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Evaluation of insecticidal and enzyme activity potentials of essential oils and extracts of *Chenopodium botrys* L. against storage products pests

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Abstract: Investigating the insecticidal properties of *Chenopodium botrys* L. plant extracts is valuable for pest control and agricultural practices. *C. botrys* L. plant extracts may exhibit various enzymatic activities, such as protease, amylase, lipase, and antioxidant activities. Investigating these enzymatic properties provides insights into the plant's biochemical composition and potential applications. This study was aimed to reveal the insecticidal activity and enzyme activity of the essential oil and extracts obtained by different solvent and extraction methods by using the aerial part of the plant. Plant material was collected from Erzincan. The essential oil of *C. botrys* L. plant was obtained by steam distillation using Neo-Clavenger device. The remaining aqueous part was extracted with the solvent ethylacetate and n-butanol, respectively. In the end, five extracts were obtained: essential oil (CB-EO), untreated aqueous extract (CB-F), ethylacetate (CB-EA), n-butanol (CB-nBu) and processed aqueous extract (CB-L). The effects of five extracts on both insecticidal (against *Sitophilus granarius* and *Tribolium castaneum*) and enzyme activities (acetylcholinesterase, xanthine oxidase (XO) and tyrosinase) were studied. It has been determined that CB-F and CB-nBu extracts have an activation effect against tyrosinase enzyme with IC_{50} values of 250 and 423 µg/mL, respectively. At the end of 48 hours, CB-EA extract was determined to cause 20% death against the adult insects of *Sitophilus granarius* as a result of contact toxicity test. As a result of GC-MS analysis of essential oil, α-Eudesmol compound was analyzed as the main component. In conclusion, studying the enzyme and insecticidal activities of *C. botrys* plant extracts is significant due to its potential applications in pest control, biotechnology, natural product discovery, and sustainable agriculture.

Keywords: Biological activity; Phenolic compounds; Spinachaceae family; Essential oils

1 Introduction

Chenopodium plant species are from the Spinachaceae family and their leaves resemble crow's feet. These plant species are grown in different parts of the world as a leafy plant and as a secondary cereal crop. This plant species can also be in the form of an annual or perennial shrub in herbaceous form. The species included in the family are generally distributed in most of Europe, Asia and North America. They spread in a wide variety of areas such as arid regions, soils rich in nitrogen potassium, coasts, roads, fields and riversides (Akman et al. 2007; Yıldırımlı 2003; Tozoğlu 2011). Recently, interest in the crowbar plant has been growing in many countries, especially in Europe, and research has been increasing. The *Chenopodium botrys* L. plant has traditionally been used for medicinal purposes. This information is based on folklore, not proven information on the threshold of scientific data. *C. botrys*, the scent of incense gives it characteristic meanings. As a result of scientific studies, it has been determined that the essential oil of the plant shows antibacterial and antifungal activity because it contains sesquiterpene compounds containing oxygen (Mahboubi et al. 2011; Andov et al. 2014; Morteza-Semnani 2017).

Plants are the main source of many bioactive compounds. Therefore, it has been reported in many studies that they

significantly affect enzyme activities both as extracts and as pure compounds. Many phycochemicals have been reported that specifically affect the enzyme activities of acetylcholinesterase (AChE), Xanthine oxidase (XO), and Tyrosinase (Kebbi et al. 2021; Yırtıcı et al. 2022). Therefore, the potential effects of the obtained extracts on these three enzymes were investigated in this study.

The world population is increasing day by day and as an effect of global warming, food supply to the population will cause great problems in the coming years. The overall pandemic conditions have made this situation even more obvious for humanity. Therefore, the supply of food, its safe transportation and limited resources are a big problem for humanity, which depends on agricultural systems. The current global agricultural production needs to be protected from many factors, from harvest to table. The biggest problem comes from warehouse pests, which also constitute a large insect team. These insects, which are harmful to cereals, can easily adapt themselves to environmental conditions and can live even in difficult conditions.

