International Journal of Academic Scientific Research
ISSN: 2272-6446 Volume 6, Issue 2 (May - June 2018), PP 01- 21
www.ijasrjournal.org

Phytochemical Screening of *Alchemilla Vulgaris, Sophora Japonica , Crataegus Azarolus*, and Their Inhibitory Activity on Lipase and α-Amylase

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Abstract: *Phytochemical screening of Alchemilla Vulgaris (leaves and flowers) ,Sophora Japonica, and Crataegus Azarolus (leaves and fruits) , have been determined, and the results showed that the different plant parts of the studied plants contained the following compounds: phenols, carbohydrates, , flavonoids, , saponins, , tannins, and glycosides. While it has shown the absence of alkaloids and cardinols, except the leaves ofCrataegus Azarolus that contained alkolois.The effect of Alchemilla Vulgaris (leaves and flowers),Sophora Japonica, and Crataegus Azarolus (leaves and fruits) on lipase activity and α –amylase activity were screened by used different extracts[MeOH; MeOH 70% EtOH ; EtOH70% ; Hexane ; Chloroform]. to test their anti-obesity activity using porcine pancreatic lipase inhibitory assay, and porcine pancreaticα –amylase. The results showedthat the extract obtained withpolarsolvents have a goodinhibitory activity, and the highest effect on lipase activity was in the MeOH extractsbut the highest effect onα –amylase activity was in the MeOH 70% extracts,on the contrary the non-polar solvents (Hexane ; Chloroform) have avery weak effect on lipase and α –amylase activity,also we showed that the polar extracts of Alchimalla Vulgaris (leaves and flowers) have the highest effect on lipase activity and α –amylase activity comparative with the extracts of Sophora Japonica , and Crataegus Azarolus (leaves and fruits)These results suggest that the chemical content of polar extracts of these plants might be of therapeutic interest with respect to the treatment of obesity.*

Keywords: *lipase, α-Amylase, inhibition , obesity, Alchemilla Vulgaris, Sophora Japonica , Crataegus Azarolus.*

1. INTRODUCTION

Obesity is a disease resulting from the excessive accumulationof body fat, and brings multiple outcomes for health, such as theprevalence and progression of cardiovascular diseases, which were the major causes of deathin 2012; Some types of cancer (endometrium, breast and colon);skeletal muscle disturbs (specially osteoarthritis – a highly incapacitating degenerative disease); hypertension and type 2 diabetes mellitus[1].One way to fight this epidemic disease is drug treatment.Medicine to fight weight gain, which has the objective to restrict absorption and cause weight loss, is widely available[2]. However, these drugs cause side effectsand are prohibited by Anvisa since 2011,anotheralternative broadly employed is the use of plant extracts. Over thelast years, there was a substantial increase in their use, by the factthat the population believes plants intake is harmless, with a low cost,and may inhibit digestive enzymes, leading to beneficial changes inmetabolism [3]. However not all natural products are beneficial and more studies are necessary to evaluate theireffects and safety. α-amylase is one of the enzymes that responsible forprocessing dietary carbohydrates, acts on starch breakdown, resulting in monosaccharide absorption by enterocytes. Therefore, its inhibition offers a promising strategy for the prevention of obesity,as well as type 2 diabetes,

by inhibiting starch breakdown and glucose absorption in the small intestine [4;5].Lipase, involved in fat metabolism, is also an important targetfor inhibitors, since its inhibition limits triacylglycerol absorption,leading to a decrease in caloric yield and weight loss.
Research has been carried out for evaluating the effects of natural products on the treatment of obesity and associated comorbidities, reinforcing the need for the search of new sources of α-amylase and lipase inhibitors[6;7]. Therefore, digestive inhibitors who assist in reducing fat and carbohydrate absorption in the small intestine may be useful helpers in the treatment of obesity. Natural products have been gaining space and importance in the pharmaceutical industry, since they have bioactive substances capable of inspiring new phytomedicines and phytotherapic products.

2. 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, Hexane, Dimethylesulfoxide (DMSO), were purchased from Merk, Germany Orlistat,Acarbose , *p*-nitrophenyl palmitate (NPP),Lipase type II and α-AmylaseEC 3.2.2.1 from porcine pancreas,Strach,3,5 dinitrosalysicacid were purchased from Sigma Aldrich.

Equipments: Ultrasonic Bath (Hwashin،Power Sonic 405), Rotary evaporator (Heidolph Laborata 4000, Germany), UV-Vis spectrophotometer (Jasco, V-650, Japan),

2.2.Plant material

The leaves and flowers of *Alchemilla Vulgaris*were collected form Hama Al Salamya in Syria, the leaves and fruits of *Crataegus Azarolus* were collected from Sarakeb-Idleb in Syria, and the leaves and fruits of *Sophora Japonica* were collected from Aleppo-Syria.Identification of plants maternal was done by the dipartites inthe Agriculture faculty- Aleppo University.
plant parts were washed , and dried in shade at ambient temperature (25-30°C) for 15-20 days , then they were ground into fine powder**.**

A **B** **C**

Figure 1. leaves and flowers of Alchemilla vulgaris , laves and fruit of *Crataegus Azarolus* and *Sophora Japonica*

A: Leaves and flowers of Alchemilla vulgaris.
B. Leaves and fruit of Crataegus Azarolus.
C: Leaves and fruit of Sophora Japonica.

2.3. Extracts Preparation

3g from each dry parts of plants were extracted with 100ml of [MeOH, MeOH 70%, EtOH, EtOH 70%, Chloroform, and Hexane], three times,then the extractscombiended vaporated 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 inhibitioncrude extracts were dissolved in DMSO that did not affect enzyme activity[8].

2.4.Chemical Content Study

Flavonoids:
2ml of each extract was mixed with 1ml of the 5% ethanolic aluminum chloride, occuring of yellow colour indicates the presence of Flavonoids [9,10].

Phenols:
2ml of each extract was mixed with 1ml of the ferric chloride solution,occuring of blue or green colour indicates the presence of phenols [11].

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 adedd to the filtrate ,shaken vigorously,then 1m of ammonia solution 10% was added ,occuring of pink colour indicates the presence of glycosides[11].

Alkaloids:
About 0.1 g of each crude extract was soaked in Hydrochloric acidsolution 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 acidsolution 1% was added and few drops of mayer reagent, formation of turbidity indicates the presence of alkaloids[12].

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[13].

Tannins:
About 0.02 g of each crude extract was dissolved in 10 ml of distilled water in atest 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[10].

Cardinols:
1ml of each extract was mixed with 2ml of benzene occuring of a brown color indicates the presence of cardinols[14].

Saponins:
About 0.02g of each crude extract was dissolved in 7 ml of distilled boiling water, and and shaken vigorously a stable persistent froth indicates the presence of saponins[15].

2.5.Pancreatic Lipase Inhibition Assay

The Porcine pancreatic lipase (PPL) inhibitory activity was measured using *p*-nitrophenyl palmitate (*p*-NPP) as a substrate the released*p*-nitrophenol, was monitored at 410nm.
1ml of one of five different concentrations(0.1 ,to 0.5 mg/ml) of the crude extract or (Orlistat as a positive control)at the concentrations (0.06, to 0.14 mg/ml) was mixed with 0.5ml lipase solution. It was incubated for 30min at 37°C. then 1ml substrate solution was added into it. After incubating the mixture for 2h at 37 °C, its absorbance was recorded at 410nm against a blank.[16]

- Enzyme solution: 20 mg/ml of PPL type II in 0.05 M Tris-HCL pH 8.5 [17].
- Substrate solution: 8mM *p*-NPP in dimethyl formamide[18].

The inhibitory activity was calculated according to the following formula:
% of lipase inhibition= [E-T/E] × 100
Where: E the absorbance of the reaction with out crude extract.
T the absorbance of the reaction with crude extract.

2.6.Pancreatic α -amylase Inhibition Assay

1ml of enzyme solution was added to 1ml of plant extracts (0.1,to 0.5 mg/ml) (or Acarbose as a positive control) at the concentrations (0.02, to 0.1 mg/ml),and incubated for 30min at 25 °C, then 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 , after that the mixture placed in a water bath at 85 °C for 15min, then the mixture was removed from abath water and cooled,9ml

of distilled water was added to the mixture, and the absorbance value determined at 540nm against a blank by spectrophotometer[19].

