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Original Article

GC–MS-Based Phytochemical Profiling and Evaluation of the Antioxidant and Nephroprotective Potential of Salvia hians Methanolic Extract: An In Vitro and In Vivo Study

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ABSTRACT

Background: Salvia hians is a Himalayan member of Lamiaceae with limited species-specific pharmacological evidence. The study evaluated its volatile-oil composition and the in vitro radical-scavenging and renal biochemical responses associated with a separately prepared methanolic extract. Methods: Shade-dried aerial material was macerated in 90% aqueous methanol, while a separate hydrodistilled oil preparation underwent gas chromatography–mass spectrometry (GC–MS). The crude extract (Sh-Crd) was examined in DPPH and ABTS assays at 62.5–1000 µg/mL and in gentamicin-exposed laboratory animals at oral doses of 100, 200, or 400 mg/kg. Serum creatinine, blood urea, and amylase were assessed descriptively. Results: Extraction yielded 178 g from 560 g of aerial material (31.79% w/w). GC–MS identified 38 oil constituents representing 96.7% of total signal, with 1,8-cineole (18.7%), camphor (16.2%), and α-thujone (13.5%) prominent. Tabulated radical inhibition increased with concentration; exact IC_{50} values could not be substantiated from the available absorbance–inhibition pairs. Mean serum creatinine and urea rose with gentamicin exposure from 0.67 ± 0.02 to 0.96 ± 0.05 mg/dL and from 29.83 ± 1.11 to 64.16 ± 2.05 mg/dL, respectively. At 400 mg/kg, Sh-Crd was associated with mean creatinine of 0.73 ± 0.05 mg/dL and urea of 40.50 ± 3.19 mg/dL. Inferential significance could not be established from the group summaries. Conclusion: Salvia hians exhibited a chemically diverse volatile oil and concentration-related cell-free radical-scavenging observations in a distinct methanolic preparation. Extract administration was associated with favorable descriptive renal biochemical differences at the higher reported doses. The findings are exploratory and do not establish a specific mechanism, statistical efficacy, or human therapeutic benefit. Keywords: Salvia hians; essential oil; GC–MS; DPPH; ABTS; gentamicin; nephrotoxicity; preclinical study.

INTRODUCTION

The genus Salvia (Lamiaceae) comprises numerous aromatic and medicinal species used in traditional medicine and investigated for chemically diverse secondary metabolites. Phenolic acids, flavonoids, monoterpenes, sesquiterpenes, diterpenoids, and triterpenoids have been reported in different Salvia species, with experimentally observed antioxidant, antimicrobial, and other pharmacological activities. However, their composition and bioactivity vary by species, plant part, geography, growing conditions, and extraction method (1,2).

The chemical diversity of the genus is not confined to the aroma-producing fraction. Phenolic acids such as rosmarinic and caffeic acids, glycosylated flavonoids, and phenolic diterpenes have been studied alongside volatile terpenoids, with variation in tissue distribution and extraction efficiency. The relative contributions of these classes depend on the chosen extraction matrix and on the biological question being examined. Comprehensive evaluations of Salvia phytochemistry therefore distinguish solvent-recovered phenolic constituents from essential-oil monoterpenes and sesquiterpenes rather than treating the two fractions as interchangeable (2,3,4).

Recent analytical studies reinforce the scale of interspecies diversity. A chemometric investigation of Iranian Salvia taxa related phenolic profiles to measured antioxidant behavior, while UHPLC/MS profiling of Romanian Salvia species identified rosmarinic and caffeic acids alongside several flavonoids in some extracts. These findings support chemically targeted investigation of an under-characterized species but do not establish that the same molecules occur in Salvia hians at comparable concentrations (5,6).

Salvia hians Royle ex Benth. is a Himalayan species for which comparatively limited pharmacological information is available. An earlier essential-oil study reported a predominance of sesquiterpene hydrocarbons and identified cadinene derivatives, caryophyllene acetate, and cubenol among its principal volatile constituents (7). Chemical work on the roots identified diterpenoid quinones, illustrating the distinction between root and aerial-part profiles (8). These

observations support systematic chemical investigation of S. hians but do not themselves establish nephroprotective activity.

The earlier Salvia hians volatile-oil work provides an especially useful species-level comparator because it identified 64 constituents representing 94.9% of the oil and a sesquiterpene-dominant profile. The present investigation, by contrast, presents an oxygenated-monoterpene–dominant profile. Differences of this magnitude are scientifically informative and call for attention to plant origin, harvesting season, plant-part selection, extraction duration, and chromatographic identification criteria. Essential-oil fingerprinting across other Salvia species has demonstrated that both between-species variation and analytical sampling strategy can alter the resulting volatile profiles (7,9,10).

Constituents isolated from the roots further caution against assuming chemical uniformity across the whole plant. Diterpenoid quinones described from Salvia hians roots represent a separate organ-specific research stream and should not be assumed to occur in its hydrodistilled aerial-part oil. Likewise, reports of antibacterial activity for essential-oil constituents in related Salvia preparations illuminate possible chemical bioactivity but cannot establish renal protection for a methanolic extract (8,11).

Extraction strategy is central to interpretation. Hydrodistillation recovers predominantly volatile and steam-distillable constituents, whereas aqueous methanol yields a different crude preparation containing solvent-extractable compounds. Comparative work in Salvia illustrates the influence of extraction and processing on chemical composition (12). It is therefore inappropriate to identify GC–MS constituents of an isolated essential oil as the active ingredients of a separately prepared methanolic extract without directly measuring those constituents in the extract.

The distinction matters analytically because hydrodistillation favors volatile compounds that can be transported with steam, whereas aqueous organic solvents extract a different range of polar and moderately polar metabolites. Comparative studies of solvent systems and Salvia extracts show that recovery of phenolics and antioxidant assay responses varies with solvent polarity and botanical preparation. Thus, a GC–MS chromatogram of the essential oil is valuable for chemotaxonomic and volatile-component characterization but is insufficient to assign the constituents responsible for biological effects observed with Sh-Crd (12,13,14). For extracts intended for pharmacological experimentation, spectrophotometric estimates of phenolics and flavonoids and targeted chromatographic analysis can provide chemical context for bioassay interpretation. HPLC-based studies have identified phenolic acids and flavonoids in methanolic extracts of other Salvia taxa, while experiments with enzyme-dependent and enzyme-independent lipid peroxidation indicate that different antioxidant assays probe different aspects of reducing or radical-interacting behavior. Such external observations provide a rationale for complementary methods; they are not surrogate measurements of the composition of the Sh-Crd batch (4,14,15).

