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ORIGINAL ARTICLE

Chemical Composition and Antifungal Effect of *Echinophora platyloba* Essential Oil against *Aspergillus flavus*, *Penicillium expansum* and *Fusarium graminearum*

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KEYWORDS

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ABSTRACT: Molds are one of the most important causes of food spoilage that produce toxic substances called mycotoxins, which endanger the consumer health. The adverse effects of synthetic food preservatives consumption made researches to focus on application of natural preservatives in order to increase shelf life of food as well as prevention of harmful effects of chemical preservatives. The present study was conducted to investigate the effects of *Echinophora platyloba* essential oil on spore growth of *Aspergillus flavus*, *Penicillium expansum* and *Fusarium graminearum*. The essential oil composition of *E. platyloba* was analyzed by gas chromatography–mass spectrometry (GC-MS) and its antifungal effect was evaluated by disk diffusion and micro dilution methods. Results revealed that the MIC values of essential oil for *A. flavus*, *P. expansum* and *F. graminearum* were 0.625 $mg.mL^{-1}$, 0.625 $mg.mL^{-1}$ and 0.3125 $mg.mL^{-1}$ and the MFC values were 0.625 $mg.mL^{-1}$, 1.250 $mg.mL^{-1}$ and 0.625 $mg.mL^{-1}$. The essential oil had the highest and the lowest anti-fungal effect on *F. graminearum* and *A. flavus* respectively. In conclusion, due to notable antifungal effects of *E. platyloba* essential oil, it can be practically applied as a natural alternative to chemical preservatives in food industry.

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INTRODUCTION

Despite new developments in the food industry, importance of food safety is increasingly growing in public health. About 30% of the citizens of industrialized countries suffer from foodborne disease at least once a year [1]. Mycotoxins are secondary metabolites produced by certain filamentous fungi, and can lead to deterioration of liver or kidney function. Some mycotoxins are neurotoxins, while others interfere in protein synthesis, and produce effects ranging from skin sensitivity or necrosis to extreme immunodeficiency. The mycotoxigenic fungi involved with the human food chain mainly belong to *Aspergillus*, *Fusarium* and *Penicillium* genera [2]. So still there is need to reduce or eliminate pathogens or toxins in food using different methods.

Today, people tend to use natural preservatives derived from herbal, animal and microbial resources because of harmful effects of chemical and synthetic preservatives in order to increase shelf life of food as well as prevention of harmful effects of chemical preservatives [3]. One of these options is to use essential oils as natural antimicrobial additives [4]. Essential oils are aromatic oily liquids that are obtained from different parts of plants (flowers, twigs, bloom, leaves, buds, bark, roots, fruits, etc.) [4, 5].

There are many reports considering antimicrobial activity of essential oils against a wide range of microorganisms, especially against common food pathogens [6, 7]. Recognition of the effects from years ago, and green consumer movement led to greater public interest in the scientific understanding of these materials [4, 8]

Genus *Echinophora* from *Apiaceae* or *Umbelliferae* family has 10 different species [9]. Four species *E. cinerea*, *E. Platyloba, E. Orientalis,* and *E. Sibthorpiana* are native in Iran and are distributed in west and northwest of this country. It is used in traditional medicine for antifungal, antimicrobial, anti-bloat, digestion and healing properties [9, 10]. It is also used as natural preservative in dairy industry [10]. *E. Platyloba* is one of important species in this genus and is known by local names of Khosharize, Tigh Touragh, Tigh Masti, Koshander, Kouzang, Tanghez or Khousharouze. Because of desire aromatic and antimicrobial properties of this species, it is traditionally added to cheeses, tomato paste and pickled cucumber as spice [11, 12].

To our best knowledge, there is no previous study focusing on antifungal activity of the oil obtained from *E. platyloba DC* against tested fungi in this study. Moreover, no data have been published on antifungal activity of the oil of this plant grown in northwest of Iran as one of the most geographically important regions of plant’s growth due to its commonly conventional consumption in various food. Thus, the purpose of the present study was to determine the chemical composition and antifungal effects of the *E. platyloba* essential oil against common foodborne fungal species such as *A. flavus, P. expansum* and *F. graminearum.*