- Enzyme solution (0.5unit/ml): 0.001g of α- amylase EC 3.2.2.1 in 100ml of 20mM sodium phosphast pH8.5 containing 6.7mM sodium chloride [19].
- Color reagent solution: (1g of 3.5 dinitro salysic 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) [20].

The inhibitory activity was calculated according to the following formula [21]:
Inhibition (%) = [Abs 540 (control) – Abs 540 (extract)]/ Abs 540(control)×100

2.7.Statistical analysis

All measurements were 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..

3. RESULTS AND DISCUSSION

3.1.Phytochemical Study

Results of phytocemical study are showin in Tables(1,2,3)

Table 1. The chemical content of leaves and flowers of *Alchemilla Vulgaris*

The extract	MeOH		MeOH70%		EtOH		EtOH70%		Hexane		Chloroform	
The part of plant	**leaves**	**flowers**	**leaves**	**flowers**	**leaves**	**flowers**	**leaves**	**flowers**	**leaves**	**flowers**	**leaves**	**flowers**
Flavonoids:	++	+	+++	++	+	+	+	+	-	-	-	-
Phenols:	+++	++	++	+	+	+	+	+	-	-	-	-
Glycosides:	++	+	+++	++	+	+	++	+	-	-	-	-
Alkaloids:	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates:	+	+	+++	++	++	++	++	+	-	-	-	-
Tannins:	+++	+++	++	++	+	+	++	+	-	-	-	-
Cardinols:	-	-	-	-	-	-	-	-	-	-	-	-
Saponins:	+++	+++	+	+	+	+	++	+	-	-	-	-

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

There was similarity in chemical compesition in both leaves and flowers of *Alchemilla Vulgaris*, contained phenols, carbohydrates, , flavonoids, , saponins, , tannins, and glycosides. Whilethe alkaloids and cardinols were absent, the quantity of flavonoids,glycosides,and carbohydrates were more in the MeOH70% extracts in compare with other extracts,while phenols,tannis, and saponins were abundant in MeOH extracts, also there was a similarity in the chemical composion between the MeOH extracts, and EtOH extracts.

Table 2. The chemical content of leaves and fruit of *Crataegus Azarolus*

The extract	MeOH	MeOH70%	EtOH	EtOH70%	Hexane	Chloroform

The part of plant	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**
Flavonoids:	+	+	++	+	+	+	+	+	-	-	-	-
Phenols:	+++	++	+	+	+	+	++	+	-	-	-	-
Glycosides:	+	+	+++	++	+	+	++	+	-	-	-	-
Alkaloids:	++	-	+++	-	+	-	+	-	-	-	-	-
Carbohydrates:	+	+	+++	++	++	++	+	+	-	-	-	-
Tannins:	+++	+++	+	+	+	-	+	-	-	-	-	-
Cardinols:	-	-	-	-	-	-	-	-	-	-	-	-
Saponins:	++	++	+	+	+	+	+	+	-	-	-	-

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

As showen in table 2 leaves and fruit of *Crataegus Azarolus*have a similarity in chemical composition, where it contained phenols, carbohydrates, , flavonoids, , saponins, , tannins, and glycosides,and the cardinols were absent,while alkaloids were found only in leaves.the quantity of flavonoids,glycosides, alkaloids and carbohydrates were larger in the MeOH70% extracts in compare with other extracts,while phenols,tannis, and saponins were abundant in MeOH extracts,the MeOH extracts, and EtOH extracts were similar in chemical composition.

Table 3. The chemical content of leaves and fruit of *Sophora Japonica*

The extract	MeOH		MeOH70%		EtOH		EtOH70%		Hexane		Chloroform	
The part of plant	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**	**leaves**	**fruit**
Flavonoids:	+	+	+	++	+	+	+	+	-	-	-	-
Phenols:	+	++	+	+	+	+	+	+	-	-	-	-
Glycosides:	+	+	+	++	+	+	+	+	-	-	-	-
Alkaloids:	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates:	+	++	++	+++	+	+	+	++	-	-	-	-
Tannins:	++	++	+	+	+	+	+	+	-	-	-	-
Cardinols:	-	-	-	-	-	-	-	-	-	-	-	-
Saponins:	++	++	+	+	-	+	+	+	-	-	-	-

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

Both Leaves and fruit of*Sophora Japonica*were contained phenols, carbohydrates, , flavonoids, , saponins, tannins, and glycosides,and the cardinols and alkaloids were absent.The quantity of flavonoids,glycosides, and carbohydrates were more in the MeOH70% extracts, in compare with other extracts, while phenols,tannis, and saponins were abundant in MeOH extracts, also we noticed that was a smilarity in a chemical composition between MeOH and EtOH extracts.

3.2.Pancreatic Lipase Inhibition

The inhibitory activity of positive control- Orlistat on lipase was determined and the IC_{50} value was 0.135 mg/ml, results are shown in Table 4.

Table 4. lipase inhibitory of Orlistat

Concentration mg/ml	Inhibition of lipase activity%
0.06	38.20±0.19
0.08	41.39±0.29
0.1	43.13±0.26
0.12	47.43±0.14
0.14	51.22±0.33

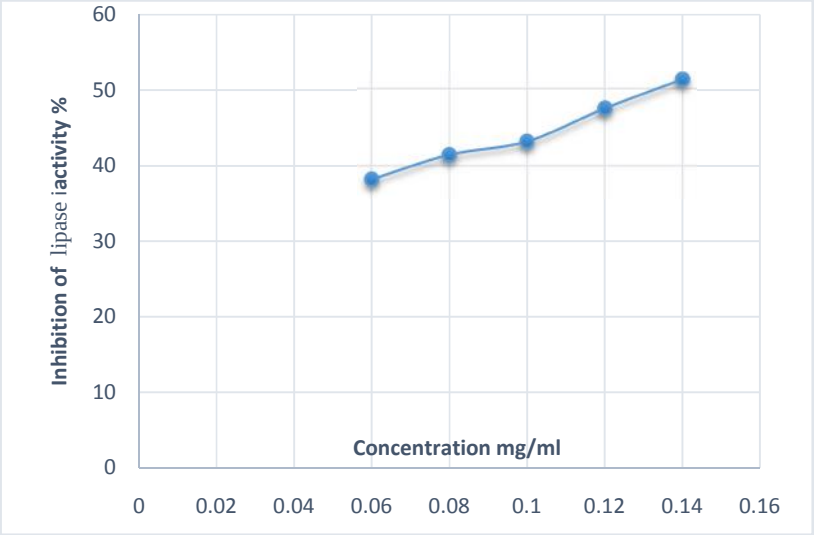

Figure 2. lipase inhibitory of Orlistat

Percent of lipase inhibition of the different extracts of the leaves and flowers of *Alchemilla vulgaris*, leaves and fruit of*Sophora Japonica* and leaves and fruit Crataegus Azarolusare showen in tables[5,6,7] . The inhibition lipase activity of extracts of leaves of *Alchemilla vulgaris*wasmore than the extracts of flowers,and the polar extracts of the leaves and flowers of *Alchemilla vulgaris* (MeOH, MeOH70%,EtOH, EtOH70%) have good inhibition of lipase activity, and the highest effect was in MeOH extracts (IC_{50} of leaves 0.26 mg/ml, IC_{50} of flowers 0.29 mg/ml) then MeOH70% extracts (IC_{50} of leaves 0.29 mg/ml, IC_{50} of flowers 0.34 mg/ml), the EtOH 70% and EtOH extracts of leaves and flowers have lower inhibition lipase activity than MeOH and MeOH70% extracts, on the other hand Chloroform and Hexane extracts showed a weak inhibition lipase activity for both leaves and flowers, also we noticedthe inhibitory of lipase activity was increased as concentration of crude extracts increased.