Gentamicin, an aminoglycoside antibiotic, can produce experimental renal tubular injury involving drug accumulation, mitochondrial disturbances, oxidative stress, and inflammation (16). In preclinical models, the resulting injury is frequently accompanied by elevated serum creatinine and urea. Antioxidant and anti-inflammatory interventions have been studied as potential modifiers of this toxicity, although the molecular pathways responsible for any observed benefit require direct tissue-level or biochemical assessment (17). Other medicinal-plant investigations have combined chemical antioxidant assays with organ-injury models, illustrating the complementary but non-equivalent information provided by these approaches (18).

Gentamicin nephrotoxicity has a complex pathogenesis in which proximal tubular accumulation can promote lysosomal abnormalities, mitochondrial dysfunction, oxidative damage, impaired transport, and inflammatory responses. These events are interconnected rather than reducible to one free-radical pathway. Integrative mechanistic reviews highlight cellular uptake and tubular cytotoxicity as central processes, while experimental work has examined changes in antioxidant defenses and TLR4-related inflammatory signaling in renal tissues (19,20,21).

Serum creatinine and urea are practical functional indicators, but they do not fully characterize tubular damage, histological injury, or changes in glomerular filtration. Animal investigations incorporating urinary injury biomarkers and tissue analyses have shown the additional information available from a multimarker approach. Accordingly, changes in serum biochemical results can identify a direction of response in an experimental exposure model while leaving the nature, anatomical distribution, and mechanism of renal injury incompletely resolved (19,22).

The DPPH and ABTS assays assess interactions between test preparations and stable radical systems by monitoring changes in visible-light absorbance. These assays are economical screening tools, but interpretation depends on substrate concentration, solvent, sample color, blank correction, incubation conditions, and the adequacy of the dose–

response curve. Results establish in vitro radical-scavenging behavior rather than demonstrating systemic antioxidant activity or reversal of renal oxidative injury (23,24). DPPH and ABTS responses are often summarized by the concentration required to suppress the radical signal by 50%, but an IC_{50} is interpretable only when the raw observations, background correction, and fitted dose–response provide adequate support. A plateau or an entire observed series above the 50% line does not identify a unique half-maximal concentration within the experimental interval. Theoretical analyses of inhibition curves and comparisons across antioxidant assays demonstrate why model selection and the coverage of the transition region affect estimated potency (24,25).

Because plant preparations can themselves be colored or turbid, sample-specific blanks are particularly important for visible-light absorbance assays. Work on DPPH signal interference has demonstrated that coexisting pigments can alter apparent radical-scavenging estimates, independent of the quantity of radical consumed. These limitations do not invalidate carefully conducted DPPH or ABTS tests; rather, they define the measurements as cell-free screening outcomes that require appropriate analytical controls and cannot independently establish a renal antioxidant mechanism (23,26). The present study therefore pursued three linked objectives: to profile the hydrodistilled volatile oil of S. hians aerial parts by GC–MS; to assess DPPH and ABTS radical-scavenging responses of the separately prepared methanolic crude extract (Sh-Crd); and to examine the extract-associated changes in serum creatinine, blood urea, and amylase in an experimental gentamicin-exposure model. By maintaining the distinction between oil composition and crude-extract activity, the investigation provides preliminary descriptive evidence for further chemical, mechanistic, and toxicological study.

MATERIALS AND METHODS

Study design and experimental preparations

The study combined essential-oil chemical profiling, in vitro radical-scavenging assays, and an in vivo gentamicin-exposure experiment. The hydrodistilled oil and crude aqueous methanolic extract were distinct preparations from S. hians aerial material. GC–MS characterized the volatile oil, whereas Sh-Crd was used in DPPH and ABTS assays and in the animal experiment.

The investigation was organized into three analytically different components: preparation and mass-yield determination for Sh-Crd; hydrodistillation followed by volatile-oil GC–MS; and biological evaluation of Sh-Crd under cell-free radical-scavenging conditions and gentamicin exposure. Comparisons among these components were intended to assess complementary chemical and biological characteristics rather than to imply that the isolated oil and crude extract had identical constituent profiles. The experimental unit for the animal biochemical comparisons was the individual laboratory animal, as described in the statistical summary.

Preparation of methanolic crude extract

Shade-dried aerial material was coarsely powdered. A 560 g portion was macerated in 5.5 L of 90% (v/v) aqueous methanol for 15 days with occasional agitation. The material was filtered through Whatman filter paper; the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator and dried in a vacuum desiccator. The resulting dark-brown semisolid extract weighed 178 g. Extraction yield was calculated as (mass of dried extract / mass of dried plant material) × 100.

The extraction calculation used the dry mass of the initial powdered material as the denominator and the mass of the concentrated, desiccated semisolid as the numerator. Accordingly, the result represents the total crude-extract mass recovered under the described maceration and solvent-removal conditions. It does not provide the proportion of a particular phenolic acid, flavonoid, or essential-oil component, and it should not be confused with the yield of hydrodistilled oil. The maceration period, aqueous methanol proportion, temperature of solvent removal, and final extract consistency are retained because they materially affect reproducibility.

Volatile-oil isolation and GC–MS profiling

A separate portion of aerial material was subjected to hydrodistillation in a Clevenger apparatus for 3 h. The recovered oil was dried with anhydrous sodium sulfate and stored in sealed vials at 4 °C. Analysis used a 30 m × 0.25 mm capillary column with a 0.25 µm film and helium carrier gas at 1.0 mL/min. The oven was held at 60 °C for 2 min, increased to 280 °C at 5 °C/min, and held for 10 min; the injector was set to 250 °C with a 1:20 split. Electron ionization was at 70 eV and mass acquisition covered m/z 40–500. Constituents were assigned using mass-spectral library comparison and reported retention indices; relative percentages were derived from chromatographic peak areas. These findings

characterize the oil, not the methanolic crude extract. The temperature program comprised an initial isothermal phase followed by a 44-minute ramp from 60 °C to 280 °C and a 10-minute final hold, based on the reported heating rate. Under electron-impact ionization, chromatographic retention information and library comparisons were used together for tentative identification of volatile compounds. Relative peak-area percentages describe the chromatographic signal distribution rather than calibrated compound concentrations. This distinction is especially important when comparing relative proportions of different chemical classes or comparing data across different laboratories (7,9).

