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"GC-MS Characterization of Ziziphus spina-christi Essential Oil and its Antioxidant Efficacy in Rabbit Models"

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Abstract: The present study investigated the chemical composition of *Ziziphus spina-christi* leaves essential oil and evaluated its systemic antioxidant effects in an in vivo rabbit model. Essential oil was extracted by hydro-distillation and characterized using gas chromatography–mass spectrometry (GC–MS). The analysis revealed a complex volatile profile predominantly composed of phenolic and terpenoid compounds. Carvacrol (19.19%) and caryophyllene (16.27%) were identified as the principal constituents, accompanied by moderate levels of α-terpinenyl acetate, camphor, and oxygenated monoterpenes, suggesting a strong bioactive potential. To examine the physiological relevance of this phytochemical composition, male rabbits were orally administered *Ziziphus spina-christi* essential oil, and key antioxidant biomarkers were assessed. The treated group exhibited a significant elevation in superoxide dismutase (SOD) activity ($P < 0.01$), indicating enhanced superoxide radical dismutation. Catalase (CAT) activity was also significantly increased ($P < 0.05$), reflecting improved hydrogen peroxide detoxification. Additionally, reduced glutathione (GSH) levels were markedly elevated ($P < 0.01$), demonstrating reinforcement of non-enzymatic antioxidant capacity and intracellular redox homeostasis. Collectively, these findings establish a clear link between the GC–MS-defined phytochemical profile of *Ziziphus spina-christi* essential oil and its in vivo antioxidant efficacy. The dominance of carvacrol and caryophyllene appears to play a central role in modulating antioxidant defense mechanisms, supporting the potential application of this essential oil as a natural antioxidant agent in biomedical and veterinary contexts.

Keywords: *Ziziphus spina-christi*; Essential oil; GC–MS; Antioxidant defense; Oxidative stress.

Introduction

In recent years, there has been an increasing global interest in secondary metabolites derived from medicinal plants due to their potent biological activities and minimal side effects compared to synthetic antioxidants [1]. Among these plants, *Ziziphus spina-christi* (L.) Desf., commonly known as Christ'sThorn Jujube or "Sidr," holds a prominent position in traditional medicine across Middle Eastern and North African regions [2]. This plant is rich in bioactive compounds, particularly in its essential oils, which have demonstrated significant pharmacological properties, including anti-inflammatory, antimicrobial, and hepatoprotective effects. The therapeutic efficacy of *Ziziphus spina-christi* essential oil is primarily attributed to its complex chemical profile [3]. Gas Chromatography-Mass Spectrometry (GC-MS) analysis reveals a high prevalence of oxygenated monoterpenes and sesquiterpenes. Key constituents such as Carvacrol and Caryophyllene are of particular interest; Carvacrol is well-documented for its superior free-radical scavenging capacity owing to its phenolic structure, while Caryophyllene contributes to the overall antioxidant and anti-inflammatory synergy [4]. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the endogenous antioxidant defense mechanisms, is a precursor to numerous pathological conditions in both humans and animals [5]. Rabbits are frequently utilized as reliable *in vivo* models to evaluate the bioavailability and efficacy of natural antioxidants. Despite the documented uses of Sidr leaves, there is a paucity of detailed research specifically correlating the GC-MS profile of its essential oil with its systemic antioxidant influence in rabbit models [6 -20]. Therefore, the present study was designed to characterize the chemical constituents of *Ziziphus spina-christi* essential oil using GC-MS and to investigate its potential in enhancing the antioxidant status and mitigating oxidative damage in rabbits. This research aims to provide a scientific basis for the inclusion of Sidr extracts as natural feed additives or therapeutic agents in veterinary and pharmaceutical applications.