Table 5. lipase inhibitory of different extracts of leaves and flowers of *Alchemilla Vulgares*

Extracts	Concentrations mg/ml	Inhibition of lipase activity% leaves	Inhibition of lipase activity% flowers
MeOH	0.1	21.56±0.43*	19.40±0.35*
	0.2	38.20±0.53	30.61±0.51*
	0.3	60.11±0.57*	52.81±0.56*
	0.4	71.31±0.71*	68.21±0.42*
	0.5	86.25±0.32*	80.56±0.47*
MeOH 70%	0.1	16.21±0.49*	14.66±0.27*
	0.2	29.53±0.55*	25.31±0.78*
	0.3	51.81±0.68*	48.53±0.44*
	0.4	67.36±0.48*	56.21±0.32
	0.5	79.01±0.52*	72.20±0.44*
EtOH	0.1	11.33±0.78*	9.96±0.78*
	0.2	19.92±0.49*	15.83±0.48*
	0.3	37.52±0.83*	28.21±0.37*
	0.4	57.77±0.54*	42.66±0.61
	0.5	64.28±0.72*	59.27±0.42
EtOH70%	0.1	13.66±0.65*	11.38±0.27*
	0.2	24.51±0.43*	19.58±0.30*
	0.3	39.96±0.39	35.01±0.24*
	0.4	61.40±0.48*	52.79±0.87*
	0.5	74.00±0.88*	68.36±0.48*
Hexane	0.1	1.56±0.23**	1.20±0.13**
	0.2	2.12±0.42**	1. 97±0.17**
	0.3	2.89±0.28**	2.25±0.21**
	0.4	3.21±0.37**	2.88±0.31**
	0.5	3.67±0.41**	3.34±0.18**
Chloroform	0.1	2.31±0.37**	1.47±0.11**
	0.2	3.10±0.26**	2.88±0.26**
	0.3	3.92±0.44**	3.11±0.21**
	0.4	4.35±0.31**	3.65±0.34**
	0.5	4.68±0.53**	4.11±0.29**

⋆p<0.05, ⋆⋆ p<0.001compared the concentrations of the extracts to the concentrations of Orlistat from the lowest concentration to the top, data were presented as mean ± SD (n=3).

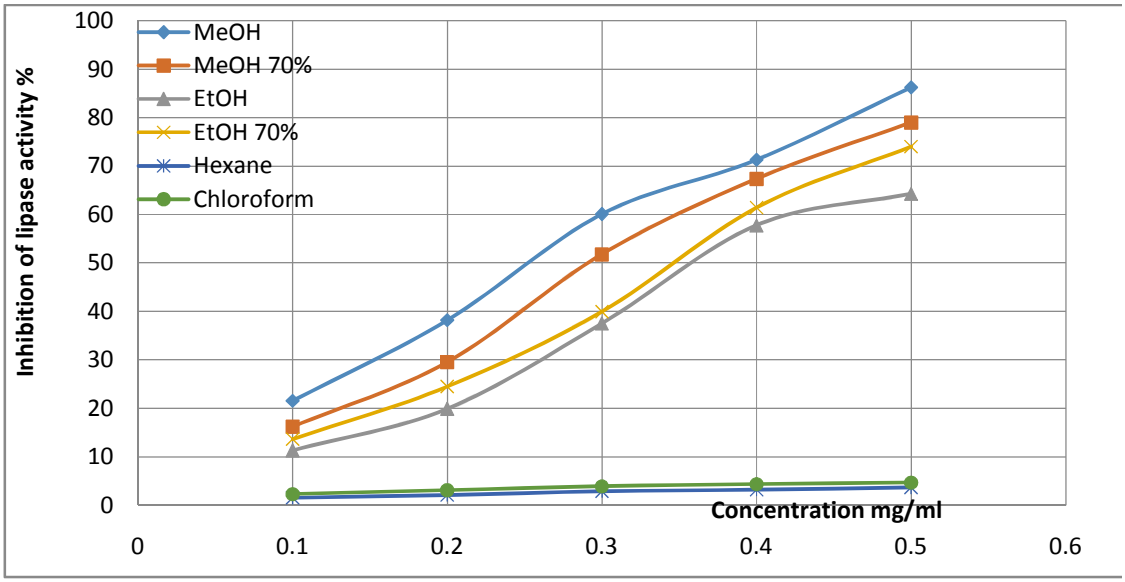

Figure 3 lipase inhibitory of different extracts of leaves of *Alchemilla Vulgares*

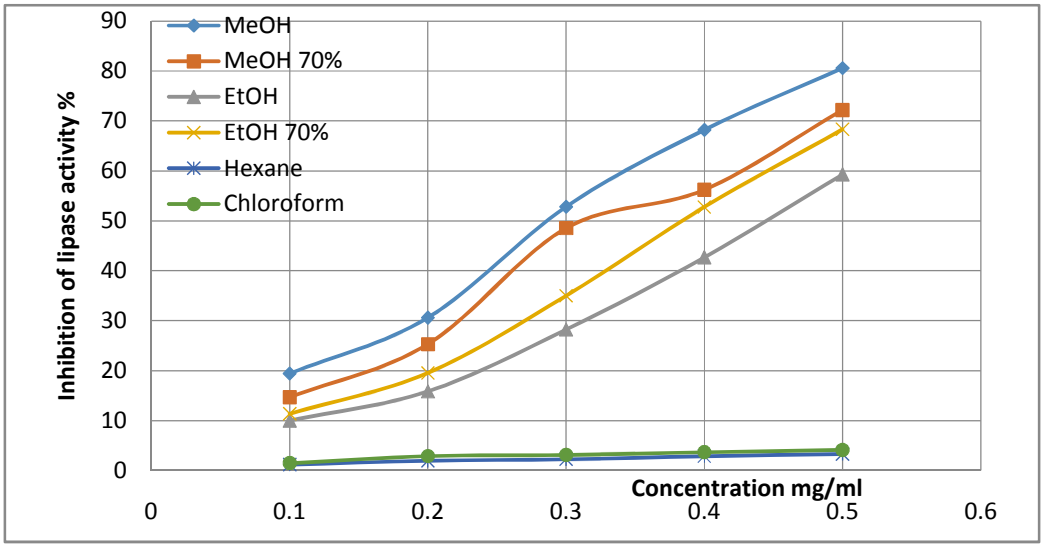

Figure 4 lipase inhibitory of different extracts of flowers of *Alchemilla Vulgares*

Table 6. lipase inhibitory of different extracts of leaves and fruit of *Crataegus Azarolus*

Extracts	Concentrations mg/ml	Inhibition of lipase activity% leaves	Inhibition of lipase activity% fruit
MeOH	0.1	16.25±0.53*	13.55±0.28*
	0.2	27.36±0.48*	20.14±0.81*
	0.3	37.72±0.28	31.05±0.62*
	0.4	52.66±0.58	44.21±0.39
	0.5	70.28±0.6*	51.09±0.43
MeOH 70%	0.1	12.08±0.37*	9.38±0.33*
	0.2	23.66±0.74*	16.69±0.63*
	0.3	31.65±0.29*	26.65±0.72*
	0.4	46.21±0.73	37.26±0.32*
	0.5	61.07±0.62*	45.21±0.51
EtOH	0.1	8.13±0.31*	5.84±0.67*
	0.2	12.86±0.18*	11.00±0.75*
	0.3	22.62±0.91*	18.31±0.50*
	0.4	36.23±0.48*	27.66±0.81*
	0.5	56.80±0.59	39.56±0.12*
EtOH70%	0.1	11.41±0.85*	7.61±0.41*
	0.2	21.38±0.39*	14.36±0.35*
	0.3	29.30±0.69*	24.46±0.77*
	0.4	43.10±0.48	33.81±0.62*
	0.5	59.11±0.61	42.10±0.19
Hexane	0.1	0.73±0.13**	0.56±0.08**
	0.2	1.08±0.19**	0.76±0.18**
	0.3	1.43±0.22**	0.92±0.13**
	0.4	1.89±0.46**	1.18±0.21**
	0.5	2.10±0.11**	1.42±0.20**
Chloroform	0.1	1.16±0.17**	0.61±0.11**
	0.2	1.62±0.25**	0.95±0.16**
	0.3	2.25±0.36**	1.28±0.25**
	0.4	2.91±0.71**	1.59±0.37**
	0.5	3.12±0.31**	1.87±0.14**

⋆ $p<0.05$, ⋆⋆ $p<0.001$compared the concentrations of the extracts to the concentrations of Orlistat from the lowest concentration to the top, data were presented as mean ± SD (n=3).

The inhibition lipase activity of The extracts of leaves of *Crataegus Azarolus*was more than the extracts of fruit, also we noticed that the polar extracts of the leveas and fruit of *Crataegus Azarolus* have good inhibition lipase activity, and the highest effect on lipase activity was in MeOH extracts(IC_{50} of leaves 0.36 mg/ml, IC_{50} of fruit 0.48 mg/ml) , then MeOH 70% extracts (IC_{50} of leaves 0.42 mg/ml, IC_{50} of fruit 0.54 mg/ml), follow them EtOH 70% extracts, then EtOH extracts of leaves and fruit , but the lowest effect on lipase activity was observed in Hexane and Chloroform extracts. also the inhibition lipase activity was increasedas concentrationof crude extracts increased.