DPPH radical-scavenging assay

A 0.1 mM DPPH solution was prepared in methanol. The extract was examined at 62.5, 125, 250, 500, and 1000 µg/mL. Extract aliquots were mixed with 2 mL of DPPH solution and incubated for 30 minutes at room temperature in the dark to reduce photodegradation. Absorbance was recorded at 517 nm using a UV–visible spectrophotometer. Methanolic DPPH without extract served as the negative control. Percentage radical inhibition was calculated as [(Acontrol – Asample)/Acontrol] × 100, where Acontrol is the DPPH control absorbance and Asample is the reaction-mixture absorbance. IC_{50} was defined as the concentration associated with 50% radical inhibition, provided that the experimental concentration range and corrected absorbances supported estimation (23,24,25). The DPPH assay was interpreted as a concentration-dependent measurement of absorbance loss rather than as an assessment of activity in living cells. In this colorimetric procedure, absorbance of the radical-containing control is the reference signal against which the sample reaction is compared. For quantitative inhibitory concentrations to be valid, experimental sample readings, extract color/blank contribution where applicable, and the corresponding control absorbance must jointly reproduce the reported percentage inhibition. A reference standard provides a contextual assay comparator only when its identity, concentration series, and experimental response are documented (23,25,26).

ABTS radical-cation scavenging assay

The ABTS radical cation was generated using 7 mM ABTS and 2.45 mM potassium persulfate, with radical formation in darkness at room temperature. Phosphate buffer (pH 7.4) was used in preparing the working solution. The Sh-Crd concentrations were 62.5, 125, 250, 500, and 1000 µg/mL, and absorbance was measured at 734 nm. The result table identifies tocopherol as comparator. Percentage inhibition was defined as [(Acontrol – Asample)/Acontrol] × 100, with IC_{50} representing the concentration associated with 50% inhibition. Assay interpretation followed established limitations of colorimetric radical-scavenging methods (23,24). ABTS and potassium persulfate generate a colored radical-cation system whose decrease in absorbance can be quantified at 734 nm following the addition of test material. The same inhibition equation was used as in the DPPH analysis, but the assays are not interchangeable because radical chemistry, reagent preparation, kinetics, and solvent environment differ. The five concentrations were kept in a consistent ascending sequence for interpretation of the recorded response. The half-maximal point is ordinarily identified from a validated dilution–response series that brackets the 50% response, or from a justified fitted model supported by raw observations (24,25).

Animal experiment and treatment schedule

Albino laboratory animals with a reported body-weight range of 22–28 g were used in the gentamicin-exposure experiment. Six experimental conditions were represented in the biochemical comparisons: normal control; gentamicin-only toxic control; gentamicin plus silymarin (500 mg/kg); and gentamicin plus Sh-Crd at 100, 200, or 400 mg/kg. Sh-Crd was administered orally over a treatment period extending up to 28 days. Gentamicin (80 mg/kg) was administered intraperitoneally once daily on days 15–21. Extract administration continued during gentamicin exposure. The protocol identifies day 28 as the terminal tissue-collection point. The species and strain cannot be established unambiguously from the reported body weight and designation and are therefore not stated as a confirmed taxon. Treatment was organized over 28 days, with the gentamicin exposure interval spanning days 15–21. Sh-Crd was administered orally at 100, 200, or 400 mg/kg, and the standard-treatment arm received silymarin at 500 mg/kg. Gentamicin (80 mg/kg) was administered intraperitoneally once daily during the stated seven-day interval. The normal control, toxic control, silymarin comparison, and three extract-dose conditions formed the six groups represented in the biochemical Results.

Serum biochemical outcomes

Blood was processed for serum analysis, and the reported variables were creatinine (mg/dL), blood urea (mg/dL), and amylase activity (U/L). Creatinine and urea served as indicators of renal functional change; amylase was considered an additional systemic biochemical observation rather than a specific marker of tubular damage. Although renal tissue retrieval was mentioned in the analytical record procedure, no interpretable kidney histopathology results or oxidative-

stress biomarkers were supplied for analysis. The renal biochemical assessment centered on creatinine and blood urea, which are routinely used as functional indices in gentamicin models. Serum amylase was an additional recorded enzyme activity; it was analyzed descriptively because it is not a selective index of renal tubular damage. Comparison of these measurements across groups provided biochemical rather than histomorphological evidence. Although kidney collection on day 28 was described in the experimental protocol, tissue microscopy, histopathology scores, and kidney homogenate oxidative-stress results were not incorporated into the available numerical outcomes (17,22).

Statistical analysis

Continuous outcomes were summarized as mean ± standard error of the mean (SEM), with n = 4 animals per group stated in the statistical description. Analysis was described as one-way ANOVA followed by Dunnett's multiple-comparison procedure using GraphPad Prism 5.0; the reported significance criterion was $p \leq 0.001$. Descriptive estimates and arithmetic contrasts are presented separately from inferential claims because exact contrast-specific test statistics, adjusted p values, and confidence intervals are not part of the group-level summaries. Reporting was evaluated with reference to ARRIVE 2.0 (27). Descriptive comparisons addressed gentamicin-only versus normal-control values and the available extract-dose and silymarin group means versus the gentamicin-only group. The original analytical approach specified one-way ANOVA followed by Dunnett's procedure; interpretation of multiplicity-adjusted contrasts requires the underlying contrast statistics. Consequently, the Results distinguish summarized outcome values from inferential efficacy statements (27).

RESULTS

Extraction yield

Extraction of 560 g of shade-dried aerial plant material with 5.5 L of 90% aqueous methanol yielded 178 g of a dark-brown semisolid crude extract after concentration and desiccation (Table 1). The calculated mass yield was 31.79% (w/w; 178/560 × 100). This represents the recovery of solvent-extractable crude material rather than the yield of isolated individual phytochemicals.The maceration solvent-to-plant ratio was approximately 9.82 mL per gram of dry material (5,500 mL/560 g). The recovered crude-extract mass was approximately 0.318 g per gram of starting material. Both derived expressions describe the same reported extraction conditions; they are not indicators of individual constituent concentration or biological potency.

