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Research Article

PHYTOCHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF *FERULA ASAFOETIDA* EXTRACTS

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ABSTRACT

Objectives: *Ferula asafoetida* ("hing") contains diverse secondary metabolites, yet the phytochemistry and antioxidant capacity of its 70% ethanolic oleo-gum-resin extract are not well described. This study profiled that extract and quantified antioxidant activity.

Methods: Oleo-gum-resin was Soxhlet-extracted with 70% ethanol. Qualitative phytochemical screening used standard colorimetric/precipitation tests (e.g., $FeCl_3$ for tannins, Dragendorff's for alkaloids, NaOH/HCl for flavonoids, foam test for saponins, Benedict's for carbohydrates). Total phenolic content (TPC) (Folin-Ciocalteu) and total flavonoid content ($AlCl_3$ colorimetry) were expressed as µg gallic acid equivalents (GAE)/mg and µg quercetin equivalents (QAE)/mg. Antioxidant activity was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS assays against gallic acid, with IC_{50} derived from concentration-response curves.

Results: Yield was 8.0% w/w. The extract contained alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, and carbohydrates; proteins and anthraquinones were absent. TPC was 29.45±6.60 µg GAE/mg and total flavonoid content was 35.96±4.69 µg QAE/mg. The extract showed dose-dependent scavenging with IC_{50} 336.78±100.0 µg/mL (DPPH) and 221.30±4.73 µg/mL (ABTS), while gallic acid was ~4.3 µg/mL.

Conclusion: The 70% ethanolic oleo-gum-resin extract of *F. asafoetida* is rich in secondary metabolites and exhibits measurable antioxidant activity, with comparatively stronger performance in ABTS than DPPH. These findings provide a practical baseline for quality control of this extract.

Keywords: *Ferula asafoetida*, Hing, Oleo-gum resin, Phenolics, Flavonoids, 2,2-diphenyl-1-picrylhydrazyl, ABTS, Antioxidant.

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INTRODUCTION

Plant extracts and essential oils contain numerous phytochemicals that help plants resist microbes and environmental stress and may confer health benefits in humans [1-3]. These compounds occur in leaves, stems, roots, and edible parts and are commonly grouped as primary metabolites (e.g., chlorophylls, proteins, sugars) and secondary metabolites (e.g., flavonoids, alkaloids, sterols, terpenoids, saponins, tannins, and volatile oils) [4].

Ferula asafoetida ("hing") is a perennial herb native to the Iran-Afghanistan region and widely used in India. Its thick roots exude a pungent oleo-gum-resin. The resin fraction contains ferulic acid and its esters, sesquiterpenes, coumarins, and terpenoids; the gum fraction comprises carbohydrates and glycoproteins; the volatile fraction includes monoterpenes and sulfur-containing compounds responsible for the characteristic odor, notably disulfides such as $C_{11}H_{20}S_2$ [5-7]. These chemical features align with traditional uses and with reports of anti-inflammatory, antibacterial, antioxidant, cytotoxic, and antiviral activities [5-7]. Palatability and formulation can be affected by the volatile-oil load and sulfurous notes [8-10].

Chemically, asafoetida typically contains about 25% gum, 40–64% resin, and 10–17% volatile oil. The resin is often dominated by ferulic-acid esters, with free ferulic acid and coumarin derivatives, whereas the volatile oil carries diverse sulfurous constituents and monoterpenes [8,9].

Despite broad literature on essential oils and other fractions, systematic data on a 70% ethanolic oleo-gum-resin extract remain limited. The present study, therefore, profiles this extract and quantifies its antioxidant activity using complementary radical systems (2,2-diphenyl-1-picrylhydrazyl [DPPH] and ABTS) with gallic acid as a comparator, providing a focused baseline for subsequent standardization work [11-15].

METHODS

Sample collection and authentication

Commercial oleo-gum-resin crystals ("hing") were procured on January 22, 2024, from Kundli, Sonipat, Haryana, India. Botanical identity was confirmed by Prof. A. Vijay Bhaskar Reddy (Department of Botany, Osmania University, Hyderabad, Telangana). Voucher specimen deposited at Herbarium/Institution, code: DB-OU/OGR/0934.

Sample preparation

Resin was air-dried at room temperature and pulverized in a cleaned, dried electric grinder to a uniform powder to minimize contamination.