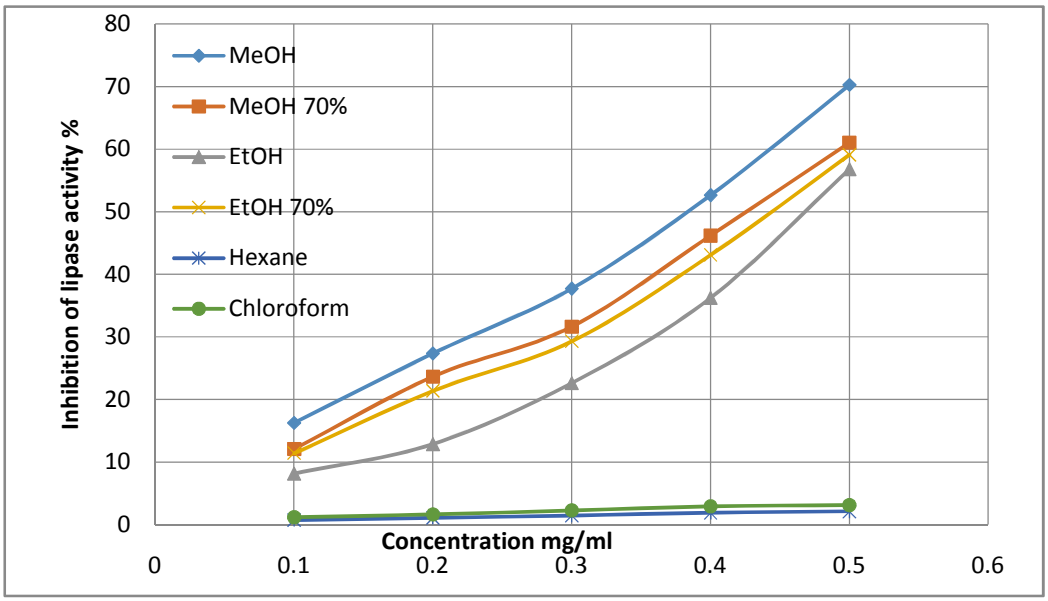

Figure 5. lipase inhibitory of different extracts of leaves of *Crataegus Azarolus*

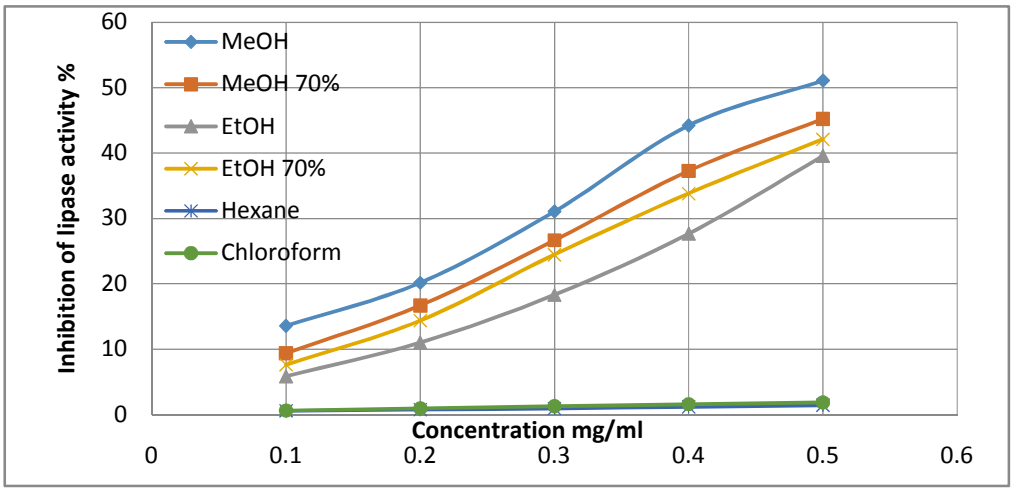

Figure 6. lipase inhibitory of different extracts of fruit of *Crataegus Azarolus*

The inhibition lipase activity of different extracts of fruit of *Sophora japonica*wasmore than the extracts of leaves, and the polar extracts of the leveas and fruit of *Sophora japonica*have good inhibition lipase activity, The highest inhibition of lipase activity was in MeOH extracts(IC_{50} of leaves 0.46 mg/ml, IC_{50} of fruit 0.40 mg/ml) then MeOH 70% extracts (IC_{50} of leaves 0.49 mg/ml, IC_{50} of fruit 0.46 mg/ml)follow them EtOH 70% and EtOH extracts of leaves and fruit, the lowest effect on lipase activity was showen in Hexane and Chloroform extracts. also it was clear that the inhibition lipase activity increased as concentration of crude extracts increased.

Table 7. lipase inhibitory of different extracts of leaves and fruit of *Sophora Japonica*

Extracts	Concentrations mg/ml	Inhibition of lipase activity% leaves	Inhibition of lipase activity% fruit
MeOH	0.1	10.21±0.64*	15.16±0.33*
	0.2	19.93±0.37*	26.21±0.45*
	0.3	27.20±0.51*	33.81±0.71
	0.4	40.21±0.55	48.06±0.78
	0.5	58.06±0.81	64.56±0.59*
MeOH 70%	0.1	9.66±0.62*	10.56±0.36*
	0.2	17.63±0.47*	20.51±0.48*
	0.3	24.83±0.23*	27.32±0.12*
	0.4	36.81±0.56*	39.22±0.82
	0.5	54.14±0.32	58.29±0.17
EtOH	0.1	5.11±0.70*	7.23±0.34*
	0.2	10.23±0.48*	16.21±0.19*
	0.3	17.07±0.37*	24.44±0.83*
	0.4	30.66±0.51*	36.61±0.51
	0.5	46.96±0.22	54.92±0.61
EtOH70%	0.1	7.81±0.66*	9.55±0.77*
	0.2	11.08±0.41*	19.81±0.85*
	0.3	20.36±0.58*	25.95±0.30*
	0.4	32.24±0.33*	36.14±0.52*
	0.5	49.00±0.28	56.02±0.23
Hexane	0.1	0.11±0.07**	0.59±0.12**
	0.2	0.24±0.02**	0.86±0.11**
	0.3	0.38±0.05**	1.17±0.23**
	0.4	0.49±0.09**	1.46±0.20**
	0.5	0.73±0.15**	1.83±0.16**
Chloroform	0.1	0.26±0.06**	0.67±0.14**
	0.2	0.31±0.12**	1.03±0.26**
	0.3	0.54±0.10**	1.61±0.17**
	0.4	0.82±0.01**	2.01±0.31**
	0.5	1.13±0.31**	2.49±0.24**

***** $p < 0.05$, ** $p<0.001$ compared the concentrations of the extracts to the concentrations of Orlistat from the lowest concentration to the top, data were presented as mean ± SD (n=3).