Storage pests are classified as primary pests and secondary pests, depending on their diet and amount. *Sitophilus granarius* and *Tribolium castaneum* are considered one of the primary pests. Due to the harmful effects of this pest, serious losses are experienced in stored products (Singh et al. 2009). The widely used approach to control warehouse pests in the world is pesticides and especially fumigants (Mutungi et al. 2014).

However, in recent years, serious concerns caused by pesticides in agricultural products, increasing demand for storage products due to diseases and increasing demand have led to the development of environmentally friendly approaches for the protection and cultivation of these products (Alkan et al. 2019). For this reason, natural origin plants and natural organic compounds obtained from them, which are naturally safe for people to consume, have started to be used for these purposes. Natural compounds, which have an insect repellent or killing effect on insects, destroy insects with many mechanisms. compounds that mostly target enzyme systems immediately stop the pest from living. If such natural compounds or enzyme inhibitors or activating compounds are found, this problem can be directly reduced to a minimum.

The mechanism of action of insecticides on insects is becoming more elucidated by the day. The importance of the enzyme system in insects has emerged. Inhibition or activation of AChE by natural insecticides can affect the movement and balance of the organism or organisms exposed to it. AChE is typically synthesized in nerve, muscle, and some blood-related cells. The enzyme is localized extracellularly in excitable tissues, both nerve and muscle. The enzyme acetylcholinesterase hydrolyzes acetylcholine to choline and acetic acid. The formed choline is transported back to the nerve centers to form new ACh molecules (Purves et al. 2008). Acetylcholine is a neurotransmitter found at the intersections of nerves and muscles, in lymph nodes in the visceral motor systems, and in various parts of the central nervous system. Studies show that acetylcholine affects the speed of individual neurons. AChE inhibitors are used in the treatment of Alzheimer's disease, glaucoma, smooth muscle weakness and various autonomic nervous system disorders (Taylor et al. 2009).

Xanthine oxidase (XO) acts as an important source of oxygen-derived free radicals that cause oxidative damage before and after biological events in living tissues. XO enzyme is among the most important factors of joint inflammation in relation to hyperuricemia in the joints, as it causes uric acid to accumulate in the joint area. Natural products provide a broad pool of XO inhibitors that can turn into critical products. Today, the potential to develop successful natural products to prevent or control XO-related diseases is still largely unexplored.

The enzyme tyrosinase, also known as phenol oxidase, is known to be a copper enzyme commonly found in plants, insects, animals and microorganisms (Yang et al. 2005, Liu et al. 2006). For vertebrates and plants it is crucial for the formation of pigmentation and for biological processes such as the browning of fruits and vegetables. It is the key enzyme involved in the formation of melanin in melanocytes. Tyrosinase catalyzes both the hydroxylation of monophenols and the oxidation of o-diphenols to o-quinones. In insects, tyrosinase is a widespread enzyme that plays an important role in normal developmental processes such as cuticular stratification, scleritization, wound healing, opsonin production, and encapsulation and nodulation to defend against foreign pathogens (Wang et al. 2005; Ma and Kanost 2000). Diphenolase is the essential insect enzyme in the oxidation of catecholamine to its corresponding kinins, which are then metabolized to melanin or crosslinking proteins in sclerotin.

In this study, *C. botrys*, known as "yapışkan kazayağı" among the people, was collected in the central district of Erzincan in our country. In this study, it was aimed to reveal the insecticidal activity and enzyme activity potential of the essential oil and extracts obtained by different solvent and extraction methods by using the aerial part of the plant. The purpose of this study can be expressed in several steps. Firstly; is to eliminate the damage of this insect species, which is a storage pest, to grains with completely natural plant-derived products. In addition, it was aimed to determine the effects on acetylcholine esterase, tyrosinase and xo enzymes and to determine the content of essential oil components by GC-MS technique.