Extraction (Soxhlet)

Powdered sample (25 g; 40–60 mesh) was loaded into a cellulose thimble and extracted with 70% (v/v) ethanol in a Soxhlet apparatus for 10 h under gentle reflux. The 10 h duration was selected a priori to standardize approximately 60–80 siphon cycles and approach exhaustion; extraction was continued until the siphonate ran near-colorless and the residue reached constant mass. The combined eluate was concentrated and dried under reduced pressure at 50°C on a rotary evaporator. The dried extract was stored in amber vials at 2–8°C; a 100 mg/mL stock in 70% ethanol was prepared for assays. No formal time-optimization study was performed in this work.

Qualitative phytochemical screening

Standard colorimetric/precipitation tests were applied to the 70% ethanolic extract: Tannins ($FeCl_3$), saponins (foam test), flavonoids

(NaOH then HCl), alkaloids (Dragendorff's), proteins (Millon's), anthraquinones (ammonia), terpenoids (chloroform/H_2SO_4 interface), and carbohydrates (Benedict's). Qualitative results are reported in Table 1.

Quantitative estimations

Total phenolic content (TPC)

TPC was measured by the Folin-Ciocalteu assay. Gallic acid standards (10–250 µg/mL in 80% ethanol) and samples were pipetted into 96-well plates, mixed with 5% Folin-Ciocalteu reagent, incubated 5 min, then with 7.5% (w/v) Na_2CO_3; plates were kept in the dark for 60 min and read at 750 nm on a Tecan multimode microplate reader. Results are expressed as µg gallic acid equivalents (GAE) per mg extract.

Total flavonoid content (TFC)

TFC was measured by the $AlCl_3$ colorimetric method. Quercetin standards (10–250 µg/mL) and samples (25 µL; 1 mg/mL) were combined with 10% (w/v) $AlCl_3$, 96% ethanol, and 10% sodium acetate, incubated for 40 min, and read at 415 nm (Tecan). Results are expressed as µg quercetin equivalents (QAE) per mg extract.

Antioxidant assays

DPPH radical-scavenging activity

Assays were done in 96-well plates: 125 µL of 125 µM DPPH was mixed with 25 µL of extract (final 7.81–1000 µg/mL) or gallic acid standard (1–8 µg/mL). Plates were incubated for 30 min at 37±2°C in the dark and read at 517 nm (Tecan). Percentage inhibition was calculated as (control-sample)/control ×100.

ABTS radical-scavenging activity

$ABTS^{\bullet+}$ was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating for 16 h in the dark, then diluting to $A_{734} \approx 0.70$. For testing, 125 µL $ABTS^{\bullet+}$ was mixed with 25 µL of extract (7.81–1000 µg/mL) or gallic acid, incubated 6 min at 37±2°C in the dark, and read at 734 nm (Tecan). Percentage inhibition values across all tested concentrations were modeled with a four-parameter logistic (4PL) curve to obtain IC_{50} with 95% confidence interval (CI). All concentrations were retained for primary analysis unless a pre-specified QC failure occurred (e.g., edge-effect artefact, pipetting error). Monotonicity was not imposed. As a robustness check, leave-one-concentration sensitivity analyses were performed to confirm that exclusion of any single point did not materially change IC_{50}.

Replicates and statistics

All plate assays (TPC, TFC, DPPH, ABTS) were performed in triplicate wells and repeated on three independent days (n=9 per data point), unless stated otherwise. Data are reported as mean±standard deviation (SD). IC_{50} values were obtained by non-linear four-parameter logistic regression with 95% CIs. Calibration curves (≥6 points) were fitted by linear regression; slope, intercept, and r^2 were recorded. Appropriate blanks (reagent only) and vehicle controls (70% ethanol) were included on every plate.

RESULTS

Percentage yield

Soxhlet extraction of the oleo-gum resin with 70% ethanol produced an 8.0% w/w extract (Table 2). For context, hydroalcoholic extractions of *Ferula* leaves/gum generally return single-digit to low double-digit yields, whereas hydrodistilled essential oil is typically ≤1% (occasionally higher by supercritical CO_2). Accordingly, the 8.0% w/w obtained here aligns with prior reports for crude hydroalcoholic extracts of *F. asafoetida* and related *Ferula* matrices.