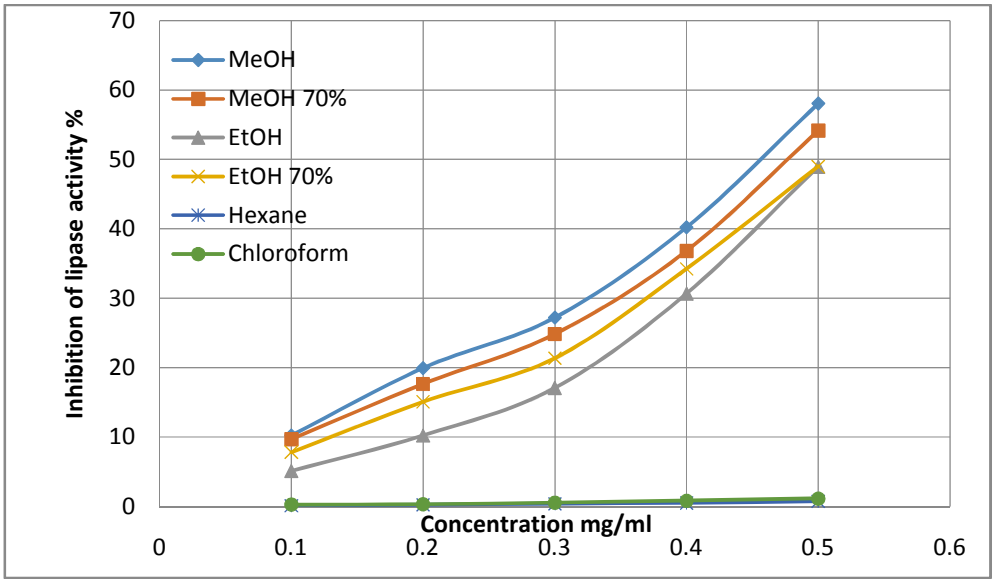

Figure 7. lipase inhibitory of different extracts of leaves of Sophora Japonica

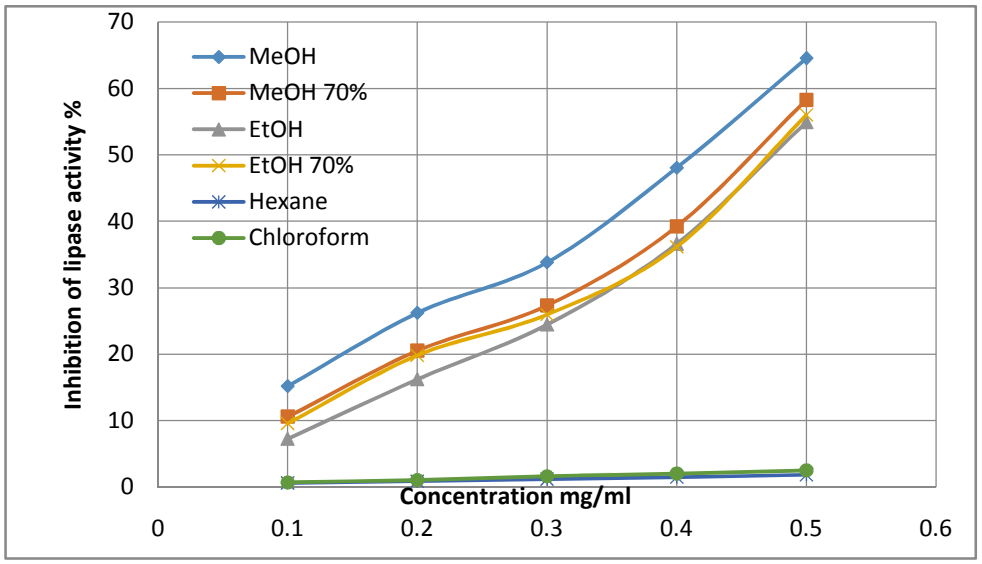

Figure 8. lipase inhibitory of different extracts offruit of Sophora Japonica

Through a comprehensive comparison of all results contained in the table 5,6,7 we found that the polar extracts of *Alchemilla Vulgaris* (leaves and flowers) ,*Sophora Japonica* , and*Crataegus Azarolus* (leaves and fruits) have a good inhibition of lipase activity and the highest effect was in MeOH extract, also we noticed the non-polar extracts (Hexane, Chloroform) have a very weak inhibition lipase activity, also we noticed that the extracts of *Alchemilla vulgaris* (leaves and flowers) have a highest effect on lipase activity comparing with *Crataegus Azarolus*and *Sophora Japonica*(leaves and fruits)

These results indicate that the extracts which prepared by polar solvents as[MeOH,MeOH70%,EtOH,EtOH70%] contain a good percentage of compounds that can have inhibitory effect of lipase activity as the phenols,polyphenols, and saponins which are known to be found in a good proportion in these extracts of various plants, which have shown many studies that these compounds have a good inhibitory lipase activity [22],[23].

3.3.Pancreatic α -amylase Inhibition

The inhibiton activity of positive control- Acarbose on α -amylase was determined and the IC50 value was 0.10 mg/ml ,the results are shown in Table 8.

Table 8. inhibitory of α -amylase Acarbose

Concentration mg/ml	Inhibition of α-amylase activity%
0.02	18.25±0.16
0.04	27.70±0.53
0.06	33.88±0.39
0.08	40.12±0.48
0.1	50.31±0.57

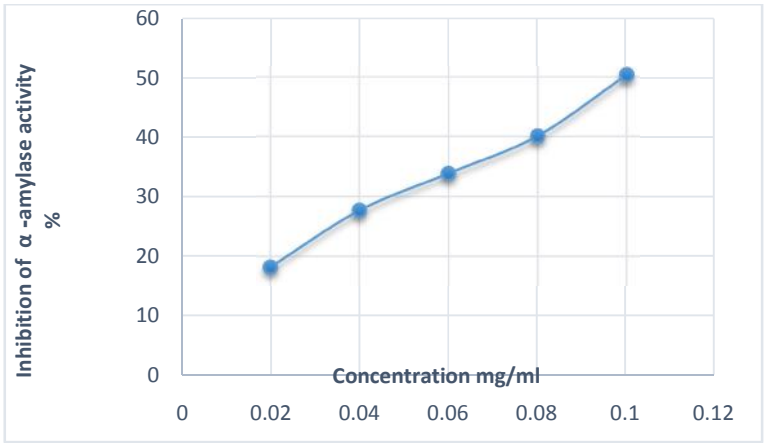

Figure 9 . inhibitory of α -amylase Acarbose

Percent of α -amylase inhibition of the different extracts of the leaves and flowers of *Alchemilla vulgaris*, leaves and fruit of *Sophora Japonica*, and*Crataegus Azarolus*are showen in tables[9,10,11].

Table 9. α- amylase inhibitory of different extracts of leaves and flowers of *Alchemilla Vulgares*

Extracts	Concentrations mg/ml	Inhibition of α-amylase activity% leaves	Inhibition of α-amylase activity% flowers
MeOH	0.1	12.50±0.37*	10.26±0.28*
	0.2	26.11±0.64	18.30±0.47*
	0.3	37.62±0.31	28.41±0.73
	0.4	63.34±0.52*	51.65±0.26*
	0.5	76.81±0.62*	67.36±0.58*
MeOH 70%	0.1	18.36±0.48	13.16±0.61
	0.2	33.11±0.34	25.51±0.53
	0.3	42.20±0.73*	36.40±0.42
	0.4	65.81±0.50*	58.20±0.81*
	0.5	82.31±0.46*	71.11±0.64*
EtOH	0.1	8.40±0.28*⋆	6.51±0.16*
	0.2	15.22±0.61*	11.46±0.36*
	0.3	29.28±0.49	21.72±0.56*
	0.4	46.34±0.66	39.63±0.44
	0.5	60.54±0.81*	52.60±0.77
EtOH70%	0.1	11.36±0.34*	9.83±0.22*
	0.2	20.87±0.30*	15.17±0.51*
	0.3	34.19±0.72	26.12±0.30
	0.4	56.32±0.68*	45.07±0.43
	0.5	68.77±0.57*	57.18±0.78
Hexane	0.1	1.16±0.13**	0.87±0.07**
	0.2	1.76±0.27**	1.21±0.13**
	0.3	2.12±0.17**	1.78±0.32**
	0.4	2.73±0.42**	2.23±0.35**
	0.5	3.16±0.31**	2.81±0.14**
Chloroform	0.1	1.42±0.29**	1.18±0.21**
	0.2	2.03±0.24**	1.87±0.31**
	0.3	2.65±0.35**	2.31±0.56**
	0.4	3.10±0.44**	2.89±0.22**
	0.5	3.72±0.39**	3.26±0.48**

⋆p<0.05, ⋆⋆ p<0.001compared the concentrations of the extracts to the concentrations of Acarbose from the lowest concentration to the top, data were presented as mean ± SD (n=3).

The inhibition α- amylase activity of leaves extracts of *Alchemilla vulgaris* was higher than the flower extracts, and the polar extracts of the leaves and flowers of *Alchemilla vulgaris* have good inhibition α-amylase activity , The highest inhibition was in MeOH70% extracts (IC_{50} of leaves 0.31 mg/ml, IC_{50} of flowers 0.36 mg/ml) then MeOH extracts (IC_{50} of leaves 0.34 mg/ml, IC_{50} of flowers 0.40 mg/ml), followed by EtOH 70% ,and finaly EtOH extracts of leavea and flowers,also we noticed that the lowest

inhibition was in non-polar extracts (Hexane and Chloroform) of leaves and flowers, and the inhibition of α- amylase activity was increasedas concentration of extracts increased.

