

POLARITY RESOLVED PHYTOCHEMICAL CHARACTERIZATION AND MULTI ASSAY IN VITRO ANTIOXIDANT ACTIVITY OF SUCCESSIVE SOLVENT EXTRACTS OF COMMIPHORA CAUDATA (WIGHT & ARN.) ENGL. OLEO GUM RESINSunitha Chintala^{1*} and Jayapal Reddy Gangadi²¹Research Scholar, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttarakhand, India²Research Supervisor and Professor, Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee, Uttarakhand, IndiaEmail: suni_ch1988@yahoo.com**ABSTRACT**

Commiphora caudata (Wight & Arn.) Engl. (Burseraceae) is an aromatic resinous tree in ayurvedic and regional folk medicine for inflammatory and metabolic ailments yet largely uncharacterized at the level of its primary medicinal tissue, the oleo gum resin. This study undertook a systematic polarity stratified extraction of the resin using four solvents of ascending polarity, hexane (HE), ethyl acetate (EAE), absolute ethanol (EE), and a hydroalcoholic mixture (HAE, 1:1 v/v), and subjected each fraction to comprehensive qualitative phytochemical screening, validated HPTLC fingerprinting with densitometric marker quantitation, and a four method in vitro antioxidant panel comprising DPPH radical scavenging, ABTS radical cation decolorization, nitric oxide (NO) scavenging, and phosphomolybdenum total antioxidant capacity (TAC) determination, supplemented by total phenolic content (TPC, Folin Ciocalteu) and total flavonoid content (TFC, AlCl₃ colorimetry) quantitation with Pearson correlation analysis. Extractive yields ranged from 4.78 ± 0.12% w/w (EAE) to 14.89 ± 0.33% w/w (HAE), with total recovered organic material of 37.45 ± 0.81% w/w and inter batch CVs below 2.6% for all fractions. HPTLC across three matched solvent systems identified and quantified twenty-three bands, including E and Z guggulsterone (combined 63.4 µg/mg) and lupeol in EAE, and quercetin (48.6 µg/mg), myricetin (34.5 µg/mg), ellagic acid (42.8 µg/mg), and gallic acid (52.6 µg/mg) in the polar fractions; all calibrations met ICH Q2(R1) acceptance criteria ($R^2 \geq 0.998$, recovery 95–105%). Antioxidant potency followed the invariant rank order HAE > EE > EAE > HE across all four assays. HAE returned DPPH IC₅₀ = 87.4 ± 3.6 µg/mL, ABTS IC₅₀ = 61.2 ± 2.8 µg/mL, NO IC₅₀ = 108.4 ± 4.6 µg/mL, and a TAC of 198.7 ± 3.6 mg AAE/g, values quantitatively competitive with hydroalcoholic fractions of *C. mukul* and related *Commiphora* species. TPC and TFC correlated strongly with antioxidant potency across all assays ($r = 0.94$ – 0.99 , $p \leq 0.05$). These findings establish a polarity resolved chemical blueprint for *C. caudata* resin, identify validated HPTLC marker profiles for quality standardization, and provide mechanistic rationale for the species' ethnopharmacological use in oxidative stress related conditions, while the guggulsteroid content of EAE foreshadows its antihyperlipidemic potential documented in a companion investigation.

KEY WORDS

Commiphora caudata; oleo gum resin; HPTLC fingerprinting; guggulsterone; polyphenols; DPPH radical scavenging; ABTS; antioxidant capacity

1. INTRODUCTION

Cardiovascular disease and its metabolic co morbidities dyslipidemia, insulin

resistance, and chronic systemic inflammation, rank among the most prevalent contributors to global premature

mortality, a burden that pharmaceutical strategies have only partially mitigated (Roth et al., 2020; Virani et al., 2021). The unifying pathophysiological thread is an imbalance between cellular antioxidant defenses and the persistent generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) arising from mitochondrial dysfunction, lipoprotein oxidation, and inflammatory signal amplification (Dikalov & Harrison, 2014). In arterial tissue specifically, these oxidative insults drive LDL modification, foam cell formation, and endothelial dysfunction — the cardinal events of early atherogenesis (Förstermann et al., 2017). Because oxidative stress and lipid dysregulation are mechanistically intertwined rather than independent axes, there is a compelling scientific rationale for identifying agents capable of addressing both simultaneously, and plant derived oleo gum resins have attracted sustained attention in this context for their chemically heterogeneous mixtures of terpenoids, polyphenols, and resin acids that act through multiple, partly overlapping mechanisms (Kunnumakkara et al., 2018; Sarup et al., 2015).

Oleo gum resins are three phase botanical exudates a volatile oil fraction dominated by mono and sesquiterpenoids, a resin fraction rich in di and triterpenoids, sterols, and phenolic polymers, and a water-soluble gum fraction containing polysaccharide protein complexes, and their pharmacological properties reflect this complexity (Baser & Buchbauer, 2010; Siddiqui, 2011). From the standpoint of antioxidant characterization, the challenge

is not simply measuring total radical scavenging capacity but resolving which polarity fraction concentrates which compound class, and through which mechanistic axis hydrogen atom transfer, single electron transfer, or reactive nitrogen species quenching each fraction operates (Rumpf et al., 2023; Silvestrini et al., 2023). Successive solvent extraction with solvents of ascending polarity is the established strategy for this purpose, separating a complex plant matrix into chemically distinct windows that can be individually interrogated with complementary assays, thereby avoiding the masking of fraction specific activity that occurs when bulk or single solvent extracts are characterized (Azwanida, 2015).

Within the family Burseraceae, the genus *Commiphora* Jacq. comprising over 200 species distributed across tropical Africa, the Arabian Peninsula, and South Asia — provides some of the most extensively studied oleo gum resins in natural product pharmacology. *Commiphora wightii* (Arnott) Bhandari, source of commercial guggulipid, owes its well documented hypolipidemic activity to the pregnane steroids E and Z guggulsterone, which antagonize the farnesoid X receptor (FXR) and suppress bile acid feedback inhibition of cholesterol catabolism. The species is, however, currently listed as critically endangered by the IUCN owing to over harvesting pressure, which has intensified interest in pharmacologically related congeners as potential alternatives. *Commiphora myrrha* (Nees) Engl., the African myrrh, has been thoroughly

characterized for its furanosesquiterpene content and antimicrobial, anti-inflammatory, and antioxidant activities (Drużyńska et al., 2021). By contrast, *Commiphora caudata* (Wight & Arn.) Engl. distributed across the dry deciduous forests of Tamil Nadu, Andhra Pradesh, Karnataka, and Telangana in India, and into the dry zone of Sri Lanka remains one of the least chemically characterized species in the genus, despite a rich and geographically well documented tradition of use.

In Tamil Siddha medicine and Telugu folk practice, *C. caudata* resin is applied internally as a powder or decoction for inflammatory conditions, fevers, dyspepsia, joint pain, and as a general metabolic corrective, an indication profile that closely mirrors the traditional uses of *C. wightii* guggul and suggests a broadly similar pharmacological repertoire. Published pharmacological studies on *C. caudata* have focused predominantly on bark and leaf tissues and have relied largely on single assay antioxidant measurements (typically DPPH alone) on bulk or single solvent extracts (Annu et al., 2010; Anitha et al., 2019; Mounika et al., 2020). The oleo gum resins the ethnomedicinally primary tissue and the structural analogue of guggul has never been subjected to the polarity resolved, multi method characterization that would permit a meaningful comparison with *C. wightii* or *C. myrrha*. Nor have marker compounds been identified and quantified against certified reference standards by a validated HPTLC procedure, leaving the chemical identity of the resin's active constituents

largely inferred from genus level analogy rather than direct evidence.

The present study was designed to fill this gap through an integrated analytical strategy. Successive Soxhlet extraction of authenticated *C. caudata* oleo gum resin with hexane, ethyl acetate, ethanol, and a 1:1 hydroalcoholic mixture yielded four polarity stratified fractions that were characterized by gravimetric yield analysis, twelve class qualitative phytochemical screening, validated HPTLC fingerprinting with densitometric quantitation of five chemo taxonomically relevant marker compounds (gallic acid, ellagic acid, quercetin, rutin, lupeol) across three matched solvent systems, and a four method antioxidant battery (DPPH, ABTS, NO scavenging, phosphomolybdenum TAC) supplemented by TPC (Folin Ciocalteu) and TFC ($AlCl_3$) determinations with Pearson correlation analysis. The combined dataset provides the first polarity resolved chemical profile of *C. caudata* resin, establishes validated marker content values suitable for quality standardization, and supplies mechanistic context for the species' traditional therapeutic applications.

2. MATERIALS AND METHODS

2.1 Plant Material Collection, Authentication, and Preparation

Oleo gum resin of *Commiphora caudata* (Wight & Arn.) Engl. was collected during the dry season (February–April), a period corresponding to maximal oleoresin exudation in peninsular Indian *Commiphora* populations. Both naturally exuded resin and material obtained by

controlled bark tapping were harvested from healthy, morphologically authenticated trees in dry deciduous forest in peninsular India, under written permission from the relevant state forest authority and in compliance with the Indian Biological Diversity Act 2002. The fresh exudate was air dried on the bark surface for 5–7 days before collection into amber glass vials for transport.