Phytochemical profile

Qualitative screening of the 70% ethanolic extract showed alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, and carbohydrates, with proteins and anthraquinones absent (Table 1). This composition reflects a secondary-metabolite-rich matrix typically associated with redox and other bioactivities.

Total phenolics (TPC)

By Folin-Ciocalteu assay [16], TPC was 29.45±6.60 µg GAE/mg extract. The gallic-acid calibration demonstrated excellent linearity (R^2=0.998; methods-anchored), supporting quantitative reliability (Table 3).

TFC

By $AlCl_3$ colorimetry, TFC was 35.96±4.69 µg QAE/mg extract, with a high-linearity quercetin calibration (R^2=0.982). The higher TFC relative to TPC is consistent with a flavonoid-enriched phenolic fraction (Table 4).

DPPH radical-scavenging

The reference gallic acid was strongly active (IC_{50}=4.22±0.17 µg/mL), while the 70% ethanolic extract was less potent (IC_{50}=336.78±100.0 µg/mL). Concentration-response values are provided for the standard (Table 5) and the extract (Table 6). Together, these data confirm dose-dependent scavenging by the extract, although requiring higher concentrations than the pure standard.

ABTS radical-scavenging [17]

In the $ABTS^{\bullet+}$ assay, gallic acid again showed strong activity (IC_{50}=4.34±0.06 µg/mL), and the extract achieved IC_{50}=221.30±4.73 µg/mL (Tables 7 and 8). Relative to DPPH, the extract exhibited lower IC_{50} in ABTS (~1.5× greater apparent potency), indicating assay-dependent differences in radical-quenching efficiency. A minor non-

Table 1: Preliminary phytochemical profile of the 70% ethanolic extract

Test	Results
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponins	Present
Steroids	Present
Phenols	Present
Proteins	Absent
Anthraquinones	Absent
Terpenoids	Present
Carbohydrates	Present

Standard qualitative tests per cited methodology; presence/absence as indicated

Table 2: Percentage yield of extract

S. No.	Extract	Percentage yield (% w/w)
1	70% ethanolic extract	8.0

Soxhlet extraction of the oleo-gum resin in 70% ethanol

Table 3: Total phenolic content (TPC) of the 70% ethanolic extract

S. No.	Sample/extract	TPC (µg GAE/mg)
1	70% ethanolic extract	29.45±6.60

Values are mean±standard deviation. Calibration (gallic acid): R^2=0.998.
GAE: Gallic acid equivalents

Table 4: Total flavonoid content (TFC) of the 70% ethanolic extract

S. No.	Sample/extract	TFC (µg QAE/mg)
1	70% ethanolic extract	35.96±4.69

Values are mean±standard deviation. Calibration (quercetin): R^2=0.982.
QAE: Quercetin equivalents

monotonic point at 62.5 µg/mL in Table 8 likely reflects typical microplate variability and does not alter the overall trend or IC_{50} estimate.

A slight drop at 62.5 µg/mL relative to 31.25 µg/mL was observed. This value fell within the expected microplate variability and replicate SDs. The 4PL fit using the full dataset yielded IC_{50}=221.30±4.73 µg/mL, and a leave-one-point sensitivity check excluding 62.5 µg/mL returned an IC_{50} estimate within the original 95% CI. The 62.5 µg/mL point was therefore retained in the primary analysis.

Overall, the extract's moderate phenolic/flavonoid burden (Tables 3 and 4) aligns with measurable free-radical scavenging across both systems (Tables 5-8), with comparatively better performance against ABTS•$^+$.

DISCUSSION

Soxhlet extraction of the oleo-gum resin with 70% ethanol yielded 8.0% w/w, which is consistent with single-digit to low double-digit returns reported for hydroalcoholic Ferula matrices and higher than typical hydrodistilled volatile-oil yields [18]. The qualitative profile of alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, and carbohydrates, with proteins and anthraquinones absent, matches the broad secondary-metabolite spectrum described for *Ferula* spp., where polyphenols and terpenoids commonly underpin bioactivity [18-20].

