THE EFFECT OF ALCOHOLIC EXTRACT OF *MYRTUS COMMUNIS* LEAF ON MAO , AChE , GOT ,AND GPT IN HUMAN SERUM IN VITRO

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Abstract:

This study was designed to show the effects of different concentrations of alcoholic extract of *M.communis* L. on the activity of MAO , AchE ,GOT, and GPT in human sera .The results showed that all concentrations of extract cause inhibition to enzymes activity ,the percentage of inhibition increased with increasing the extract concentration. Kinetic parameters were studied and the results showed that alcoholic extract of *m.communis* l. acted as competitive inhibitor with MAO ,AchE ,GOT, and GPT .

key words :*Myrtus communis* leaves ,Monoamine oxidase, Acetylcholine esterase, Glutamate oxaloacetate transaminase, Glutamate pyruvate transaminase

INTRODUCTION:

Myrtus communis leaf commonly named as myrtle is a wild evergreen plant, is from myrtaceae family, species of myritriflora .it is one of the important aromatic and medicinal species from this family [1] [2].*M. Communis* L. contain various chemical compounds such as tannins, flavonoids, saponins and terpenoids[3]. Several studies have indicated the property of this plant as strong antibacterial, anti-inflammatory, and antioxidant.[4].

Monoamineoxidase(MAO) (EC 1.4.3.4) is a metabolically important enzyme which catalyzes the deaminating oxidation of amines to corresponding aldehyde producing hydrogen peroxide and free amine [5] .MAO is found in all tissues.There are two major isoforms of MAO , MAO –A and MAO- B.[6].

Acetyle Choline Esterase(AChE) (EC 3.1.1.7) hydrolyses acetylcholine to choline and acetate. AChE regulates the transport of nerve impulse from nervous system[7]. [8] . Inhibition of AchE is considered approach for the treatment of (AD) and myasthenia gravis[9].

Glutamate Oxaloacetate Transaminase is hydrolase enzyme that catalyzes the transfer of amine group of aspartate to α-keto glutarate. The clinical significance of this enzyme is diagnosis of heart and liver disease[10].

Glutamate Pyruvate Transaminase is hydrolase enzyme that catalyzes the transfer of amine group of amino acid (alanine) to α-keto glutarate .The clinical significance of this enzyme is diagnosis of liver disease. [10]

Materials and methods:

extraction: Alcoholic

M.communis leaves powder gotten where purchased from local markets. 50 g of the leaf powder were soaked with 500 ml ethyl alcohol(96%),then the mixture was stirred in a shaker for 48 hours in the dark. After that the contents of the flask were passed through filter paper and then were poured into a vacuum rotating vaporizer machine. The concentrated extract was then poured into Petri dishes and was oven dried ,the dried powder was then collected .

Qualitative tests of active compounds in *Myrtus communis* leaf :

Qualitative tests of alkaloids, saponines , tannins , flavanoids, phenols ,and resins in *m.communis* leaf were achieved by using the method in [11] [12].

Determination of monoaminoxidase activity:

MAO activity was assayed by using Mcwen and Cohen method[13].

Different concentrations of crude extract of *m.communis* l. were prepared (0.1,0.04,0.02,0.01,0.001)(mg/ml).the inhibition percentage was calculated by using the method in [14].

0.1mg/ml of alcoholic extract of *m.communis* l. was used with different concentrations of substrate (0.008, 0.006, 0.004, 0.002, 0.001) M to determine type of inhibition and by using lineweaver-Burk Equation the following values were calculated: Vmap, Kmap, and inhibition type.

Determination of acetylcholinesterase activity:

AChE activity in human sera was assayed by using modified Ellman method[15] .

Different concentrations of crude extract of *m.communis* l. were prepared (0.1,0.04,0.02,0.01,0.001)(mg/ml). the inhibition percentage of AChE activity was measured in healthy human serum by using the method in[14].

0.1mg/ml alcoholic extract of *m.communis* l. was used with different concentration of substrate (0.1,0.06,0.04,0.02,0.01)M to determine type of inhibition and by using line weaver-Burk Equation the following values were calculated : Vmap ,Kmap, and inhibition type.