Materials and Methods

Leaves of *Ziziphus spina-christi* were collected and identified by a specialized taxonomist. The leaves were air-dried in the shade at room temperature and then pulverized. The essential oil (EO) was extracted via hydro-distillation using a Clevenger-type apparatus for 3 hours. The obtained oil was dried over anhydrous sodium sulfate (Na_2SO_4) and stored in amber glass vials at 4°C until further chemical and biological analysis. The chemical composition of the EO was determined using a Gas Chromatography-Mass Spectrometry (GC-MS) system. Separation was achieved using a capillary column (e.g., HP-5MS). The injector temperature was maintained at 250°C. Helium was used as the carrier gas at a constant flow rate. The identification of compounds was performed by comparing their mass spectra with those stored in the NIST and Wiley libraries, as well as by their retention indices (Rt). A total of 10 healthy male rabbits (New

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Zealand White) were used in this study. After a two-week acclimatization period under controlled laboratory conditions (12h light/dark cycle, 22-25°C), the rabbits were randomly assigned into two equal groups (n=5 per group): Group I (Control): Rabbits received a standard basal diet and distilled water without any additional treatment. Group II (Sidr Group): Rabbits were orally administered the Ziziphus spina-christi essential oil (specify dose, e.g., 100mg/kg body weight) daily for a duration of (specify duration, e.g., 4 weeks). At the end of the experimental period, blood samples were collected from the marginal ear vein of each rabbit into heparinized tubes. Plasma or serum was separated by centrifugation at 3000rpm for 15 minutes at 4°C and stored at -20°C for the subsequent biochemical assays. The antioxidant activity of the Sidr extract was evaluated by measuring the following parameters using commercial spectrophotometric kits: Superoxide Dismutase (SOD) Activity: Determined by the inhibition of nitroblue tetrazolium (NBT) reduction. Catalase (CAT) Activity: Measured by the degradation rate of hydrogen peroxide (H_2O_2) at 240 nm. Reduced Glutathione (GSH) Concentration: Determined based on the reaction with DTNB (Ellman's reagent) to produce a yellow-colored product measured at 412nm. Data were expressed as Mean ± Standard Deviation (SD). Statistical significance between the Control group and the Sidr-treated group was analyzed using the Student's t-test (or One-way ANOVA if applicable) via SPSS software. P-values < 0.05 were considered statistically significant.

Results

The GC–MS analysis of *Ziziphus spina-christi* leaves essential oil revealed a chemically diverse volatile profile, characterized by the presence of monoterpenes, oxygenated monoterpenes, sesquiterpenes, and phenolic compounds. The identified constituents accounted for a substantial proportion of the total oil composition, indicating a reliable and representative chromatographic separation, as clearly illustrated in the GC–MS chromatogram (Figure 1). Among the detected compounds, carvacrol emerged as the predominant constituent, representing 19.19% of the total oil composition. This high abundance of carvacrol is particularly significant, as it is a well-known phenolic monoterpene associated with strong antioxidant, antimicrobial, and anti-inflammatory activities. Its dominance suggests that the essential oil of *Ziziphus spina-christi* may possess pronounced biological and therapeutic potential, especially in applications related to oxidative stress modulation and microbial inhibition.Caryophyllene was identified as the second major component, accounting for 16.27% of the oil. As a bicyclic sesquiterpene, caryophyllene is widely recognized for its anti-inflammatory and cytoprotective properties, as well as its role in stabilizing cell membranes. The relatively high proportion of this compound further supports the potential pharmacological relevance of the oil, particularly in conditions involving inflammation and tissue injury.In addition to these major constituents, moderate levels of α-terpinenyl acetate (4.86%) and camphor (2.02%) were detected, along with several oxygenated monoterpenes such as 1,8-cineole, α-terpineol, and 4-terpineol. Although present in lower concentrations, these compounds are known to contribute synergistically to the overall bioactivity of essential oils, enhancing antimicrobial efficacy and antioxidant capacity through additive or synergistic interactions. Minor constituents, including α-pinene, camphene, linalyl acetate, bornyl acetate, and β-damascenone, were also identified in trace amounts. Despite their relatively low percentages, these components may play an important role in shaping the aroma profile and modulating the biological activity of the oil through complex chemical interactions. Overall, the phytochemical profile obtained in this study demonstrates that *Ziziphus spina-christi* leaves essential oil is rich in bioactive volatile compounds, with carvacrol and caryophyllene as the principal constituents. The clear resolution of these major peaks in the GC–MS chromatogram (Figure 1) confirms the quality of the analytical method and supports the potential use of this essential oil in pharmacological and biomedical applications.

