



Evaluation of Phytochemical Properties and Antioxidant Activity of Methanol Extract of *Ziziphus Spina-Christi*, *Satureja Khuzistanica*, and *Salvia Rosmarinus* Using FT-IR Method

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ABSTRACT

Introduction: Medicinal plants have shown special application in the pharmaceutical and food industries. Chemical analysis and determination of medicinal compounds of medicinal plants attract great attention throughout the world. The present study was designed to evaluate the phytochemical properties and antioxidant activity of methanol extract of three species of aromatic plants by FTIR method.

Methods: Three species of aromatic plants including Konar (*Ziziphus spina-christi*), rosemary (*Salvia rosmarinus*), and Marzeh khuzestani (*Satureja khuzistanica*) were prepared and studied from the cities of Dehlan and Ilam locating in the west of Iran. These medicinal plants were dried and then powdered. The active groups of the medicinal plants were identified by the FT-IR method. Total antioxidant capacity was measured based on divalent iron reducing ability (FRAP) and by a single electron transfer mechanism.

Results: The FT-IR results displayed that *Z. spina-christi* surrounds 11 functional groups, rosemary has 13 functional groups, and *Khuzestani* pepper contains 12 functional groups. The antioxidant potential of the methanolic extract of *Z. spina-christi* was 7 mmol Fe²⁺/L, rosemary was 1.93 mmol Fe²⁺/L, and Marzeh khuzestani was 12.99 mmol Fe²⁺/L.

Conclusion: *Ziziphus spina-christi*, *Salvia rosmarinus*, and *Satureja Khuzestani* have strong antioxidant properties due to their chemical compounds and can be used for medicinal purposes.

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Intorduction

The application of herbal medicines for the treatment of numerous diseases and for modifying non-pathological states has significantly increased throughout the world

(Palombo et al., 2011). According to a World Health Organization report, most privates in developing countries use herbal traditional medicines for primary

healthcare (Organization, 1998; Fadaei Raieni et al., 2020; Ebrahimi et al., 2022; khajehpour et al., 2020). Iran has a precious history in the use of traditional medicine that has been customary since the Babylonian-Assyrian civilization. One of the most important legacies is the sophisticated expertise of private practitioners who for millennia have tried to explore beneficial plants to provide health, with each generation promoting this tradition with its knowledge and experience. Due to the vast biodiversity of traditional herbs and their phytochemical compounds, a large number of different species are employed in traditional healthcare and traditional medicine in Iran (Naghibi et al., 2022). Beneficial features of herbal drugs are because of secondary herbal metabolites (Fürstenberg-Hägg et al., 2013). Therefore, it is necessary to study and explore traditional medicinal herbs for antioxidant and free radical scavenging potential and also for bioactive components such as polyphenol, and flavonoids as a basis for additional research (Saki et al., 2023; Altememy et al. 2022; Baniesmaeili et al. 2023; Eftekhari et al., 2022; Ebrahimi et al., 2022). Recently, the application of natural antioxidants has been promoted dramatically because synthetic antioxidants may have carcinogenic effects on food products (Bahmani et al. 2022; Darvishi et al., 2022). Moreover, the antioxidant effect of various phytochemicals in the medicinal plant has been reported (Dokhani et al., 2022; Falahi et al., 2019). Antioxidant activity is defined as biological property, which may preserve different foods from the toxic impacts of oxidants (Shakeri et al., 2022). The polyphenols showed antioxidant, antiproliferative activities, and apoptosis-inducing, which can be greatly effective in the prevention of various illnesses, mainly cancer (Ramos et al., 2008). Also, flavonoids displayed anticancer, antimicrobial, anti-inflammatory, and anti-allergic properties (De Sousa et al., 2007). This study is now aimed to discover natural plant-based antioxidants.

