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THE ANTIMICROBIAL AND ANTIOXIDANT POTENCIES OF *SATUREJA KHUZISTANICA* ESSENTIAL OIL FOR PRESERVING OF VEGETABLE OILS

Behnaz Bahrami[1], *Mohaddese Mahboubi*[2*], *Reza Yari*[1], *Mehdi Mehran*[3]

Address(es):
[1]Biology Department, Faculty of Sciences, Boroujerd Branch, Islamic Azad University, Boroujerd, Iran.
[2]Microbiology Department, Medicinal Plant Research Center of Barij, 87135-1178 Kashan, Iran.
[3]Phytochemistry Department, Medicinal Plants Research Center of Barij, 87135-1178 Kashan, Iran.

*Corresponding author: mahboubi1357@yahoo.com ; mahboubi@barijessence.com

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ABSTRACT

In recent years, the use of natural preservatives for protection of vegetable oils against microbial and chemical deterioration is one of the interesting issues. The purpose of this study was to evaluate the preservative activity of *Satureja khuzistanica* essential oil (SKEO) against microbial and chemical deterioration in sesame and flaxseed vegetable oils. Chemical composition of SKEO, chemical profiles, antioxidant and preservative potencies of inoculated vegetable oils with different concentration of SKEO were determined. Carvacrol was the main component of SKEO. The chemical profile of vegetable oils in presence of SKEO had no changes. Sesame and flaxseed vegetable oils had the IC_{50} equal to 26 and 22 µg/ml, respectively. Inoculation the SKEO (1%v/v) in vegetable oils decreased the IC_{50} for vegetable oils. SKEO showed promised antimicrobial activity against food microorganisms. Inoculation the SKEO (0.75%v/v) in sesame oil inhibited completely the bacteria and fungi after 14 days. Flaxseed oil inoculated with SKEO (1% v/v and lower concentrations) decreased the bacteria and fungi populations after 28 days. Therefore, the use of SKEO as natural preservative can protect vegetable oils from deterioration; also it gives the vegetable oils the other pharmacological effects such as anti-inflammatory and analgesic effects with applications in different industries.

Keywords: Preservative, Essential oil, *Satureja khuzistanica*, Antioxidant, Vegetable oil

INTRODUCTION

Inadequate drying process of oilseeds or poor situation in extracting the vegetable oils from oilseeds usually leads to microbial contamination in final products **(Okpokwasili and Molokwu, 1996)**. Microbial contaminations can make considerable changes in vegetable oils and finally affect on quality of these vegetable oils.
In contrast, the difference in intrinsic properties of vegetable oils can change the microbial load of vegetable oils. The fatty acid profile, phenolic compounds, tocopherol and sterol contents of vegetable oils have critical role in their intrinsic properties **(Gromadzka and Wardencki, 2011)**.
In addition to microbial contaminations in vegetable oils, the oxidants can affect on their shelf life **(Aluyor and Ori-Jesu, 2008)**. Benzoic acid, nitrites, sulfites as antimicrobial agents and butylated hydroxyltoluene (BHT), butylated hydroxyanisole (BHA), tocopherols and ascorbic acid as antioxidants are used for prevention the vegetable oils from spoilage. The application of chemical antioxidants and preservatives are associated with major health hazardous problems and toxicity **(Parke and Lewis, 1992)**. Furthermore, replacement the chemical and synthetic ones with natural agents in vegetable oils has great importance for many consumers of food, pharmaceutical and cosmetic industries in different parts of the worlds.
In these years, the use of essential oils as natural preservatives and antioxidants has increased as a new approach to overcome on these adverse effects (**Davidson and Taylor., 2007; Lambert *et al.*, 2001; Mahboubi *et al.*, 2014; Smith-Palmer *et al.*, 2001**).
Satureja khuzistanica essential oil (Lamiaceae family) has been known for its antiseptic effects in traditional medicines. The antimicrobial activities of *S. khuzistanica* essential oil have been determined against a large number of bacteria and fungi *in vitro* conditions **(Akbari-Shahabi *et al.*, 2014; Sadeghi-Nejad *et al.*, 2010; Zarrin *et al.*, 2010**). Other biological effects of *S. khuzistanica* such as anti-inflammatory (**Ghazanfari *et al.*, 2006**), antinociceptive and analgesic (**Saberi *et al.*, 2013**) activities have been confirmed. Therefore, inoculation of *S. khuzistanica* essential oil into vegetable oils may be proposed it as suitable candidate for different industries especially in aromatherapy related therapies.
So, the aim of this study was to evaluate the preservative and antioxidant potencies of *S. khuzistanica* essential oil in sesame and flaxseed oils. These vegetable oils are two popular vegetable oils in different industries. Due to a positive relation between the chemical composition of essential oil and biological activities, we analyzed the chemical composition of *S. khuzistanica* essential oil and fatty acid profiles of vegetable oils in presence of this essential oil as natural preservative.