MATERIALS AND METHODS

Preparation and GC/MS analysis of E. platyloba essential oil

Aerial parts of *E. platyloba* plant was collected at flowering stage in summer from Maraghe City, East Azerbaijan Province, Iran, and was confirmed by the Herbarium Department of Jahad Agriculture and Natural Resources Center of West Azerbaijan, Iran (Voucher no: 6502). Essential oils of dried plant were extracted by hydrodistillation method using a Clevenger apparatus [13]. Chemical composition of the essential oil was analyzed by gas chromatography. The gas chromatograph (Agilent 6890, Swindon, UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50 °C, temperature ramp 5 °C per min, 240 °C min to 300 °C (holding for 3 min), and injector temperature at 290 °C. The carrier gas was helium and the split ratio was 0.8 mL-1 per min. For confirmation of the results, essential oil was also analyzed by gas chromatography mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent, Swindon, UK) and the same capillary column and analytical conditions were used as mentioned above. The MS was run in electron ionization mode with ionization energy of 70 eV using library of Wiley-VCH 2001, Weinheim, Germany [13, 14].

Evaluation of Antifungal activity of E. platyloba essential oil

Preparation of fungal spore suspension

Aspergillus flavus (PTCC 5006), *P. expansum* (ATCC 20331) and *F. graminearum* (ATCC 20466) were prepared from Department of Mycology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, and were cultured on potato dextrose agar (PDA) for 5-7 days at 28 °C. The spores were counted by neubauer slide and numbers of spores were adjusted with Tween 80 (0.5%) achieving approximately 10^6 spores.ml^{-1} [15].

Agar disk diffusion assay

The essential oil was first sterilized by filtration via 0.45 µm millipore filters.Then sterile paper disks (diameter: 6 mm) impregnated with 10 mg.ml^{-1} of essential oil in methanol were placed on PDA plates inoculated with 0.1 ml of fungal spores (*A. flavus*, *P. expansum* and *F. graminearum*) to achieve final concentration of 10^5 spores.ml^{-1}. Plates were incubated at 28 °C for 72 h and inhibition zones were measured by a caliper [15]. Essential oil free paper discs containing methanol solution were used as control and the experiment was performed in triplicate.

Micro-dilution method:

Broth micro dilution susceptibility test was performed to determine MIC and MFC values of the essential oil against tested fungal strains. The essential oil was dissolved in 10% dimethyl sulfoxide and diluted to the highest concentration (100 mg.ml^{-1}) as a stock solution. Then serial two-fold dilutions were made in a concentration range from 1.56- 100 mg.ml^{-1}. Aliquots of 160 µl of potato dextrose broth (PDB) and 20 µl of fungal spores were dispensed into the 96-well micro plates. Amounts of 20 µL from serial dilutions of the essential oil were added into each well as well. The experiment was performed in triplicate for each concentration. The last wells were considered as positive controls consisted of inoculated PDB without essential oil and the negative controls consisted of un-inoculated PDB containing the essential oil. The final volume of each well was 200 µL and the final concentrations of fungal spores and the essential oil were approximately 10^5 spores.ml^{-1} and 0.156- 10 mg.ml^{-1} respectively. The plates were sealed using parafilm and mixed on a micro-plate shaker (Boeco, Hamburg, Germany) at 300 rpm for 30 sec, and then incubated at 28 °C for 72 h.

The MIC values were determined as the lowest concentration of the samples, where the microorganism does not show visible growth. The MFC values were determined by inoculating 10 µL of none turbid wells on PDA and the lowest concentration with no visible bacterial growth on the agar was regarded as the MFC values of the essential oil [16, 17].

RESULTS

Chemical composition

GC-MS analysis of *E. platyloba* essential oil identified 28 components representing 93.29% of total contents of the essential oil (Table 1). Chemical analysis showed that the main components of *E. platyloba* essential oil were Ocimene (26.51%), 2, 3-Dimethyl-1, 3-cyclohexadiene (9.87%), Gamma dodecalactone (9.12%), and Alpha –Pinene (7.69%).

Table 1. Chemical compositions of *E. platyloba* essential oil by GC/MS analysis

NO	Kováts Index	Components	Total %
1	804	Hexanal	1.25
2	863	2,3-Dimethyl-1,3-cyclohexadiene	9.87
3	948	Alpha –Pinene	7.69
4	1011	3-Carene	0.84
5	1043	Ocimene	26.51
6	1098	Linanool	1.8
7	1289	Benzopyran	1.18
8	1105	Cyclohexene, 2-ethenyl-1,3,3-trimethyl	1.95
9	1583	Globulol	0.78
10	2112	2,5-Octadecadiynoic acid, methyl ester	2.3
11	1418	Caryophyllene	2.48
12	1460	Dihydropseudoionone	1.48
13	1684	Gamma dodecalactone	9.12
14	1475	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-1	1.13
15	1564	Nerolidol	5.66
16	1722	Trans-Farnesol	3.3
17	1531	Cis-Z-.alpha.-Bisabolene epoxide	1.11
18	3942	1-Heptatriacotanol	1.25
19	1846	Hexahydrofarnesyl acetone	0.85
20	1560	Limonen-6-ol, pivalate	0.51
21	1927	Farnesyl acetone	0.75
22	2103	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol	0.24
23	2561	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	0.64
24	2238	Retinol	2.89
25	3289	Fenretinide	0.37
26	3331	2-Butenoic acid	0.55
27	2700	Heptacosane	2.1
28	2904	Nonacosane	4.69
		Total	93.29

