Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 2410 - 2415
Received 20 January 2021; Accepted 08 February 2021.

Influence of Capparis Spinosa Crude Extract on the Amylase Activity in Vitro

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ABSTRACT:

The present study is designed to Study the effect of some extracted chemical components from C. spinosa leaves and fruits on □- amylase activity in vitro.

Subjects: In the method extracted the active substances in the biology of the leaves and fruits of this plant using two solvents, water and ethanol . The biological compounds were estimated by means of the chemical reagents detection method and the optical density spectrum method under the flesh Behind.The effect of these extracts on inhibiting amylase enzyme has also been studied in different concentrations (25 , 12.5 , 6.25 , 3.125 , 1.5625 mg / L).

Results: There is no significant difference between the product in ethanol extraction and the extraction product in distilled water. As the result showed that both the leaves and fruits have an effect on the enzyme activity , since they are all inhibited the enzyme function . This result can be useful for referring functional effect on the digestion of carbohydrates , which may be has a significant in the treatment of diabetes.

Conclusion: There is not that much difference in the effect of the extracts with different solvents because both solvents used are polar , it is noted that leaf extracts and fruits have effect in inhibiting the alpha amylase enzyme.

Keywords:C. spinosa, □- amylase ,Leaves,fruits.

INTRODUCTION:

The natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. Higher plants contribute no less than 25% of the total [1] . The interest in natural products for use as medicine has acted as the catalyst for exploring methodogies involved in obtaining the required plant materials for pharmacological screening and drug development [2] . This has led to the belief that natural products are safe because they are more harmonious with biological systems [3] . Since old days, plants were used to treat many ailments. India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties [4] .

The relationship between human and other elements of nature is as old as the history of the traditional systems of medicine [5]. Alternative therapy for all the civilizations throughout the world

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is based more on plants than on animals [6]. Plants are reputed in the indigenous systems of medicine for the treatment of various diseases [7]. Though plants play important roles in metabolic activities for example photosynthesis and respiration. The selection of plants for food and drugs is predicated on the presence of phytochemicals [8]. Medicinal plants are prescribed widely even when their biologically active compounds are un know , because of their safety, effectiveness and availability [9]. Several herbs have been reported to exhibit antioxidant activity [10]. The herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in their required, a need of hour is to shift towards the different endogenous plant and herbal formulations [11]. The World Health Organisation is not only encourage the use of plant medicines, but also recommended scientific evaluation of the hypo glycaemic properties of plant extracts [12]. Several plant extracts with a potential therapeutic properties for the treatment of hypertension and complications such as coronary heart disease, angina, arrhythmias and congestive heart failure have been identified [13].

Capparis spinosa L. is well known with its common name 'Capers' in different countries [14].*Capparis spinosa* belonging to the family Capparidaceae is a xerophytic plant growing in a broad range of climatic conditions, varying from dry deserts to cooler altitudes of mountains [15]. This plant also known as the caper bush, is a perennial winter deciduous species that bears rounded, fleshy leaves and large white to pinkish flowers [16]. Plants provide the predominant ingredients of medicines in most traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs [17].

Many species of *Capparis* are reported from Iraq, from northern to southern plateau of the country [18]. Extracts of different parts of *C. spinosa* have been found possess biological activity against a large number of pathogens [19] . Antifungal , antibacterial , anti-amoebic , anti-worm [20] , antidiabetic , antihyperlipidemic [21] , anti hypertensive , poultice [22] antileishmania , antihepatotoxic , and antiallergic activities have been demonstrated [23].

Method and Materials:

The experimental work of the present study has been carried out in the laboratories of chemistry / College of science, university of Al Muthanna , and biological department-college of science at University of Thi qar in Iraq .

Plant Collection :

*Capparis spinosa L.*plant was collected in Oct- 2018 from Al Samawa city at Iraq .

The leaves and fruits were cleaned , washed by distilled water, dried at room temperature for two weeks, ground as powder and kept in glass containers for further use.

Soxhlet Extraction :

100 grams of a finely ground sample is placed in exceedingly porous thimble made up of a robust filter paper, in thimble chamber of the Soxhlet equipment. Extraction solvents (ethanol and distled water) is heated within the bottom flask, vaporizes into the sample(thimble), condenses within the condenser and drip back. Once the liquid content reaches the siphon arm. the liquid contents emptied into the bottom flask again and the process is continued 24 hours at 40C°. The extracts are filtered , and concentrated by rotary evaporator. The extract kept at round bottom flask away from light and moisture for used [24].

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Assessment of □-amylase activity :

1: Assessment of □-amylase activity [25]by premixing addition method :

1- Preparation of different concentration of the extract (25 , 12.5 , 6.25 , 3.125 ,1.5625) mg/L .
2- Preparation of premixed solutions from constant concentration of amylase enzyme with the different concentrations of the extract as above .
3- Add 500 µl of each premixed solution that prepared in step 2 to the volume of starch solution (2 ml) with a concentration of 8 mg/ml.
4- Measure the absorbance at 405 nm of the blank in comparison with the sample included the different concentrations of the premixed solution .