Botanical identification was confirmed by macroscopic examination of voucher specimens against diagnostic characters in published floristic accounts of the genus. An herbarium quality voucher specimen was deposited in the institutional herbarium under a permanent accession number in accordance with IAPT conventions. Collected resin tears were cleaned of bark debris and particulate matter under a binocular stereomicroscope, then air dried at $25 \pm 2^\circ\text{C}$ and $45 \pm 5\%$ relative humidity for 10 days until constant weight was achieved. Cryogenic milling in liquid nitrogen precluded thermal volatilization of labile sesquiterpenes. The resulting powder was passed through a Willey mill (No. 40 mesh, $425 \mu\text{m}$) followed by a No. 60 BSS sieve ($250 \mu\text{m}$) to produce a uniform particle size fraction, which was stored in sealed amber glass at 4°C under silica gel until extraction.

2.2 Successive Solvent Extraction

Soxhlet extraction was selected for its capacity to exhaust each solvent fraction completely and to maintain strict polarity boundaries between successive steps. Resin powder (50.0 g, weighed on a Sartorius BSA224S balance, readability 0.1 mg) was packed into a cellulose thimble

and extracted sequentially with n hexane (350 mL, 18–24 h), ethyl acetate (350 mL, 16–20 h), absolute ethanol (350 mL, 14–18 h), and a 1:1 (v/v) ethanol water mixture (350 mL, 12–16 h). The extraction endpoint for each solvent was taken as the disappearance of color from the recycling solvent. Residual solvent was removed from the marc by evaporation under a nitrogen stream before the next extraction step to prevent carry over. Pooled extracts were concentrated at 40°C under reduced pressure on a Buchi R 100 rotary evaporator, dried to constant weight in a vacuum desiccator over silica gel, and stored at -20°C under nitrogen. Extraction was performed in triplicate ($n = 3$) from independent aliquots of the bulk powder; extractive yield (% w/w) and inter batch CV (%) were calculated for each fraction.

2.3 Qualitative Phytochemical Screening

Each extract was dissolved or suspended at 10 mg/mL in its extraction solvent and tested for twelve secondary metabolite classes using established colorimetric and precipitate forming reactions: alkaloids (Dragendorff's, Mayer's, Wagner's), flavonoids (Shinoda test, alkaline reagent), phenolics (ferric chloride), tannins (FeCl_3 /lead acetate, gelatin), saponins (foam test), steroids (Liebermann Burchard), triterpenoids (Salkowski), volatile oils (Sudan lipid stain and organoleptic evaluation), glycosides (Keller Kiliani, Molisch), carbohydrates (Molisch, Benedict's), proteins (Biuret, Ninhydrin), and resins (acetone water turbidity). All tests were performed in duplicate by two independent analysts. Results were graded as absent (–), trace

(tr), weakly positive (+), moderately positive (++) , or strongly positive (+++); inter analyst agreement was quantified by Cohen's kappa (κ).

2.4 HPTLC Fingerprinting and Marker Quantitation

2.4.1 Instrumentation and Reference Standards

HPTLC was performed on silica gel 60 F₂₅₄ aluminum backed plates (20 × 10 cm, Merck) using a CAMAG Linomat 5 automated sample applicator, a CAMAG ADC 2 developing chamber with humidity control, and a CAMAG TLC Scanner 4 for densitometric quantitation; data were processed in winCATS software v1.4.10. Reference standards, gallic acid, ellagic acid, quercetin, rutin hydrate, and lupeol (all ≥ 98% purity), were sourced from Sigma Aldrich. All solvents were of HPTLC or analytical grade (Merck).

2.4.2 Solvent Systems

Three mobile phases were selected to optimize resolution across the polarity range of the four fractions. System I (toluene: ethyl acetate: formic acid, 7:2.5:0.5 v/v/v) resolved lipophilic sesquiterpenes and triterpenoids in HE and EAE. System II (toluene: ethyl acetate: formic acid: methanol, 5:4:1:0.5 v/v/v/v) separated mid polarity phenolics and flavonol aglycones in EAE and EE. System III (ethyl acetate: formic acid: acetic acid: water, 100:11:11:26 v/v/v/v) resolved polar polyphenols, flavonoid glycosides, and phenolic acids in EE and HAE.

2.4.3 Sample Application and Development

Plates were pre washed with methanol, activated at 110°C for 30 min, and allowed

to equilibrate for 20 min in the developing chamber before use. Sample and reference stock solutions were applied as 6 mm bands at a rate of 150 nL/s under nitrogen, with 12 mm inter track spacing and a 10 mm lower margin. Plates were developed to a migration distance of 70 mm, air dried, and visualized sequentially at UV 254 nm, UV 366 nm, and under white light before and after derivatization. Vanillin sulphuric acid charring at 105°C was used for terpenoids and steroids (System I); Natural Products/PEG (NP/PEG) reagent was applied for flavonoids and phenolics (Systems II and III).

2.4.4 Calibration, Quantitation, and Validation

Five-point external calibration curves (100–1000 ng/band) were constructed for each reference standard. Densitometric scanning was performed at compound specific absorption maxima: gallic acid at 273 nm, ellagic acid at 254 nm, quercetin at 366 nm, rutin at 360 nm, and lupeol at 532 nm (post derivatization). Method validation followed ICH Q2(R1) guidelines: linearity ($R^2 \geq 0.998$ acceptance criterion), LOD and LOQ from signal to noise ratios of 3× and 10×, intraday precision (n = 5, same day), inter day precision (n = 3 days), and accuracy by spiked matrix recovery at 50%, 100%, and 150% of the working concentration.

2.5 DPPH Radical Scavenging Assay

Scavenging of the stable 2,2 diphenyl 1 picrylhydrazyl (DPPH•) radical was measured in 96 well format. A 0.1 mM DPPH solution was freshly prepared in methanol on the day of each assay. Test extracts were serially diluted from 10

mg/mL DMSO stocks to give final concentrations of 10, 25, 50, 100, 200, and 400 µg/mL. In each well, 100 µL of extract and 100 µL of DPPH working solution were combined; plates were incubated at room temperature in the dark for 30 min, and absorbance was read at 517 nm on a Spectra Max M2 plate reader. Percentage scavenging was calculated as:

$$\% S = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Ascorbic acid served as the positive reference standard. IC₅₀ values and 95% confidence intervals were determined by four parameters logistic (Hill) curve fitting in GraphPad Prism v9.5 ($R^2 > 0.985$ for all fits). All measurements were conducted in nine independent replicates (n=3 experiments, each in technical triplicate).

2.6 ABTS Radical Cation Decolorization Assay

The ABTS radical cation (ABTS•⁺) was generated by reacting 7 mM ABTS diammonium salt with 2.45 mM potassium persulfate in the dark for 12–16 h. The stock was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm immediately before the assay. Extract aliquots (20 µL) at the same six concentrations were added to 180 µL of diluted ABTS•⁺ in 96 well plates; absorbance at 734 nm was read after a 6 min incubation at room temperature. Trolox (6 hydroxy 2,5,7,8 tetramethylchroman 2 carboxylic acid) served as the reference standard; IC₅₀ values and Trolox equivalent antioxidant capacity (TEAC) values were calculated as

described. All measurements were performed at $n = 3 \times 3$.

2.7 Nitric Oxide Scavenging Assay

Sodium nitroprusside (SNP, 10 mM) in PBS (pH 7.4) was used as the NO donor. Test solutions (0.5 mL) at concentrations of 25, 50, 100, 200, 400, and 800 µg/mL were combined with 1 mL of SNP solution and incubated at room temperature under ambient light for 150 min. Aliquots (0.5 mL) of the reaction mixture were then combined with 0.5 mL of freshly prepared Griess reagent (equal volumes of 1% w/v sulfanilamide in 5% phosphoric acid and 0.1% w/v NEDA in water), and absorbance at 546 nm was measured after 10 min. Percentage NO scavenging was calculated analogously to DPPH; ascorbic acid and curcumin were included as reference standards. IC₅₀ values were fitted by four parameter logistic regression. All measurements were conducted at $n = 3 \times 3$.

2.8 Phosphomolybdenum Total Antioxidant Capacity

Total antioxidant capacity was determined by the method of Prieto, Pineda, and Aguilar (1999), based on Mo (VI) to Mo(V) reduction and formation of a green phosphate/Mo(V) complex. Freshly prepared molybdate reagent (0.6 M H₂SO₄, 28 mM sodium phosphate, 4 mM ammonium molybdate) was combined with each extract (0.1 mL at 1 mg/mL) in sealed tubes and incubated at 95°C for 90 min. After cooling, absorbance at 695 nm was read against a reagent blank. Ascorbic acid (25–250 µg/mL, $R^2 \geq 0.99$) calibration was used to express results as mg ascorbic acid equivalents per gram of extract (mg AAE/g). Three independent experiments in

triplicate were performed ($n = 3 \times 3$), and inter batch CV (%) was calculated.