Determination of GOT and GPT activities:

GOT and GPT activities were assayed by colorimetric method according to the following equations:

L-aspartate+ α –ketoglutarate _GOT_ oxaloacetate + glutamate

Alanine +α-ketogltarate_GPT_____ pyruvate+glutamate

Oxaloacetate and pyruvate formed was measured in form dinitrophenylhydrazone ,which obsorbed at 505 nm[16].

Different concentrations of crude extract of M.Communis L. were prepared (0.1,0.04,0.02,0.01,0.001)(mg/dl).percentage of inhibition of GOT and GPT activities were measured in human serum by using the method in [10].

0.1mg/ml of alcoholic extract of *m.communis* l. was used with different concentration of substrate (200,180,150,100,50 mmole/L) to determine type of inhibition and by using lineweaver-

Burk Equation the following values were calculated : Vmap ,Kmap, and inhibition type.

Results and Discussion:

The results obtained showed that *m.communis* leaf contains active compounds like tannins ,phenols ,flavonoids ,resins ,glycosides , and saponins with absence of alkaloidsin this plants as shown in table(1).this result was in agreement with the results that obtained by alsalami [12].

The results obtained in table (2)(3)(4) and (5) showed that different concentrations of alcoholic extract (0.1, 0.04, 0.02 ,0.01 ,0.001 mg\ml) cause inhibitory effects on enzymes activity MAO ,AChE ,GOT ,and GPT respectively.low concentration of extract (0.001mg/dl) exhibited low percentage of inhibition with MAO(35.02%), ACHE(25.04%), GOT(14.28%), and GPT(13.04%), MAO (26.5%), ACHE (24.8%), GOT (36.8%), GPT (28.6%) but this percentage begins to increase with elevating concentration of extract ,the high concentration of extract 0.1 mg\ml showed 85.08% with MAO ,76.28% with AChE ,85.71% with GOT ,and 82.61% with GPT as shown in figure (1) and figure (3)..

Different concentrations of the substrate were used to study the type of inhibition, the results obtained from line weaver-burke plots indicated that *m.Communis* L. extract acted as competitive inhibitor for MAO,AchE,GOT,and GPT, the kinetic parameters (km,vm,and type of inhibition) were also determined by using line weaver- Burk plot as shown in Table(1) and figure (2)

. The alcoholic extract of *m.communis* L. inhibit the activity of MAO because it is rich in poly phenolic compounds such as tannins, which is able to inhibit the enzyme [17].

The results obtained showed that *m.Communis* L. extract has an inhibition effect on AChE activity because the alc. extract of this plant has carbonyl group (phenolic compounds) ,therefore the hydroxyl group of the amino acid series residue attacks the carbonyl group of inhibitor instead of attacking the carbonyl group of acetyl choline and forms inhibitor-enzyme complex which causes inhibition AChE activity.

this research proved that alc. extract of of *m.Communis* l. inhibit GOT and GPT activities ,therefore ,catabolism of amino acids will decrease.

Table 1: Essential active compounds in *m.communis* leaf (qualitative tests)

Test	**result**
Alkaloids	-ve
Saponins	+ve
Taninns	+ve
Glycoside	+ve
Phenols	+ve
Flavanoids	+ve
Resins	+ve

+ve : mean *m.communis* leaf contain this compounds

-ve : mean *m.communis* leaf not contain this compounds

Table 2 :The effect of different concentrations of *m.communis* l. on the activity of monoaminoxidase (MAO) in healthy human sera.

Alcohol extract of *m.communis* l.(mg\ml)	MAO activity (µmol\ml\min)	%inhibition
Nil	29.55	
o.oo1	19.20	35.02
o.o1	16.12	45.44
o.o2	12.98	56.07
o.o4	1o.o1	66.13
o.1	4.41	85.08

Table 3:The effect of different concentrations of *m.communis* l. on the activity of acetylcholinesterase(AchE) in healthy human sera.

