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Scientific Research

Identification of chemical compounds of *Foeniculum vulgare* essential oil, antioxidant power, and its antifungal effect on postharvest grape spoilage molds

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ABSTRACT

Grape is a very perishable fruit that has a short shelf life. Due to the increase in consumer awareness, the tendency to use natural preservative compounds instead of pesticides and chemical preservatives has increased. Plant essential oils are considered to be the most appropriate natural compounds due to their potentially effective compounds. Therefore, the aim of this research was to identify the chemical compounds of *Foeniculum vulgare* essential oil, determine its phenolic and flavonoid content, and investigate the antioxidant and antifungal activity of this essential oil against a number of molds that cause spoilage after grape fruit harvesting. The compounds identified in *F. vulgare* essential oil included limonene, fenchone, p-allylanisole and anethole, which made up 97.60% of the total essential oil. The total phenol content of *F. vulgare* essential oil was equal to 26.29 mg GAE/g and the total flavonoid content was equal to 19.23 mg QE/g. The antioxidant properties of the essential oil of this plant were obtained by DPPH, ABTS and beta-carotene-linoleic acid decolorization methods, respectively, 38.56, 42.12, and 30.30%. Also, , it was found that *Foeniculum vulgare* essential oil is capable of inhibiting and controlling *Rhizopus stolonifera*, *Aspergillus niger*, and *Botrytis cinerea*. Due to the antimicrobial and antioxidant compounds present in *F. vulgare*, the essential oil of this plant can be used as a compound with practical properties to increase the shelf life of agricultural products, including grapes.

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1- Introduction

Ensuring the food security of the world population requires further development in the agricultural sector. Therefore, it is necessary to use high-quality and long-lasting agricultural and garden cultivars. But the presence of diseases and pests in products is one of the most important limiting factors in this field [1].

Grapes are non-secreting fruits that contain carbohydrates, vitamins, minerals and antioxidants, which have a very short shelf life due to drop of pods, loss of water, low firmness, change in the color of the tail of the cluster and fungal rot [2]. Fungal diseases such as mold and cluster rot caused by*Botrytis cinehra*، *Aspergillus*، *Alternaria*، *Rhizopus* It reduces the yield and quality of grapes. In addition, the presence of fungi in grapes may have adverse effects on volatile compounds and thus on the taste of grape products, which is one of the most important factors affecting consumer acceptance [3]. Using fungicides on a weekly basis is the best way to control fungal diseases. But chemical pesticides have caused the production of disease-resistant species. Therefore, more of these fungicides are used to control this problem. Also, due to their temporary effect, they may be used several times, and all these factors are effective in environmental pollution and damage to humans and other creatures [4]. In addition, the sublimation of sulfur gas in the form of rounds is one of the most common methods of controlling the rotting of grapes stored in cold storage, which causes damage to the grapes and the wood of the grape cluster [5]. Therefore, nowadays, non-chemical control of pests and plant diseases using natural compounds and physical treatments is highly considered. In this context, environmentally compatible agents, including essential oils and plant extracts, are suitable alternatives to synthetic fungicides in controlling disease and maintaining product quality. Besides being safe, plant extracts have important compounds and have anti-cancer, anti-inflammatory, antimicrobial and antioxidant properties [6].

Fennel with scientific name **(***Common fennel***)** It is an aromatic and herbaceous plant from the parsley family. This plant is similar in appearance to dill, which has yellow umbrella flowers [7]. Fennel contains protein, calcium, potassium, iron, fat, phosphorus, a little mucilage sugar, about 4-5% oil and vitamins.A AndB is [8]. Fennel has been used in traditional medicine as expectorant, laxative, analgesic, carminative and anti-inflammatory. In the food industry, this plant is also used as a chocolate flavoring agent [9]. Among the most important effective substances of this plant, we can mention sterols, phenol, trans-anthole, phenolic glycosides, fencones and sterols [10]. The antimicrobial and antioxidant effects of this plant have been reported in various studies [9-12].

Considering the antimicrobial and antioxidant effects of fennel plant, the aim of this study was to identify the chemical compounds, antioxidant power and antifungal effect of the essential oil of this plant on a number of molds that cause grape fruit spoilage.