Table 5: 2,2-diphenyl-1-picrylhydrazyl assay-gallic acid concentration versus % inhibition

S. No.	Concentration (µg/mL)	Percentage inhibition	SD
1	1	15.19	0.32
2	2	27.66	1.40
3	4	50.85	2.62
4	6	68.87	2.00
5	8	82.39	4.92

Values represent mean % inhibition (replicates pooled; standard deviation [SD] not displayed in table). IC_{50} (gallic acid)=4.22±0.17 µg/mL

Table 6: 2,2-diphenyl-1-picrylhydrazyl assay-70% ethanolic extract concentration versus % inhibition

S. No.	Concentration (µg/mL)	Percentage inhibition	SD
1	7.81	2.64	0.15
2	15.625	4.59	0.05
3	31.25	5.29	0.32
4	62.5	9.83	0.42
5	125	30.82	1.12
6	250	46.24	0.71

Values represent mean % inhibition (replicates pooled; standard deviation [SD] not displayed in table). IC_{50} (extract)=336.78±100.0 µg/mL (nonlinear fit)

Table 7: ABTS assay-gallic acid concentration versus % inhibition

S. No.	Concentration (µg/mL)	Percentage inhibition	SD
1	1	13.00	0.73
2	2	27.89	0.85
3	4	49.36	3.46
4	6	67.50	2.09
5	8	82.30	1.80

Values represent mean % inhibition (replicates pooled; standard deviation [SD] not displayed in table). IC_{50} (gallic acid)=4.34±0.06 µg/mL

Table 8: ABTS assay-70% ethanolic extract concentration versus % inhibition

S. No.	Concentration (µg/mL)	Percentage inhibition	SD
1	7.81	12.15	0.75
2	15.625	15.67	1.13
3	31.25	32.83	0.86
4	62.5	26.65	0.40
5	125	40.99	2.29
6	250	59.46	4.10
7	500	82.73	3.72

SD: Standard deviation

The quantitative totals (TPC=29.45±6.60 µg GAE/mg; TFC=35.96±4.69 µg QAE/mg) fit the pattern usually seen with mixed hydroalcoholic systems, which extract mid-polar phenolics and flavonoids more efficiently than water alone and more broadly than absolute alcohols. In this context, 70% ethanol is expected to recover both glycosidic and aglycone flavonoids along with simple phenolics; therefore, the higher TFC relative to TPC is plausible and indicates a flavonoid-enriched phenolic pool. Analytical credibility is supported by strong calibration linearities (gallic acid R^2=0.998; quercetin R^2=0.982), in line with Folin-Ciocalteu and $AlCl_3$ colorimetry good practice [21-24]. Mechanistically, phenolics and flavonoids act as electron/hydrogen donors and stabilize radicals through resonance, and they are frequently linked with antimicrobial and host-defense actions, aligning with traditional uses of *F. asafoetida*.

Antioxidant findings mirror this chemistry. The extract showed dose-dependent scavenging with IC_{50}=336.78±100.0 µg/mL in DPPH and 221.30±4.73 µg/mL in ABTS, while gallic acid, as a potent single phenolic, lay near 4.3 µg/mL in both systems. The comparatively stronger performance in ABTS than DPPH (≈1.5× lower IC_{50}) is expected because ABTS•$^+$ is soluble in both aqueous and alcoholic media and is less sterically hindered, allowing better access for bulky or polymeric phenolics; DPPH• is more sensitive to solvent composition and reacts preferentially through single-electron transfer with smaller donors. Differences in radical type, reaction kinetics, and phase behavior therefore explain the assay divergence [21-25]. The gap between crude extract and pure standard reflects matrix complexity, normalization by mass rather than active equivalents, and possible intra-matrix interactions that can blunt the apparent activity of any single class in chemical assays.

Placing these data against the wider Ferula literature, hydroalcoholic extracts frequently report richer phenolic/flavonoid signals than aqueous fractions and display stronger ABTS than DPPH responses, whereas essential-oil-focused methods emphasize volatile terpenes with limited impact on colorimetric phenolic totals. The present pattern, moderate yield, flavonoid-skewed phenolic burden, and ABTS-leaning activity fits this solvent-dependent landscape and supports the choice of 70% ethanol for balanced recovery of redox-active constituents [21-25]. Tannins likely add high-equivalent reducing capacity; saponins may influence membrane interactions and immune tone; and terpenoids/steroids provide complementary anti-inflammatory effects converging on oxidative-stress pathways [18-20].