Table 1. Methanolic extraction conditions and yield of S. hians aerial material

Parameter	Recorded value
Dried aerial material	560 g
Extraction solvent	90% aqueous methanol
Solvent volume	5.5 L
Maceration period	15 days
Crude extract obtained	178 g
Appearance	Dark-brown semisolid
Calculated yield	31.79% (w/w)

Volatile-oil composition by GC–MS

Chromatographic analysis yielded 42 features, of which 38 were assigned compound identities, together representing 96.7% of the recorded volatile-oil peak area. Oxygenated monoterpenes constituted 58.3% of the profile, followed by monoterpene hydrocarbons (22.1%) and sesquiterpenes (16.3%). Eleven named constituents are presented in Table 2. The principal identified components were 1,8-cineole (18.70%), camphor (16.20%), α-thujone (13.50%), α-pinene (9.04%) and β-caryophyllene (7.10%). Their relative abundances describe the hydrodistilled oil and cannot be assumed to represent the chemical composition of the methanolic extract used for biological evaluation.

The three reported chemical-class percentages total 96.7%, matching the stated overall assigned area. Within the selected constituent list, oxygenated monoterpenes included 1,8-cineole, linalool, α-thujone, β-thujone, and camphor; monoterpene hydrocarbons included α-pinene, β-pinene, and limonene; and the listed sesquiterpenes comprised β-caryophyllene, α-humulene, and germacrene D. The named components collectively represented 90.35% of the chromatographic peak area. The complete set of 38 identified constituents was not individually tabulated.

Table 2. Selected volatile constituents reported for S. hians hydrodistilled oil

Compound	Class	RT (min)	RI	Peak area (%)
α-Pinene	Monoterpene hydrocarbon	7.07	936	9.04
β-Pinene	Monoterpene hydrocarbon	7.98	983	4.09
Limonene	Monoterpene hydrocarbon	10.11	1033	5.12
1,8-Cineole	Oxygenated monoterpene	11.86	1030	18.70

Linalool	Oxygenated monoterpene	13.05	1095	4.80
α-Thujone	Oxygenated monoterpene	14.62	1114	13.50
β-Thujone	Oxygenated monoterpene	12.94	1113	4.70
Camphor	Oxygenated monoterpene	13.89	1145	16.20
β-Caryophyllene	Sesquiterpene	21.22	1415	7.10
α-Humulene	Sesquiterpene	22.05	1452	3.90
Germacrene D	Sesquiterpene	21.53	1483	3.20

RT, retention time; RI, retention index; relative peak area is a chromatographic signal proportion and not an absolute concentration. Table 2 presents the 11 constituents for which individual entries were available.

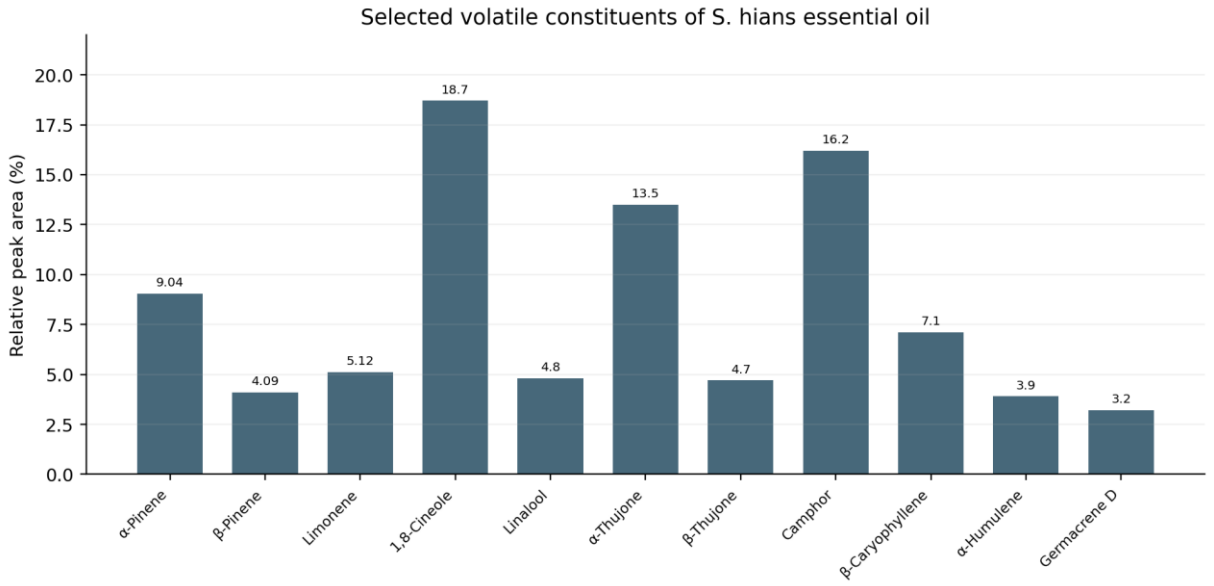

Figure 1. Relative peak-area percentages of the 11 individually listed volatile-oil constituents (reconstructed from Table 2).

ABTS radical-scavenging response

ABTS inhibition values increased from 40.52% at 62.5 µg/mL to 78.83% at 1000 µg/mL (Table 3; Figure 2), with intermediate responses of 54.28%, 68.51%, and 72.98% at 125, 250, and 500 µg/mL, respectively. The observed percentages cross 50% inhibition between 62.5 and 125 µg/mL. The listed absorbances do not yield a single consistent control absorbance when substituted into the stated inhibition equation; a numerical IC_{50} is therefore not reported as an established result. Tocopherol was associated with a comparator IC_{50} of 26.02 µg/mL, but a full comparator dilution series was not part of the present tabulation.

The absolute inhibition difference between the highest and lowest concentrations was 38.31 percentage points (78.83% minus 40.52%). The highest concentration thus showed a larger recorded response than the lowest, but the percentage changes are descriptive and do not quantify precision or between-replicate variability. The absorbance series itself changed only from 0.993 at 62.5 µg/mL to 0.878 at 1000 µg/mL; without a consistent control and valid blank correction, that pattern cannot be mapped reliably to the separately listed percentage values.