2 Materials and Method

2.1 Plant Material and Extraction

C. botrys plant was collected from the Central region of Erzincan province during the flowering period of June-July. Prof. Dr. Ali Kandemir validated the taxonomic identity of the plant material, and a voucher specimen (EBYU 11627) has been deposited at the Herbarium of the Faculty of Pharmacy, Erzincan Binali Yıldırım University. The aerial parts of 1000 g *C. botrys* plant were cut with scissors and boiled in water for approximately 3 hours at 110 °C in the Neo clavenger apparatus. The essential oil collected in the collection chamber of the Clavenger apparatus was taken into

glass vials and a small amount of sodium sulfate was added into it to remove the water (CB-EO). Total of 650 µl of essential oil was obtained. On the other hand, the remaining aqueous part was separated from the pulp with the help of filter paper. Slightly separated from the supernatant portion and this was recorded as the untreated aqueous portion (CB-F). The aqueous fraction was first extracted with ethylacetate and the solvent of the extract was evaporated (CB-EA, yield % 2.7, 2.7 gr). The remaining aqueous was then extracted with n-butanol and its solvent was evaporated (CB-nBu, yield % 4.6, 4.6 gr). At the end of these processes, the remaining aqueous portion was placed in a separate container (CB-L). Especially CB-F and CB-L extracts were dehydrated in the lyophilizer. Extracts were stored in the dark and at +4 °C until study time.

2.2 Production of insect cultures

Adult individuals belonging to *S. granarius* and *T. castaneum* insect species were used for insecticide activity studies. The cultivation of both insect species was carried out by modifying the methods of Karakoç et al (2006). In order to obtain the same stage adults from *S. granarius* to be studied, 1/3 of wheat was placed in 1 L glass jars and 500 adults were released on average and they were expected to lay eggs for 7 days. Adult individuals released after this period were collected. For obtaining *T. castaneum* eggs, nutrient medium consisting of sifted 70% flour and 30% dry yeast was prepared. Approximately 500 adults were placed in a 1-liter spawning jar containing a mixture of sifted flour and yeast. The eggs obtained as a result of the sieving process made from the laying jar with an interval of 3 days were transferred to the nutrient medium containing cracked wheat and yeast. The culture was incubated at 27±2 °C, 50±10% humidity and dark conditions, and adults 2-4 weeks old were used in the experiments.

Application of insecticidal activity tests; The extracts were prepared with acetone as a 10% plant extract/acetone (weight volume) mixture. This mixture was applied to the insect with a micro applicator at a dose of 1 µl/insect for each insect. In the control group, 1 µl of pure acetone was applied to each insect. In all treatments, 10 adult beetles were used in all replicates. The treated adult insects were transferred to 60 mm plastic petri dishes and incubated at 27±2 °C. After 24 hours, the data obtained were recorded. Analysis of variance was performed with the results obtained. Differences between treatments were determined by Tukey multiple comparison test.

2.3 GC/MS analysis

The analysis of the essential oil components was determined by Thermo Scientific brand Trace 1310 model GC-MS (gas chromatography-mass spectrometry) device. A DB-5MS capillary column (30 m x 0.25 mm inner diameter and 0.25 µm) was used on the device. 100 µl of EO sample was prepared by dissolving in 1.5 ml of chloroform. The carrier gas was helium at a flow rate of 1 ml/min and injections were in split mode (50:1). The mass-spectrometer interface temperature was set at 280 ºC. The temperature of the ion source was 250 ºC, electron energy 70 eV. Initial temperature for column oven temperature started at 60°C and held there for 2 minutes, increased to 200°C at a rate of 8°C/min and held for 3 minutes and increased to 250°C at a rate of 8°C/min. and hold for 3 minutes. The total run time was 32 minutes. The percentages of the essential oil components were calculated using peak areas without any correction factors. Identification and accuracy of compounds were confirmed using current Wiley, NIST and MS libraries.

