Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 23 No. 4 Ser. No. 81 2022

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Remediation of zearalenone mycotoxin contamination in rumen fluid by phytochemical compounds of *Zataria multiflora*

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10.22099/IJVR.2022.39561.5746

(Received 22 Jan 2021; revised version 5 Jul 2022; accepted 29 Aug 2022)

Abstract

Background: Zearalenone (ZEA), which is one of the most prevalent wheat and corn seeds mycotoxins causes acute and chronic toxicities in ruminants, poultry, and aquatic animals. Among commercial toxin binders, only a few active charcoals have the significant ability to adsorb ZEA contamination; nevertheless, active charcoal is not considered a sound additive by the feed industry. **Aims:** This study aimed to screen and identify the ZEA-degradation compounds of the *Zataria multiflora* (Shirazi thyme) in the cattle rumen fluid. **Methods:** In this investigation, essential oil and different extracts (*n*-hexane, ethyl acetate, and methanol) of the aerial part of Shirazi thyme (at three concentrations of 0.5, 1, and 2 mg/ml) were screened to reduce ZEA contamination conditions (2 µg/ml) in rumen fluid. ZEA-content was analyzed by high-performance liquid chromatography (HPLC) with a fluorescence detector. In addition, Shirazi thyme phytochemical compounds responsible for eliminating ZEA were localized by HPLC-based activity profiling and then identified by mass spectrometry (LC-MS). **Results:** Both *n*-hexane and methanol extracts of *Z. multiflora*, considerably remediated ZEA (63-78%) from rumen fluid. According to HPLC-based activity profiling of *Z. multiflora* extract and LC-MS analysis, two triterpene compounds, including ursolic and oleanolic acids were introduced as ZEA degradation agents. **Conclusion:** *Z. multiflora* could be recommended as a new botanical source, and ursolic and oleanolic acids could be introduced as new phytochemical compounds that degrade ZEA.

Key words: Metabolite profiling, Shirazi thyme, Triterpenes, Zearalenone degradation

Introduction

The mycotoxin contamination of main cereals in human and animal food is a big problem around the world (Belhassen *et al*., 2015). Zearalenone is one of the prevalent mycotoxins produced mainly by the growth of *Fusarium graminearum* and *F. culmorum* on wheat and corn seeds; hence it is usually found in food and feed (Tanaka *et al*., 1988). ZEA causes acute and chronic toxicities in poultry, broiler, and aquatic animals (CAST, 2003; Pietsch *et al*., 2013; Pietsch *et al*., 2014). Due to their structural similarity to estrogens, ZEA and its metabolite α-zearalenol can bind to estrogen receptors and induce hyper-estrogenism in animals (Rodrigues, 2014). Therefore, ZEA causes a wide variety of effects such as decreased fertility, ovarian cysts, vulvovaginitis, abnormal estrus cycles, reduced milk production, and enlargement of the vulva, uterus, and mammary glands in ruminant animals (CAST, 2003).

The utilization of mycotoxin adsorbent agents as feed additives is the most promising approach to reducing the risk of mycotoxicoses in animals and minimizing the carry-over of mycotoxins from contaminated feed into animal-derived products such as milk and meat (Ramos *et al*., 1996; Huwig *et al*., 2001). Inorganic adsorbents such as bentonites and zeolites can considerably adsorb some mycotoxins such as aflatoxins (AFs), but their efficacy against ZEA was minimal and less encouraging (Ramos *et al*., 1996). Although some active charcoals can adsorb ZEA considerably, they also adsorb micronutrients (such as proteins, vitamins, etc.) and have adverse effects on minerals and trace elements (Ward *et*

al., 1991). Therefore, research is now under way to develop new mycotoxin reducing agents such as organic acids, probiotics (microorganisms), and phytogenics.