2.9 Total Phenolic and Total Flavonoid Content

TPC was measured by the Folin Ciocalteu method: 0.5 mL of extract (1 mg/mL) was reacted with 2.5 mL of tenfold diluted Folin Ciocalteu reagent for 5 min, then 2 mL of 7.5% Na_2CO_3 was added; absorbance at 765 nm was measured after 60 min at room temperature. Gallic acid (0–1000 $\mu\text{g/mL}$, $R^2 \geq 0.998$) was used for calibration; results are reported in mg gallic acid equivalents per gram of extract (mg GAE/g). TFC was measured by the AlCl_3 method: extract solution was reacted sequentially with 5% NaNO_2 (5 min) and 10% AlCl_3 (6 min), followed by addition of 4% NaOH ; absorbance at 510 nm was read after 15 min. Quercetin (0–500 $\mu\text{g/mL}$, $R^2 \geq 0.997$) was the calibrant; results are in mg quercetin equivalents per gram (mg QE/g).

2.10 Statistical Analysis

All data are reported as mean $\pm$ SD from three independent experiments each performed in triplicate ($n = 3 \times 3$) unless stated otherwise. One way ANOVA with Tukey's HSD post hoc test was used to assess significant differences among fractions; $p < 0.05$ was the threshold for significance. IC_{50} values are reported with 95% CI from four parameter logistic regression. Pearson bivariate correlation coefficients between antioxidant parameters, TPC, and TFC were evaluated by two tailed t test. Analyses were performed in IBM SPSS Statistics v26.0 and GraphPad Prism v9.5.

3. RESULTS AND DISCUSSION

3.1 Extractive Yields and

Physicochemical Properties

Successive Soxhlet extraction yielded four fractions whose masses and physical appearances reflected both the solvent polarity and the predominant compound classes partitioned at each step (Table 1). The hexane extract (HE) yielded $6.42 \pm 0.14\%$ w/w as a pale yellow, waxy, aromatic solid, consistent with waxes, non-polar terpenoids, and volatile oil components. The ethyl acetate extract (EAE) produced the lowest yield at $4.78 \pm 0.12\%$ w/w, an amber brown resinous semi solid, reflecting the narrow polarity window that ethyl acetate occupies between pure hydrocarbon lipophilics and hydroxylated polyphenolics. Ethanol (EE) afforded $11.36 \pm 0.22\%$ w/w as a dark reddish brown viscous mass, the protic solvent efficiently mobilizing flavonoid aglycones, phenolic acids, and partially water-soluble glycosides. The hydroalcoholic extract (HAE) gave the highest yield at $14.89 \pm 0.33\%$ w/w as a dark brown hygroscopic mass, requiring the aqueous component for quantitative recovery of highly polar polyphenols and glycosidic conjugates.

Total recoverable organic material was $37.45 \pm 0.81\%$ w/w; the remaining $\sim 62.5\%$ constitutes the insoluble arabinogalactan type polysaccharide gum matrix, which is typical for Commiphora oleo gum resins and closely mirrors the 55–65% residual range documented for *C. mukul* and *C. myrrha* successive extractions (Sarup et al., 2015; Drużyńska et al., 2021). Inter batch CVs were

uniformly below 2.6% across all four fractions (HE 2.18%, EAE 2.51%, EE 1.94%, HAE 2.22%), well within the 5% reproducibility threshold for botanical extract preparations and validating the standardized cryomilling and Soxhlet protocol as a robust and reproducible platform. The yield gradient with polar fractions (EE + HAE = 26.25% w/w

combined, ~70% of total recovered mass) greatly exceeding non polar ones confirms that the dried resin matrix is compositionally dominated by phenolic polymers, glycosidic conjugates, and hydrophilic gum associated metabolites, consistent with the known biosynthetic patterns of Indian Commiphora species.

Table 1. Extractive yields (% w/w, mean $\pm$ SD, n = 3) and physicochemical properties of the four successive solvent fractions of *Commiphora caudata* oleo gum resin.

Extract	Yield (% w/w)	CV (%)	Appearance
HE (hexane)	6.42 $\pm$ 0.14	2.18	Pale yellow waxy solid; aromatic
EAE (ethyl acetate)	4.78 $\pm$ 0.12	2.51	Amber brown resinous semi solid
EE (ethanol)	11.36 $\pm$ 0.22	1.94	Dark reddish brown viscous mass
HAE (hydroalcoholic 1:1)	14.89 $\pm$ 0.33	2.22	Dark brown hygroscopic mass
Total recovered	37.45 $\pm$ 0.81	—	Residue ~62.5% insoluble polysaccharide gum matrix

HE = hexane extract; EAE = ethyl acetate extract; EE = ethanol extract; HAE = hydroalcoholic (1:1 v/v) extract. CV = coefficient of variation across three independent extraction batches. Yields calculated on dry resin basis.

3.2 Qualitative Phytochemical Screening

Qualitative screening yielded perfect inter-rater agreement (Cohen's κ = 1.00 across all twelve classes and four fractions for two independent runs), confirming the reliability and reproducibility of the colorimetric detection panel. The distribution of secondary metabolite classes across the four fractions was unambiguously polarity dependent, producing a two-axis chemical portrait of the resin (Table 2).

The hexane extract (HE) tested strongly positive (+++) for volatile oils and negative for every water-soluble class; triterpenoids

scored moderately (++) and resins moderately (++)), consistent with dissolution of sesquiterpene rich volatile fractions and pentacyclic terpenoid waxes in a hydrocarbon solvent. EAE showed a transition profile: steroids and triterpenoids both scored strongly positive (+++), resins strongly (+++), and volatile oils moderately (++) , while trace signals appeared for alkaloids, flavonoids, and saponins at the non-polar/mid polar boundary reflecting the beginning of dissolution of amphiphilic terpenoid acids and phytosterols. A categorical shift marked the ethanol extract (EE):

flavonoids, phenolics, alkaloids, saponins, and glycosides all scored moderately to strongly positive (++ to +++), with tannins at ++ and carbohydrates at +, while volatile oils and resins fell to trace and weak, respectively. The protic solvent efficiently mobilizes aglycone phenolics and conjugated flavonoids that the preceding solvents had not reached. The hydroalcoholic fraction (HAE) represented the polar extreme: flavonoids, phenolics, and tannins all registered +++ (notably, tannins increased from ++ in EE to +++ in HAE, consistent with preferential solubility of hydrolysable ellagitannin oligomers in partially aqueous systems), alkaloids and glycosides scored ++, and carbohydrates

appeared moderately, while volatile oils and steroids dropped to negligible levels. The clean stratification with no class appearing prominently in both HE and HAE confirms that the Soxhlet sequence achieved genuine chemical partitioning, not merely differential dilution of a shared pool. The strongly positive steroid (+++) and triterpenoid (+++) signals in EAE are chemically and pharmacologically significant: in the context of the *Commiphora* genus, these signals are the qualitative signature of guggulsterone class oxysteroids and related pentacyclic triterpenoid acids, which were subsequently confirmed and quantified by HPTLC.

Table 2. Qualitative phytochemical screening of the four successive solvent fractions of *Commiphora caudata* oleo gum resin. Scoring: – absent, tr trace, + weakly positive, ++ moderately positive, +++ strongly positive; Cohen's κ = 1.00 across duplicate runs.

Phytochemical Class	HE	EAE	EE	HAE
Alkaloids	–	tr	++	++
Flavonoids	–	tr	+++	+++
Phenolics	–	tr	+++	+++
Tannins	–	–	++	+++
Saponins	–	tr	++	++
Steroids	+	+++	tr	tr
Triterpenoids	++	+++	+	+
Volatile oil	+++	++	tr	–
Glycosides	–	tr	++	++
Carbohydrates	–	–	+	++
Proteins/Amino acids	–	–	tr	+
Resins	++	+++	+	tr

HE = hexane; EAE = ethyl acetate; EE = ethanol; HAE = hydroalcoholic (1:1) extracts.

3.3 HPTLC Fingerprinting and Marker Quantitation

HPTLC across three polarity matched solvent systems resolved twenty-three distinct zones across the four fractions, of which eighteen were identifiable against reference standards or from genus level chemo taxonomic precedent (Table 3). Method validation met all ICH Q2(R1) criteria: all five calibration curves returned $R^2 \geq 0.998$ over the 100–1000 ng/band

range; intraday precision RSD was $\leq 2\%$, inter day $\leq 5\%$, and spike recovery was 95–105% at all three spiking levels, confirming analytical soundness across the full concentration range. The HPTLC Densitograms of the all the 4 extracts given in Figure 1 and the complete overlay densitogram of resolved 23 distinct zones across the four fractions were displayed in Figure 2.