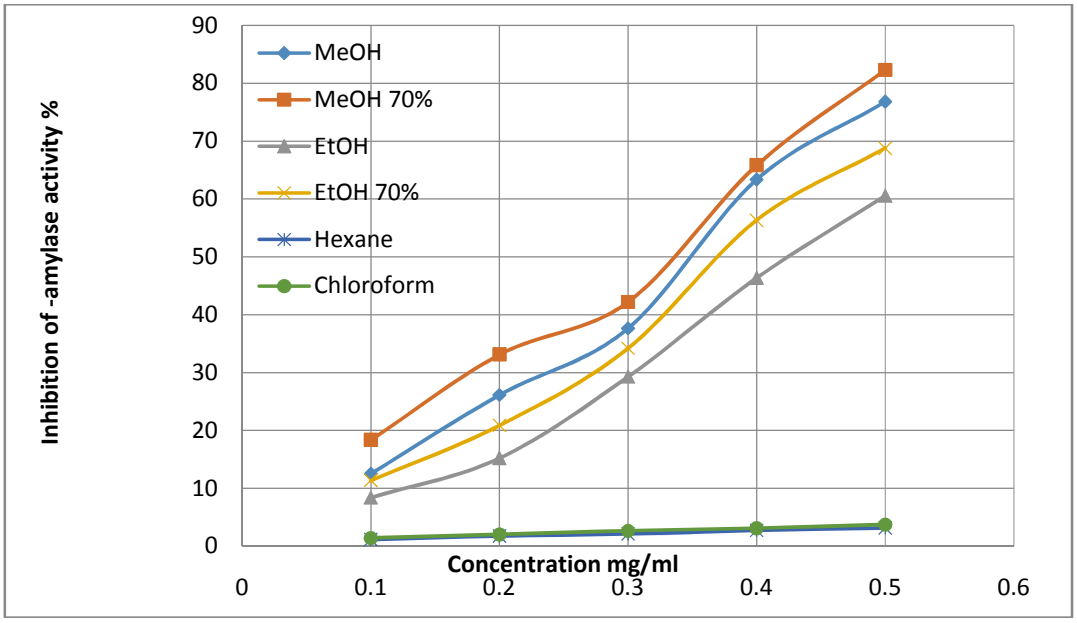

Figure 10 α – amylase inhibitory of different extracts of leavesof *Alchemilla Vulgares*

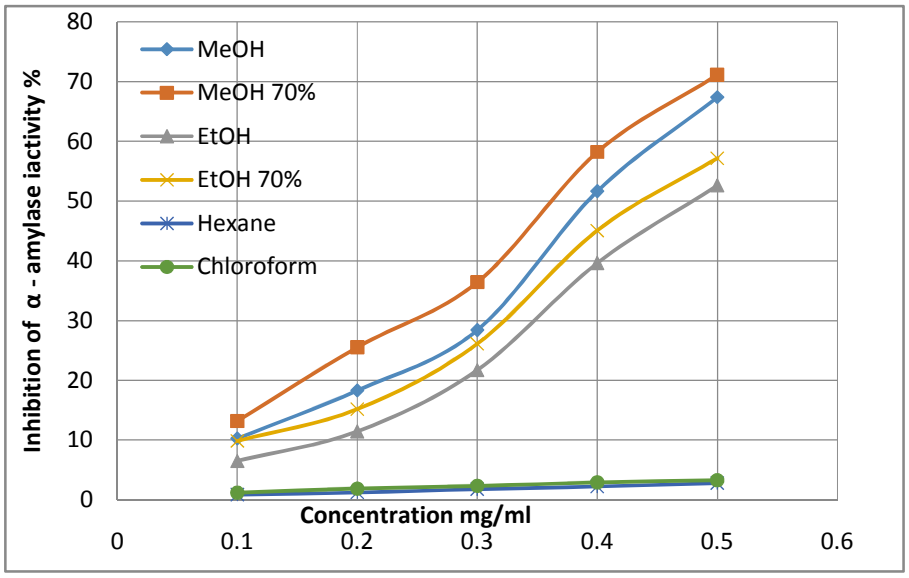

Figure11. α – amylase inhibitory of different extracts of flowers of *Alchemilla Vulgares*

Table 10. α- amylase inhibitory of different extracts of leaves and fruit of *Crataegus Azarolus*

Extracts	Concentrations mg/ml	Inhibition of α-amylase activity% leaves	Inhibition of α-amylase activity% fruit
MeOH	0.1	8.13±0.54*	7.16±0.11*
	0.2	18.71±0.47*	14.82±0.35*
	0.3	26.25±0.62	23.67±0.57*
	0.4	39.15±0.58	37.22±0.64
	0.5	53.41±0.39	49.65±0.41
MeOH 70%	0.1	11.61±0.71*	9.50±0.19*
	0.2	21.38±0.68	18.12±0.31*
	0.3	30.26±0.41	27.41±0.56
	0.4	48.56±0.84	43.55±0.63
	0.5	62.13±0.56*	57.31±0.49
EtOH	0.1	5.03±0.30*	4.33±0.24*
	0.2	9.32±0.67*	7.25±0.38*
	0.3	17.45±0.35*	13.12±0.46*
	0.4	31.21±0.79*	28.61±0.51*
	0.5	47.56±0.53	40.21±0.33*
EtOH70%	0.1	7.10±0.48*	5.17±0.24*
	0.2	16.62±0.74*	11.27±0.77*
	0.3	24.40±0.55*	18.50±0.45*
	0.4	38.45±0.43	31.66±0.26*
	0.5	50.13±0.61	45.18±0.80
Hexane	0.1	1.11±0.17**	0.76±0.03**
	0.2	1.30±0.21**	0.98±0.12**
	0.3	1.67±0.19**	1.36±0.29**
	0.4	1.87±0.28**	1.67±0.45**
	0.5	2.10±0.22**	1.88±0.30**
Chloroform	0.1	1.31±0.34**	0.89±0.18**
	0.2	1.70 ±0.16**	1.17±0.24**
	0.3	2.21±0.25**	1.56±0.47**
	0.4	2.73±0.33**	1.86±0.39**
	0.5	3.15±0.53**	2.13±0.31**

⋆$p<0.05$, ⋆⋆ $p<0.001$compared the concentrations of the extracts to the concentrations of Acarbose from the lowest concentration to the top, data were presented as mean ± SD (n=3).

The inhibition α-amylase activity of different extracts of leaves of *Crataegus Azarolus*was higher than the extracts of fruit, and the polar extracts of the leaves and fruit of *Crataegus Azarolus* have a good inhibition α- amylase activity,in contrast the non-polarextracts which have verey weak effect on α- amylase activity, The highest effect on α-amylase activity was in MeOH70% extracts (IC_{50} of leaves 0.41 mg/ml, IC_{50} of fruit 0.45 mg/ml) then MeOH extracts (IC_{50} of leaves 0.48 mg/ml, IC_{50} of fruit 0.51 mg/ml), followed by EtOH70% and EtOH extracts of leaves and fruit, and we noticed that the lowest inhibition was in non-polar extracts (Hexane and Chloroform). the inhibition of α-amylase activity was increased as concentration of crude extracts increased.

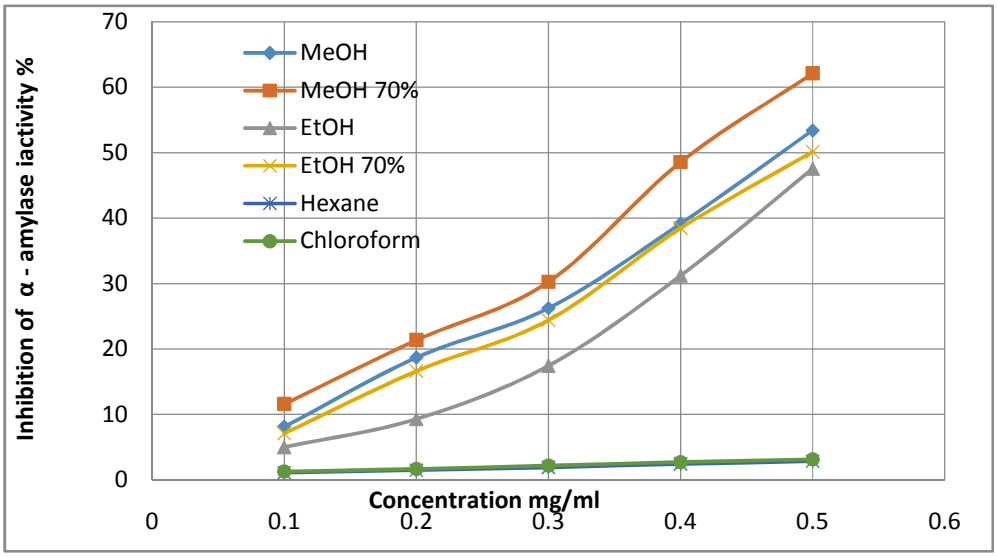

Figure 12. α – amylase inhibitory of different extracts of leaves of *Crataegus Azarolus*