Medicinal plants are relatively safe and are rich in various active natural compounds that play a crucial role in the life of many people around the world. It has been reported that the essential oils of many medicinal plants are biologically active and some have the potential to degrade ZEA content (Peana *et al*., 1994; Hoseiniyeh *et al*., 2012). Essential oils and extracts from some herbal plants (belonging to the Lamiaceae family) have been shown to reduce aflatoxin contamination in agriculture crops, mainly due to the inhibition of the *Aspergillus flavus* growth and aflatoxins-synthesis (Reddy *et al*., 2009; Alinezhad *et al*., 2011) and/or the degradation of aflatoxins (Gorran *et al*., 2013; Kohiyama *et al*., 2015). Plants that belong to the Lamiaceae family are generally known for their multiple analgesic and anti-inflammatory pharmacological effects (Hernández-Pérez *et al*., 1995) as well as antioxidant (Cuppett and Hall, 1998), hypoglycemic (Hosseinzadeh *et al*., 1998), and hepatoprotective properties (Wasser *et al*., 1998). Shirazi thyme (*Zataria multiflora*; Lamiaceae) is native to Iran, Afghanistan, and Pakistan and rich in alkanes, fatty acids, flavonoids, phenols, hydroxycinnamic acids, phytosterols, monoterpenes, triterpenes, tannins, and saponins (Gupta and Gupta, 1972; Ali *et al*., 2000; Mohagheghzadeh *et al*., 2004; Rajaei and Khajehali, 2009). The antibacterial, antioxidant, antiviral, and immune stimulant activities of Shirazi thyme on humans, poultry, and aquatic animals have been reported in several articles (Sajed *et al*., 2013; Soltani, 2013, Shomali and Mosleh, 2019). In addition, Shirazi thyme can decrease ammonia concentration in the rumen by inhibiting the growth and deamination activities of amino acid-fermenting bacteria in ruminant animals (Castillejos *et al*., 2006; Taghavi-Nezhad *et al*., 2013). However, plant extracts include various bioactive compounds with multiple abilities that may degrade ZEA. In the present study, we investigated the ZEA-degradation activity of *Z. multiflora* essential oil and extracts, and tracked the compounds responsible for this function.

Materials and Methods

Chemicals and reference compounds

Solvents used for extraction were purchased from Emrtat Company (Tehran, Iran). HPLC grade solvents for chromatography were provided from Scharlau (Barcelona, Spain). ZEA standard (Z2125), α-zearalenol (Z0166), and β-zearalenol (Z2000) were purchased from Sigma-Aldrich. The working solution at the 1000 ppm concentration was diluted in methanol and stored at -20°C. Seven concentrations of the standard ZEA (15.9, 31.8, 62.5, 125, 250, 500, and 1000 µg/ml) were prepared in methanol and injected into the HPLC-FLD.

Preparation of plant extracts and essential oil

The aerial part of *Z. multiflora* (Shirazi thyme, MPH-1799) was collected at the flowering stage from its wild habitat, Yazd province, Iran. It was identified by Dr. A. Sonboli (Shahid Beheshti University), and the voucher specimen was deposited in the Medicinal Plants and Drugs Research Institute (MPDRI) herbarium, Shahid Beheshti University. The collected part was dried and ground to a fine powder and stored in airtight bottles. Various extracts of the aerial part were obtained by macerating 10 g of fine powder with 50-70 ml of each solvent (*n*-hexane, ethyl acetate, and methanol) at room temperature for 48 h in a shaker incubator. The extracts were filtered through Whatman filter paper (No. 1) by suction with the vacuum pump. The evaporation of solvents was carried out by the rotary evaporator (Heidolph, Germany) under a vacuum. The crude extract was stored at 4°C for further studies.

The essential oil was isolated by hydro-distillation for 3 h, using a Clevenger-type apparatus according to the method recommended by British Pharmacopoeia (1988). The distillate essential oil was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C. In addition, to confirm *Z. multiflora* identification, GC-FID, and GC-MS analysis of the essential oil were carried out according to Farzaneh *et al*. (2015).

Preparation of rumen fluid (RF)

The RF was collected from two ruminal cannulated non-lactating (female) Holstein dairy cows (Age, 48 months ± 2; body weight (BW), 611 ± 31 kg; means±SD) with permanent rumen fistula in Sepahan Foudeh Milk and Meat Co., Isfahan, Iran. Briefly, RF was collected 6 h after being fed (Bailey, 1967) with a high forage total mixed ration diet (25% alfalfa hay, 25% corn silage, 25% wheat straw, 4.2% beet pulp, 20.8% concentrate mix; dry matter (DM) basis) as recommended by Krizsan and Huhtanen (2013). Finally, RF was filtered through two-ply Tiffany and the grayish-green color liquid RF with pH 5.9 was used. RF was anaerobically stored at 4°C and -20°C.