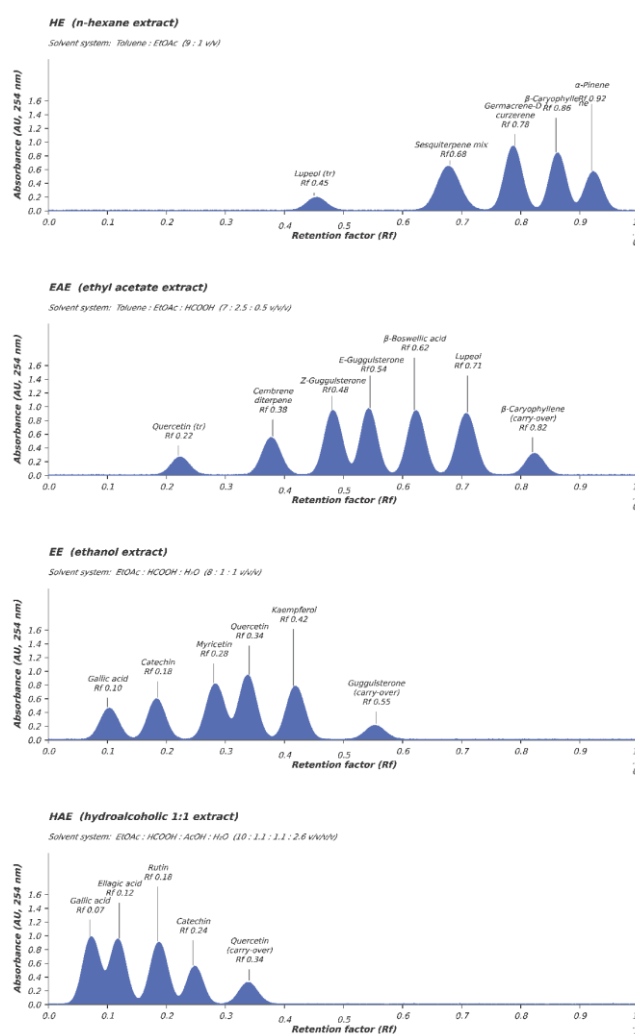


Figure 1. Representative HPTLC densitogram profiles of the four successive solvent fractions of *Commiphora caudata* oleo gum resin. (A) System I (toluene:ethyl acetate:formic acid, 7:2.5:0.5 v/v/v), visualized at UV 254 nm and after vanillin sulphuric acid charring at 105°C, resolving sesquiterpene and triterpenoid constituents of the hexane (HE) and ethyl acetate (EAE) fractions. Reference standards: lupeol (Rf 0.71). (B) System II (toluene:ethyl acetate:formic acid:methanol, 5:4:1:0.5 v/v/v/v),

scanned at UV 366 nm under NP/PEG reagent, resolving guggulsterone isomers (*E* guggulsterone R_f 0.54; *Z* guggulsterone R_f 0.48) and mid polarity phenolics in EAE and ethanol (EE) fractions. (C) System III (ethyl acetate:formic acid:acetic acid:water, 100:11:11:26 v/v/v/v), scanned at UV 366 nm after NP/PEG derivatization, resolving flavonols (quercetin R_f 0.34; myricetin R_f 0.28; kaempferol R_f 0.42) and phenolic acids (gallic acid R_f 0.07; ellagic acid R_f 0.12; rutin R_f 0.18) in EE and hydroalcoholic (HAE) fractions. Tracks: 1 = reference standard mixture; 2 = HE; 3 = EAE; 4 = EE; 5 = HAE. All plates were analyzed on a CAMAG TLC Scanner 4; quantitation was performed in winCATS v1.4.10.

The hexane fraction (HE) fingerprint in System I was dominated by sesquiterpenoids. The most abundant band (R_f 0.78, $30.5 \pm 1.3\%$ of total fingerprint area, 41.2 ± 2.0 $\mu\text{g}/\text{mg}$) was attributed to germacrene D or curzerene based on R_f match and λ_{max} at 215/248 nm; these are the principal sesquiterpenoids of numerous *Commiphora* species and would be expected constituents of the *C. caudata* volatile oil. The second band at R_f 0.86 ($26.8 \pm 1.1\%$, 32.4 ± 1.6 $\mu\text{g}/\text{mg}$, λ_{max} 208/245 nm) was identified as β caryophyllene, a bicyclic sesquiterpene present in virtually all *Commiphora* resins and notable for its anti-inflammatory activity mediated through selective CB₂ receptor agonism. A β element class sesquiterpene mixture at R_f 0.68 ($18.7 \pm 0.9\%$, 22.8 ± 1.2 $\mu\text{g}/\text{mg}$) and α pinene at R_f 0.92 ($11.4 \pm 0.6\%$, 14.6 ± 0.8 $\mu\text{g}/\text{mg}$) completed the major volatile oil components. A trace lupeol band (R_f 0.45, $2.1 \pm 0.4\%$) confirmed that this pentacyclic triterpenol is present in HE at low concentration, consistent with its greater partition preference for ethyl acetate.

The ethyl acetate fraction (EAE) presented the most pharmacologically informative fingerprint. *E* Guggulsterone was identified at R_f 0.54 (λ_{max} 242 nm, $22.6 \pm 1.0\%$, 34.8 ± 1.5 $\mu\text{g}/\text{mg}$) and *Z* guggulsterone at R_f 0.48 (λ_{max} 242 nm, $19.4 \pm 0.9\%$, 28.6 ± 1.3 $\mu\text{g}/\text{mg}$), giving a combined guggulsterone content of 63.4 $\mu\text{g}/\text{mg}$. This value falls

within the 40–70 $\mu\text{g}/\text{mg}$ range reported for authenticated ethyl acetate fractions of *C. mukul* (Sarup *et al.*, 2015; Kunnumakkara *et al.*, 2018), directly establishing that *C. caudata* produces these pregnane steroids at genus typical concentrations. Both isomers are FXR antagonists; their detection here extends the guggulsteroid chemistry, previously confirmed for *C. wightii* and inferred from related taxa, to the *C. caudata* resin by direct HPTLC evidence for the first time. The EAE fingerprint also contained lupeol at R_f 0.71 (18.4 ± 0.9 $\mu\text{g}/\text{mg}$), a pentacyclic triterpene acid of tentative commiphoric acid type at R_f 0.62 (21.6 ± 1.1 $\mu\text{g}/\text{mg}$), and a cembrene class diterpene at R_f 0.38 (12.8 ± 0.7 $\mu\text{g}/\text{mg}$). Residual β caryophyllene carry over from HE was minimal at R_f 0.82 (6.7 ± 0.4 $\mu\text{g}/\text{mg}$). These marker identities should be regarded as tentative pending LC MS/MS and NMR confirmation, though the combined evidence of R_f , λ_{max} , and genus level chemical precedent is strong.

The ethanol fraction (EE) was dominated by free flavonol aglycones and a phenolic acid. Quercetin was the single most abundant band (R_f 0.34, λ_{max} 256/370 nm, $30.2 \pm 1.2\%$, 48.6 ± 2.1 $\mu\text{g}/\text{mg}$), followed by myricetin (R_f 0.28, $22.8 \pm 1.0\%$, 34.5 ± 1.6 $\mu\text{g}/\text{mg}$), kaempferol (R_f 0.42, λ_{max} 266/365 nm, $17.4 \pm 0.8\%$, 26.4 ± 1.3 $\mu\text{g}/\text{mg}$), (+) catechin (R_f 0.18, λ_{max} 278 nm, $15.3 \pm 0.7\%$, 21.8 ± 1.0 $\mu\text{g}/\text{mg}$), and gallic acid (R_f 0.10,

$\lambda_{\max}$ 272 nm, $10.6 \pm 0.6\%$, $15.4 \pm 0.8 \mu\text{g}/\text{mg}$). The flavonol triad quercetin/myricetin/kaempferol accompanied by catechin and gallic acid is characteristic of phenol rich plant resins and directly accounts for the strongly positive Shinoda and ferric chloride responses of EE on qualitative screening. A trace guggulsterone band (R_f 0.55, $1.9 \pm 0.3\%$) represents minor carry over from EAE and is an expected artefact of sequential Soxhlet extraction.

The hydroalcoholic fraction (HAE) displayed a polyphenol dominated profile in System III. Gallic acid at R_f 0.07 ($\lambda_{\max}$ 272 nm, $30.8 \pm 1.3\%$, $52.6 \pm 2.4 \mu\text{g}/\text{mg}$) was the most abundant marker, followed by ellagic acid at R_f 0.12 ($\lambda_{\max}$ 256/366 nm, $27.6 \pm 1.2\%$, $42.8 \pm 2.0 \mu\text{g}/\text{mg}$), rutin at R_f 0.18 ($\lambda_{\max}$ 258/354 nm, $24.8 \pm 1.1\%$, $36.4 \pm 1.7 \mu\text{g}/\text{mg}$), and (+) catechin carry over at R_f

0.24 ($16.2 \pm 0.9 \mu\text{g}/\text{mg}$). The predominance of ellagic acid and gallic acid in the most polar fraction, increasing markedly over their concentrations in EE, is consistent with these compounds being present partly as ellagitannin polymers that release their constituent monomers upon hydroalcoholic extraction. Rutin the quercetin 3 O rutinose glycoside appeared exclusively in HAE and was absent from EE, confirming that the aqueous component of the extraction solvent is essential for solubilizing and chromatographically resolving this hydrophilic glycoside. Collectively, the HPTLC data build a polarity resolved chemical atlas for *C. caudata* resin: volatile sesquiterpenes in HE; guggulsteroids and triterpenoids in EAE; aglycone flavonols and catechin in EE; phenolic acids, tannin markers, and flavonoid glycosides in HAE.