Alcohol extract of *m.communis* l.(mg\ml)	AchE activity (µmol\3min \ml)	%inhibition
Nil	6.o3	
o.oo1	4.52	25.04
o.o1	4.14	31.34
o.o2	3.97	34.16
o.o4	3.77	37.48
o.1	1.43	76.28

Table 4:The effect of different concentrations of *m.communis* l. on the activity of glutamate oxaloacetate transaminase GOT in healthy human sera.

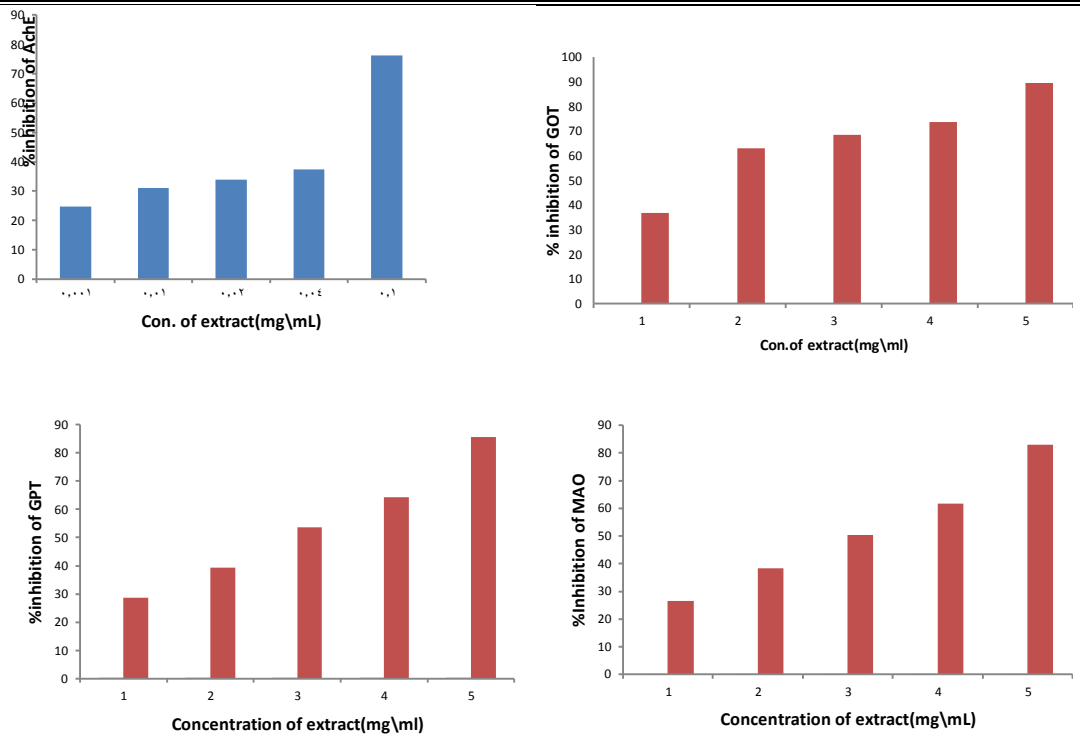
Alcohol extract of *m.communis* l.(mg\ml)	GOT activity (µmol\ml\min)	%inhibition
Nil	14	
o.oo1	12	14.28
o.o1	7	50.00
o.o2	5	64.28
o.o4	5	64.28
o.1	2	85.71

Table 5 :The effect of different concentrations of *m.communis* l. on the activity glutamate pyruvate transaminase(GPT) in healthy human sera.