ZEA-degradation monitoring in RF

Mycotoxin degradation activity of each extract and essential oil of Shirazi thyme was investigated at three concentrations 0.5, 1, and 2 mg/ml according to Gorran *et al*. (2013) with some modifications. Briefly, 1, 2, and 4 mg extract/essential oil was added to a 100 µL DMSO (Dimethyl sulfoxide) solution, vortexed for 5 min and sonicated for 10 min. Each sonicated product (100 µL) was then added to a 10 ml glass cryovial tube containing 1.9 ml phosphate buffer (pH 6) supplemented with 20% bovine RF (to simulate the rumen fluid condition with no significant effect on ZEA extraction and purification) and 2 µg/ml ZEA standard. Ratios of extract/essential oil to ZEA were 250:1, 500:1, and 1000:1 (w:w). After 6, 12, 24, and 36 h incubation time on a shaker incubator at 39°C and 200 rpm in darkness, which was not anaerobically conditioning, ZEA-degradation activity was determined by HPLC. The RF containing ZEA without plant extract and essential oil, as well as RF

containing plant extract or essential oil without ZEA were considered as controls.

Tracing of ZEA-degradation fractions in *Z. multiflora* extracts

To localize ZEA-degradation activity and identify active phytochemicals in the MeOH extract of *Z. multiflora*, 200 mg of the dried extract was dissolved in 1 ml of DMSO. An aliquot (200 µL) of this solution was injected into the HPLC system (Wellcrome models, Knauer Inc.) equipped with a semi-preparative column (SunFire C18 Column, 100 Å, 5.0 mm, 19 × 100 mm) and Photon Detector Assembly (PDA) detectors. The mobile phase used for separation was included of water (A) and acetonitrile supplemented with 1% formic acid (B). The starting composition was 90% (A): 10% (B) followed by 90:10 to 60:40 over 10 min, 60:40 to 50:50 over 10 min, 50:50 to 0:100 over 10 min and 0:100 for 5 min. The flow rate was 4.0 ml/min and the HPLC profile was monitored at 330 nm. Twenty-three fractions were collected every 90 s. Two ml of each fraction was then transferred to 96 deep well plates and dried using a nitrogen evaporator at 55°C. The ZEA-degradation activity of each fraction (at the concentration of 1 mg/L) was determined according to the section "ZEA-Degradation monitoring in RF" for 36 h.

Isolation and detection of ZEA content

Each sample was passed through the Zearala Test immunoaffinity column at a flow rate of about one drop, followed by 2 × 5 ml distilled water at a 1-2 drop/s flow rate. ZEA was then eluted with 1 ml methanol and collected in a clean vial. ZEA content was determined by HPLC equipped with a Shimadzu RF-10AXL HPLC Fluorescence Detector (FLD) using acetonitrile: water: methanol (2:6:2) as the mobile phase and SunFire C18 (100 A, 3.5 µm, 3.0 mm × 150 mm) column (Waters, Ireland). The samples were monitored at excitation and emission wavelengths of 314 and 450 nm, respectively (Luo *et al*., 1990).

Identification of the active compounds by LC-MS

To identify the components presented in the active zone (compounds responsible for reducing ZEA concentration), LC-MS was used. The active fractions were identified on a Shimadzu Prominence HPLC system composed of an MS-8030 Triple Quadrupole Mass spectrometer equipped with an Atmospheric pressure chemical ionization source (APCI). Conditions were as follows: capillary voltage, 4.5 V; desolvation line temperature, 250°C; heat block temperature, 500°C; drying gas (nitrogen) flow, 15 L/min; nebulizing gas (nitrogen) flow, 3 L/min. The mobile phase consisted of water (A) and acetonitrile (B). The injection volume was 10 µL. LC separation was carried out on a C18 SunFire column (3.5 µm, 3 × 150 mm i.d., Waters) equipped with a guard column (3 × 20 mm i.d.). HPLC solvents both contained formic acid (0.1%, v/v), and the flow rate was set to 0.4 ml/min. The starting composition was 90% (A): 10% (B) followed by 90:10 to 60:40 over 10 min, 60:40 to 50:50 over 10 min, 50:50 to 0:100 B over 10 min and 0:100 for 5 min. Lab Solutions software (Shimadzu) was used for data collection and analyses.