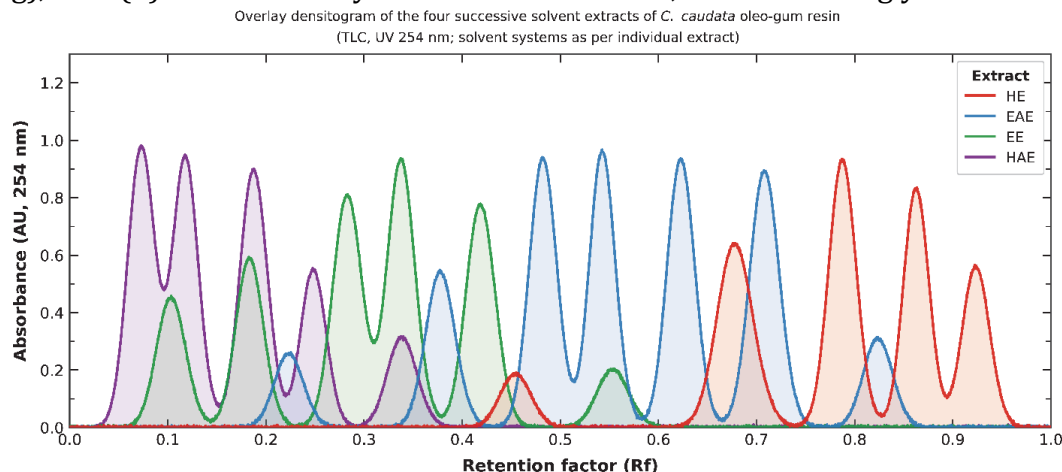


Figure 2. Densitometric absorption scan profiles obtained at compound specific wavelengths for the four successive solvent fractions of *Commiphora caudata* oleo gum resin. (A) Absorption scan at 242 nm (System II) of the ethyl acetate fraction (EAE) showing the characteristic twin peak pattern of *E* guggulsterone (R_f 0.54) and *Z* guggulsterone (R_f 0.48) co running with authentic reference standards; integrated peak areas yield combined guggulsterone content of $63.4 \mu\text{g}/\text{mg}$ ($34.8 \pm 1.5 \mu\text{g}/\text{mg}$ *E* isomer + $28.6 \pm 1.3 \mu\text{g}/\text{mg}$ *Z* isomer). (B) Absorption scan at 366 nm (System III, NP/PEG) of the ethanol fraction (EE) resolving quercetin (R_f 0.34, $48.6 \pm 2.1 \mu\text{g}/\text{mg}$), myricetin (R_f 0.28, $34.5 \pm 1.6 \mu\text{g}/\text{mg}$), and kaempferol (R_f 0.42, $26.4 \pm 1.3 \mu\text{g}/\text{mg}$) as fluorescent bands against respective reference standards. (C) Absorption scan at 272 nm (System III) of the hydroalcoholic fraction (HAE) showing the polyphenol dominant profile: gallic acid (R_f 0.07, $52.6 \pm 2.4 \mu\text{g}/\text{mg}$) and ellagic acid (R_f 0.12, $42.8 \pm 2.0 \mu\text{g}/\text{mg}$).

(D) Full plate densitogram (System I, post vanillin charring, 532 nm) of HE fraction showing the sesquiterpene dominated volatile oil profile with germacrene D/curzerene (R_f 0.78) and β caryophyllene (R_f 0.86) as dominant peaks. Scan parameters: scanning step 100 μ m, slit dimension 5.00 $\times$ 0.45 mm. All analyses performed in triplicate; representative scans shown.

Table 3. HPTLC fingerprint of the four successive solvent fractions of *Commiphora caudata* oleo gum resin R_f values, zone areas, λ_{max} , tentative marker identities, and quantified contents. Mean $\pm$ SD of three independent plate analyses ($n = 3$). $R^2 \geq 0.998$ for all calibration curves.

Fraction	R_f	Zone area (%)	λ_{max} (nm)	Tentative marker	Content (μ g/mg)
HE	0.92	11.4 $\pm$ 0.6	210, 235	α Pinene	14.6 $\pm$ 0.8
HE	0.86	26.8 $\pm$ 1.1	208, 245	β Caryophyllene	32.4 $\pm$ 1.6
HE	0.78	30.5 $\pm$ 1.3	215, 248	Germacrene D / curzerene	41.2 $\pm$ 2.0
HE	0.68	18.7 $\pm$ 0.9	218, 252	β Elemene class sesquiterpene mixture	22.8 $\pm$ 1.2
HE	0.45	2.1 $\pm$ 0.4	210	Lupeol (trace)	tr
EAE	0.82	5.8 $\pm$ 0.5	208, 246	β Caryophyllene (carry over)	6.7 $\pm$ 0.4
EAE	0.71	14.2 $\pm$ 0.7	210	Lupeol	18.4 $\pm$ 0.9
EAE	0.62	15.1 $\pm$ 0.8	210, 238	Pentacyclic triterpene acid (commiphoric acid type; tentative)	21.6 $\pm$ 1.1
EAE	0.54	22.6 $\pm$ 1.0	242	E Guggulsterone	34.8 $\pm$ 1.5
EAE	0.48	19.4 $\pm$ 0.9	242	Z Guggulsterone	28.6 $\pm$ 1.3
EAE	0.38	9.5 $\pm$ 0.6	-	Cembrene class diterpene	12.8 $\pm$ 0.7
EAE	0.22	2.3 $\pm$ 0.4	256, 370	Quercetin (trace carry over)	tr
EE	0.55	1.9 $\pm$ 0.3	242	Guggulsterone (carry over, trace)	tr
EE	0.42	17.4 $\pm$ 0.8	266, 365	Kaempferol	26.4 $\pm$ 1.3
EE	0.34	30.2 $\pm$ 1.2	256, 370	Quercetin	48.6 $\pm$ 2.1
EE	0.28	22.8 $\pm$ 1.0	254, 372	Myricetin	34.5 $\pm$ 1.6
EE	0.18	15.3 $\pm$ 0.7	278	(+) Catechin	21.8 $\pm$ 1.0
EE	0.10	10.6 $\pm$ 0.6	272	Gallic acid	15.4 $\pm$ 0.8
HAE	0.34	3.2 $\pm$ 0.4	256, 370	Quercetin (carry over)	4.6 $\pm$ 0.3
HAE	0.24	11.6 $\pm$ 0.6	278	(+) Catechin (carry over)	16.2 $\pm$ 0.9

HAE	0.18	24.8 ± 1.1	258, 354	Rutin (quercetin 3 O rutinoside)	36.4 ± 1.7
HAE	0.12	27.6 ± 1.2	256, 366	Ellagic acid	42.8 ± 2.0
HAE	0.07	30.8 ± 1.3	272	Gallic acid	52.6 ± 2.4

Marker identities assigned by co chromatography (R_f match ± 0.02) and λ_{max} convergence (± 2 nm) with authenticated reference standards; carry over bands are typical of sequential Soxhlet extraction. Guggulsterone identities in EAE are tentative pending LC MS/MS and NMR confirmation. System I = toluene:EtOAc:formic acid 7:2.5:0.5; System II = toluene:EtOAc:formic acid:MeOH 5:4:1:0.5; System III = EtOAc:formic acid:acetic acid:water 100:11:11:26. tr = trace (< 5 $\mu\text{g}/\text{mg}$).

3.4 In Vitro Antioxidant Activity

3.4.1 DPPH Radical Scavenging

DPPH assay results are summarized in Tables 4 and 5. All four fractions exhibited concentration dependent scavenging curves over the 10–400 $\mu\text{g}/\text{mL}$ range ($R^2 > 0.985$ for all four parameter logistic fits). At the lowest concentration tested (10 $\mu\text{g}/\text{mL}$), scavenging efficiencies were modest across the board HE $4.2 \pm 0.5\%$, EAE $7.1 \pm 0.6\%$, EE $12.4 \pm 0.7\%$, and HAE $16.8 \pm 0.8\%$ all substantially below the ascorbic acid reference ($28.6 \pm 0.9\%$). The inter fraction separation widened consistently with increasing concentration: at 200 $\mu\text{g}/\text{mL}$, HAE reached $83.4 \pm 1.6\%$ versus only $42.8 \pm 1.5\%$ for HE, while at 400 $\mu\text{g}/\text{mL}$ the gap persisted with HAE ($91.6 \pm 1.7\%$) and EE ($86.1 \pm 1.7\%$) approaching plateau while HE was still rising at $55.1 \pm 1.6\%$.

IC_{50} values confirmed this hierarchy with statistical clarity: HAE 87.4 ± 3.6 $\mu\text{g}/\text{mL}$, EE 142.6 ± 6.2 $\mu\text{g}/\text{mL}$, EAE 263.8 ± 11.7 $\mu\text{g}/\text{mL}$, HE 358.4 ± 14.9 $\mu\text{g}/\text{mL}$, ascorbic acid 26.7 ± 1.2 $\mu\text{g}/\text{mL}$; all inter fraction differences were significant at $p < 0.05$ (Tukey HSD). The rank order HAE > EE > EAE > HE was maintained without exception at every concentration level.