Table 3. Recorded ABTS absorbance and percentage inhibition for Sh-Crd

Concentration (µg/mL)	Absorbance	Reported inhibition (%)
62.5	0.993	40.52
125	0.939	54.28
250	0.922	68.51
500	0.897	72.98
1000	0.878	78.83

Percentages and absorbances are retained as recorded measurements; their arithmetic reconciliation is necessary before quantitative IC_{50} estimation.

DPPH radical-scavenging response

DPPH inhibition increased from 82.72% at 62.5 µg/mL to 98.96% at 1000 µg/mL, with 90.17%, 92.14%, and 93.60% at the three intermediate concentrations (Table 4; Figure 2). All five tabulated responses exceeded 50% inhibition. Accordingly, the tested concentration series does not bracket the DPPH IC_{50}, which can only be described as below 62.5 µg/mL if the inhibition values are valid and monotonically ordered.

Table 4. Recorded DPPH absorbance and percentage inhibition for Sh-Crd

Concentration (µg/mL)	Absorbance	Reported inhibition (%)
62.5	0.902	82.72
125	0.772	90.17
250	0.721	92.14
500	0.653	93.60
1000	0.611	98.96

The absorbance-to-inhibition relationship was also internally inconsistent. A separate comparator IC_{50} below 13.7 µg/mL was listed, without sufficient measurements for independent verification. The total rise across the observed DPPH concentration range was 16.24 percentage points, substantially smaller in absolute percentage-point terms than the corresponding ABTS change because DPPH inhibition was already high at the lowest concentration. The recorded absorbance fell from 0.902 to 0.611 as concentration increased, while the reported inhibition percentages rose. These data show the direction of the documented spectrophotometric response but do not independently define relative potency against a validated reference antioxidant.

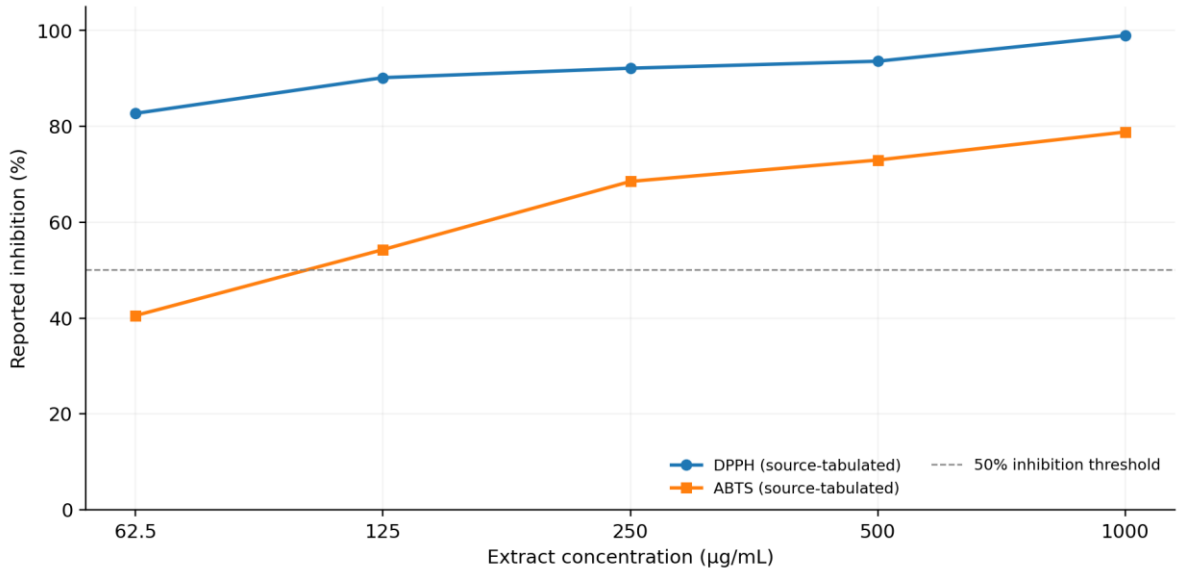

Figure 2. Recorded concentration-related DPPH and ABTS percentage inhibition of the methanolic extract. Lines connect the five measured concentrations for visualization; the dashed reference denotes 50% inhibition. Curves are descriptive rather than fitted IC_{50} models.

Renal biochemical outcomes

Relative to the normal control, gentamicin exposure was associated with higher mean serum creatinine (0.96 ± 0.05 versus 0.67 ± 0.02 mg/dL) and blood urea (64.16 ± 2.05 versus 29.83 ± 1.11 mg/dL), alongside lower amylase activity (30.30 ± 2.82 versus 63.50 ± 2.54 U/L). In the silymarin comparison group, the corresponding means were 0.71 ± 0.04 mg/dL, 31.33 ± 1.34 mg/dL, and 52.66 ± 2.29 U/L (Table 5). At 200 mg/kg, Sh-Crd-treated animals had creatinine of 0.78 ± 0.04 mg/dL, blood urea of 48.55 ± 2.51 mg/dL, and amylase activity of 49.55 ± 2.34 U/L. At 400 mg/kg, the corresponding values were 0.73 ± 0.05 mg/dL, 40.50 ± 3.19 mg/dL, and 51.76 ± 2.37 U/L (Table 5). Numerical results were not provided for the 100 mg/kg condition. The available treatment means are therefore presented descriptively without treatment-equivalence or significance claims.

Table 5. Reported serum biochemical outcomes across experimental groups

Group	Creatinine (mg/dL)	Blood urea (mg/dL)	Amylase (U/L)
Normal control	0.67 ± 0.02	29.83 ± 1.11	63.50 ± 2.54
Gentamicin (GM)	0.96 ± 0.05	64.16 ± 2.05	30.30 ± 2.82
GM + silymarin (500 mg/kg)	0.71 ± 0.04	31.33 ± 1.34	52.66 ± 2.29
GM + Sh-Crd (100 mg/kg)	Not tabulated	Not tabulated	Not tabulated
GM + Sh-Crd (200 mg/kg)	0.78 ± 0.04	48.55 ± 2.51	49.55 ± 2.34
GM + Sh-Crd (400 mg/kg)	0.73 ± 0.05	40.50 ± 3.19	51.76 ± 2.37

Values are means ± SEM; four animals per group were described in the statistical methods. No inferential significance symbols are applied to these descriptive summaries.