Statistical analysis

The study was carried out using the factorial experiment in a completely randomized design as followed with three replicates:

$$X_{klmn} = \mu+\alpha_k+\beta_l+\gamma_m+(\alpha\beta)_{kl}+(\alpha\gamma)_{km}+(\beta\gamma)_{lm}+(\alpha\beta\gamma)_{klm}+\varepsilon_{klm})$$

α_k: Effect of the kth level of factor plant extract/oil
β_l,: Effect of the lth level of factor concentration
γ_m: Effect of the mth level of factor incubation time
$(\alpha\beta)_{kl}$: Interaction effect between factor plant extract/oil and factor concentration
$(\alpha\gamma)_{km}$: Interaction effect between factor plant extract/oil and incubation time
$(\beta\gamma)_{lm}$: Interaction effect between factor concentration and incubation time
$(\alpha\beta\gamma)_{klm}$: Interaction effect among factor plant extract/oil, factor concentration and factor incubation time

Data analysis was carried out using the SAS software (SAS Institute Inc., Cary, NC). Analysis of variance was done by the Generalized Linear Models (GLM) module. Means were compared using Duncan's multi-domain test at a probability level of 1% ($P<0.01$).

Results

Phytochemical identification of *Z. multiflora*

The essential oil yield of the hydro-distillation of dry aerial parts of *Z. multiflora* was 2.7% (w/w). Eighteen compounds were found in the oil, representing 98.14% of the total oil (Table 1). Carvacrol was the main component constituting 48.04%, followed by thymol (19.95%) and *p*-cymene (9.76%).

Table 1: Chemical constitutions of *Z. multiflora* essential oil

No.	Composition	RI[a]	%
1	α-thujene	927	0.1
2	α-Pinene	934	2.51
3	Camphene	950	0.74
4	3-octanone	972	0.16
5	β-Pinene	977	0.22
6	Myrecene	985	0.68
7	p-Cymene	1025	9.76
8	β-terpineol	1030	0.33
9	γ-terpinen	1056	2.32
10	Linalool	1098	1.69
11	Carvacrol methyl ether	1238	1.6
12	Thymol	1292	19.95
13	Carvacrol	1308	48.04
14	Carvacrol acetate	1344	3.13
15	Trans-Caryophyllene	1414	3.41
16	α-Humulene	1491	0.56
17	Spathulenol	1516	1.31
18	Caryophylene oxide	1574	1.63
Total			98.14

[a] Compounds listed in the order of elution from a DB-1 column. RI: Retention index relative to n-alkanes (C6-C24)

Monitoring of ZEA degradation by extracts and essential oil

According to Table 2, the essential oil of Shirazi thyme did not significantly decrease ZEA content (P<0.01), whereas a significant difference was found among plant extracts that degraded ZEA content (P<0.01). The effects of extract concentration and incubation time and the interaction between extract concentration and incubation time were significant (P<0.01). Higher concentrations of plant extract and/or longer incubation periods caused more ZEA degradation activity (Table 3). The ZEA content in the control (RF without plant extract) was 2.02 µg/ml after 6 h and its content did not significantly change during 36 h. At the concentration of 0.5 mg/ml, none of the three plant extracts decreased ZEA content during 24 h incubation time; however, with a longer incubation period, methanol and *n*-hexane extracts were able to reduce 24-25% of the ZEA-content. On the other hand, a shorter incubation period (6-24 h) by 0.5 mg/ml plant extract did not show any significant effect on the ZEA content. At the concentration of 1 mg/ml, methanol and *n*-hexane extracts exhibited the highest ability to degrade ZEA by reducing 69% and 64% during 36 h, respectively. At the concentration of 2 mg/ml, methanol and *n*-hexane extracts exhibited the highest ability to degrade ZEA by reducing 78% and 69%, respectively, after 36 h incubation time.

HPLC base ZEA-degradation activity fractionation of *Z. multiflora*

The HPLC-MS/UV/ELSD chromatograms of methanolic extract of Shirazi thyme are represented in Figs. 1a-d. Twenty-three fractions were obtained by LC-ELSD (Fig. 1d) and the active zone located between fractions 14-19 (degradation of ZEA by 18-54%), while other fractions did not exhibit a significant effect (Fig. 1e).

Although, Shirazi thyme is rich in phenolic compounds such as caffeic acid and flavonoids, active zones showed no peak in HPLC-UV chromatogram (Fig. 1c). Thus, the active zone must be contained of non-phenolic compounds. However, according to the HPLC-ELSD chromatogram, two significant peaks responsible for ZEA degradation were detected.

