentrations of Acarbose data were presented as mean ± SD (n=3)

The different extracts of *Salvia OfficinalisL* **.**have good inhibition α- amylase activity , The highest inhibition was in MeOH70% extract (IC_{50}0.31 mg/ml, then MeOH extract (IC_{50}0.34 mg/ml,), followed by EtOH 70%extract,and finally EtOH extract, also we noticed that the inhibition of α- amylase activity was increased as concentration of extracts increased.

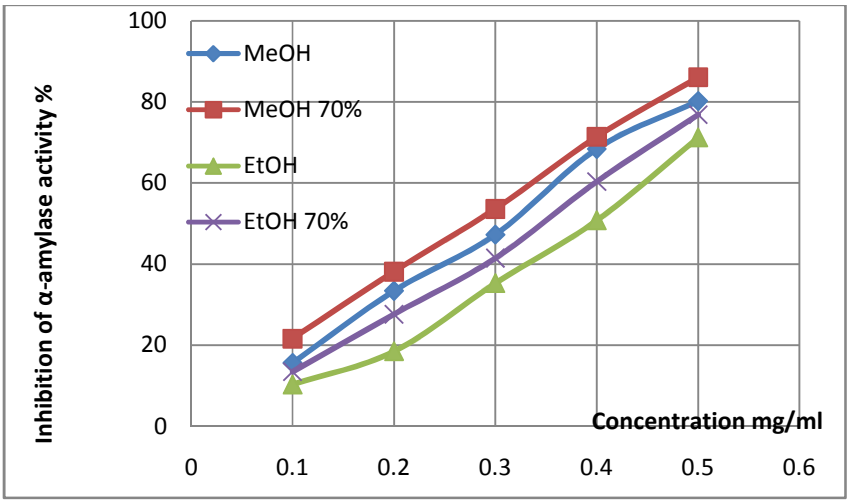


Figure (8): α – amylase inhibitory of different extracts of leaves of *Salvia Officinalis L.*

The results in table (8) indicate that the extracts [MeOH,MeOH70%,EtOH,EtOH70%] of *Salvia OfficinalisL.* contain a high percentage of compounds that can have inhibitory effect of α – amylase activity as the phenols, flavonoids, polyphenols, tannis and saponins which have shown many studies that these compounds have a good inhibitory lipase activity [36].
The results in table(4) showed that theMeOH70% extract rich in flavonoids, therefore these compounds have a inhibitory effect on α – amylase activity more than other compounds such as phenols.

Conclusions

The study of leaves of *Salvia Officinalis L.* has indicated that; this plant is rich with phenols, carbohydrates, , flavonoids, , saponins, , tannins, Steroids, Terpenoids, and glycosides. While it has shown the absence of alkaloids and cardinols, also we noticed that the leaves of *Salvia OfficinalisL.* have a good percentage of antioxidants compounds, phenols, and flavonoids, itcan be concluded that the leaves of *Salvia Officinalis L.* has a good anti-obesity effect and can be used as a natural sources for slimming instead of using other chemical products, which are known with their many harmful effect on health.

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