For quality control, pairing TPC/TFC with dual-assay read-outs gives a practical specification starting point. Next steps should include marker-guided standardization (sentinel phenolic/flavonoid indices) and chromatographic–spectrometric profiling (HPLC-DAD/LC-MS) to resolve dominant constituents against authentic standards [21,23-25]. Because DPPH and ABTS are non-physiological, biological validation in cells and *in vivo* is needed to confirm the mechanism (ROS attenuation, lipid-peroxidation control, or Nrf2/ARE activation) and define dose-response in realistic exposure windows [17-19]. Overall, the 70% ethanolic extract shows a chemistry-to-function pattern consistent with published solvent effects and provides a defensible base for standardization and translational testing.

Limitations and Future Directions

This study employed a single-batch, single-solvent (70% ethanol) Soxhlet extract and relied on preliminary qualitative tests alongside colorimetric totals (TPC/TFC) and chemical antioxidant assays (DPPH, ABTS). While these methods are appropriate for first-line screening,

they are non-specific (TPC/TFC) and non-physiological (DPPH/ABTS), and can be influenced by matrix constituents that either inflate or mask true antioxidant capacity. The use of a crude extract without chromatographic resolution limits attribution of activity to particular molecules or classes, and the absence of multi-lot replication, solvent comparisons, volatile profiling, and stability/toxicity assessments constrains generalizability and translational inference. Furthermore, comparisons to a pure standard (gallic acid), though conventional, cannot account for potential synergy or antagonism within complex plant matrices.

Future work should prioritize marker-based standardization and molecular characterization: (i) perform HPLC-DAD/LC-MS/MS to quantify sentinel phenolics/flavonoids (e.g., ferulic acid, coumarins) and GC-MS for sulfurous volatiles; (ii) conduct bioassay-guided fractionation to link chemical fractions with activity; and (iii) expand the antioxidant panel to include FRAP, ORAC, and metal-chelating assays. To address biological relevance, incorporate cell-based models (e.g., ROS suppression, lipid peroxidation, mitochondrial protection, Nrf2/ARE signaling) and *in vivo* oxidative-stress models, alongside dose-response, pharmacokinetics, acute/sub-chronic safety, and storage stability. Method-development studies comparing green extraction approaches and cross-batch reproducibility will strengthen QC, while synergy analyses (fraction recombination) can clarify matrix effects. Collectively, these directions will translate the present chemistry-to-function signal into mechanistically grounded, standardized, and biologically validated evidence suitable for preclinical development.

CONCLUSION

The 70% ethanolic Soxhlet extract of *F. asafoetida* oleo-gum resin yielded a chemically rich, secondary-metabolite dominated profile (alkaloids, flavonoids, tannins, saponins, steroids, phenols, terpenoids, carbohydrates) with proteins and anthraquinones absent, an architecture consistent with the plant's ethnopharmacology and with redox-active phytochemical classes. Quantitative estimations confirmed a credible phenolic and flavonoid burden, supported by robust assay calibration. Functional testing demonstrated reproducible free-radical scavenging. In line with assay chemistry, the extract showed relatively stronger performance in ABTS than in DPPH, while the expected superiority of a pure comparator (gallic acid) underscored the influence of matrix complexity on mass-normalized potency.

Taken together, the chemistry-to-function coherence supports *F. asafoetida* 70% ethanolic extract as a plausible natural antioxidant source and provides a defensible baseline for quality control. Building on these results, marker-based standardization and molecular characterization (e.g., chromatographic-spectrometric profiling) should be prioritized, followed by biologically relevant validation in cell-based and *in vivo* models to confirm mechanism and dose-response. The planned antimicrobial evaluation will complement the current antioxidant evidence and help define broader translational potential for food, nutraceutical, or phytotherapeutic applications.

ETHICS STATEMENT

This study was approved by the ethical committee after submitting an application to conduct clinical research with the number ref: TBPL/FART/009/2024. Dated on August 17, 2024.

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AUTHORS CONTRIBUTIONS

Thulluri Sravani Priya conceptualized and designed the study, supervised the experimental work, and prepared the manuscript draft. Karthick Selvaraj assisted with laboratory analyses and data interpretation. Divya Prafulla Yerraguntla contributed to methodology validation and critical manuscript review. S. Saravana Kumar provided technical guidance, supervised data analysis, and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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