2.4 Enzyme activity tests

Acetylcholine esterase (AChE) enzyme activity measurements of the extracts were made in vitro under laboratory conditions by making some changes in the Ellman method (Ellman et al. 1961). Accordingly, the reaction mixture consists of 0.1 M Tris-HCl (pH 8.0), 0.5 mM acetylcholine iodate, 0.5 mM EDTA, 0.025 mM DTNB and 0.05% sodium citrate solution. AChE enzyme activity was evaluated by measuring the absorbance of the yellow 5-thio-2-nitrobenzoate anion at 412 nm with the enzyme solution added to the medium.

The tyrosinase inhibitory activity properties of the extracts and essential oil were determined by the dopachrome method used by Sarıkurkcu et al. (Sarıkurkcu et al. 2018). Accordingly, 25 mL of each extractant was taken from the stock extract solutions prepared in DMSO at a ratio of 1:1 and 40 mL of tyrosinase solution and 100 mL of sodium phosphate buffer (pH 6.8) were added and mixed. The mixture was incubated at 25°C for 15 minutes. After adding 40 mL of L-DOPA, the mixture was incubated again at 25°C for 10 minutes. Absorbance was measured at 492 nm. The inhibitory effect of the scutellarin compound, which was used as a positive control, on the tyrosinase enzyme was determined. For this, 1 mg of the scutellarin compound was weighed and dissolved in 1mL of DMSO, then diluted ten times with distilled water. Enzyme activity was tested at five different scutellarin concentrations.

The inhibition activity measurements of the extract and essential oil against the Xanthine oxidase (XO) enzyme were determined by performing minor modifications to the methods mentioned in the literature (Bustanji et al. 2010; Mohammed et al. 2010; Sweeney et al. 2001; Chiang et al. 1994). For each experiment, the solution and substrate containing the enzyme were freshly prepared just before the experimental studies. The solution mixture contains 80 mM sodium pyrophosphate buffer (pH = 8.5), 0.120 mM xanthine and 0.1 unit of XO enzyme and the reaction takes place in this mixture. Absorbance was measured for the formation of uric acid at 295 nm at 25°C. Thus, the initial rate of the reaction was calculated. Each extract was first dissolved in buffer solution and added to the reaction mixture to determine the inhibitory effect at a concentration of 200 µg/ml.

3 Results

Five essential oils (CB-EO), untreated aqueous extract (CB-F), ethylacetate (CB-EA), n-butanol (CB-nBu), and processed aqueous extract (CB-L) from the aerial parts of *C. botrys* plant extract was obtained. Insecticidal activities of these extracts were determined by contact toxicity tests against *Sitophilus granarius* and *Tribolium castaneum* storage pests. It was determined that only CB-EA extract caused 19.31% death to

adult insects of *Sitophilus granarius* (F= 37.23; df = 5,12; P<0.05). The other extracts, especially the essential oil, did not show any effect for the two insects. At the same time, acetylcholinesterase, xanthine oxidase (XO) and tyrosinase enzymes of the same extracts were examined in the concentration range of 0-1 mg/mL. As a result of the enzyme activity test, it was determined that only the CB-F coded extract had an activation effect on the tyrosinase enzyme. Content analysis of essential oil was done with GC-MS device. GC-MS chromatogram of CB-EO extract is given in Figure 1 and analysis result is given in Table 1. A total of 58 component analyzes were determined according to the GC-MS analysis result. α-Eudesmol compound was analyzed as the main component. The results were evaluated by comparing with the results of the Wiley and NIST library.