Antifungal activity of E. platyloba essential oil

According to the diameter of inhibition zone obtained by results of disk diffusion method and the MIC and MFC values obtained from results of microdilution method, *F. graminearum* and *A. flavus* were the most sensitive and the most resistant fungus to the antifungal activity of *E. platyloba* essential oil. The MIC values of essential oil for *A. flavus*, *P. expansum* and *F. graminearum* were 0.625 mg.ml^{-1}, 0.625 mg.ml^{-1} and 0.3125 mg.ml^{-1} and the MFC values were 0.625 mg.ml^{-1}, 1.250 mg.ml^{-1} and 0.625 mg.ml^{-1}, respectively.

Table 2. Antifungal Activity of *E. platyloba* essential oil by agar disk diffusion assay

Microorganisms	**Diameter of inhibition zone (mm)**	
	Essential oil (10 mg/ml)	**Negative control**
Aspergillus flavus	8	0
Penicilium expansum	11	0
Fusarium graminearum	12	0

Table 3. Antifungal activity of *E. platyloba* essential oil by micro-dilution method

Microorganisms	**MIC(mg/mL)**	**MFC(mg/mL)**
Aspergillus flavus	0.625	0.625
Penicilium expansum	0.625	1.25
Fusarium graminearum	0.3125	0.625

DISCUSSION

The main components of the *E. platyloba* essential oil were Ocimene (26.51%), 2, 3-Dimethyl-1, 3-cyclohexadiene (9.87%), Gamma dodecalactone (9.12%) and Alpha –Pinene (7.69). In a former study, ocimene (38.9%) and alpha-flandren (29.4%) composed the main components of *E. platyloba* essential oil collected from Maraghe City, East Azarbayjan, Iran [18]. In another study on aerial parts of *E. platyloba* in Esfahan, the main components of obtaibed essential oil were ocimene (26.71%), delta-3- caren (16.16%) and limonen (6.59%) [19]. Trans-β-ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%), linalool (3.1%), and cis-β-ocimene (2.3%) were the main constituents of the *E. platyloba* [20].

These reported results were completely consistent with results obtained from the present study. Although there are some other studies which are not completely in agreement with results of this study. Ocimen (27.19%), thymol (27.19%) and carvacrol (7.22%) were the main components of *E. platyloba* essential oil [10] and also Moghaddam et al. identified 29 components including p-cymene (22.15%), α-pinene (18.52%), β-phellandrene (14.40%) and α-phellandrene (9.69%) as the main components of *E. platyloba* seeds [21]. Thymol and carvacrol are known phenolic compounds not detected in the current study. The composition of plants essential oil may vary greatly depending upon changes in geographical area, soil composition, climate, age of the plant, harvest season, part of the plant which were used to obtain essential oil, extraction method and type of solvent [22].

Antifungal activity of *E. platyloba* essential oil was evaluated by disk diffusion and micro-well dilution methods. According to the results of this study, both methods were in agreement with each other and showed the following order based on the sensitivity to the antifungal activity of the essential oil: *F. graminearum* < *P. expansum* < *A. flavus*.

Heretofore, there is no published data on antifungal activity of the oil obtained from *E. platyloba* against the fungi which were tested in this study, but in a study antifungal activities of its extract were evaluated against *A. flavus* and *P. expansum* and *A. flavus* showed more resistance to the extract which was similar to results of the present study [23]. Also, results of other studies suggested higher sensitivity of *F. graminearum* and *P. expansum* in comparision with *A. flavus* to the antifungal activity of essential oils [15, 24].

CONCLUSIONS

Results of this study proved inhibitory effect of *E. platyloba* essential oil on *A. flavus*, *P. expansum* and *F. graminearum*, *E. platyloba* essential oil can be introduced as an alternative antifungal agent to chemical preservatives in food as well as therapeutic and industrial utilization. More researches can be conducted on isolation and identification of sub-fractions of *E. platyloba* essential oil as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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