2- Materials and method

2-1- Chemicals and microbial strains

Quercetin solution, Folin-Ciocalcio reagent, solutionDPPH And a solution ABTS From Sigma (USA) and Sabrose Dextrose Broth and Disk Blank culture medium from Merck (Germany).

strains*Aspergillus niger* ،*Rhizopus astolonifera* And *Botrytis cinehra* It was prepared from the microbial collection of the Department of Food Industry Science and Engineering, Khuzestan

University of Agricultural Sciences and Natural Resources.

2-2- Extraction of fennel essential oil and determination of its chemical composition

After grinding the dried fennel plant, its essential oil was extracted through water distillation with the help of a Cloninger machine at a temperature of 100 degrees Celsius for 3 hours. Quantification of essential oil components was done using gas chromatography [13].

2-3- Measurement of total phenol

To measure total phenol content Using the reagentFolin-Ciocalcho, first, 20 microliters of essential oil was added to 110 microliters of fresh Folin-Ciocalcio reagent. In the next step, 70 microliters of sodium carbonate solution was added to it. Then the solution was kept for 30 minutes at room temperature and its absorption in the wavelength 765 nm was recorded.The amount of total phenol was reported in mg of gallic acid per gram of essential oil [14].

2-4- Total flavonoid measurement

For this purpose, 1 milliliter of essential oil or quercetin solution with concentrations of 0.5-0.5 mg per milliliter was added to 0.3 milliliters of sodium nitrite solution (5 percent). Then 0.3 milliliters of aluminum chloride (10 percent by weight/volume) was added to The samples were added and the mixture was stirred for 6 minutes. After that, 2 ml of sodium hydroxide (1 M) was added. At the end of the absorption of the solution at a wavelength of 510 nm, the total flavonoid content was measured based on milligrams of quercetin per gram of essential oil [15].

5-2- Investigating the antioxidant property of essential oil

2-5-1- Measurement of free radical inhibition activityDPPH

To determine free radical scavenging activityDPPH, 50 microliters of essential oil or control, with 5 milliliters of solution DPPH Ethanol (0.12 mM) was mixed. The resulting solution was kept at 25°C for 30 minutes and its absorption (A) was read at a wavelength of 517 nm. Then free radical scavenging activityDPPH It was measured as follows [16].

$$\text{Radical absorption percentage DPPH} = \frac{(\text{Witness sample absorption} - \text{Absorption essential oil})}{\text{Witness the absorption sample}} \times 100$$

2-5-2- Inhibition of free radicals ABTS

Cation radicalsABTS By mixing a 7 mM solutionABTS and 2.45 millimolar solution of sodium persulfate in water, with a ratio of 1:1 and It was produced by keeping it in a dark place for 16 hours. The produced solution was diluted using methanol until the absorbance of 0.7 at 734 nm wavelength was reached. After that, 3.9 ml of diluted solution of cationic radicalsABTS, mixed with 0.1 ml of essential oil. Finally, the absorbance of the sample at 734 nm wavelength was read after 6 minutes at room temperature. The amount of radical scavenging activityABTS It was calculated using the following formula [17].

$$\text{Radical absorption percentage ABTS} = \frac{(\text{Witness sample absorption} - \text{Absorption essential oil})}{\text{Witness the absorption sample}} \times 100$$

2-5-3- beta-carotene-linoleic acid dyeing method

During this test, the antioxidant power of the essential oil was determined by recording the color changes of beta-carotene by a spectrophotometer over time. The higher the antioxidant power of the essential oil, the faster the color change of beta-carotene due to the reaction with free radicals decreases [18].

2-6- Investigating the antifungal properties of the essential oil

2-6- 1- Disc diffusion method

Activated microbial strains were cultured on Sabrose dextrose agar medium. After absorbing the strains on the medium, the blank discs soaked in essential oil were fixed on the medium. After 72 hours of greenhouse at 27°C, the diameter of non-growth halos was measured in millimeters [19].

2- 6- 2- Diffusion of wells in agar

After surface cultivation of each of the studied mushrooms on sabrose dextrose agar medium, the prepared essential oil was poured into the created wells (with a diameter of 6 mm). Then, the plates were kept for 72 hours at a temperature of 27 degrees Celsius and the halo of non-growth was measured in millimeters [20].