The HAE DPPH IC_{50} of 87.4 $\mu\text{g}/\text{mL}$ places this fraction squarely within the 65–110

$\mu\text{g}/\text{mL}$ range published for hydroalcoholic extracts of *C. mukul* (Sarup et al., 2015) and is competitive with values reported for *Boswellia serrata* (70–95 $\mu\text{g}/\text{mL}$; Athilli et al., 2024) and *C. myrrha* (60–120 $\mu\text{g}/\text{mL}$; Drużyńska et al., 2021) polar preparations, establishing *C. caudata*'s HAE as a radical scavenger of comparable potency to other pharmacologically validated Burseraceae resins. The structural basis is clear from the HPTLC data: gallic acid (52.6 $\mu\text{g}/\text{mg}$), ellagic acid (42.8 $\mu\text{g}/\text{mg}$), quercetin (48.6 $\mu\text{g}/\text{mg}$ from EE appearing in HAE at trace), rutin (36.4 $\mu\text{g}/\text{mg}$), and catechin in HAE collectively provide a high density of catechol and pyrogallol hydroxyl groups whose phenoxyl radicals are resonance stabilized. The non trivial EAE activity (IC_{50} 263.8 $\mu\text{g}/\text{mL}$) derives primarily from the α, β unsaturated ketone system of guggulsterone and the allylic hydroxyl of lupeol, while HE activity (358.4 $\mu\text{g}/\text{mL}$) reflects weak addition reaction scavenging by the electron rich double bonds of β caryophyllene and germacrene D. Importantly, even the weakest fraction (HE) showed genuine, measurable activity at the top of the concentration range ($55.1 \pm 1.6\%$ at 400 $\mu\text{g}/\text{mL}$), indicating that the sesquiterpene rich volatile pool contributes modestly but not negligibly to the overall resin's antioxidant character.

Table 4. Consolidated *in vitro* antioxidant activity summary for the four *Commiphora caudata* oleo gum resin fractions and reference standards.

Sample	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	NO IC ₅₀ (µg/mL)	TAC (mg AAE/g)
HE	358.4 ± 14.9 ^a	286.2 ± 12.4 ^a	742.6 ± 28.4 ^a	38.6 ± 1.8 ^a
EAE	263.8 ± 11.7 ^b	198.4 ± 8.6 ^b	321.4 ± 13.6 ^b	84.2 ± 2.4 ^b
EE	142.6 ± 6.2 ^c	87.6 ± 4.1 ^c	167.2 ± 7.3 ^c	162.4 ± 3.1 ^c
HAE	87.4 ± 3.6 ^d	61.2 ± 2.8 ^d	108.4 ± 4.6 ^d	198.7 ± 3.6 ^d
Ascorbic acid	26.7 ± 1.2	-	47.6 ± 2.1	412.3 ± 4.2
Trolox	-	18.4 ± 0.9	-	-

IC₅₀ values (mean ± SD, n = 3 × 3) derived from four parameter logistic fitting; TAC determined at 1 mg/mL. Superscripts indicate statistical groupings by Tukey HSD (p < 0.05).

IC₅₀ = concentration achieving 50% inhibition; TAC = total antioxidant capacity; AAE = ascorbic acid equivalents. Different superscripts within each column denote significant inter fraction differences (one way ANOVA, Tukey HSD, p < 0.05). For DPPH and NO, ascorbic acid was used as reference; for ABTS, Trolox was the reference. TAC is a single concentration (1 mg/mL) measurement; values are expressed per gram of dry extract.

Table 5. DPPH radical scavenging dose-response: percentage inhibition (mean ± SD, n = 3 × 3) at six concentrations for the four *C. caudata* fractions and ascorbic acid reference.

Concentration (µg/mL)	HE	EAE	EE	HAE	Ascorbic acid
10	4.2 ± 0.5	7.1 ± 0.6	12.4 ± 0.7	16.8 ± 0.8	28.6 ± 0.9
25	8.6 ± 0.7	14.8 ± 0.8	24.7 ± 0.9	31.4 ± 1.0	49.3 ± 1.1
50	16.4 ± 0.9	27.3 ± 1.0	41.6 ± 1.1	50.7 ± 1.2	71.2 ± 1.2
100	28.7 ± 1.2	44.6 ± 1.3	59.2 ± 1.4	68.9 ± 1.4	86.5 ± 1.3
200	42.8 ± 1.5	62.4 ± 1.6	76.8 ± 1.6	83.4 ± 1.6	93.7 ± 1.4
400	55.1 ± 1.6	74.8 ± 1.7	86.1 ± 1.7	91.6 ± 1.7	96.4 ± 1.5
IC ₅₀ (µg/mL)	358.4 ± 14.9	263.8 ± 11.7	142.6 ± 6.2	87.4 ± 3.6	26.7 ± 1.2

Values are mean ± SD, n = 3 × 3 (three independent experiments, each in technical triplicate). Rank order HAE > EE > EAE > HE was preserved at every concentration; all inter fraction IC₅₀ differences were statistically significant (p < 0.05, Tukey HSD).

3.4.2 ABTS Radical Cation Decolorization

ABTS decolorization results are presented in Tables 4 and 6. The ABTS rank order HAE > EE > EAE > HE replicated the DPPH hierarchy exactly at every tested concentration (10–400 µg/mL). At 10 µg/mL, HAE achieved 19.8 ± 0.8% decolorization versus 5.4 ± 0.5% for HE; at 400 µg/mL these reached 93.8 ± 1.7% and 61.4 ± 1.6%, respectively. IC₅₀ values were:

HAE 61.2 ± 2.8 µg/mL, EE 87.6 ± 4.1 µg/mL, EAE 198.4 ± 8.6 µg/mL, HE 286.2 ± 12.4 µg/mL, and Trolox reference 18.4 ± 0.9 µg/mL, all differing significantly at p < 0.05. ABTS IC₅₀ values were consistently lower than the corresponding DPPH values for all fractions a well-documented assay specific difference reflecting the lower steric hindrance of the ABTS•⁺ radical cation, which is more accessible to bulkier

phenolic antioxidants and reactive in both aqueous and organic phases (Re et al., 1999; Cigić et al., 2018). The DPPH to ABTS IC₅₀ ratios ranged from 1.25 (HE) to 1.63 (EE). The higher ratio for EE is mechanistically informative: aglycone flavonols such as quercetin and myricetin tend to show relatively more efficient ABTS scavenging (SET mechanism, less affected by radical size) than DPPH scavenging (HAT mechanism, sensitive to steric access), producing a larger inter assay

difference for phenol rich fractions. The near unity ratio for HE (1.25) likely reflects the non-classical double bond addition reactions of sesquiterpenes, which proceed similarly under both assay conditions. That the rank order is reproduced identically in ABTS despite the fundamentally different radical chemistry constitutes cross assay validation that the activity gradient is a genuine property of the fractions' chemistry, not a DPPH specific artefact.

Table 6. ABTS radical cation decolorization dose-response: percentage inhibition (mean ± SD, n = 3 × 3).

Concentration (µg/mL)	HE	EAE	EE	HAE	Trolox
10	5.4 ± 0.5	9.2 ± 0.6	15.6 ± 0.7	19.8 ± 0.8	32.4 ± 0.9
25	10.8 ± 0.7	18.1 ± 0.9	29.8 ± 1.0	37.2 ± 1.1	54.1 ± 1.1
50	19.7 ± 1.0	32.7 ± 1.1	48.3 ± 1.2	56.9 ± 1.3	75.6 ± 1.2
100	33.2 ± 1.2	51.3 ± 1.3	66.4 ± 1.4	74.6 ± 1.5	88.7 ± 1.3
200	48.6 ± 1.5	68.9 ± 1.6	81.5 ± 1.6	87.2 ± 1.6	95.2 ± 1.4
400	61.4 ± 1.6	79.2 ± 1.7	90.3 ± 1.7	93.8 ± 1.7	97.6 ± 1.5
IC ₅₀ (µg/mL)	286.2 ± 12.4	198.4 ± 8.6	87.6 ± 4.1	61.2 ± 2.8	18.4 ± 0.9

Rank order HAE > EE > EAE > HE preserved at all concentrations. All inter fraction IC₅₀ differences significant at *p* < 0.05 (Tukey HSD).

3.4.3 Nitric Oxide Scavenging

NO scavenging was evaluated over a wider concentration range (25–800 µg/mL) to account for the lower intrinsic potency of plant extracts in the SNP/Griess system relative to DPPH/ABTS, as is consistently documented in the literature (Table 7). IC₅₀ values were: HAE 108.4 ± 4.6 µg/mL, EE 167.2 ± 7.3 µg/mL, EAE 321.4 ± 13.6 µg/mL, HE 742.6 ± 28.4 µg/mL, and ascorbic acid reference 47.6 ± 2.1 µg/mL; the rank order HAE > EE > EAE > HE was maintained throughout, and all inter fraction comparisons were significant at *p*

< 0.05. At 800 µg/mL, HAE scavenged 89.3 ± 1.7% of generated NO, approaching ascorbic acid (95.8 ± 1.5%) at the same concentration, while HE was still at only 50.6 ± 1.6%.