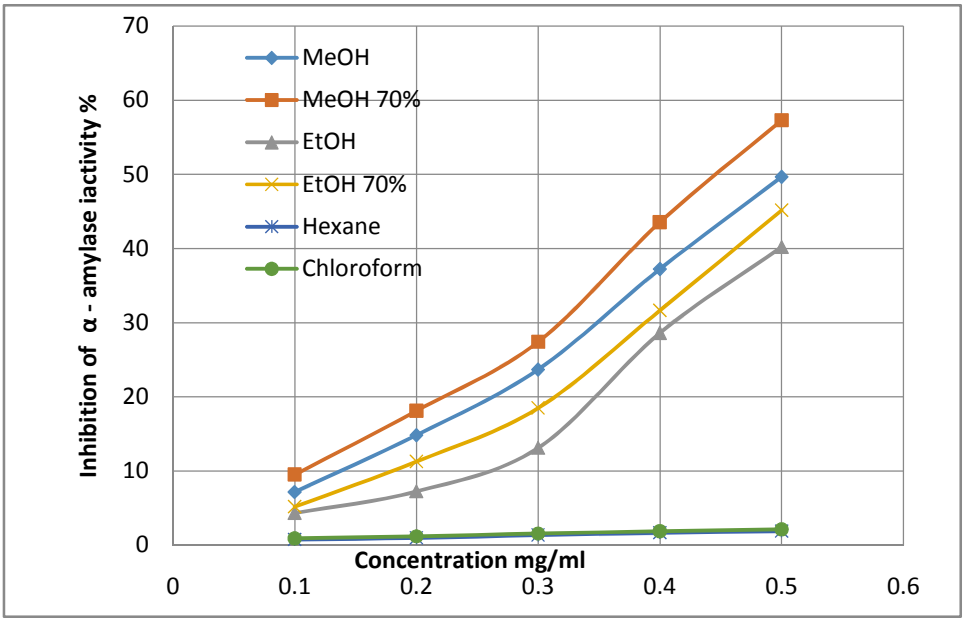

Figure13. α– amylase inhibitoryof different extracts of fruitof *Crataegus Azarolus*

Table 11 α- amylase inhibitory of different extracts of leaves and fruit of *Sophora Japonica*

Extracts	Concentrations mg/ml	Inhibition of α-amylase activity% leaves	Inhibition of α-amylase activity% fruit
MeOH	0.1	5.81±0.35*	7.31±0.19*
	0.2	10.67±0.60*	17.20±0.3*
	0.3	19.42±0.51*	26.46±0.71
	0.4	32.11±0.32	39.66±0.38
	0.5	44.56±0.29	52.00±0.69
MeOH 70%	0.1	7.34±0.38*	11.88±0.61
	0.2	13.11±0.55*	20.36±0.57*
	0.3	23.26±0.31*	29.48±0.30
	0.4	40.04±0.69	47.09±0.37
	0.5	52.10±0.47	60.36±0.41*
EtOH	0.1	3.77±0.17*	6.11±0.46*
	0.2	6.80±0.25*	9.56±0.70*
	0.3	11.31±0.50*	17.30±0.71*
	0.4	25.21±0.63*	30.11±0.55*
	0.5	37.68±0.28*	44.09±0.27
EtOH70%	0.1	4.20±0.39*	7.81±0.15*
	0.2	9.71±0.66*	15.87±0.19*
	0.3	13.21±0.37*	24.27±0.75*
	0.4	26.47±0.71*	37.16±0.42
	0.5	39.11±0.54*	49.11±0.64
Hexane	0.1	0.22±0.05**	0.38±0.13**
	0.2	0.56±0.11**	0.69±0.21**
	0.3	0.87±0.17**	0.97±0.30**
	0.4	1.11±0.31**	1.31±0.28**
	0.5	1.38±0.25**	1.53±0.21**
Chloroform	0.1	0.43±0.12**	0.55±0.08**
	0.2	0.67 ±0.19**	0.91±0.17**
	0.3	0.93±0.15**	1.26±0.33**
	0.4	1.26±0.23**	1.45±0.27**
	0.5	1.58±0.13**	1.88±0.40**

⋆$p<0.05$, ⋆⋆ $p<0.001$compared the concentrations of the extracts to the concentrations of Acarbose from the lowest concentration to the top, data were presented as mean ± SD (n=3).

The inhibition α- amylase activity of the different extracts of the leaves of *Sophora japonica* were lower than the different extracts of fruit, and the polar extracts of the leaves and fruit of *Sophora japonica* have a good inhibition α- amylase activity. Also we showed that the highest inhibition was in MeOH70% extracts (IC_{50} of leaves 0.49 mg/ml, IC_{50} of fruit 0.43 mg/ml) then MeOH extracts (IC_{50} of leaves 0.57 mg/ml, IC_{50} of fruit 0.49 mg/ml), followed by EtOH70%,and finally EtOH extracts of both leaves and fruit, and the lowest inhibition was in non-polar extracts (Hexane and Chloroform).The inhibition of α-amylase activity was increased asconcentration of crude extracts increased.

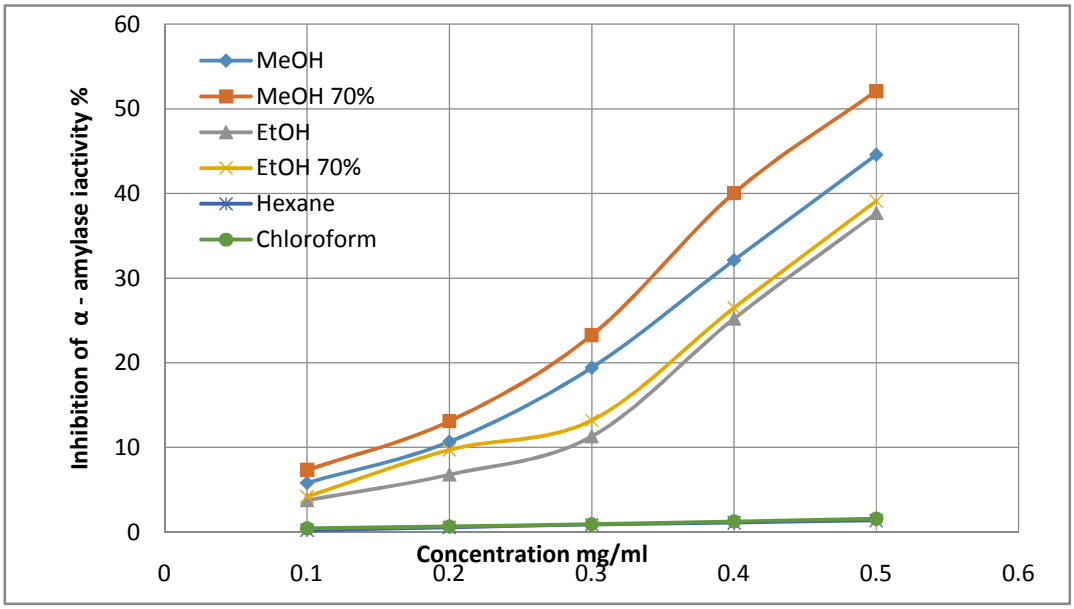

Figure 14. α – amylase inhibitory of different extracts of leaves of *Sophora Japonica*