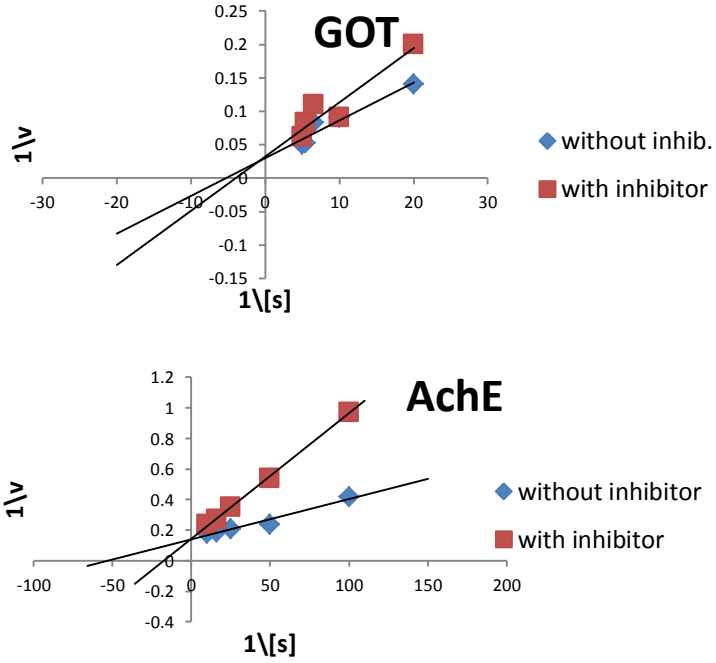
Alcohol extract of *m.communis* l.(mg\ml)	GPT activity (µmol\ml\min)	%inhibition
Nil	23	
o.oo1	20	13.04
o.o1	17	26.09
o.o2	13	43.48
o.o4	1o	56.52
o.1	4	82.61

Table 6:The kinetic properties of MAO,AchE,GOT, and GPT with alcoholic extract of *M.Communis* L.

enzymes	Vmax	km	Type of inhibition
MAO	28	0.02	competitive
AchE	8	0.05	competitive
GOT	25	0.142	competitive
GPT	33	0.5	competitive



Figure(1) :%inhibition of MAO ,AchE , GOT, and GPT with different concentrations of alcoholic extract of *M.Communis L.*