The difference between the 400 mg/kg group and the toxic control was −0.23 mg/dL for creatinine, −23.66 mg/dL for blood urea, and +21.46 U/L for amylase. Relative to the toxic-control mean, these differences correspond to approximately 24.0% lower creatinine, 36.9% lower blood urea, and 70.8% higher amylase activity, respectively. These derived percentages describe group-mean differences only and cannot be interpreted as individual responses,

validated protection fractions, or statistically significant effects. Comparison with silymarin remained descriptive: its reported creatinine and urea means were 0.71 and 31.33 mg/dL, respectively, compared with 0.73 and 40.50 mg/dL for the 400 mg/kg extract group.

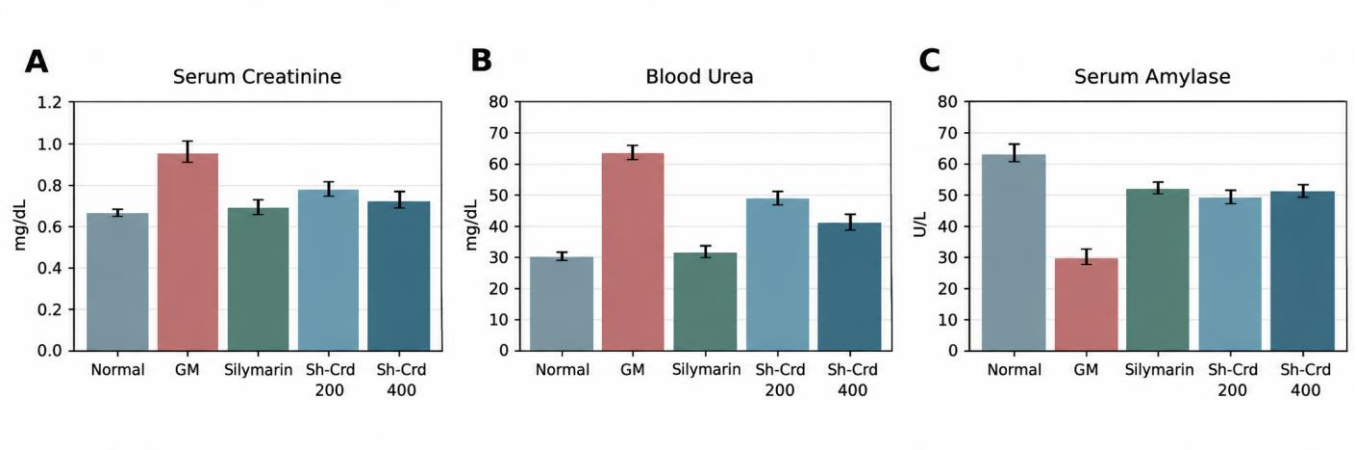

Figure 3A–C visualizes mean ± SEM values for the five conditions with numerical results. The 100 mg/kg condition is retained in Table 5 but not plotted because numeric summaries are unavailable. Renal histopathology is not represented by these biochemical figures.

DISCUSSION

This study combined volatile-oil characterization, chemical radical-scavenging assays, and an experimental gentamicin-exposure model to explore the pharmacological potential of Salvia hians. The reported oil profile was rich in oxygenated monoterpenes, whereas the separate aqueous methanolic extract generated concentration-related values in both in vitro assays and showed numerically more favorable creatinine and urea means at 200 and 400 mg/kg than the gentamicin-only control. The observations support a preliminary research rationale, but assay and statistical inconsistencies prevent a definitive potency or efficacy conclusion.

A central strength of this combined design is that it asks three complementary questions about one botanical species. The GC–MS analysis addresses which volatile constituents occur in the hydrodistilled oil; the DPPH and ABTS assays address how a separate methanolic extract interacts with stable radical systems; and the animal comparison addresses whether extract exposure is associated with a more favorable renal biochemical profile during gentamicin challenge. These are related but not interchangeable endpoints. Their combined interpretation therefore depends on maintaining the chemical identity of each test preparation and on resisting mechanistic conclusions that have not been directly tested. The volatile-oil composition merits comparison with the species-specific literature. In the earlier S. hians study by Melkani et al., 64 constituents accounted for 94.9% of the oil, and sesquiterpene hydrocarbons predominated (52.5%), with cadinenes, caryophyllene acetate, and cubenol among the principal components (7). The present source summary, in contrast, records 58.3% oxygenated monoterpenes and places 1,8-cineole, camphor, and α-thujone among the largest individual constituents. Such marked differences could reflect geographical origin, phenological stage, ecological conditions, postharvest handling, analytical conditions, or genuine chemical variability. Similar variability was documented by Pitarokili et al. across geographically distinct S. fruticosa collections, with 1,8-cineole, thujones, camphor, and (E)-caryophyllene varying in relative abundance (28). These possibilities require direct compositional verification rather than assuming that all S. hians populations share one essential-oil chemotype.

In relation to the wider Salvia genus, the prominence of 1,8-cineole, camphor, and thujones in the present oil is not chemically unusual. Pitarokili et al. identified the same major volatile families across Salvia fruticosa populations and observed considerable geographic variability. Earlier chemotaxonomic profiling of multiple species likewise identified pinene, camphor, and related volatile markers, but the diagnostic pattern differed by species. The divergence from the sesquiterpene-rich S. hians profile previously described by Melkani et al. may therefore reflect population, organ, growth phase, local environment, or analytical differences, rather than indicating that one dataset is necessarily false (9,10,28).

Nevertheless, the magnitude of this contrast is important. Melkani et al. reported sesquiterpene hydrocarbons comprising 52.5% of S. hians oil, whereas the current profile assigns 58.3% to oxygenated monoterpenes. These findings represent contrasting chemotypes across the two datasets, rather than a confirmed compositional agreement. Seasonal and organ-dependent investigations of other sage oils demonstrate how harvest context can influence composition, although authenticated material and the underlying chromatograms would be necessary to

identify the explanation in S. hians. Complete constituent identification, library scores, retention-index calibration, and reference-standard information would improve interpretability of the current fingerprint (7,9,10).