The inhibition percentage of □-amylase was assessed by the following formula:

$$I\ \alpha - amylase\ \% = \frac{\Delta\ A\ Control - \ \Delta\ A\ Sample}{\Delta\ A\ Control} \times 100$$

2:Assessment of □-amylase activity by sequential addition method:

1- Preparation of different concentration of the extract (25 , 12.5 , 6.25 , 3.125,1.5625) mg/L .
2- Add and mix 400 µl of each extract concentration that prepared in the step 1 to 1600 µl of starch solution (8 mg/ml).
3- Add 500µl of amylase enzyme to each 2ml of the mixed solutions that prepared in step 2.
4- Measure the absorbance at 405 nm of the samples in comparison with the blank solution (starch + buffer).
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The inhibition percentage of □-amylase was assessed by the following formula:

$$I\ \alpha - amylase\ \% = \frac{\Delta\ A\ Control - \ \Delta\ A\ Sample}{\Delta\ A\ Control} \times 100$$

Statistical Analysis: The statistical analysis used in this study is done using Microsoft Excel 2010 .

RESULTS AND DISSCUSSION:

Table 1,2 and figure 1,2 show there is a direct relationship between the concentration of the extract and the enzyme inhibition .

As it evident from table 1 and 2 , there is no significant differences in □-Amylase Inhibitory Percentage between premixing and sequential methods , This indicates that there is no competition for the active site of the enzyme

The inhibition of pancreatic alpha-amylase is one of the therapeutic targets for delaying oligosaccharide digestion to absorbable monosaccharides in the intestinal brush border, resulting in reduced postprandial hyperglycemia [26]. Phenolic compounds such as phenolic acids and flavonoids bind covalently to alpha-amylase and change its activity due to the ability to form quinones or lactones that react with nucleophilic groups on the enzyme molecule [27].

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Table 1: □-Amylase Inhibitory Percentage of Fruits .

Comments	Concentration mg/L	Ethanol+Fruits (Seq.add)	Water+Fruits (Seq.add)	Ethanol+Fruits (Premix.add)	Water+Fruits (Premix.add)
1	25	59	52	62	55
2	12.5	57.74648	50.26455	60	53.86243
3	6.25	46.92737	47.28477	49.72067	44.37086
4	3.125	28.26087	30.92593	37.5	37.22222
5	1.5625	23.69565	24.68354	27.82609	28.90295

Table 2: □-Amylase Inhibitory Percentage of Leaves.

Comments	Concentration mg/L	Ethanol+Leaves (Seq.add)	Water+ Leaves (Seq.add)	Water+ Leaves (Premix.add)	Ethanol+ Leaves (Premix.add)
1	25	82	72	75	90.54746
2	12.5	80.8046	71.49028	73.65011	86
3	6.25	73.70968	68.45426	69.40063	75.16129
4	3.125	66.14699	65.21739	58.69565	66.81514
5	1.5625	38.07531	34.48276	31.03448	39.74895

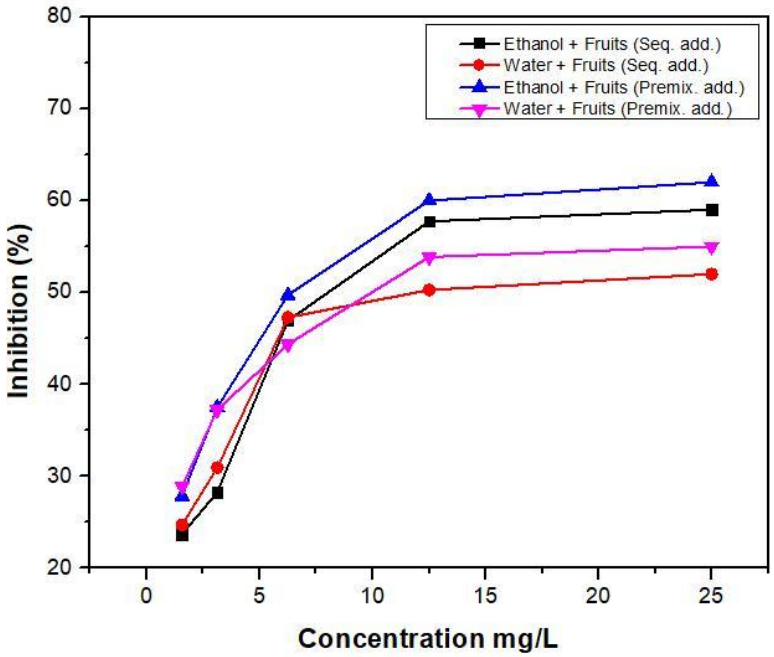

Figure1: □-Amylase Inhibitory Percentage of Fruits .

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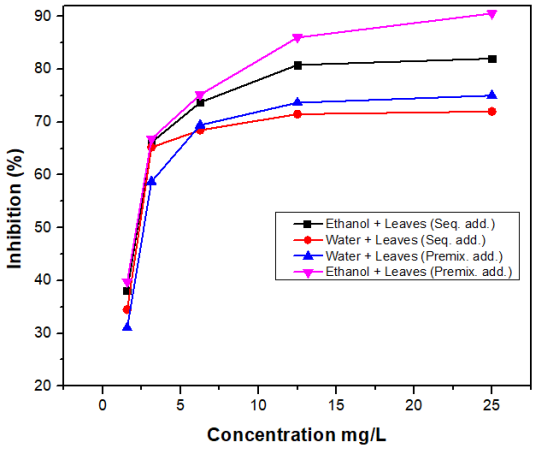

Figure2: □-Amylase Inhibitory Percentage of Leaves **.**

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