MATERIAL AND METHODS

S. khuzistanica essential oil and analysis of its chemical composition by Gas Chromatography (GC) and Gas chromatography–mass spectrometry (GC-MS)

Satureja khuzistanica essential oil with pale yellow color had been dedicated by Barij Essence Pharmaceutical Company of Iran. The chemical composition of essential oil were conducted on coupled Agilent technology (HP) 6890 with capillary column of HP-1MS (30 m × 0.25 mm, film thickness 0.25 µm) and Agilent technology (HP) 6890 with 5973 network mass selective detector system using GC and GC-MS apparatuses. The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3 °C /min, held for 10 min. Helium as the carrier gas at a flow rate of 1.0 ml/min with a split ratio equal to 1/50 injector were used. The detector and injector temperatures were 250 and 230 °C, respectively. Components of essential oil were identified by comparison with Retention indices (RI) relative to homologous series of n-alkanes (injected in conditions equal to samples ones) and by computer search using libraries of Wiley 275.L and Wiley 7n.1, as well as comparison of the fragmentation pattern of the mass spectra with data published in the literature **(Adams, 2001)**.

Microbial strains and the antimicrobial activity evaluation of essential oil

The microbial strains were including: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404. The inhibition zones (IZ) diameters, the minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) values of essential oil were evaluated by disc diffusion and micro broth dilution assay as it reported elsewhere **(Mahboubi *et al*., 2014**).

Table 1 GC and GC-MS analysis of *Satureja khuzistanica* oil

compound	RI	%
α-terpinene	929	0.2
p-cymene	938	0.5
β-phellandrene	941	0.1
γ-terpinene	971	0. 4
α-terpinolene	999	0.1
linalool	1013	0.1
carvacrol methyl ether	1142	0.1
thymol	1200	1.0
Carvacrol	1257	**84**
Eugenol	1295	0.2
carvacryl acetate	1303	0.02
trans-caryophyllene	1327	0. 4
α-bergamotene	1336	0.1
neryl acetone	1350	0.1
β-Bisabolene	1387	2.7

RI-retention index

Vegetable oils

Two samples of vegetable oils (sesame oil, flaxseed oil) were used. Vegetable oils were extracted from the seeds of *Sesamum indicum* (sesame oil) and *Linum usitatitissimum* (flaxseed) by cold press procedures in Glkaran Company, Kashan, Iran. The chemical properties of vegetable oils were determined as below. 0.2 ml of 1 M potassium hydroxide and 10 ml of methanol were added to 300 mg of each vegetable oil, then, it was refluxed for 10 min at 90 ºC. After cooling of this solution, the components of vegetable oils were extracted by hexane. 1 µl of extracted components was injected to GC-CP3800 (Varian) with a column of CP-Wax 52CB (50 m × 0.32 mm, film thickness 0.2 µm). Nitrogen was used as carrier gas (pressure 7 psi, flow rate 1.0 ml/min). The temperatures of detector and injector were 250 °C with split ratio of 10. The oven temperature program was initiated at 170 °C, held for 10 min, and then raised up to 250 °C with a rate of 3 °C /min. After that, it was held for 30 min at 250 °C.