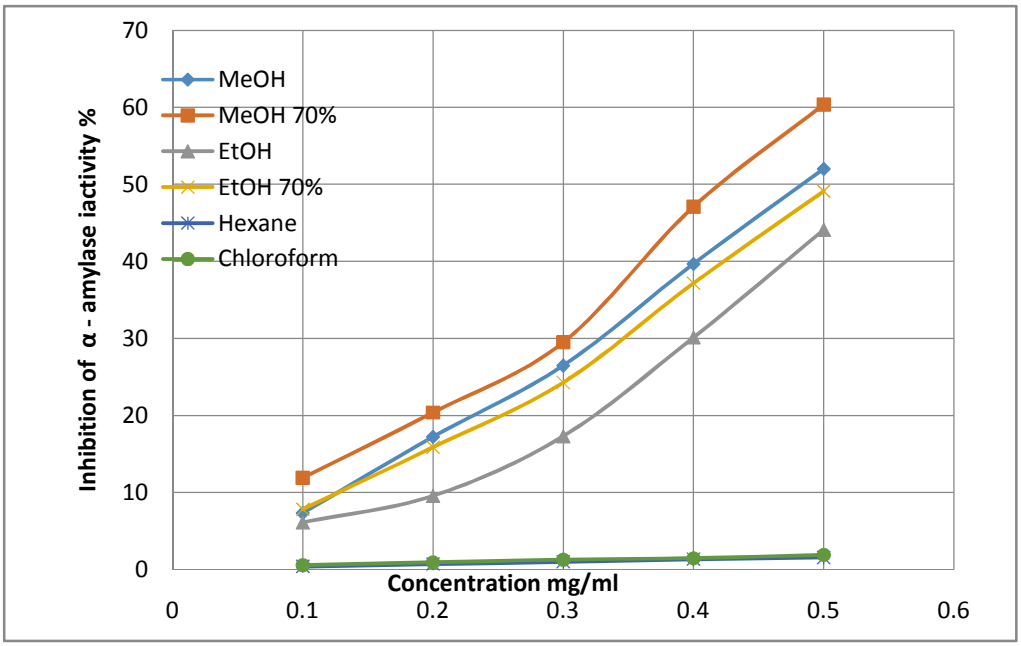

Figure 15. α – amylase inhibitory of different extracts of fruit of *Sophora Japonica*

The results in the tables [9,10,11] showed that the *Alchemilla vulgaris* (leaves and flowers) have a highest effect onα – amylase activity comparing with *Crataegus Azarolus* and *Sophora Japonica* (leaves and fruits).

These results indicate that the extracts which prepared by polar solvents as[MeOH,MeOH70%,EtOH,EtOH70%] contain a high percentage of compounds which have a good inhibitory effect of α – amylase activity as the phenols,saponins and flavonoids which are known to be found in good proportion in these extracts of various plants,as indecated in previous studies these compounds have a good inhibition of α – amylase activity [20],[24].

Conclusions

The study of plants *Alchemilla Vulgaris* (leaves and flowers) ,*Sophora Japonica*, and *Crataegus Azarolus* (leaves and fruits)has indicated that; these plants are rich withphenols, carbohydrates, , flavonoids, , saponins, , tannins, and glycosides. While it has shown the absence of alkaloids and cardinols, except the leaves of *Crataegus Azarolus* that contained alkolois, also itcan be concluded that the studied plants *Alchemilla Vulgaris* (leaves and flowers) and *Sophora Japonica* , *Crataegus Azarolus* (leaves and fruits) have 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.

References

[1] E Wanderley, V Ferreira.Obesity: a plural perspective. *Cienc. SaúdeColetiva*, 15, 2010,185–194.
[2] C Boniglia, B Carratù, S Di Stefano, S Giammarioli, M Mosca, E Sanzini.Lectins, trypsin and α-amylase inhibitors in dietary supplements containingPhaseolus vulgaris. *Eur. Food Res. Technol*, 227, 2008, 689–693.
[3] A Simao, A Correa,.P Chagas.Inhibition of digestive enzymes bymedicinal plant aqueous extracts used to aid the treatment of obesity. *J. Med.Plants Res*., 6, 2012,5826–5830.
[4] YKwon,. E Apostolidis, K Shetty. Inhibitory potential of wine and teaagainst α-amylase and α-glucosidase for management of hyperglycemia linkedto type 2 diabetes. *J. Food Biochem*,32, 2008,15–31.
[5] V Balasubramaniam, S Mustar, N Khalid, A Rashed, M Noh, M Wilcox, I Peter, I Brownlee, J Pearson.Inhibitory activities ofthree Malaysian edible seaweeds on lipase and α-amylase. *J. Appl. Phyco*, 25, 2013, 1405–1412.
[6] C Pereira, L Pereira, A Corrêa, P Chagas, S Souza, C Santos. Inhibition of digestive enzymes by commercial powder extracts of Hoodiagordonii. *Rev. Bras. Biocienc*, 9,2009, 265–269.
[7] .L Pereira, C Santos, L Sátiro, S Marcussi, C Pereira, S Souza. Inhibitory activity and stability of the white bean flour extract on digestiveenzymes in the presence of simulated gastric fluid. *Rev. Bras. Farm*, 92,2011, 367–372.
[8] C Roh,U Jung,. Screening of crude plant extracts with anti-obesity activity.*International journal of Molecular Science*,13, 2012, 1710-1719.
[9] A Khan, R Qureshi, F Ullah, S Gilani , A Nosheen , S Sahreen, M Laghari, M Laghari, S Ur-Rehman, I Hussain, W Murad. Phytochemical Analysis of Selected Medicinal Plants of Margalla Hills and Surroundings. Journal of Medicinal Plants Research; 5(25),2011, 6017-6023.
[10] H Monajjed, IHasan Aga.. Phytochemistry and Extraction. Practical Issue.(The Office of Books and University Publications, Damascus University, Syria, 1997).
[11] M Hajnos, JSherma, T Kowalska.Thin Layer Chromatography in Phytochemistry**. (**CRC Press Taylor & FrancisGroup, LLC, Boca Raton, FL the USA.,2008).
[12] **S** Balbaa , S Hilal S, A Zaki .Medicinal plant constituent. (General Or. ganization for university and schoolbook), 1981.
[13] J Ahuja, J Suresh, A Deep,M Ravi .Phytochemical Screening of Aerial Parts of *Artemisia parviflora*Roxb.: A Medicinal Plant**.***Der Pharmacia Lettre*, 3 (6),2011,116-124.
[14] C Chandrappa , M Govindappa,N Kumar.Phytochamical screening and antibacterial property of Carmona Retusa (VAHL) Masam. .*International Journal of Pharma ,Medicine and Biological Sciences*, 1(2),2012..
[15] .AKhan, R Quresh,F Ullah, S Gilani, A Nosheen,S Sahreen, M Laghari, M Laghari, S Ur-Rehman, I Hussain, W Murad.Phytochemical Analysis of Selected Medicinal Plants of Margalla Hills and Surroundings.*Journal of Medicinal Plants Research*, 5(25), 2011, 6017-6023.
[16] M Maqsood,W Malik.Lipase inhibitory activity of *Lagenaria siceraria* fruit as a strategy to treat obesity..*Asian pacific journal of Tropical Medicine*,10, 2017, 305-310.
[17] C Kaewpiboon,.K Lirdpramongkol, C Srisomsap, P Winayanuwattikum, T Yongvanich, P Puwaprisirisan, J Svasti, W Assavalapsakul.Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected the medicinal plants**.** *BMC Complementary and Alternative Medicine* 12, 2012, 217.

[18] ODanis, A Ogan, D Anbar, B Dursun, S Demir, U Salan. Inhibition of pancreatic lipase by culinary plant extracts..*International Journal of plant Biology &Research*, 3(2),2015,1038.
[19] .Z Dastjerdi, F Namjoyan, M Azemi.Alpha amylase inhibition activity of some plants extract of *Teucrium* species.*European Journal of Biological Sciences* 7 (1), 2015, 26-31.
[20] GKeerthana, M Kalaivani, A Sumathy.In-vitro alpha amylse inhibitory and anti-oxidant activities of ethanolic leaf extract of *Croton bonplandianum*.*Asian J Pharm Clin Res*, Vol 6, 2013, 32-36**.**
[21] .**S** Nair, V Kavrekar, A Mishra,In vitrostudies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts.*Euro. J. Exp. Bio.*, 3(1), 2013, 128-132.
[22] C Santos,A Souza, L Pereira, S Souza. Inhibition of pancreatic lipase by extracts of Baccharis trimera: evaluation of antinutrients and effect on glycosidases**.** *Brazilian Journal of Pharmacognosy*,21(3), 2011, 450-455.
[23] M Karamac, R Amarowicz, . Inhibition of pancreatic lipase by phenolic acids-Examination in vitro**. .***Z.Naturforsch*,51, 1996, 903-905.
[24] V Malathi, S Dem, K Revathi. Anti diabetic activity bt the in vitro alpha amylase and alpha- glucosidase inhibitory activity of Catharanthus roseus**..***the Biascan* 5(4) , 2010, 655-659.