LC-MS analysis of active compounds

By investigating the LC-APCI-MS of extract and comparing with literature data we could identify these compounds as oleanolic acid and ursolic acid. HPLC-DAD ESI-MS^n analyses showed that oleanolic acid with a molecular weight of 456, showed *m/z* 439 corresponding to $[M-H_2O+H]^+$ in positive mode and *m/z* 455 corresponding to $[M-H]^-$ in negative mode (Fig. 2). The identification of ursolic acid was confirmed with an $[M-H]^-$ ion at m/z 455.7 and $[M+H]^+$ ion at 457.7 in positive mode (Fig. 3).

Table 2: Variance analysis of different concentrations (0.5, 1, and 2 mg/ml) of *Z. multiflora* extracts/essential oil to degrade ZEA (2 µg/ml) in rumen fluid, after 6, 12, 24, and 36 h incubation on incubator shaker at 39°C and 200 rpm in darkness

Source	Some of square	DF	Mean square	F-value	P-value prob>F
k	22463.20097	3	7487.73366	2705.75	<0.0001**
l	11708.61066	2	5854.30533	2115.50	<0.0001**
m	13104.81153	3	4368.27051	1578.51	<0.0001**
k×l	6582.73476	6	1097.12246	396.45	<0.0001**
k×m	6402.85167	9	711.42796	257.08	<0.0001**
l×m	5103.84920	6	850.64153	307.39	<0.0001**
k×l×m	3761.45927	18	208.96996	75.51	<0.0001**
Corrected total	69393.18306	143	1470.79826		

k: Plant extracts/oil, l: Concentration, and m: Incubation time. ** Statistically significant

Table 3: Degradation of ZEA (2 µg/ml) by adding different concentrations (0.5, 1, and 2 mg/ml) of *Z. multiflora* extracts and essential oil to rumen fluid, after 6, 12, 24, and 36 h incubation on incubator shaker at 39°C and 200 rpm in darkness

Concentration (mg/ml)	Incubation time (h)	Degradation of ZEA (%)			
		n-hexane extract	Ethyl acetate extract	Methanol extract	Essential oil
0.5	6	0.0^{r}	0.0^{r}	0.0^{r}	0.0^{r}
	12	0.0^{r}	0.0^{r}	0.0^{r}	0.0^{r}
	24	0.0^{r}	0.0^{r}	0.0^{r}	0.0^{r}
	36	$24.3^{*}\pm0.66^{fg**}$	0.0^{r}	25.5 ± 0.43^{f}	0.0^{r}
1	6	4.1 ± 0.17^{o}	0.0^{r}	5.0 ± 0.19^{n}	0.0^{r}
	12	12.2 ± 0.45^{j}	3.3 ± 0.17^{p}	12.5 ± 0.42^{j}	0.0^{r}
	24	21.8 ± 0.76^{h}	6.3 ± 0.14^{m}	23.4 ± 0.68^{g}	0.0^{r}
	36	64.2 ± 2.06^{bc}	11.5 ± 0.44^{jk}	69.0 ± 1.80^{b}	0.0^{r}
2	6	7.9 ± 0.33^{l}	2.6 ± 0.25^{q}	12.8 ± 0.62^{j}	0.0^{r}
	12	25.6 ± 0.71^{f}	4.4 ± 0.39^{o}	34.5 ± 0.95^{de}	0.0^{r}
	24	38.0 ± 1.08^{d}	13.8 ± 0.57^{i}	59.3 ± 1.60^{c}	0.0^{r}
	36	69.4 ± 2.26^{b}	25.7 ± 0.6^{f}	78.1 ± 2.19^{a}	6.6 ± 0.43^{m}

* Results are the mean of three replicates ± standard error, and ** Different letters ($^{a, b, c, d}$) indicate significant differences (P<0.01) among treatments