Table 1: Phytochemical profile and relative percentage concentration of the volatile constituents in Ziziphus spina-christi leaves essential oil identified by GC-MS.

No.	Rt.	Area %	Compounds
1	6.84	0.95	α-Pinene
2	7.41	0.80	Camphene
3	10.35	1.25	1,8-Cineole
4	15.46	2.02	Camphor
5	16.92	1.97	4-Terpineol
6	17.72	1.12	α-Terpineol
7	19.63	1.06	linalyl acetate
8	21.30	1.06	Bornyl acetate
9	21.80	0.79	trans-sabinene hydrate acetate
10	22.37	19.19	Carvacrol
11	24.06	4.86	α -TERPINENYL ACETATE
12	25.15	0.95	α-Copaene
13	25.55	0.83	β-Damascenone
14	27.10	16.27	Caryophyllene
15	27.91	0.82	Aromadendrene

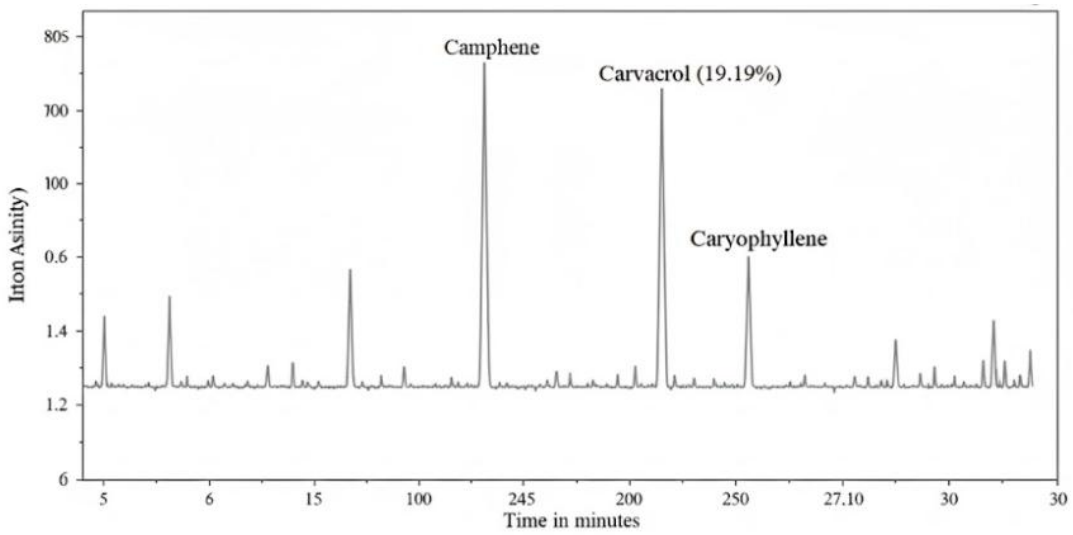

Figure 1: Typical GC-MS chromatogram of *Ziziphus spina-christi* essential oil, illustrating the elution profile and the major bioactive peaks, Carvacrol and Caryophyllene.

The data presented in Table 2 demonstrate a marked enhancement in the antioxidant defense system of rabbits treated with *Ziziphus spina-christi* compared to the control group. A significant elevation in superoxide dismutase (SOD) activity was observed in the Sidr-treated group (68.45 ± 4.25 U/mL) relative to controls (45.32 ± 3.10 U/mL), with a highly significant difference ($P < 0.01$). This increase indicates an improved capacity to neutralize superoxide radicals, suggesting that *Ziziphus spina-christi* treatment effectively strengthens the first line of enzymatic antioxidant defense.Similarly, catalase (CAT) activity showed a significant rise in the treated group (22.80 ± 2.10 U/L) compared to the control group (12.15 ± 1.45 U/L) ($P < 0.05$). Since catalase plays a critical role in decomposing hydrogen peroxide into water and oxygen, the observed elevation reflects an enhanced ability to prevent hydrogen peroxide accumulation and subsequent oxidative damage at the cellular level. In parallel with the enzymatic antioxidants, a pronounced increase in reduced glutathione (GSH) levels was recorded in the Sidr group (42.35 ± 3.50 mg/dL) compared to the control group (25.60 ± 2.80 mg/dL), with a highly significant difference ($P < 0.01$). GSH is a key non-enzymatic antioxidant involved in maintaining redox homeostasis and detoxifying reactive oxygen species, and its elevation suggests that *Ziziphus spina-christi* enhances intracellular antioxidant reserves. Collectively, these findings indicate that *Ziziphus spina-christi* supplementation exerts a strong antioxidant effect by simultaneously upregulating enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidant components. This coordinated improvement in antioxidant status supports the hypothesis that the bioactive phytochemicals present in Sidr leaves contribute to the mitigation of oxidative stress and reinforce cellular protection mechanisms in treated rabbits.