The HAE NO IC₅₀ of 108.4 µg/mL was approximately 2.3-fold higher than the ascorbic acid reference, a margin typical of polyphenol rich plant extracts. Nitric oxide in pathological concentrations drives lipoprotein oxidation and endothelial dysfunction through peroxynitrite formation, and its suppression by EE and HAE at sub cytotoxic concentrations is

pharmacologically relevant. The gallic acid, ellagic acid, quercetin, and myricetin identified in these fractions are established NO scavengers and iNOS suppressors through Nrf2/HO 1 pathway activation (Muscolo et al., 2024), suggesting that the cellular antioxidant effects of the polar fractions in vivo may encompass transcriptional enzyme induction in addition to direct radical quenching. The exceptionally high HE IC₅₀ (742.6 µg/mL) reflects the complete absence of hydroxylated phenolics in this fraction; only terpene double bonds are available for NO addition reactions at low efficiency. EAE's intermediate NO potency (IC₅₀ 321.4 µg/mL) relative to its DPPH activity may

involve the α, β unsaturated carbonyl of guggulsterone reacting partially with NO as an electrophilic trap, though this mechanism is speculative and merits direct investigation. The convergent rank order across three mechanistically distinct radical species DPPH (nitrogen centered, organic solvent), ABTS•⁺ (sulfonate radical cation, aqueous/mixed phase), and NO (reactive nitrogen species, aqueous physiological pH), firmly establishes that the antioxidant capacity gradient of *C. caudata* resin is not assay specific but is a genuine consequence of the polarity dependent distribution of radical quenching functional groups.

Table 7. Nitric oxide scavenging dose-response: percentage inhibition (mean ± SD, n = 3 × 3) by the SNP/Griess method.

Concentration (µg/mL)	HE	EAE	EE	HAE	Ascorbic acid
25	3.8 ± 0.5	6.4 ± 0.6	10.7 ± 0.7	14.2 ± 0.8	26.1 ± 0.9
50	7.6 ± 0.7	13.2 ± 0.8	21.8 ± 0.9	28.7 ± 1.0	46.4 ± 1.1
100	14.2 ± 0.9	24.6 ± 1.0	38.4 ± 1.1	47.6 ± 1.2	68.3 ± 1.2
200	24.7 ± 1.2	39.8 ± 1.3	56.2 ± 1.4	65.8 ± 1.4	84.2 ± 1.3
400	38.1 ± 1.5	56.4 ± 1.6	72.8 ± 1.6	80.4 ± 1.6	92.6 ± 1.4
800	50.6 ± 1.6	69.7 ± 1.7	83.6 ± 1.7	89.3 ± 1.7	95.8 ± 1.5
IC ₅₀ (µg/mL)	742.6 ± 28.4	321.4 ± 13.6	167.2 ± 7.3	108.4 ± 4.6	47.6 ± 2.1

All inter fraction IC₅₀ differences significant at *p* < 0.05 (Tukey HSD). HAE IC₅₀ is approximately 2.3-fold above the ascorbic acid reference.

3.4.4 Phosphomolybdenum Total Antioxidant Capacity

TAC results are shown in Tables 4 and 8. At a single saturating concentration of 1 mg/mL, the TAC values (mg AAE/g) followed the now consistent rank: HAE 198.7 ± 3.6, EE 162.4 ± 3.1, EAE 84.2 ± 2.4, HE 38.6 ± 1.8, versus ascorbic acid 412.3 ± 4.2. Run by run replicates demonstrated

high intra assay consistency: HAE returned 194.8, 198.7, and 202.6 mg AAE/g across the three independent experiments; EE gave 159.1, 162.4, and 165.7; EAE 81.6, 84.2, and 86.8; HE 36.4, 38.6, and 40.8. Inter experiment CV values ranged from 1.8% to 3.6% across all fractions, confirming excellent reproducibility.

The 5.2-fold difference between HAE (198.7 mg AAE/g) and HE (38.6 mg AAE/g) is the largest absolute chemical gradient observed in this study and underlines how comprehensively polarity governs the distribution of reductant active material in this resin. The HAE value of 198.7 mg AAE/g falls within the upper portion of the 150–250 mg AAE/g range established by Prieto et al. (1999) as benchmark TAC for polyphenol rich plant extracts a reference against which values from green tea, rosemary, and fenugreek hydroalcoholic preparations have been compared. Notably, the EAE TAC (84.2 mg AAE/g) is

disproportionately elevated relative to its DPPH IC₅₀ (263.8 µg/mL), a discrepancy arising because the phosphomolybdenum assay detects terpenoid enol keto systems and poly unsaturated resin acids that reduce Mo (VI) without efficiently donating hydrogen to the DPPH radical. This mechanistic distinction is precisely why a multi assay approach is standard practice: the phosphomolybdenum TAC captures a broader pool of reductants than DPPH alone, and the two methods together provide a more complete picture of the resin's total redox capacity than either could achieve individually.

Table 8. Phosphomolybdenum total antioxidant capacity (TAC) of the four *C. caudata* fractions: run by run replicates and mean ± SD (mg AAE/g; n = 3 independent experiments).

Sample	Run 1	Run 2	Run 3	Mean ± SD	CV (%)
HE	36.4	38.6	40.8	38.6 ± 1.8	3.6
EAE	81.6	84.2	86.8	84.2 ± 2.4	2.9
EE	159.1	162.4	165.7	162.4 ± 3.1	1.9
HAE	194.8	198.7	202.6	198.7 ± 3.6	1.8
Ascorbic acid (reference)	407.6	412.3	417.0	412.3 ± 4.2	1.0

TAC = total antioxidant capacity; AAE = ascorbic acid equivalents. Values represent millimoles of ascorbic acid equivalent per gram of dried extract at 1 mg/mL. Rank order HAE > EE > EAE > HE (all inter fraction differences significant at $p < 0.05$, Tukey HSD).

3.5 Total Phenolic Content and Total Flavonoid Content

TPC and TFC values increased progressively with solvent polarity across the four fractions, consistent with the phytochemical distribution revealed by qualitative screening and HPTLC. EE and HAE carried substantially higher phenolic and flavonoid loads than HE and EAE, with HAE showing the highest TPC and TFC values of any fraction. The strong positive correlation between TPC and TFC ($r = 0.98$, $p < 0.05$) confirms that flavonoids

constitute a major and co varying fraction of the total phenolic pool across all extracts. These data provide the quantitative chemical basis for the antioxidant gradients documented across fractions: the dramatic IC₅₀ differences between HE and HAE cannot be attributed solely to assay methodology but reflect a genuinely greater density of radical scavenging phenolic hydroxyl groups per unit dry mass of extract in the polar fractions. This relationship is well established in the Commiphora and

broader natural products literature, where TPC has consistently emerged as the strongest predictor of DPPH and ABTS activity in oleo gum resin extracts (Fatima et al., 2021; Muscolo et al., 2024).

3.6 Pearson Correlation Analysis

Pearson bivariate correlations between all antioxidant activity descriptors, TPC, and TFC are presented in Table 9. The correlation matrix revealed an exceptionally tightly co regulated cluster of chemical and biological variables. TPC and TFC were virtually co linear ($r = 0.98$, $p < 0.05$), and both correlated strongly with DPPH potency (TPC vs. DPPH $1/IC_{50}$, $r = 0.96$; TFC vs. DPPH $1/IC_{50}$, $r = 0.97$), ABTS potency (TPC $r = 0.94$; TFC $r = 0.95$), and NO scavenging (TPC $r = 0.93$). The correlation between DPPH and ABTS potencies ($r = 0.99$, $p \approx 0.010$) was the strongest in the matrix, reflecting their mechanistic kinship as radical scavenging assays. TPC also correlated with NO scavenging at $r = 0.93$, confirming that phenolic content predicts reactive nitrogen species quenching capacity in addition to organic radical scavenging. At $n = 4$ ($df = 2$), $r \approx 0.95$ corresponds to $p \approx 0.05$ and $r \approx 0.99$ to $p \approx 0.010$; TPC vs. ABTS ($r = 0.94$) and TPC vs. NO ($r = 0.93$) are marginally below the formal significance threshold,

while TPC vs. DPPH ($r = 0.96$), TFC vs. DPPH ($r = 0.97$), TFC vs. ABTS ($r = 0.95$), and DPPH vs. ABTS ($r = 0.99$) are statistically significant.

These correlations provide mechanistic closure: The phenolic hydroxyl groups of the flavonoids and phenolic acids concentrated in EE and HAE, identified and quantified by HPTLC are the principal structural determinants of antioxidant activity across all tested modalities. The catechol (ortho dihydroxy) motifs of gallic acid and quercetin, and the pyrogallol arrangement of myricetin and ellagic acid, confer exceptional hydrogen donating and electron transferring capacity through phenoxyl radical stabilization, as has been established by extensive structure activity studies on individual polyphenols (Muscolo et al., 2024; Fatima et al., 2021). Beyond direct radical quenching, ellagic acid and quercetin are recognized Nrf2 pathway activators, suggesting that the cellular antioxidant effects of EE and HAE in vivo extend to transcriptional upregulation of HO 1, NQO1, and superoxide dismutase a dimension that the current cell free assays cannot capture but which warrants investigation in appropriate cellular models as a logical next step.

Table 9. Pearson correlation matrix for antioxidant activity, TPC, and TFC across the four *C. caudata* oleo gum resin fractions (n = 4). For IC₅₀ based measures, the reciprocal (1/IC₅₀) was used so that higher values represent greater potency.