Aromatic herbs are well-known for aromatic properties and also antiseptic agents, which are employed as spices and natural preservatives in food products (Sureshjani et al., 2013). *Ziziphus spina-christi* (L.) from *Rhamnaceae* family is very popular as Christ's thorn or Jerusalem-thorn in English and Sidr in Arabic. It is endemic in subtropical and warm-temperate areas, like the south and east of Asia and the Middle East. *Ziziphus Spina-christi* is a very common traditional medicine across the gulf district and is broadly used in the relieving of pain and inflammatory disorders (Waggas et al., 2009). Based on pharmacological investigations, *Ziziphus Spina-christi* has shown hypotensive, antimicrobial, hepatoprotective, hypoglycemic, antioxidant, and antitumor effects as well as immune stimulatory attributes (Avizeh et al., 2010). Several photochemical components have been extracted from *Ziziphus spina-christi* consist of flavonoids, sterols, cyclopeptide alkaloids, tannins, and saponins (Shahat et al., 2001; Tripathi et al., 2001; Yu et al., 2012). The fruit of *Z. spina-christi* is highly nutritious and is usually eaten fresh. Also, the *Z. spina-christi* plants have been valuable as food and medicine in southwestern Asia (Adeyemo et al., 2011). *Satureja khuzestanica* (*Lamiaceae* family), is a native medicinal herb that grows in southern Iran. *Satureja khuzestanica* is renowned as a folk medicine due to its therapeutic applications as herbal tea, an antiseptic and

analgesic agent. This plant contains bioactive compounds such as flavonoids, phenolics, steroids terpenoids, and tannins (Jamzad et al., 19943). According to previous studies, useful properties such as antioxidant, antibacterial, antidiabetic, antibiofilm, antifungal, anti-inflammatory, antihyperlipidemic, antispasmodic, antinociceptive, antidiarrhea, and also triglyceride-lowering effects have been presented for *S. khuzestanica* (Hosainzadegan and Delfan, 2009). Additionally, no negative side effects, as well as toxicity, were reported (Haeri et al., 2006). The essential oil composition of *S. khuzistanica* primarily consists of carvacrol, p-cymene, myrcene, γ -terpinene, and terpinene-4-ol, respectively. Essential oils of *khuzestanica* extracts and carvacrol have revealed antimicrobial and antioxidant activities (Vosough-Ghanbari et al., 2010).

Lamiaceae plants are grown throughout the world, typically utilize as culinary and medicinal plants, and are widely studied as valuable sources of natural antioxidants including polyphenols. *Salvia rosmarinus* Spenn. (or *Rosmarinus officinalis* L.) belongs to the *Lamiaceae* family and is native to the Mediterranean district, commonly called rosemary. *S. Rosmarinus* leaves are conventionally used for treating different diseases. The antioxidant, hepatoprotective, anticancer, anti-inflammatory, hypoglycaemic, antibacterial, and antithrombotic effects have been informed for rosemary (Pérez-Fons et al., 2010). Furthermore, rosemary extracts contain biologically active constituents, consisting of polyphenols (rosmarinic acid and carnosic acid), and also phenolic diterpenes (carnosol) (Erkan et al., 2008). On the other hand, the bioactive content varies remarkably relying on ecological conditions. Rosemary leaf is traditionally utilized in medicinal teas. Rosemary is considered a good source of phenolic compounds, flavonoids, di- and triterpenoids. Carnosic acid, carnosol and rosmarinic acid are the predominant antioxidant compounds of rosmary (Cuvelier et al., 1994). *Salvia rosmarinus* extract has revealed anti-proliferative activity on several tumor cell lines (Cheung et al., 2007). Carnosic acid and carnosol that obtain from rosemary leaves have also shown anticancer properties, as recognized in HL-60 cells (Bai et al., 2010). The present study aimed to evaluate the phytochemical properties by FTIR method and to investigate the antioxidant activity of the methanol extract of the medicinal plants *Ziziphus Spina-christi*, *Satureja khuzistanica*, and *Salvia rosmarinus*.