Vegetable oils were inoculated by different concentrations of *S. Khuzestanica* essential oil (0.25, 0.5, 0.75, and 1% v/v). Vegetable oils alone were used as negative controls. Carvacrol as *S. Khuzestanica* essential oil indicator was detected in inoculated vegetable oil.
For this purpose, 1.5 g of each vegetable oil was mixed with 4.5 g ethanol (90 ºC) and 0.5 ml of hexanol (10 µg/ml). The mixture was put in water bath (60 ºC) for 5 min and then was put in cold water bath (4 ºC) for 10 min. Then, the mixture was centrifuged. 1 µl of supernatant was injected to GC-Sil 8CB (Varian) with column: CP-Wax52CB (60 m × 0.32 mm, film thickness 0.45 µm), nitrogen was used as the carrier gas (pressure 7 psi, flow rate of 1.0 ml/min, split ratio of 10). The temperatures of injector and detector were 230 and 250 °C, respectively. The oven temperature program was initiated at 50 °C, held for 10 min, then it was raised up to 230 °C at a rate of 3 °C /min and then was held for 70 min **(Pharmacopoeia, 2015a, b**).

Table 2 Chemical profiles of vegetable oils and inoculated *S. khuzistanica* essential oil

Sample	IC_{50}	Essential oil	Vegetable oil (w/w %)				
	µg	carvacrol	Palmetic acid	Estearic acid	Oleic acid	Linoleic acid	α- linoleic acid
S	26	0.00	9.4	5.6	44.1	38.7	0.4
SS (0.25%)	21	0.21	9.4	5.6	44.1	38.7	0.4
SS (0.5%)	17	0.42	9.4	5.6	44.1	38.7	0.4
SS (0.75%)	14	0.58	9.4	5.6	44.1	38.7	0.4
SS (1%)	12	0.77	9.4	5.6	44.1	38.7	0.4
F	22	0.02	5.5	5.1	19.7	13.5	54.9
FS (0.25%)	14	0.20	5.5	5.1	19.7	13.5	54.9
FS (0.5%)	13	0.36	5.5	5.1	19.7	13.5	54.9
FS (0.75%)	10	0.56	5.5	5.1	19.7	13.5	54.9
FS (1%)	7	0.7	5.5	5.1	19.7	13.5	54.9

SS- Sesame oil+*S.khuzestanica* oil, FS- Flaxseed oil+ *S.khuzestanica* oil

Table 3 The antimicrobial activity of *Satureja khuzistanica* Essential Oil

	Disc diffusion (mm)	Broth dilution (µg/ml)	
	0.5 µl	MIC	MLC
S. aureus	18.4±0.12	85	170
E. coli	13.8±0.33	170	170
P. aeruginosa	11.5±0.1	340	340
C. albicans	23.4±0.19	43	43
A. niger	27.3±0.61	170	170

MIC=Minimal Inhibitory Concentration; MLC=Minimal Lethal Concentration

Evaluation the preservative efficacy of *S. khuzistanica* essential oil in vegetable oils

The preservative efficacy of *S. khuzistanica* essential oil in vegetable oils was evaluated by evaluating the antimicrobial effectiveness testing. Inoculated vegetable oils and negative controls were contaminated with predetermined number of microorganisms that mentioned above (10^5–10^6 CFU/ml). At time intervals 0, 7, 14, 21, 28 days, the CFU/ml of microorganisms were determined by inserting the aliquots of inoculated vegetable oils into neutralizing broth media at room temperature, then they serially were diluted and were cultured on specified media cultures. The log CFU/ml of each microorganism for each sample were calculated and the results were reported **(Sutton and Porter, 2002**).

Evaluation the antioxidant activity of *S. khuzistanica* essential oil in vegetable oils

Radical scavenging potency of vegetable oils containing different concentrations of *S. khuzistanica* essential oil (0, 0.25, 0.5, 0.75 and 1% w/w) were determined by free radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Briefly, 40 µl of serial diluted different samples of vegetable oils in methanol was mixed with DPPH. After 70 min incubation period at room temperature, the absorbance of solutions was read against a blank at 517 nm. Inhibition percent of free radicals was calculated and reported. Vegetable oils were used as controls. All experiments were performed in triplicates (**Mahboubi *et al*., 2013**).