2-6-3- minimum inhibitory and lethal concentration

Successive dilutions of fennel essential oil (128, 256 and 512 mg/ml) were mixed with microbial suspensions in the test tube. The samples were kept in a greenhouse for 72 hours at a temperature of 27 degrees Celsius. The created turbidity was evaluated visually and the first tube in which no turbidity was observed was recorded as the minimum inhibitory concentration.

In order to determine the minimum concentration of lethality, tubes without turbidity were cultured on sabrose dextrose agar medium. After greenhouse, the first concentration in which no colony grew was recorded as the minimum lethal concentration of the essential oil [21].

2-7- Statistical analysis

from one-way analysis of variance and Duncan's test at a confidence level of 95% (p<0.05) was used for data analysis. All the tests were done in 3 repetitions and the results are as standard deviation± The average was reported.

3. Results and Discussion

3-1- Chemical compounds of essential oil

The compounds identified in fennel essential oil using gas chromatography are shown in Figure 1. It can be seen that 4 identified compounds includelimonene، fenchone، p-allylanisole And anetholeIn total, they made up 97.60% of the total essential oil andanetholetook the most amount. In a study, the percentage of total extracted compounds of fennel seed essential oil has been reported as 95.8%anetholewith 68.53 percent,spoil with 10.42 percent,limonene with 6.24 percent andfenchone With 5.45%, they accounted for the highest amount respectively [22].Telci et al (2009), Compoundsanethole(65.59 percent),spoil(13.11 percent),limonene (8.54 percent) andfenchone reported (8.54%) as the main compounds in Egyptian fennel essential oil [23]. The difference in the type and amount of fennel essential oil compounds is due to differences in geographical regions, the type of cultivated varieties, the methods used for extraction, the stage of plant harvesting and the conditions of analysis [9].

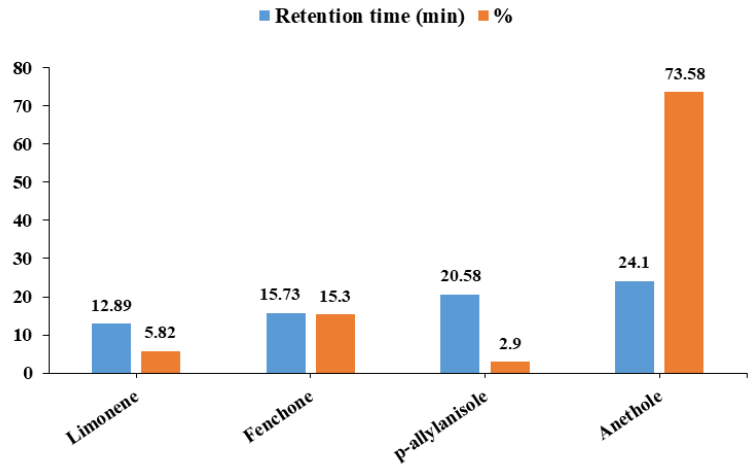

Fig 1 The chemical composition of *Common fennel*essential oil.

3- 2- Phenol and flavonoid essential oil

Total phenol content of fennel essential oil is 26.29 The milligram of gallic acid per gram of essential oil and its total flavonoid content was equal to 19.23 milligrams of quercetin per gram of essential oil (Figure 2). In studies, total phenolic and flavonoid content of fennel essential oil has been reported as 12.87 milligrams of gallic acid per gram of essential oil and 39.67 milligrams of quercetin per gram of essential oil [9].

Anwar et al. (2009), reported the amount of total phenol for the ethanolic extract of Pakistani fennel as 967.50 mg of gallic acid per gram of extract [11]. Also, values of 100 and 150 mg of chlorogenic acid per gram of extract have been reported for total phenolic extracts produced from cultivated and wild fennel in Italy [24]. These differences can be attributed to different extraction techniques and plant parts used for essential oil extraction. In addition, the origin, variety, growth rate during harvesting and the quality of raw materials in combination with pretreatments and extraction modes can affect the yield and compounds. to affect the essential oil produced [17]. However, phenolic and flavonoid compounds, which constitute a major part of the secondary metabolites of plants, play a role in the emergence of various biological properties such as antioxidant and antimicrobial properties [25].