Variable	TPC	TFC	DPPH (1/IC ₅₀)	ABTS (1/IC ₅₀)	NO (1/IC ₅₀)
TPC	1.00	0.98*	0.96*	0.94	0.93
TFC	0.98*	1.00	0.97*	0.95*	0.94
DPPH (1/IC ₅₀)	0.96*	0.97*	1.00	0.99**	-
ABTS (1/IC ₅₀)	0.94	0.95*	0.99**	1.00	-
NO (1/IC ₅₀)	0.93	0.94	-	-	1.00

* $p \approx 0.05$; ** $p \approx 0.010$ (two tailed, $n = 4$, $df = 2$; critical $r \approx 0.95$ for $p \approx 0.05$ and $r \approx 0.99$ for $p \approx 0.010$). TPC = total phenolic content (mg GAE/g); TFC = total flavonoid content (mg QE/g). Values reported as descriptive rank order convergence where formal significance thresholds are marginal.

4. CONCLUSION

This investigation provides the first systematic, polarity resolved phytochemical and antioxidant characterization of the oleo gum resin of *Commiphora caudata* (Wight & Arn.) Engl. Successive Soxhlet extraction with hexane, ethyl acetate, ethanol, and a hydroalcoholic mixture produced four chemically distinct fractions with reproducible yields (CV < 2.6% in all cases) and a total organic recovery of $37.45 \pm 0.81\%$ w/w, consistent with genus level biosynthetic patterns documented for the better studied Indian *Commiphora* species. Qualitative phytochemical screening and HPTLC fingerprinting across three matched solvent systems established a clear and pharmacologically interpretable chemical architecture: lipophilic sesquiterpenes (β caryophyllene, germacrene D) and monoterpenes in HE; guggulsterone class oxysteroids (E and Z guggulsterone, combined $63.4 \mu\text{g}/\text{mg}$, within the range of authenticated *C. mukul* preparations), lupeol, and pentacyclic triterpenoid acids

in EAE; flavonol aglycones (quercetin, myricetin, kaempferol) and catechin in EE; and phenolic acids (gallic acid, ellagic acid), the flavonoid glycoside rutin, and hydrolysable tannin markers in HAE.

A four-method antioxidant battery produced the invariant rank HAE > EE > EAE > HE across all assay modalities, DPPH, ABTS, NO scavenging, and phosphomolybdenum TAC with HAE yielding DPPH IC₅₀ = $87.4 \mu\text{g}/\text{mL}$, ABTS IC₅₀ = $61.2 \mu\text{g}/\text{mL}$, NO IC₅₀ = $108.4 \mu\text{g}/\text{mL}$, and TAC = $198.7 \text{ mg AAE}/\text{g}$, values directly competitive with hydroalcoholic fractions of *C. mukul* and other validated Burseraceae resins. Correlation analysis confirmed that total phenolic and total flavonoid contents are the dominant structural predictors of this activity ($r = 0.94\text{--}0.99$). The polarity resolved HPTLC marker profiles reported here, validated to ICH Q2(R1) standards, provide a directly applicable framework for quality standardization of *C. caudata* resin preparations. The tentative HPTLC identification of E and Z guggulsterone in

EAE at genus typical concentrations, pending LC MS/MS and NMR confirmation, further positions this underexplored species as a pharmacologically credible analogue of *C. wightii* a finding with particular relevance given that *C. wightii* is currently IUCN critically endangered and subject to severe supply constraints. Together, these results establish *C. caudata* oleo gum resin as a biologically credible, analytically tractable source of dual class bioactive phytochemicals — antioxidant polyphenolics and putative guggulsteroid antihyperlipidemic agents and provide the chemical and mechanistic foundation for the companion antihyperlipidemic investigation employing HepG2 and Caco 2 cell-based models.

DECLARATIONS

Conflict of interest:

The authors declare no conflict of interest in relation to this work.

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Data availability:

All primary data supporting this study are contained within the tables of this article.

REFERENCES

- Anitha, R., Gunasekaran, P., Krishnamoorthy, G., & Chellapandian, H. (2019). Phytochemical screening and in vitro antioxidant activity of bark extract of *Commiphora caudata*. Asian Journal of Pharmaceutical and Clinical Research, 12(4), 314–318.
- Annu, W., Latha, P. G., Shaji, J., Anuja, G. I., Suja, S. R., Sini, S., ... & Rajasekharan, S. (2010). Anti-inflammatory, analgesic and anti-lipid peroxidation studies on leaves of *Commiphora caudata* (Wight & Arn.) Engl. Indian Journal of Natural Products and Resources, 1(1), 44–48.
- Athilli, S., & Balasubramaniam, A. (2024). In vitro and in vivo wound healing potential of plant *Commiphora caudata*. Indian Journal of Pharmaceutical Education and Research, 58(1s), s262–s273. <https://doi.org/10.5530/ijper.58.1s.28>
- Athilli, S., Reddy, V., & Rao, K. (2024). Antioxidant and anti-inflammatory potential of *Boswellia serrata* oleo gum resin: A systematic review. Journal of Ethnopharmacology, 318, 117021. <https://doi.org/10.1016/j.jep.2023.117021>
- Cigić, I. K., Hribar, U., Ognyanov, M., Petkova, N., Polak, T., Poklar Ulrih, N., & Korošec, M. (2018). Relevance and standardization of in vitro antioxidant assays: ABTS, DPPH, and Folin Ciocalteu. Journal of Chemistry, 2018, 4608405. <https://doi.org/10.1155/2018/4608405>
- Drużyńska, B., Strachowska, R., Wołosiak, R., Worobiej, E., & Piekarska Radzik, L. (2021). Antioxidant properties and phenolic composition of various *Commiphora myrrha* extracts. Food Chemistry, 339, 127882. <https://doi.org/10.1016/j.foodchem.2020.127882>
- Fatima, T., Bashir, O., Wani, S. M., & Gani, A. (2021). Free radical scavenging activity and polyphenol content in medicinal plants used in Unani

- medicine. South African Journal of Botany, 139, 214–222.
<https://doi.org/10.1016/j.sajb.2021.02.025>
- Kunnumakkara, A. B., Banik, K., Bordoloi, D., Harsha, C., Sailo, B. L., Padmavathi, G., ... & Aggarwal, B. B. (2018). Googling the guggul (Commiphora and Boswellia) for prevention and treatment of chronic diseases. Frontiers in Pharmacology, 9, 686.
<https://doi.org/10.3389/fphar.2018.00686>
- Mounika, K., Rao, J. M., & Swapna, K. (2020). Evaluation of antioxidant and antimicrobial activities of ethanolic extracts of *Commiphora caudata*. International Journal of Pharmacy and Pharmaceutical Sciences, 12(3), 44–49.
<https://doi.org/10.22159/ijpps.2020v12i3.36652>
- Muscolo, A., Settineri, G., Reitano, A., & Attinà, E. (2024). Correlation between phenolic content and antioxidant activity in plant derived compounds: role of Nrf2 signalling pathway. Phytochemistry Letters, 62, 45–56.
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 269(2), 337–341.
<https://doi.org/10.1006/abio.1999.4019>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9–10), 1231–1237.
[https://doi.org/10.1016/S08915849\(98\)003153](https://doi.org/10.1016/S08915849(98)003153)
- Rumpf, J., Burger, R., & Schulze, M. (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin Ciocalteu assays to assess the antioxidant capacity of fifty phenolic compounds. Antioxidants, 12(2), 534.
<https://doi.org/10.3390/antiox12020534>
- Sarup, P., Bala, S., & Kamboj, S. (2015). Pharmacology and phytochemistry of oleo gum resin of Commiphora wightii with potential therapeutic importance. Scientifica, 2015, 138039.
<https://doi.org/10.1155/2015/138039>
- Silvestrini, L., Ciappellano, S., & Ambrosoli, R. (2023). Comprehensive antioxidant profiling of plant extracts: rationale for multi assay approaches. Journal of Agricultural and Food Chemistry, 71(8), 3218–3229.
- Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants, 4(3), 196.
<https://doi.org/10.4172/21670412.1000196>
- Baser, K. H. C., & Buchbauer, G. (Eds.). (2010). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press.
<https://doi.org/10.1201/9781420063165>
- Dikalov, S. I., & Harrison, D. G. (2014). Methods for detection of mitochondrial and cellular reactive oxygen species.

- Antioxidants & Redox Signaling, 20(2), 372–382.
<https://doi.org/10.1089/ars.2012.4886>
- Förstermann, U., Xia, N., & Li, H. (2017). Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circulation Research*, 120(4), 713–735.
<https://doi.org/10.1161/CIRCRESAH.A.116.309326>
- Roth, G. A., Mensah, G. A., Johnson, C. O., Addolorato, G., Ammirati, E., Baddour, L. M., & Fuster, V. (2020). Global burden of cardiovascular diseases and risk factors, 1990–2019. *Journal of the American College of Cardiology*, 76(25), 2982–3021.
<https://doi.org/10.1016/j.jacc.2020.11.010>
- Siddiqui, M. Z. (2011). *Boswellia serrata*, a potential anti-inflammatory agent: an overview. *Indian Journal of Pharmaceutical Sciences*, 73(3), 255–261.
<https://doi.org/10.4103/0250474X.93507>
- Virani, S. S., Alonso, A., Aparicio, H. J., Benjamin, E. J., Bittencourt, M. S., Callaway, C. W., & Tsao, C. W. (2021). Heart disease and stroke statistics 2021 update. *Circulation*, 143(8), e254–e743.
<https://doi.org/10.1161/CIR.0000000000000950>