Table 4 The preservative potency of *Satureja khuzistanica* Essential Oil against pathogens in vegetable oils

Sample	*S. aureus*					*E. coli*					*P. aeruginosa*					*C. albicans*					*A. niger*				
Day	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
S	6.3	0	0	0	0	5.9	6.6	6.7	6.8	6.8	6.2	6.2	6.8	6.8	6.4	4.7	4.7	4.8	4.8	4.8	3.4	2.4	2.3	2.3	2.3
SS (0.25%)	6.3	0	0	0	0	5.9	6.5	6.7	6.8	6.8	6.2	5.9	5.9	5.9	5.3	4.7	4.5	4.1	3.9	2.4	3.4	2.4	2	2	1.2
SS (0.5%)	6.3	0	0	0	0	5.7	6.5	6.7	6.7	6.8	6.2	5.6	5.6	5.4	5.2	4.7	4.4	4	3.8	3.6	3.3	1.8	0	0	0
SS (0.75%)	6.3	0	0	0	0	5.9	0	0	0	0	6.2	2.1	0	0	0	4.7	3.3	1.1	0	0	3.3	1.6	0.8	0	0
SS (1%)	6.3	0	0	0	0	5.9	0	0	0	0	6.3	1.0	0	0	0	4.7	2.1	0.7	0	0	3.4	1.1	0.6	0	0
F	6.9	4.7	0	0	0	6.9	6.2	5.9	5.7	5.6	6.2	7.9	8.8	8.6	8.6	4.7	3.9	3.8	3.7	3.7	3.6	2.6	2.5	2.4	2.4
FS (0.25%)	6.8	4.3	0	0	0	6.9	5.7	5.6	5.6	5.2	6.2	7.7	8.7	8.6	8.4	4.7	3.8	3.7	3.6	3.5	3.6	2.3	2.4	2.4	2.2
FS (0.5%)	6.7	4.1	0	0	0	6.7	5.4	5.2	5.1	4.8	6.1	7.7	8.2	7.9	7.6	4.7	3.6	3.5	3.4	3.2	3.6	2.3	2.3	2.3	2.0
FS (0.75%)	6.3	3.8	0	0	0	5.9	0	0	0	0	6.2	4.1	2.8	2.6	2.4	4.7	4.7	3.8	2.8	1.4	3.4	2.4	2.3	2.3	2.1
FS (1%)	6.3	2.8	0	0	0	5.9	0	0	0	0	6.2	3.9	1.8	1.8	1.8	4.7	4.5	3.2	2.1	1.6	3.4	2.4	2	2	1

SS- Sesame oil+*S.khuzestanica* oil, FS- Flaxseed oil+ *S.khuzestanica* oil

RESULTS AND DISCUSSION

Chemical composition of *S. khuzistanica* essential oil

Thirty five different components were identified in *S. khuzistanica* oil, representing 93.2% of total essential oil composition. Carvacrol (84%), β-bisabolene (2.7%), thymol (1%) and p-cymene (0.5%) were the main components of *S. khuzistanica* essential oil **(Tab 1)**.

The effects of *S. khuzistanica* essential oil on chemical profile of vegetable oils

Oleic acid (44.1%), linoleic acid (38.7%), palmitic acid (9.5%) and stearic acid (5.6%) were the major fatty acids of sesame oil while α- linoleic acid (54.9%), oleic acid (19.7%), linoleic acid (13.5%), palmitic acid (5.5%) and stearic acid (5.1%) were the main fatty acids of flaxseed oil **(Tab 2)**.
Inoculation the *S. khuzistanica* essential oil in vegetable oils had no effect on chemical profiles of vegetable oils as the **Table 2** has been shown. In inoculated vegetable oils with essential oil, the amounts of carvacrol were related to the amounts of carvacrol in inoculated essential oil into vegetable oils.

The effects of *S. khuzistanica* essential oil on antioxidant activity of vegetable oils

The antioxidant evaluation of vegetable oils by DPPH assay showed that sesame oil had IC_{50} equal to 26 µg/ml and this IC_{50} was higher than the IC_{50} for flaxseed oil (22 µg/ml). Inoculation of *S. khuzistanica* essential oil in sesame and flaxseed oils increased the antioxidant potencies of inoculated vegetable oils by reduction in IC_{50} of vegetable oils, dose dependently **(Figure 1, Tab 2).** Inoculation the *S. khuzistanica* essential oil (1% v/v) into vegetable oils decreased the IC_{50} of sesame and flaxseed oils from 26, 22 µg/ml to 12 and 7 µg/ml, respectively.