Materials and Methods

Plant Preparation

In April 2022, *Ziziphus Spina-christi* and *Satureja khuzistanica* were collected from Dehrlan city, and rosemary medicinal plant from Ilam city in Ilam province located in the west of Iran. The plant was identified and confirmed as a species by using the morphological keys of the book of plant flora of Ilam province in the research center of biotechnology and medicinal plants of Ilam University of Medical Sciences. The dried plants were powdered and they used for antioxidant test. The characteristics of the medicinal plant used in this study are shown in Table 1.

Table 1. Medicinal plant details of the present study

Scientific name	Family	Collection area	.Geographical coordinates
<i>Ziziphus spina-christi</i>	Rhamnaceae	Dehloran	32° 41' 28" North, 47° 15' 58" East
<i>Salvia rosmarinus</i>	Lamiaceae	Iilam	32° 41' 28" North, 47° 15' 58" East
<i>Satureja khuzistanica</i>	Lamiaceae	Dehloran	32° 41' 28" North, 47° 15' 58" East

Table 2. Ferric reduces the capacity of different plant extracts

Common name	Scientific name	Total antioxidant capacity
Konar	<i>Ziziphus spina-christi</i>	7 mmol Fe ²⁺ /L
Rosemary	<i>Salvia rosmarinus</i>	1.93 mmol Fe ²⁺ /L
Marzeh khuzestani	<i>Satureja khuzistanica</i>	12.99 mmol Fe ²⁺ /L

Table 3. Functional groups of *Ziziphus spina-christi* plant

Wavelength	Functional group	Wavelength range	Type
3422	OH	3550-3200	Stretching - alcohol
2923	CH	2850-3000	Alkanes
2359	O=C=O	2000-2400	Carbon dioxide-elastic
1734	C=O	1700-1780	Ketones
1622	C=C	1626-1662	Stretch-alkene
1446	CH	1450	Bending-alkane
1321	OH	1310-1390	Bending-phenol
1247	Co	1275-1200	Aromatic stretch-ester
1056	Co	1020-1075	Stretchy-Vanilla Ether
777	CH	20±770	Bending
606	C-Br	515-690	Holliday stretching-combination

Table 4. Functional groups of the *Satureja khuzistanica* plant

Wavelength	Functional group	Wavelength range	Type
3863	O-H	4000-3000	Stretching-alcohol
3429	O-H	3550-3200	Stretching-alcohol
2927	O-H	3200-2700	Stretching-alcohol
1632	C=O	1750-1735	Stretch-ester
1511	C=C	1658-1648	stretch-alkene
1451	C-H	1450	Bending-alkane
1363	O-H	1420-1330	Bending-alcohol
1249	C-N	1250-1020	Stretchy-amine
1027	C-O	1075-1020	Stretchy-Vanilla Ether
821	C=C	840-790	Bending-Alkene
770	C-H	770±20	-
621	C-I	600-500	Holliday stretching-combination

Table 5. *Salvia rosmarinus* plant functional groups

Wavelength	Functional group	Wavelength range	Type
3430	O-H	3550-3200	Stretching-alcohol
2925	N-H	3000-2800	Tensile-amino salt
2857	C-H	3000-2840	Stretching-alkane
2358	N=C=O	2275-2250	Tensile-isocyanate
1733	C-H	2000-1650	Bending-aromatic compounds
1650	C=N	1690-1640	Stretch-imine/oxime
1455	C-H	1465-1400	Bending-alkane
1377	C-H	1370-1365	Stretching-alkane
1255	C-O	1275-1200	Aromatic stretch-ester
1162	C-O	1210-1163	Stretch-ester
1041	C-O	1124-1087	Stretching-alcohol of the second type
821	C=C	840-790	Bending-Alkene
665	C-Br	690-515	Holliday stretching-combination

Preparation of plant sample

After drying, we homogenize 1 gram of the dry powder using 100 mL of methanol solution and shake it in the same solution for 6 hours. Then the obtained solution was poured into a plastic falcon and centrifuged at 6000 rpm for 10 minutes.