An important interpretation boundary is that GC–MS analysis was conducted on hydrodistilled oil, not on Sh-Crd. Although monoterpenes and sesquiterpenes were identified in the oil, equivalent concentrations of those compounds have not been demonstrated in the crude methanolic extract administered during the antioxidant and animal experiments. Likewise, phenolic acids such as rosmarinic acid and individual flavonoids, although well described in other Salvia preparations, were not quantified in Sh-Crd. Attributing the observed biochemical response to 1,8-cineole, camphor, thujone, rosmarinic acid, or a specific mixture would therefore exceed the chemical characterization actually performed (1,2,12).

Evidence from methanolic or hydroalcoholic extracts of other Salvia species indicates that polar fractions can contain rosmarinic acid, caffeic acid derivatives, and flavonoids, but the relative contribution of these molecules differs between extracts. Al-Jaber et al. directly profiled phenolic constituents by HPLC in three Salvia species, while Grzegorczyk-Karolak and Kiss compared aqueous and hydroethanolic extract chemistry. These papers illustrate an appropriate next analytical pathway for Sh-Crd, not proof of its current composition. Assigning the observed biological response to a specific polyphenol without analyzing the administered material would create an unsupported causal chain (6,13,14). The species-level diversity of phytochemicals also limits uncritical extrapolation from S. officinalis, which has been investigated more extensively than S. hians. Reviews and recent chemometric analyses show that related taxa may share broad classes of metabolites yet differ markedly in concentration and composition. A useful interpretation is therefore that the present oil analysis demonstrates volatile diversity, while the extract assays establish a distinct, preliminary signal for further targeted identification of active extract components (1,2,3,5). The tabulated DPPH and ABTS responses increased with Sh-Crd concentration, a pattern compatible with a concentration-dependent interaction with both chemical radical systems. However, the recorded absorbances do not reconstruct the reported percentages using a common assay control. Furthermore, the DPPH series was above 50% inhibition at every tested concentration, while the ABTS series crossed that threshold between 62.5 and 125 µg/mL. These characteristics preclude treating the precise earlier IC_{50} values as validated potency estimates. Antioxidant assay literature emphasizes blank correction, optical interference, appropriate concentration ranges, and suitable curve fitting (23,24,26).

Antioxidant assays are especially susceptible to overinterpretation when they are converted into exact potency rankings without complete dilution curves. A numerical IC_{50} reflects the relationship between concentration and response under a particular assay, not a general measure of biological efficacy. Here, the ABTS inhibition percentages cross the nominal 50% point between tested levels, whereas all DPPH values already exceed that threshold. The DPPH result is therefore left-censored relative to the tested interval; even if every percentage were fully validated, it would not justify an exact value below 62.5 µg/mL without measurements over a lower range. Likewise, a monotonic ABTS response does not support the initially reported value below its lowest tested level (24,25). The mismatch between absorbance and inhibition percentages is an analytically separate concern from model fitting. The standard inhibition expression assumes that the sample absorbance has been appropriately related to a control signal, with the matrix's optical contribution managed. When the reported concentration-specific absorbances imply different control readings for a single assay, numerical precision may have been affected by transposition, missing blank correction, separate runs, or an unreported normalization step. It would be inappropriate to select one explanation without the instrument data. Methodological evaluations of antioxidant assays emphasize inter-method differences, kinetic effects, and the susceptibility of DPPH absorbance to pigmented extracts (24,26).

Gentamicin exposure increased mean creatinine and blood urea relative to normal control, consistent with biochemical renal dysfunction seen in experimental aminoglycoside toxicity models. The 400 mg/kg Sh-Crd group showed the largest favorable change in creatinine and urea among extract-dose conditions with available numerical values. These are descriptive differences rather than demonstrated statistically significant treatment effects. Gentamicin-induced renal injury involves proximal tubular accumulation, oxidative processes, mitochondrial dysfunction, and inflammatory signaling, but none of those mechanisms was directly quantified in this experiment (16,17,19).

The renal biochemical response is consistent in direction with experimentally induced aminoglycoside toxicity: gentamicin was associated with higher creatinine and urea, whereas the available extract groups had means closer to the normal-control values. Gentamicin renal injury involves tubular accumulation and can include cell death, energetic disruption, oxidative damage, and changes in local inflammation. Mechanistic reviews emphasize that these processes

are interrelated and that a change in serum creatinine alone cannot specify their relative contributions in a particular experiment (16,19,20).

The value of a multi-pathway interpretation becomes clear when comparing experiments that measured tissue-level markers. Pakfetrat et al. investigated oxidative and TLR4-related inflammatory signaling in gentamicin-exposed rats. Adil et al. evaluated oxidative stress, apoptosis, mitochondrial alterations, and inflammatory processes in animals given berberine, while Sahu et al. studied similar mechanistic domains with naringin. These studies establish that the proposed pathways are experimentally measurable, not that the present Sh-Crd exposure changed those pathways. Confirmation would require kidney oxidative-stress measurements, inflammatory proteins or transcripts, and tissue morphology within this study (21,29,30). Serum creatinine and blood urea, although appropriate exploratory outcomes, are influenced by processes beyond the severity of direct tubular injury. In animal nephrotoxicity research, additional biomarkers and quantitative histopathology can improve sensitivity and anatomical interpretation. Udupa and Prakash evaluated urinary biomarkers in a gentamicin renal-injury model, illustrating how reliance on two serum biochemical variables may miss earlier or more localized injury. Without measured filtration, urinary protein or injury markers, and tissue examination, the present results should be interpreted as alterations in biochemical profiles rather than definitive structural nephroprotection (17,22).

Existing evidence from related Salvia species supports biological plausibility but should not be conflated with direct evidence for S. hians. Ashtiani et al. examined a hydroalcoholic S. officinalis leaf extract in a gentamicin-exposed rat model, providing a species-adjacent experimental comparator (31). Other plant-derived preparations have also been evaluated in renal-injury models. Mestry et al. described renal biochemical and oxidative-stress responses to Punica granatum in gentamicin-exposed rats (32). More recently, Ayusso et al. reported anti-inflammatory effects of green tea extract in gentamicin-related kidney injury (33), while Abed et al. investigated renal-function, oxidative-stress, and histological outcomes in a related rat model (34). These studies illustrate how direct tissue and biochemical measurements can strengthen mechanistic interpretation, but they cannot establish that Sh-Crd operates by the same pathways.