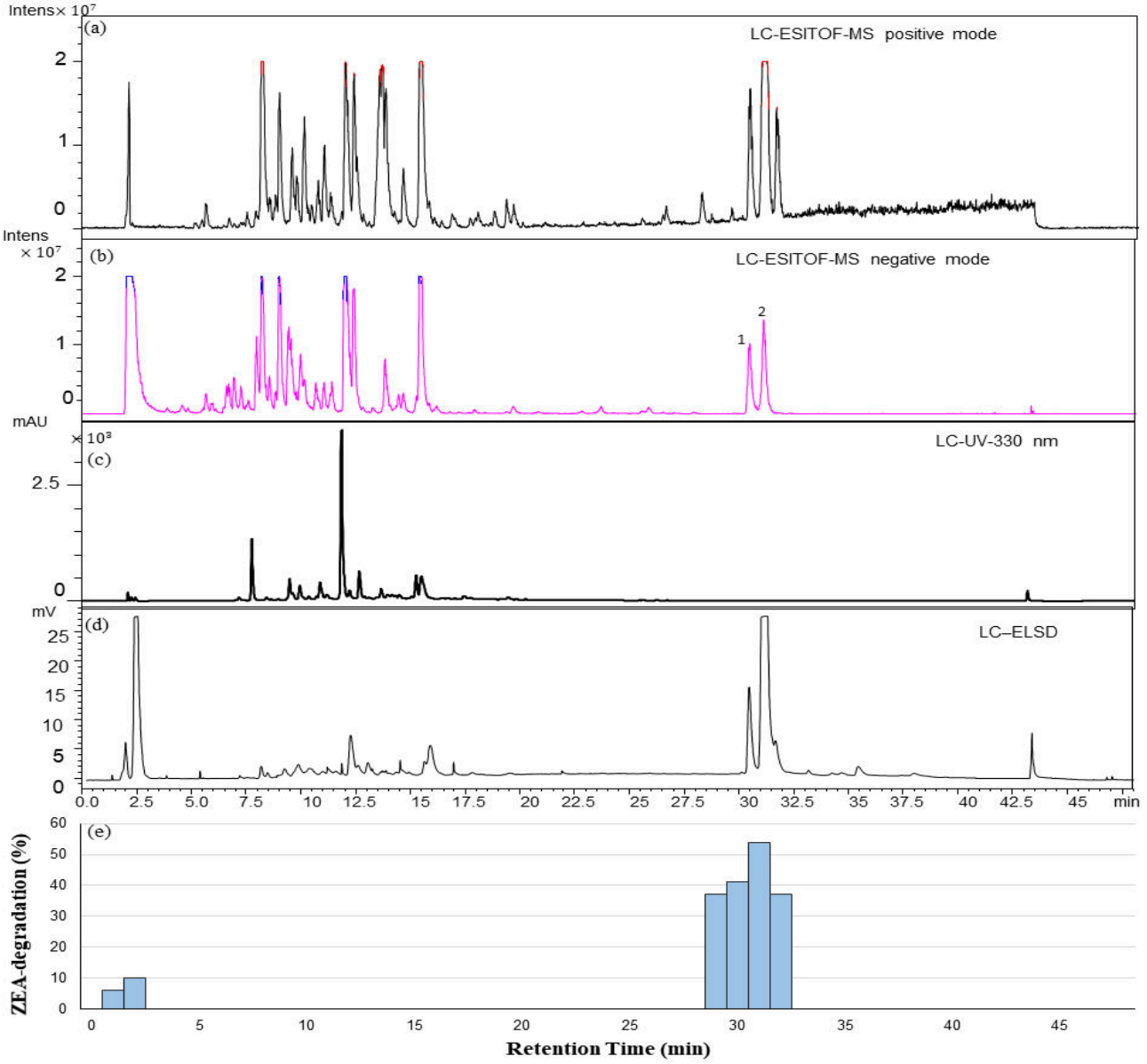

Fig. 1: LC-MS chromatogram of methanolic extract of *Z. multifloria*. (**a**) Positive mode, (**b**) Negative mode, (**c**) HPLC-UV (254 nm), (**d**) HPLC-ELSD, and (**e**) HPLC activity profiling of collected fractions tested for degradation of ZEA