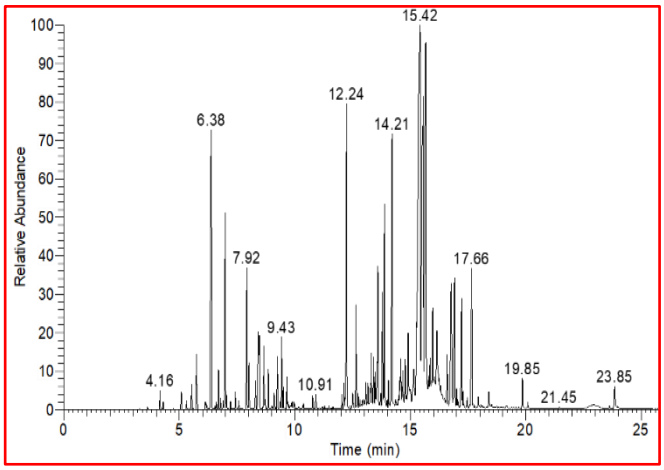

Fig. 1 GC-MS chromotogram of CB-EO

Table 1 Chemical compositions of *C. botrys* essential oils (CB-EO).

RT	Compounds Name	% Area
4.16	4-Hydroxy-4-methyl-2-pentanone	0.22
5.08	Bornylene	0.28
5.53	α-pinene	0.43
5.74	α-Fenchene	1.10
6.13	Sabinene	0.22
6.37	α-Myrcene	7.52
6.69	3-Carene	0.47
6.98	Limonene	2.92
7.42	γ-Terpinene	0.23
7.92	Fenchone	2.41
8.01	Linalool	0.77
8.30	Fenchyl alcohol	0.44
8.45	Trans-2-pinanol	2.61
8.66	Cis-sabinene hydrate	1.02
8.85	9-hydroxy-linalool	0.49
9.09	Borneol	0.20
9.24	4-Terpineol	0.74
9.50	cis-Piperitol	0.24
9.66	trans-Piperitol	0.48
10.77	α-Fenchyl acetate	0.18
10.91	p-Menth-1-en-9-ol	0.22
12.12	(-)-α-Elemene	0.33
12.23	β-Eudesmol acetate	5.78
12.50	α-Gurjunene	0.28
12.64	Caryophyllene	1.36
13.07	α-Humulene	0.29
13.17	δ-Cadinene	0.29
13.31	Eremophilene	0.90
13.40	Germacrene-D	0.56
13.48	α-Selinene	0.37
13.59	α-Muurolene	2.58
13.79	α-Amorphene	1.76
13.88	Germacrene B	3.17
14.05	Spathulenol	0.31
14.20	Elemen	5.70
14.58	Alloaromadendrene oxide	1.13
14.67	Caryophyllene oxide	0.51
14.77	Veridiflorol	0.65
14.90	Globulol	1.30
14.99	Cubenol	0.28
15.14	Spathulenol	0.81
15.26	α-Guaiene	0.27
15.40	α-Eudesmol	13.2
15.55	Veridiflorol	8.43
15.66	Elemol	8.91
15.85	Isoaromadendrene epoxide	0.33
15.97	Juniper camphor	1.01
16.15	γ-himachalene	1.49
16.59	Aromadendrene oxide	0.72
16.76	(Z)-valerenyl acetate	3.38
16.91	γ-Gurjunene	2.75
17.00	Farnesene	0.23
17.22	Isospathulenol	2.06
17.65	trans-Longipinocarveol	3.03
17.94	α-copaene-11-ol	0.23
18.40	Hexadecanoic acid	0.30
19.85	Phytol	0.39
23.85	Hexadecanoic acid, methyl ester	0.54

The effects of the extracts on the enzyme activities are shown in Table 2. While CB-L and CB-nBu extracts showed an inhibitory effect on AChE enzyme activity, the same extracts activated the activity of XO and tyrosinase enzymes.

Table 2 Effects of extracts on Acetylcholine esterase (AChE), Xanthine oxidase (XO), and Tyrosinase enzymes activities.