Evidence from Salvia officinalis is particularly relevant because it investigates another member of the same genus in the same gentamicin exposure context. Ashtiani et al. tested a hydroalcoholic sage leaf extract in rats receiving gentamicin at 80 mg/kg/day and examined renal and oxidative outcomes. That design supports the broader biological rationale for testing sage preparations in experimental nephrotoxicity, but differences in species identity, plant chemistry, dose, timing, animal strain, and measured endpoints preclude treating the magnitude of their effect as directly transferable to S. hians (31). The broader phytomedicine literature offers both supportive and cautionary comparators. Mestry et al. evaluated Punica granatum in gentamicin-associated kidney dysfunction and assessed oxidative-stress endpoints, while Shahani et al. examined a watercress-derived hydroalcoholic extract in gentamicin-exposed animals. More recently, Ayusso and colleagues described anti-inflammatory effects of green-tea extract in a renal-injury model, and Abed et al. combined renal biochemical outcomes with inflammatory, oxidative, and histological assessments. Together these studies suggest sensible pathways for future testing, while underscoring that nephroprotection should not be inferred solely from scavenging activity in cell-free chemical assays (32,33,34,35).

The reported silymarin arm provides a contextual comparison rather than an equivalence benchmark. Hilmi et al. separately studied silymarin in a gentamicin-induced rat nephrotoxicity model and evaluated renal biochemical and oxidative-stress outcomes (36). In the present data, the 400 mg/kg Sh-Crd means for creatinine and urea did not exactly match those of the silymarin group; no formal equivalence or noninferiority analysis was provided. Differences in plant preparation, protocol timing, species, dosing, and outcome measurement preclude assuming a shared effect size with other animal investigations. The use of silymarin is understandable in the context of published experimental studies. Hilmi et al. evaluated silymarin during gentamicin exposure and measured renal biochemistry and oxidative-stress outcomes, supplying a pertinent model-level comparator. In the present group means, the 400 mg/kg Sh-Crd condition approached—but did not duplicate—the creatinine mean of the silymarin condition, whereas the residual elevation of urea was larger. Observational proximity between means should not be described as equivalence: equivalence or noninferiority requires prespecified margins, appropriate statistical design, and confidence intervals, none of which were established in these group summaries (36).

The measured serum amylase change should also be interpreted conservatively. Amylase is not a specific biomarker of renal tubular injury, and its concentration can vary for reasons independent of kidney pathology. Although the recorded amylase means increased in the extract-treated groups relative to the toxic control, these observations cannot independently verify nephroprotection. The most directly renal-relevant outcomes in the supplied dataset are serum

creatinine and blood urea, and these too reflect limited group-level biochemical information rather than a complete assessment of renal function.

The direction of the amylase response warrants separate consideration rather than automatic inclusion in a composite nephroprotective narrative. Serum amylase originates from more than one tissue source, its clearance can be affected by renal function, and its activity is not a validated stand-alone measure of proximal tubular injury. The recorded decreases during gentamicin exposure and increases with extract treatment may be biologically interesting, but they are not readily interpretable as an index of renal tissue preservation. For this reason, the most informative renal biochemical results in the current dataset remain creatinine and urea, interpreted within their own limitations (22).

Several limitations qualify these findings. The animal designation was inconsistent with the stated weight range, and details of sex, age, housing, husbandry, allocation, masking, humane endpoints, and ethical authorization were incomplete. The stated sample size was small (n = 4 per group), and numerical values at 100 mg/kg were not available, preventing a complete dose-response assessment. The timing of blood collection in relation to days 15–21 gentamicin exposure and day-28 termination was not fully resolved. Although ANOVA with Dunnett's procedure was described, exact contrast-specific results and confidence intervals were not available to substantiate statistical significance. Finally, no analyzable renal histopathology, direct oxidative-stress markers, inflammatory mediators, or extract-specific phenolic concentrations were included. These limitations constrain the interpretation to exploratory biochemical evidence (27). The next experimental phase should combine documented botanical authentication and voucher deposition with targeted chemical fingerprinting of the methanolic extract, for example by appropriately validated HPLC or LC–MS methods. A fully specified animal protocol should reconcile the actual species, sex, age, housing, treatment chronology, group allocation, sample-size reasoning, and renal tissue assessment. Direct measurements of oxidative injury, inflammatory pathways, or mitochondrial function would permit testing of proposed mechanisms rather than inferring them from cell-free scavenging assays. Independent toxicity and dose-ranging work would also be required before therapeutic translation (21,22,27).

CONCLUSION

The hydrodistilled volatile oil of Salvia hians was characterized by a predominance of oxygenated monoterpenes, particularly 1,8-cineole, camphor, and α-thujone. The separate aqueous methanolic crude extract produced concentration-related DPPH and ABTS responses and was associated with lower mean serum creatinine and blood urea values in gentamicin-exposed animals, most clearly at 400 mg/kg among the reported extract-dose groups. The inconsistent antioxidant calculations, incomplete dose-specific renal data, uncertain animal characteristics, and unavailable inferential contrasts limit the strength of the efficacy inference. These exploratory biochemical findings support more complete phytochemical, toxicological, and mechanism-directed investigations, but do not demonstrate a specific renal antioxidant pathway or human clinical benefit.

DECLARATIONS

Author Contributions:	Concept: AA, MI, FH²; Design: AA, FH¹, MI, HSA; Data Collection: FH¹, MZ, MMK, KA, MP, YI, AK; Analysis: AA, KA, MI, HSA; Drafting: AA, MZ, MP, MI, FH², HSA.
Ethical Approval:	The study involved experimental animals subjected to gentamicin-induced nephrotoxicity. Details of approval by the relevant institutional animal ethics committee of Abdul Wali Khan University Mardan, Pakistan.
Informed Consent:	Not Applicable
Conflict of Interest:	The authors declare no conflict of interest.
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Data Availability:	The experimental data supporting the findings are presented in the manuscript. Additional original experimental records may be obtained from the corresponding author upon reasonable request, subject to their availability.
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AI Declaration:	Generative AI tools were used solely for language and grammatical refinement. The authors verified the final manuscript and remain fully responsible for its accuracy, integrity, and scientific content.

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