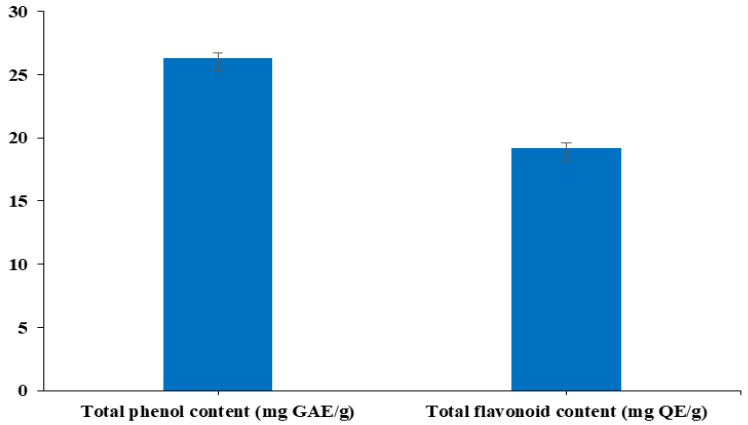

Fig 2 The total phenol and flavonoid content of *Common fennel*essential oil.

3-3- Antioxidant activity

Since the effective compounds of plants have complex reactive activity, it is recommended to use at least two antioxidant tests to confirm and determine their antioxidant activity [25]. Therefore, in this research, the antioxidant property of fennel essential oil was measured by 3 methods of free radical inhibitionDPPHFree radical inhibition ABTS‹ **Beta-carotene-linoleic acid discoloration was evaluated, which was equal to 38.56%, 42.12% and 30.30%, respectively (Figure 3). In studies, the amount of antioxidant property of fennel essential oil in different concentrations (150-1200 mg/ml) has been reported as 16-57% [26].** The difference in antioxidant power reported in different studies can be due to ecological differences and different methods used to determine antioxidant properties. In addition, the position and composition of hydroxyl groups and the presence of functional and ketone groups can also play a role in the occurrence of antioxidant properties. Also, the difference in the amount and type of phenolic compounds and other main compounds of essential oils of different varieties is effective in the amount of antioxidant properties [25, 27]. Phenolic compounds are able to donate hydrogen atoms to free radicals and thus stop the chain reaction of the propagation step during the oxidation process [28].

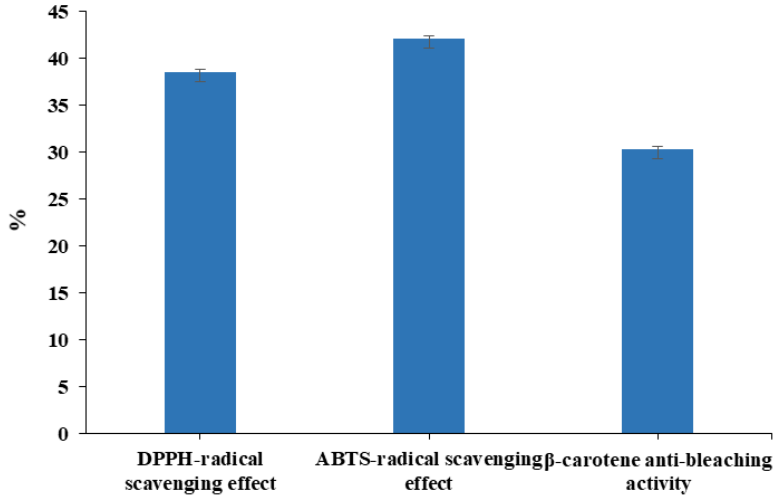

Fig 3 The antioxidant activity of *Common fennel*essential oil.

3- 4- Antifungal properties of essential oil

The results of the antimicrobial effect of fennel essential oil on the fungi that cause spoilage of grape fruit by two methods, agar disk diffusion and well diffusion, are shown in Figure 4. It can be seen that there is a significant difference in the diameter of non-growth halos in both methods, and the highest diameter of the non-growth halo is related to mushroom.*Rhizopus astolonifera* and the smallest halo diameter related to the mushroom*Botrytis cinehra*Was. The higher antimicrobial effect of the essential oil in the diffusion method in the well compared to the disk diffusion method is due to the direct contact of the essential oil with the fungi in this method, while in the disk diffusion method, the essential oil shows its antimicrobial effect after passing through the disk [6].