Preparation of solutions

2.2 mL of the R2b solution was added to the R2a stock bottle and vortexed until complete dissolution, and the R2 solution was obtained. Then R2 solution was mixed with a ratio of 1:1 and after vortexing, 5 times its volume was added to the R1 solution. The obtained solution is the working solution of the antioxidant kit.

$$Y = 0.2447X + 0.0988 \quad \& \quad R^2 = 0.9997$$

Preparation of standard solution

Standard solution with concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1 were also prepared.

Antioxidant activity

To evaluation of antioxidant activity, 5 microliters of the solution containing the plant was added to each well. In the next step, 250 microliters of the working solution of the kit was added to each well containing the solution. Incubate this mixture in the kit for 30 minutes at 40 degrees Celsius. Finally, the kit was read by Elizar Reader at a wavelength of 570 nm.

Results

The results of evaluation of antioxidant activity of the plants exhibited total antioxidant capacity of the plants. Antioxidant results for medicinal plants are shown in Table 2.

Also, the FTIR results of the medicinal plants has been presented in table 3, 4, and 5. Based on the results obtained from FTIR, Konar has 11 functional groups, marzeh has 12 functional groups, and rosemary has 13 functional groups.

Discussion

Antioxidant activity has been used broadly in the food industry to improve the shelf life of food products. The antioxidant is attributed to a component that can prevent or lessen oxidation reactions. Most extracts obtained from traditional plants have been studied for their antioxidant activities (Al-Ghamdi et al., 2017). The antioxidant effect of *S. khuzistanica* (Named Marzeh khuzestani in Persian) was 12.99 mmol Fe²⁺/L (the ferric reducing antioxidant power), which was greater than *Z. spina-christi* (Konar), and *S. rosmarinus* (rosemary). Saidi (2014) showed that the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of *S. khuzistanica* extract is 60.5% before flowering and 58.1% after full blooming. In addition, the average ferric reducing capacity was detected at about 5.1 in terms of mMol Fe²⁺/μL essential oil. Carvacrol was determined as the predominant essential oil component of *S. khuzistanica* before flowering (with 93.7%) and after full blooming (94.2 %). Total phenolic contents of the *S. khuzistanica* extract were reported in the range of 81.2 to 96.3 mg pyrocatechol/essential oil (Saidi et al., 2014).

Phenolic constituents especially, carvacrol can quench free radicals. The mechanism of action of natural antioxidants might be attributed to hydrogen-donating ability and their capability as hydrogen peroxide, superoxide, and free radical scavengers (Gülçin et al., 2005). The outcomes acquired from several types of research have presented that *S. khuzistanica* essential oils can be recognized as a rich source of natural antioxidant compounds. Additionally, flavonoids, p-cymene, and carvacrol have been considerable compounds in the *S. khuzistanica*, their antioxidant activity is because of their capability of chelating redox-active metals, preventing lipid peroxidation, and decreasing further processes such as reactive oxygen species. Also, carvacrol displayed notable antioxidant activity (Safarnavadeh et al., 2011). In a study conducted by Hasheminya et al. (2019) identified twenty four essential oil compounds in *S. Khuzestanica*, which the main ones consist of carvacrol (80.5%), p-cymene (6.4%), beta-bisabolene (3.2%), citronellal (1.8%) and linalool (1.3%) (Hasheminya et al., 2019). This study showed some similarities to previous research. The study completed by Farzaneh et al. (2015) informed that the predominant essential oils components of *S. Khuzestanica* include carvacrol, p-cymene, and alpha-terpinene. The author stated that genetic differences and the climatic conditions of the place of growth can cause variety in *S. Khuzestanica* essential oils compositions (Farzaneh et al., 2015). Moreover, the antioxidant activity of *S. Khuzestanica* extract was reported by Soltanzadeh et al., 2018. In detail, the total phenolic content of water, methanol, and ethanol extracts were about 210.7 ppm, 218.4ppm, and 222.5ppm, respectively. The ethanolic extract contained the highest amount of total phenolic content in comparison to others (Soltanzadeh et al., 2018). According to several studies, *Satureja* species have strong antioxidant activities, and the findings from the present study proved that *S. khuzestanica* has a valuable source of antioxidant compounds.