Antimicrobial activity of *S. khuzistanica* oil against microorganisms

The antimicrobial evaluation of *S. khuzistanica* essential oil against tested microorganisms showed that, in disc diffusion method, the most sensitive microorganism was *A. niger* (27.3 mm), followed by *C. albicans* (23.4 mm), and *S. aureus* (18.4 mm), respectively. *E. coli* and *P. aeruginosa* showed the lower inhibition zone diameters than the others (lower than 15 mm).
Antimicrobial evaluations of *S. khuzistanica* essential oils by micro broth dilution assays exhibited the microorganisms had different behavior in broth media in comparison with solid media. *C. albicans* with MIC and MBC values of 43 µg/ml exhibited more sensitivity to *S. khuzistanica* oil, followed by *S. aureus* (MIC, MLC= 85, 170 µg/ml). *A. niger* and *E. coli* had the same sensitivity to *S. khuzistanica* oil. *P. aruginosa* was the less sensitive microorganisms to *S. khuzistanica* oil (MIC, MLC= 340 µg/ml) **(Tab 3)**.

The preservative potency of *S. khuzistanica* essential oil in vegetable oils

Sesame and flaxseed oils alone inhibited the growth of *S. aureus*, therefore, inoculation of *S. khuzistanica* essential oil in vegetable oils for inhibition the growth of *S. aureus* was meaningless. The log CFU/ml of other bacteria such as *E. coli* and *P. aeruginosa* in vegetable oils did not inhibited by vegetable oils after 28 days. The log CFU/ml of *P. aeruginosa* particularly increased. Inoculating the essential oil (0.25% and 0.5% v/v) into vegetable oils did not exhibit any preservative effects against bacteria and molds. Sesame oil with *S. khuzistanica* essential oil at concentration of 0.75% and 1% (v/v) completely inhibited the growth of tested microorganisms. Flaxseed oil also inhibited the growth of *E. coli* after 7 days in presence of 0.75% (v/v) of *S. khuzistanica* essential oil but the log CFU/ml of *P. aeruginosa*, *C. albicans* and *A. niger* decreased to 2.4, 1.4 and 2.1 after 28 days in inoculated vegetable oil with *S. khuzistanica* essential oil (0.75% v/v). *P. aeruginosa* was the most resistant microorganisms to inoculated vegetable oils **(Tab 4)**.
As the results are shown, carvacrol was the main components of *S. khuzistanica* essential oil according to the results of other investigators **(Saidi, 2014; Siavash Saei-Dehkordi *et al.*, 2012)**. Furthermore, the antimicrobial activity of *S. khuzistanica* essential oil **(Akbari-Shahabi *et al.*, 2014; Siavash Saei-Dehkordi *et al.*, 2012)**, carvacrol (**Cacciatore *et al.*, 2015**) and the mechanism of action for carvacrol **(Ait-Ouazzou *et al.*, 2013; Chueca *et al.*, 2014)** have been confirmed. Also, the antioxidant activity of *S. khuzistanica* essential oil has been reported (**Ahmadvand, 2014**). Although, there are many investigations on biological activities of *S. khuzistanica* essential oil but to now, there is no study that evaluates the preservative and antioxidant potency of *S. khuzistanica* essential oil in valuable vegetable oils with therapeutic potencies. Nowadays, the use of natural products among the consumers has increased and natural products have good features in different part of the World. Therefore, finding the natural preservatives with other pharmacological potency such as anti-inflammatory and analgesic effects (**Esmaeili-Mahani *et al.*, 2015**) can enhance the therapeutic potencies of vegetable oils, meanwhile it can protect vegetable oils against deterioration. *S. khuzistanica* essential oil by preservative and antioxidant potency protects sesame and flaxseed oils from deterioration and also enhances the therapeutic potency of these oils.