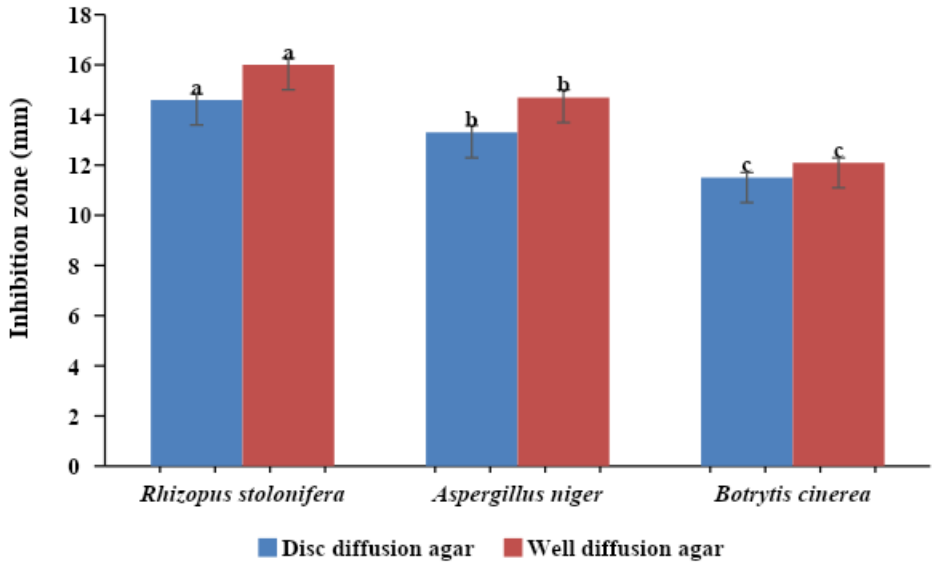

Fig 4 The antifungal activity of *Common fennel*essential oil, according to disk diffusion agar and well diffusion agar methods.

The minimum inhibitory and lethal concentration of the essential oil is also shown in Figure 5. It is observed that the minimum inhibitory concentration for fungi*Aspergillus niger* And*Rhizopus astoloniferae*qual to 128 mg/ml and for mushrooms*Botrytis cinehra* It was equal to 256 mg/ml. In addition, the minimum lethal concentration of Aspergillus niger fungi and*Rhizopus astolonifera* equal to 256 mg/ml and the minimum lethal concentration of the fungus*Botrytis cinehra* It was equal to 512 mg/ml.

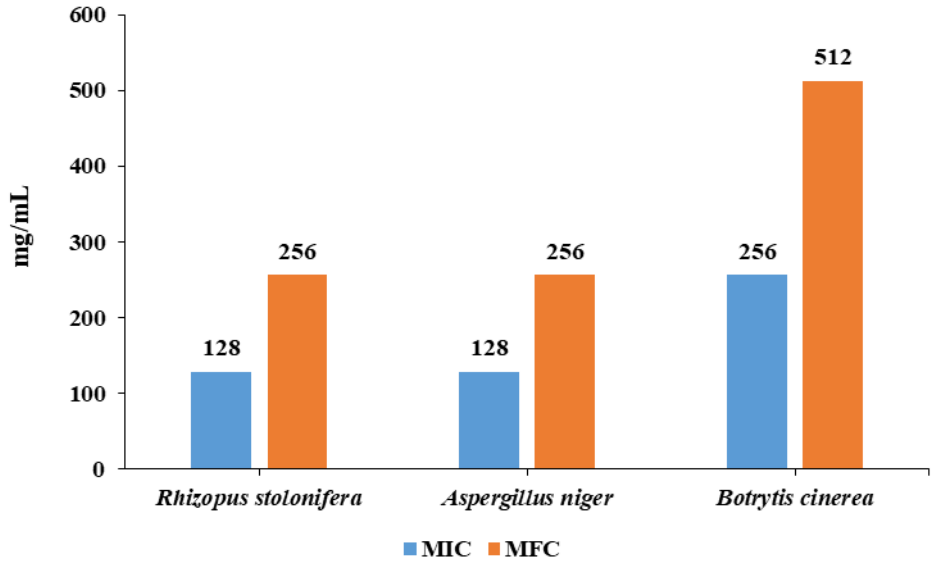

Fig 5 The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Common fennel*essential oil.