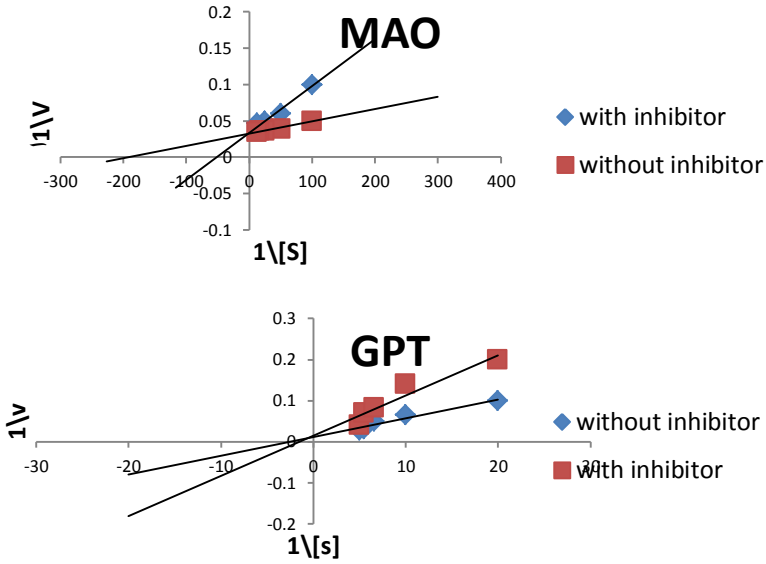
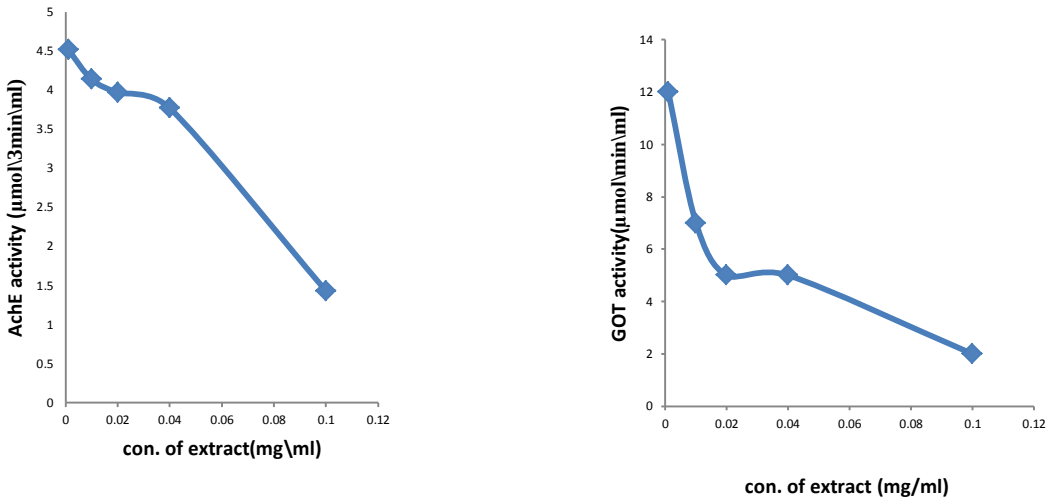
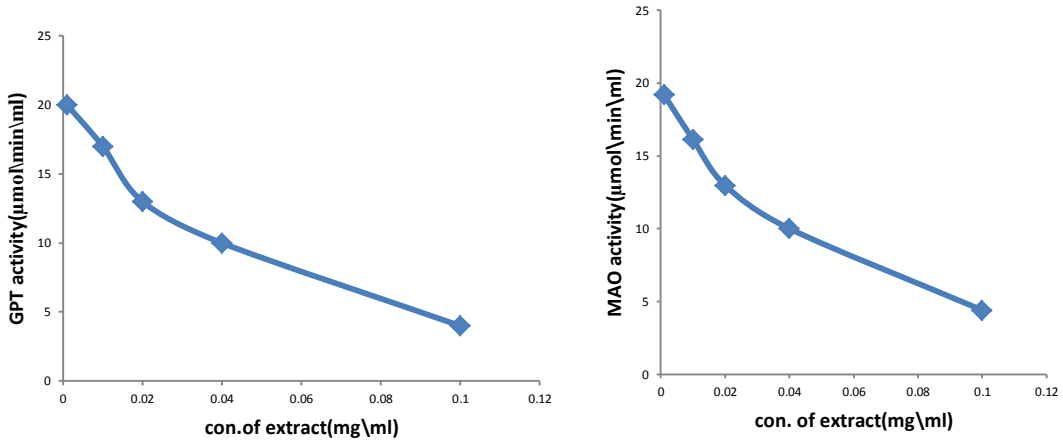


Figure (2): Line weaver –Burk plots for alcoholic extract of *M.Communis L.* on MAO,AChE,GOT,andGPT.





Figure(3):The effect of different concentrations of *m.communis* l. extract on MAO,AchE,GOT, and GPT activity

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تأثير المستخلص الكحولي لنبات الياس على الفعالية الانزيمية للمونواميناوكسيديز، اسيتيل كولين استيريز،كلوتاميت اوكزالواسيتيت ترانساميناز ، وكلوتاميت بايروفيت ترانساميناز في مصل الدم البشري

الخلاصة:

تم في هذا البحث تحضير المستخلص الكحولي لنبات الياس ودراسه تأثير تراكيز مختلفه من هذا المستخلص على الفعاليه الانزيميه للانزيمات التاليه : (مونواميناوكسيديز، اسيتيل كولين استيريز،كلوتاميت اوكزالواسيتيت ترانساميناز ، وكلوتاميت بايروفيت ترانساميناز) في مصل الدم البشري.وقد اظهرت النتائج ان جميع تراكيز المستخلص الكحولي لنبات الياس لها تأثير تثبيطي على الانزيمات وانه كلما زاد تركيز المستخلص ازداد التأثير التثبيطي على الانزيم.كما تم دراسه الخواص الحركيه للمستخلص مع الانزيمات قيد الدراسه اذ وجد ان المستخلص الكحولي لنبات الياس يسبب تثبيطا تنافسيا