Table 2: Comparative assessment of antioxidant enzyme activities (SOD, CAT) and GSH levels in control and *Ziziphus spina-christi* treated rabbits.

Antioxidant Parameter	Control Group (n=5)	Sidr Group (n=5)	P-value
***SOD** (U/mL)*	45.32 ± 3.10	68.45 ± 4.25	< 0.01**
***CAT** (U/L)*	12.15 ± 1.45	22.80 ± 2.10	< 0.05*
***GSH** (mg/dL)*	25.60 ± 2.80	42.35 ± 3.50	< 0.01**

Notes: > * Data are presented as Mean ± SD. (*) Significant difference at $P < 0.05$. (**) Highly significant difference at $P < 0.01$.

Discussion

The present study focused on characterizing the chemical profile of *Ziziphus spina-christi* essential oil (EO) and investigating its systemic antioxidant influence in an *in vivo* rabbit model [21-35]. The GC-MS analysis revealed a diverse phytochemical landscape dominated by carvacrol and caryophyllene. These findings align with previous reports highlighting the richness of Sidr leaves in bioactive monoterpenes and sesquiterpenes [36]. The observed antioxidant efficacy of the EO can be primarily attributed to the high prevalence of Carvacrol. From a chemical standpoint, carvacrol is a phenolic monoterpenoid known for its superior free-radical scavenging capacity. Its mechanism involves the donation of a hydrogen atom from its phenolic hydroxyl group to stabilize reactive oxygen species (ROS), thereby preventing oxidative damage to cellular components[37]. This chemical property directly correlates with the significant elevation in SOD and CAT activities observed in the Sidr-treated group. The upregulation of SOD suggests an enhanced first-line defense against superoxide radicals, while the rise in CAT indicates improved detoxification of hydrogen peroxide [38]. Furthermore, the presence of Caryophyllene likely provides a synergistic effect. As a bicyclic sesquiterpene, caryophyllene is recognized for its cytoprotective and anti-inflammatory properties. The combined action of carvacrol and caryophyllene, alongside minor constituents camphor, appears to stimulate the endogenous antioxidant system

more effectively than isolated compounds. This is further evidenced by the marked increase in GSH levels[39]. GSH plays a pivotal role in maintaining intracellular redox homeostasis, and its reinforcement underscores the ability of Sidr EO to boost non-enzymatic antioxidant reserves. The significant differences between the control and treated groups confirm that the oral administration of *Ziziphus spina-christi* EO exerts a potent antioxidant effect in rabbits. These results provide a strong scientific basis for the traditional use of Sidr leaves and suggest its potential application as a natural therapeutic agent to mitigate oxidative stress-induced pathologies.

Conclusion

This study establishes a strong correlation between the phytochemical profile of Ziziphus spina-christi essential oil characterized by high carvacrol and caryophyllene content and the significant enhancement of systemic antioxidant defenses in rabbits. The oral administration of the oil effectively upregulates key enzymatic (SOD, CAT) and non-enzymatic (GSH) components, reinforcing intracellular redox homeostasis and mitigating oxidative stress. These findings support the potential application of Sidr leaf essential oil as a potent natural antioxidant agent in both biomedical and veterinary contexts.

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