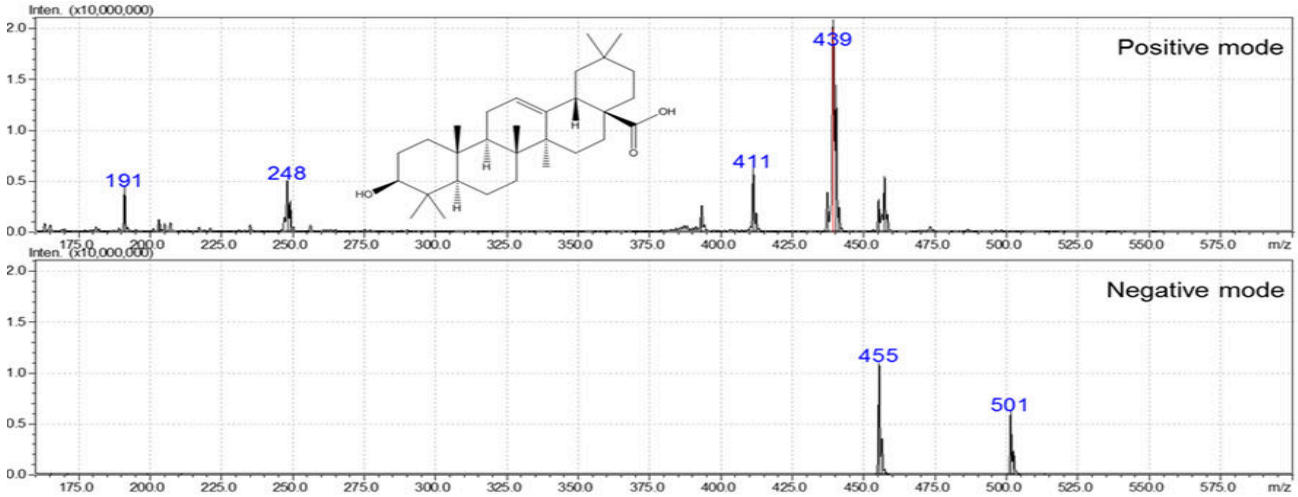

Fig. 2: Chemical structures of oleanolic acid and it's MS spectra in positive and negative modes

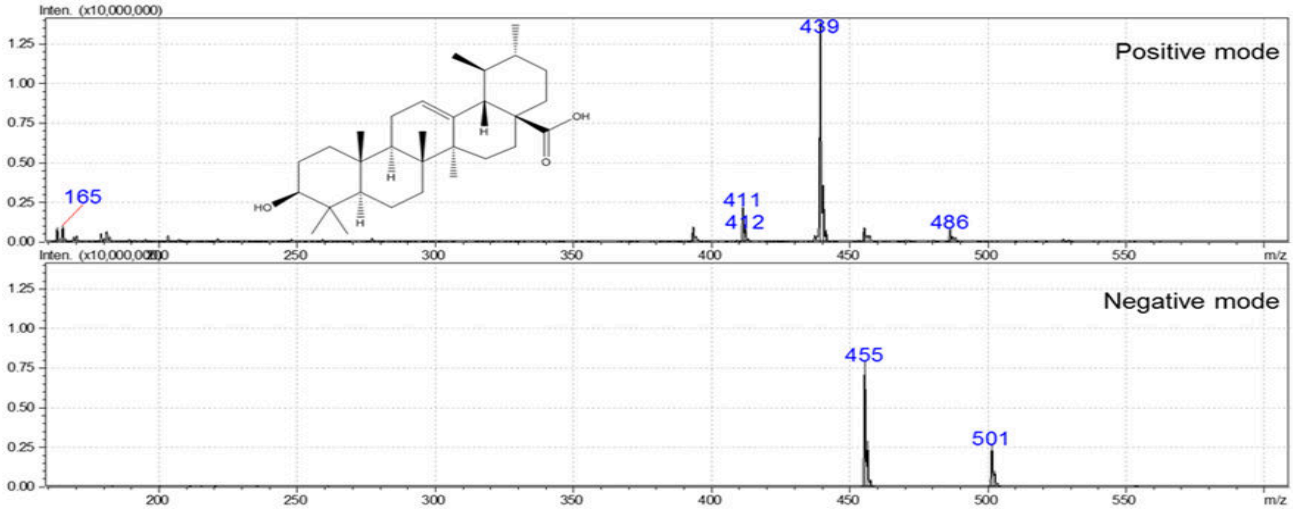

Fig. 3: Chemical structures of ursolic acid and it's MS spectra in positive and negative modes