Extracts	AChE (IC_{50})	XO (AC_{50})	Tyrosinase (AC_{50})
CB-L	460 µg/mL	604 µg/mL	250 µg/mL
CB-nBu	376 µg/mL	493 µg/mL	423 µg/mL
CB-F	-	641 µg/mL	-
CB-EA	-	-	-
CB-EO	-	-	-

The contact toxicity effects of essential oil and extracts obtained from *C. botry* plant on *S. granarius* and *T. castneum* insects after 24 hours are given in Table 3. It was determined that CB-EA extract had a mortality rate of approximately 20% against *S. granarius*, while it had a mortality rate of approximately 4.5% against *T. castneum*. However, the other extracts and essential oil showed no effect.

Table 3 Contact effect of different extracts of *C. botrys* against S. granarius and T. castneum after 24 hours.

Extracts	% Mortality±StDev	
	S. granarius	***T. castaneum***
Control	0.00±0.00b[1]	0.00±0.00b
CB-EO	0.00±0.00b	0.00±0.00b
CB-F	0.00±0.00b	0.00±0.00b
CB-EA	19.31±1.66a	4.53±3.41a
CB-nBu	0.00±0.00b	0.00±0.00b
CB-L	0.00±0.00b	0.00±0.00b

[1]Different letters in the same column indicate that the means are statistically significantly different. (Anova P<0,05, Tukey test).

4 Discussion

In studies on the essential oil analysis of the *C. botry* plant, the essential oil differs in terms of amount and composition. Bicyclic sesquiterpenoids were found in *C. botrys* (Kokanova-Nedialkova et al. 2009). The smell that gives the plant its unique smell is due to monoterpenes and sesquiterpenes (Kletter et al. 2001). As a result of *C. botrys* of GC-MS analysis; monoterpenes (camphor, δ-3-caren, fenchone, linalool, menthone, nerol, β-pinene, pulegone, terpineol-4, and thujone) and sesquiterpenes (β-elemene, elemol, and β-eudesmol) compounds, as well as mucus anserine of anserine compound. It has been found to be responsible for its aromatic, herbaceous, earthy, dull, heavy and pine-like odor (Buchbauer et al. 1995). The first essential oil analysis of the *C. botry* plant mentions the ascaridiol compound and is a bicyclic monoterpene species with a very unusual bridging peroxide functional group. It was determined that this plant species, also known as crow's feet in our country, contains the compound 2-(4a.8-dimethyl-1.2.3.4.4a.5.6.7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol (Karabörklü et al. 2011). In their study conducted in Özer et al (2017) determined the essential oil content of the *C. botry* plant they collected from the provinces of Isparta, Konya and Afyon. unlike us, they determined ledol, elemol, germacrene D-4-ol and eudesm-7(11)-en-4-ol compounds as the main components. In addition, this group examined the effects of essential oils on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase enzymes and found a little activity against tyrosinase enzyme (Özer et al. 2017). These results are important in that they show similarities with our study results. In another study, the effect of essential oil obtained from the same plant species on three different storage pests of *T. castaneum* beetle was investigated by a fumigant effect experiment. They found a high inhibitory effect there (Kumar and Pandey 2021).

Tyrosinase, xanthine oxidase, and acetylcholinesterase are enzymes that play an important role in normal insect development. For this reason, it may be possible to control the damages that will be caused by inhibiting or activating enzymes, as disrupting the working potential will directly affect the life of the insect. Although CB-L and CB-nBu extracts are very strong, they appear to have moderate enzyme activities. With further studies, compounds that cause changes in enzyme activities can be determined. The low activity values may be due to the low amount of effective compounds.

5 Conclusion

This study is about the insecticide and enzyme activity properties of *C. botrys*, which is used for its pleasant aromatic odor and medicinal properties. Scientific studies support the medical potential of *C. botrys* in developing new drugs. Different isomers of ascaridole of different origins have been identified in *C. botrys* oil. The absence of ascaridol compound in this plant species proves that it has negative effects on both insecticide and enzyme activity. In conclusion, research into the enzyme and insecticidal properties of *C. botrys* plant extracts is important because of its potential uses in biotechnology, sustainable agriculture, pest management, and the creation of new natural products.

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All authors contributed equally to this work.

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