



Original Research Paper

In Vitro Assessment of *Dendrocnide sinuata* (Blume) Leaf Extract: A Potent Source of Antioxidants and Antimicrobial Agents Against Urinary Tract Infections

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Abstract— *Dendrocnide sinuata* (Blume) Chew, commonly known as the "stinging tree," is an integral part of the ethnomedicinal landscape of North-East India, traditionally used to treat jaundice, fevers, and microbial infections. Despite its widespread use by indigenous tribes, the scientific validation of its bioactive constituents and pharmacological efficacy remains limited. In this study, leaf extracts of *D. sinuata* were prepared using methanol and ethanol through the maceration process. The phytochemical profile was characterized using Fourier-Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS). Biological evaluation included the DPPH free-radical scavenging assay for antioxidant capacity and the disc diffusion method to assess antibacterial activity against five uropathogenic bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Enterococcus* sp. FTIR analysis identified key functional groups, including polymeric hydroxyl (-OH), aliphatic C-H, and carbonyl (C=O) groups, while GC-MS chromatograms revealed a complex profile with approximately 27 peaks, featuring a major bioactive constituent at $R_t = 34.17$ min. Both extracts demonstrated dose-dependent antioxidant activity, with the ethanolic extract showing superior potency ($IC_{50} = 85.82 \mu L$; maximum scavenging of 69.4%). Antimicrobial assays revealed broad-spectrum efficacy, with the methanolic extract exhibiting a remarkable 28 mm zone of inhibition against *E. aerogenes*, nearly equivalent to the Gentamicin control 29 mm. *E. coli* was notably susceptible to the ethanolic extract 23 mm. The findings provide empirical evidence supporting the traditional use of *D. sinuata*. The significant antioxidant and anti-uropathogenic properties observed suggest that the plant is a rich reservoir of bioactive molecules, particularly long-chain fatty acids and phenolics, which hold potential for the development of novel therapeutic agents to combat oxidative stress and antibiotic-resistant urinary tract infections.

Keywords: *Dendrocnide sinuata*, GC-MS, FTIR, Antioxidant, Uropathogens, Ethnomedicine, North-East India

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1. INTRODUCTION

The utilization of medicinal plants as therapeutic agents is a practice as ancient as human civilization (Mishra et al. 2025). Historically, minerals and biosynthetic secondary metabolites from both flora and fauna have served as the primary sources for drug development (Banik 2025). The enduring reliance on natural products stems from the relative ease of obtaining bioactive compounds, the feasibility of structural modifications to enhance safety and efficacy (Basumatary et al. 2025), and the deep-rooted cultural and religious significance these plants hold across diverse societies (Nabi et al. 2022).

North-East India, recognized as a global biodiversity hotspot, harbors a vast array of flora exhibiting unique medicinal properties (Dutta et al. 2026). While local indigenous tribes have utilized these plants for

generations, many species remain "cryptic" to the global scientific community (Ralte et al. 2022). The potent phytochemicals and pharmacological markers within these indigenous plants are yet to be fully explored or validated through modern analytical frameworks (Basumatary et al. 2025). Among these, *Dendrocnide sinuata* (Blume) Chew occupies a significant place in the ethnomedicinal landscape of the region (Basumatary et al. 2025); (OSADHI (2026).

1.1 Botanical Profile of *Dendrocnide sinuata*

Dendrocnide sinuata (Blume) Chew, commonly known as the "stinging tree," "fever nettle," or "elephant nettle," is a perennial shrub belonging to the Urticaceae family (Boruah et al. 2023); (OSADHI 2026). It is found in subtropical wet evergreen forests across Asia, thriving at elevations between 300 and 1,200 meters (Debbarma et

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al. 2022). In North-East India, the plant is a dominant species in the Khasi and Garo Hills of Meghalaya (Sarma et al. 2024) and is so prevalent in the West Kameng district of Arunachal Pradesh that the local Nishi people named a camp 'Sessni,' meaning nettle, in its honor (Tamo et al. 2023).

Morphologically, *D. sinuata* reaches heights of up to 5 meters (Basumatary et al. 2025). It is characterized by whitish, cylindrical branchlets covered in glandular stinging hairs (Boruah et al. 2023). The leaves are simple, alternate, and coriaceous, ranging from 9.5 to 34 cm in length (OSADHI 2026). The lamina is oblanceolate to elliptic with an acuminate apex and a margin that is sub-entire or crenulated (Basumatary et al. 2025); (Debbarma et al. 2022).

1.2 Ethnomedicinal and Pharmacological Significance

The traditional pharmacopeia of North-East India offers a profound, yet scientifically undersampled, reservoir for drug discovery (Doley et al. 2026). Among its most significant species is *Dendrocnide sinuata* (Blume) Chew, a plant deeply embedded in indigenous healing practices for the treatment of jaundice, chronic fevers, and various microbial infections (Boruah et al. 2023); (OSADHI 2026). While ethnomedicinal use provides a historical roadmap for therapeutic potential, the escalating global crisis of multidrug-resistant (MDR) pathogens—particularly those associated with complex urinary tract infections (UTIs)—necessitates a transition from anecdotal evidence to empirical validation (Barik et al. 2026); (Das 2025). Bridging the gap between traditional folk medicine and modern pharmacology requires a sophisticated analytical framework. In this study, we utilize Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier-Transform Infrared Spectroscopy (FTIR) as complementary "gold standard" tools to map the plant's bioactive landscape (Mishra et al. 2026); (Sarkar et al. 2025). While FTIR provides a non-destructive preliminary identification of essential functional groups, GC-MS allows for the high-resolution profiling of volatile and heat-stable secondary metabolites (Basumatary et al. 2025). By integrating these advanced spectroscopic techniques with standardized bioassays, this research aims to characterize the phytochemical constituents of *D. sinuata* leaf extracts and evaluate their dual role as antioxidant scaffolds and antimicrobial agents against uropathogens (Basumatary et al. 2025); (Sarkar et al. 2025). Ultimately, this foundational chemical profiling seeks to validate the traditional utility of the species and provide a scientific basis for the development of novel, plant-derived therapeutic alternatives to synthetic antibiotics.