The ferric reducing antioxidant power (FRAP) assay is defined as the capability of antioxidants in reducing ferric iron (Jemal et al., 2011). In this study, the FRAP value was measured for *Z. spina-christi* extract (7 mmol Fe²⁺/L). DPPH radical scavenging test is largely used to evaluate in-vitro antioxidant activity (Philips et al., 2010). In research conducted by Alhakmani et al (2014), *Z. spina-christi* fruit (at a concentration of 200 μg/mL) inhibited 54.1% of the DPPH radical, but the seed could exhibit 42.59% inhibition (Dokhani et al., 2022).

In another study, the phenolic content (approximately 82 mg Gallic Acid /g) was detected for methanol extract of *Z. spina-christi*. The total *Z. spina-christi* extract revealed high antiradical activity (DPPH scavenging activity 89%). The total flavonoid content of the variety *Z. spina-christi* extracts was determined between 24.6 to 36.2 mg/g. *Z. spina-christi* has a good potential antioxidant activity among medicinal plants. Overall, *Z. spina-christi* is a natural source of flavonoids and polyphenols, which may be the leading reason for its strong antioxidant activity. Although, Boroomand et al. (2018) reported that only caffeic acid and rosmarinic acid were determined in rosemary (Boroomand et al., 2018).

Furthermore, the free radical scavenging activity of *S. Rosmarinus* extracts from different areas was reported in the range of 8.8 to 14.7 µg/mL. FRAP results of *S. Rosmarinus* extracts that were collected from different zones were between 76.7-97.2 µM Fe (II)/g. Outcomes displayed higher FRAP values than our results (Table2). Rosemary extract consists of numerous types of bioactive compounds with great therapeutic impacts. The extract includes essential oils, triterpenes (e.g. oleanolic acid and ursolic), phenolic acids (e.g. rosmarinic and caffeic acid), and diterpenes (e.g. carnosol and carnosic acid) (de Macedo et al., 2020). Rosemary extract exhibited a strong antioxidant effect, which is typically in correlation with phenolic content. Antioxidant activity is associated with free radical scavenging, nevertheless, secondary metabolites might have the key biological role in regulating apoptosis and transduction of cell signals (Lamaison et al., 1991). Kontogianni et al. (2012) evaluate the phytochemical profile of *R. officinalis* extract and its antioxidant properties. The total phenolic and flavonoid contents of the extract were quantified at 54.6 mg CfA/g (Caffeic acid/g) and 24.6 mg R/g d, respectively. The antioxidant activity was evaluated by using the DPPH radical-scavenging assay which was about SC50 = 40.6 µg/mL for the rosemary. The antioxidant activity of rosemary extracts is because of various constituents such as flavonoids, triterpenoids, phenolic abietane diterpenes including carnosic acid, carnosol, and rosmanol isomers, especially rosmarinic acid. These findings are consistent with our findings. Hence, this could provide a reasonable cause for utilizing *R. officinalis* in food industries and therapeutic applications as a natural antioxidant (Kontogianni et al., 2013).

Conclusions

The present study used the FTIR method to evaluate the antioxidant activity of traditional Iranian plants including, *Z. spina-christi*, *S. rosmarinus*, and *S. khuzistanica*. Our results suggest that these medicinal plants might have great potential to be employed in the food industry as a natural antioxidant to prolong shelf life and also for medicinal usage. Among the three extracts, the high antioxidant activities of *S. khuzestanica* (12.99 mmol Fe²⁺/L) reveals the potential application value of this extract.

Declarations

Conflict of interest

There is no conflict of interest.

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Consent for publications

The authors approved the manuscript for publication.

Funding/support

None.

Authors' contributions

FFMA and AJK conceived the research idea. HA and SSH designed the work. SSH carried out the experiment. FFMA and AJA wrote the first draft of the manuscript. SSH carried out the literature search. AJA carried out the statistical analysis. AJA supervised the study. All authors read and approved the final manuscript for publication.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the author.

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