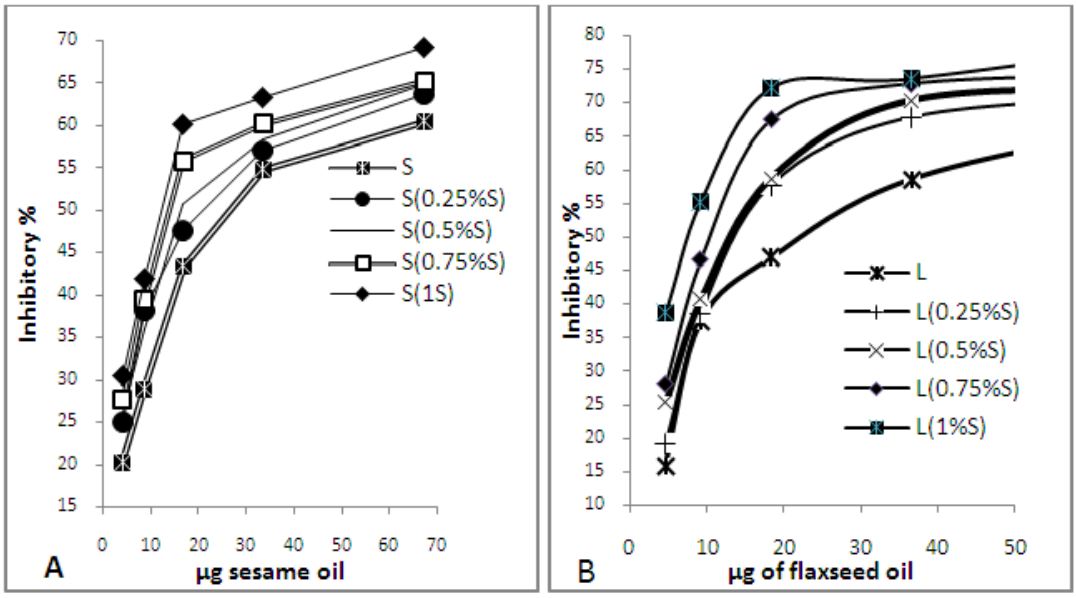

Figure 1 The antioxidant activity of Sesame (A), flaxseed (B) oils with different concentration of *S. khuzistanica* oil by DPPH method

According to our results, sesame oil has shown the better antimicrobial potency than that of flaxseed oil, while its antioxidant activity was lower than the flaxseed oil. The difference in antimicrobial and antioxidant activities of vegetable oils is related to their fatty acid profiles. Oleic acid, linoleic acid (unsaturated fatty acids) as the main components of sesame oil have shown the antimicrobial activity against Gram positive bacteria with MIC 0.01-0.1 mg/ml and also they have shown the synergistic effects with each other (**Dilika *et al.*, 2000**). In fact, the inhibition of *S. aureus* growth by vegetable oils particularly by sesame oil is related to oleic acid, linoleic acid and their synergistic effects between oleic acid and linoleic acid.
Furthermore, it has been reported, among different identified fatty acids in vegetable oils, palmitic acid and stearic acid has exhibited a weak antibacterial activity against *S. aureus*, while oleic acid and linoleic acid has shown high antibacterial activity against *S. aureus*. The antibacterial activity of oleic acid has been reported higher than that of linoleic acid (**Zheng *et al.*, 2005**). In fact, the presence of higher antibacterial agents in sesame oil makes it as stronger antimicrobial agents. In total, identified unsaturated fatty acids in sesame oil were

83% of total fatty acids, while the corresponding amounts in flaxseed oil were 88%. Identified saturated fatty acids were 15.1 and 10.6% for sesame and flaxseed oils, respectively. The interaction between saturated and unsaturated fatty acids makes flaxseed oils as a valuable antioxidant, although, the antioxidant potency of sesame oil was good. Other valuable finding of our study was traceability the essential oil in vegetable oils. In other word, carvacrol did not have any effects on chemical profiles of vegetable oils and the amount of carvacrol in vegetable oils was related to the inoculated essential oil into vegetable oil.

CONCLUSION

In conclusion, the use of *Satureja khuzistanica* essential oil in sesame and flaxseed oils as preservative and antioxidant agents can protect the vegetable oils from deterioration and also donates it other biological activity such as anti-inflammatory effect and analgesic potency, without any changes in fatty acid profile of vegetable oils, whereas the use of this natural agent help to remove the adverse effects of chemical antioxidant and antimicrobial agents from the life of humans. The limitation of our study was no assessing the organoleptic effects of *S. khuzistanica* in vegetable oils for oral applications.

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