In studies of the antimicrobial properties of fennel essential oil against various microorganisms, including*Escherichia coli*، *Staphylococcus aureus* And*Candida albicans* to the compounds found in essential oils, includingspoil، fenchone Andlimonene attributed [29]. Antimicrobial

effect of a combination likelimonene It is related to its ability to disturb the integrity of the membrane of bacterial or fungal strains and inhibit the process of ion transport and respiration [30].Anwar et al. (2009), in the investigation of the antimicrobial effect of fennel essential oil, reported that among different microorganisms, Aspergillus niger with a diameter of 28 mm and a minimum inhibitory concentration equal to 80.6 mg/ml is one of the most sensitive microorganisms to the essential oil [11]. alsoSingh et al. (2006), high antifungal activity of different concentrations of fennel essential oil against different species of fungi*Aspergillus*، *Fusarium*، *Penicillium*And*Corollary* attributed to the phenolic compounds and other effective compounds in the essential oil [28].

4 - Conclusion

According to the results of this research, it was determined that fennel essential oil has a favorable phenolic and flavonoid content. The presence of these compounds has been effective in the manifestation of the antioxidant and antimicrobial properties of the essential oil of this plant, so that it is able to inhibit the growth of fungi that cause grape spoilage, including*Rhizopus astolonifera*، *Aspergillus niger* And*Botrytis cinehra* is. Therefore, if fennel extract and essential oil are used as natural preservatives, the safety and quality of these products can be increased by controlling the growth of many micro-organisms that contaminate fruits and vegetables.

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6- Resources

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شناسایی ترکیبات شیمیایی اسانس رازیانه، قدرت آنتی اکسیدانی و اثر ضدقارچی آن بر کپک‌های عامل فساد پس از برداشت میوه انگور

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چکیده

انگور میوه‌ای بسیار فسادپذیر است که دارای عمر نگهداری کوتاهی می‌باشد. به دلیل افزایش سطح آگاهی مصرف‌کنندگان تمایل به استفاده از ترکیبات نگهدارنده طبیعی به جای آفت‌کش‌ها و نگهدارنده‌های شیمیایی افزایش یافته است. اسانس‌های گیاهی به دلیل ترکیبات مؤثره بالقوه از مناسب‌ترین ترکیبات طبیعی مورد استفاده به شمار می‌آیند. از این رو، هدف از این پژوهش، شناسایی ترکیبات شیمیایی اسانس رازیانه، تعیین میزان فنول و فلاونوئید آن، بررسی فعالیت آنتی‌اکسیدانی و فعالیت ضد قارچی این اسانس بر آن بر تعدادی از کپک‌های عامل فساد پس از برداشت میوه انگور بود. ترکیبات شناسایی شده در اسانس رازیانه شامل limonene، fenchone، p-allylanisole و anethole بود که در مجموع ۹۷/۶۰ درصد کل اسانس را تشکیل دادند. میزان فنول کل اسانس رازیانه برابر با ۲۶/۲۹ میلی‌گرم گالیک اسید در گرم اسانس و میزان فلاونوئید کل آن برابر ۱۹/۲۳ میلی‌گرم کوئرستین در گرم اسانس به دست آمد. خاصیت آنتی اکسیدانی اسانس این گیاه در روش مهار رادیکال‌های آزاد DPPH، ABTS و **رنگ‌بری بتاکاروتن- لینولئیک اسید** به ترتیب ۳۸/۵۶، ۴۲/۱۲ و ۳۰/۳۰ درصد به دست آمد. همچنین در بررسی اثر ضد قارچی بر کپک‌های عامل فساد مشخص گردید که اسانس رازیانه قادر به مهار و کنترل کپک‌های *ریزوپوس استولونیفر*، *آسپرژیلوس نایجر* و *بوتریتیس سینه‌را* می‌باشد. با توجه به ترکیبات ضد میکروبی و آنتی اکسیدانی موجود در رازیانه، اسانس این گیاه می‌تواند به عنوان یک ترکیب با خاصیت عملگرایی در افزایش عمر نگهداری محصولات کشاورزی از جمله انگور مورد استفاده قرار گیرد.