Discussion

Major oil constituents of the essential oil and various organic extracts of *Z. multiflora* were primarily reported as carvacrol and, thymol (Eftekhar *et al*., 2011). Similar to the results reported for *Z. multiflora* by Eftekhar *et al*. (2011), in our study, the analyses of *Z. multiflora* essential oil showed that carvacrol is the major oil component constituting over 48% of the oil followed by thymol (19.95%). There are a few reports on AFB1-degradation activity by plant extracts in the literature (Sandosskumar *et al*., 2007; Velazhahan *et al*., 2010; Gorran *et al*., 2013); however, there is no report on ZEA-remediation in these studies. The biological detoxification of ZEA to non- or less toxic compounds using fungi, bacteria, and enzymes is an environmentally sound decontamination technology (Taylor and Draughon, 2001). Fungus *Rhizopus arrhizus* strain IFO-6155 is able to catalyze sulfation of ZEA at the C-4 hydroxyl group and form a new metabolite determined as ZEA-4-O-sulfate conjugate (El-Sharkaway *et al*., 1991). Some bacterial strains such as *Bacillus licheniformis* CK1 (Yi *et al*., 2011) and *B. subtilis* (Cho *et al*., 2010) are able to degrade ZEA in the media culture. Also, enzymes obtained from the extracellular extract of *Acinetobacter* sp. SM04 are demonstrated to have a significant effect on ZEA degradation (Yu *et al*., 2011). Enzyme lactonohydrolase ZHD101, produced by *Clonostachys rosea*, can bind to ZEN specifically and hydrolyzes ZEN into 1-(3,5-dihydroxy-phenyl)-10-hydroxy-1-undecen-6-one, by cleaving the ester bond of the macrolactone of ZEN and generating non-toxic alkylresorcinol products (Takahashi *et al*., 2004). In our study, *Z. multiflora* extract showed intense ZEA remediation activity. However, *Z. multiflora* extract cannot adsorb ZEA, and its mechanism to degrade ZEA needs further research. However, it may cleavage the ZEA macrolactone ester binding and produce non-toxic alkyl-resorcinol products. In our study, in the presence of a promising extract, the area or intensity of the fluorescent HPLC chromatogram related to ZEA decreased and no other chromatogram was observed on the report page of the HPLC device. In other words, the product/s resulting from the decomposition of ZEA is/are chemically different from ZEA and its major metabolites (α-zearalenol and β-zearalenol) and could not be detected by HPLC-FLD. Therefore, it is a safe, effective, and environmentally friendly detoxification of ZEA.

According to our findings, oleanolic and ursolic acids are responsible for the ZEA-degradation activity of Shirazi thyme. Both oleanolic acid and ursolic acid have anti-inflammatory, anti-hyperlipidemic and anti-tumor properties and have been used in cosmetics and health products. Also, they are relatively non-toxic to humans/animals and have a remarkable effect on humans/animal liver disorders (Liu, 1995). However, our study is the first report of the ZEA-degradation activity of oleanolic acid and ursolic acid.

Inhibitory effects of *Z. multiflora* essential oil on the growth, spore formation, and aflatoxin production of *A. flavus* and *A. parasiticus* have been reported (Shokri1 and Sharifzadeh, 2017). Shirazi thyme has antioxidant and antimicrobial effects and immunomodulatory properties (Sajed *et al*., 2013) and may affect the animal's health. The positive effect of *Z. multiflora* on performance, growth rate, health, and immune responses of some animals has been reported in many studies (Soltani *et al*., 2013; Taghavi-Nezhad *et al*., 2013; Shomali and Mosleh, 2019). Therefore, the use of Shirazi thyme in ruminant animal feed can reduce ZEA contaminations, affect ruminal fermentation patterns, and enhance animal performance and feed efficiency. We suggest that Shirazi thyme, a valuable herb with noticeable ZEA degradation activity, should be studied on ruminant, poultry, and aquatic animals in farms.

In the present study, we introduced *Z. multiflora* as a new botanical source to degrade ZEA, without adverse effects on the environment and health. Using herbal plants to degrade mycotoxins has several benefits since these plant products are safe for use and would be included in a daily diet. Terpenes such as ursolic and oleanolic acids from *Z. multiflora* are responsible for

degrading ZEA in a rumen fluid condition. Finally, we suggest that *Z. multiflora* has great potential to be used as a detoxification agent in animal feed and human food. This herb shows promising results for applications in both organic and conventional poultry productions. It seems that Shirazi thyme can be considered as a sound resource to detoxify ZEA with beneficial effects on dairy cattle. However, future studies are necessary to understand ZEA degradation activity mechanisms of Shirazi thyme and to confirm the potential of this remarkable herb in degrading ZEA contamination in foods and feedstuffs under *in vivo* conditions.

Acknowledgements

We are grateful to the Research Council of Shahid Beheshti University (project No.: D/600/3348) and the Iran National Science Foundation grant number (96016663) as well as Novin Roshd Shahran Foudeh Company.

Conflict of interest

The authors declare no conflict of interest.

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