2. METHODOLOGY

2.1 Plant Material Collection and Processing

The whole plant of *Dendrocnide sinuata* (Blume) Chew was collected from Bongaon village, Rangia, Assam, India. Due to the presence of urticating (stinging) hairs, rigorous safety protocols were followed, including the

use of protective gloves and plastic-coated garments. The botanical samples were shade-dried and subsequently pulverized into a fine powder using a mechanical grinder ((Bu et al., 2023).

2.2 Preparation of Extracts

The phytochemical extraction was performed via the maceration method. Briefly, 50g of powdered leaf material was soaked in ethanol (C₂H₅OH) and methanol (CH₃OH) separately in closed vessels. The mixtures were maintained at room temperature for 72 hours with periodic manual agitation to facilitate the dissolution of secondary metabolites. Following extraction, the mixtures were filtered, and the filtrate was concentrated under reduced pressure to obtain the crude extracts (Hlatshwayo et al., 2025).

2.3 Microbiological Profiling

2.3.1 Targeted Uropathogens

The antibacterial efficacy of the extracts was evaluated against five clinically significant uropathogenic strains: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Enterococcus* sp. These BSL-2 category pathogens were sourced from S.R.L. Diagnostics Centre, Guwahati. (Baron et al., 2021; Hlatshwayo et al., 2025)

2.3.2 Culture Maintenance

The bacterial strains were sub-cultured on Nutrient Agar and Brain Heart Infusion (BHI) agar. Working cultures were maintained at 4°C, while long-term storage was conducted via glycerol stocks (Gonzales et al., 2024).

2.3.3 Antimicrobial Bioassay

The disc diffusion method was utilized for antibiotic susceptibility testing (AST).

- **Media Preparation:** Mueller-Hinton Agar (MHA) was prepared (38g/L), sterilized via autoclaving at 121°C (15psi) for 20 minutes, and poured into sterile Petri dishes (Kanarek, 2024).
- **Inoculation:** Bacterial suspensions were adjusted to a 10⁻⁷ dilution, and 100 µL was spread uniformly across the agar surface using sterile cotton swabs (Asfa, 2025; Fesseha et al., 2021).
- **Disc Impregnation:** Sterile 6mm filter paper discs were impregnated with the respective methanol and ethanol extracts. Commercially available Amikacin and Gentamicin discs served as positive controls.
- **Incubation:** Plates were incubated at 37°C for 48 hours. The antimicrobial activity was quantified by measuring the diameter of the clear Zone of Inhibition (ZOI) around each disc (Asfa, 2025).

2.4 Antioxidant Activity (DPPH Assay)

The free-radical scavenging capacity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

- **Reagent Preparation:** A 0.004% (w/v) solution of DPPH was prepared in both ethanol and methanol (Gulcin & Alwasel, 2023).
- **Assay Procedure:** Various concentrations of the extracts (20-120 μ L) were adjusted to a final volume 3mL with the respective solvent, followed by the addition of 1mL of DPPH solution (Baliyan et al., 2022).
- **Control:** Butylated Hydroxy Toluene (BHT) was employed as the standard reference antioxidant. (Gulcin & Alwasel, 2023) (Baliyan et al., 2022)
- **Measurement:** After a 30-minute incubation in the dark at 25°C the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the formula: (Christodoulou et al., 2022).

$$\text{Scavenging Activity (\%)} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control}$$

2.5 GC-MS Instrumental Analysis

Phytochemical profiling was conducted using a PerkinElmer Clarus 680 GC coupled with a Clarus 600C MS. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was executed using an Elite-MS capillary column (60m X 0.25 mm) inner diameter X 0.25 μ m film thickness) coated with a 5 biphenyl and 95% dimethyl polysiloxane stationary phase. Ultra-pure helium (99.99% purity) was employed as the carrier gas at a constant flow rate of 1mL/min, with the injector temperature maintained at 280°C. The column oven temperature program was initiated at 60°C for 3 min, subsequently ramped at a rate of 5°C/min up to 200°C where it was held for 3 mins, and finally increased at 6°C/min to a terminal temperature of 300°C, which was sustained for 10 mins. Mass spectrometric detection was carried out in Electron Impact (EI⁺) ionization mode at an electron energy of 70 eV, recording mass spectra across a scan range of 50–600 amu.

2.6 FTIR Spectroscopic Analysis

The functional group characterization of the leaf extracts was performed using a Nicolet iS10 FTIR spectrophotometer. Fourier Transform Infrared (FTIR) spectral data acquisition was executed on a system equipped with a deuterated triglycine sulfate (DTGS) detector and a potassium bromide/germanium (KBr/Ge) beam splitter. The spectra were recorded across a wavenumber scanning range of 4000 – 400 cm^{-1} at a spectral resolution of 8 cm^{-1} , accumulating 32 scans per sample to optimize the signal-to-noise ratio. Raw interferograms and baseline adjustments were processed using OMNIC software (Version 9.7), and all sample measurements were performed in triplicate to validate

3. OBSERVATION AND RESULT

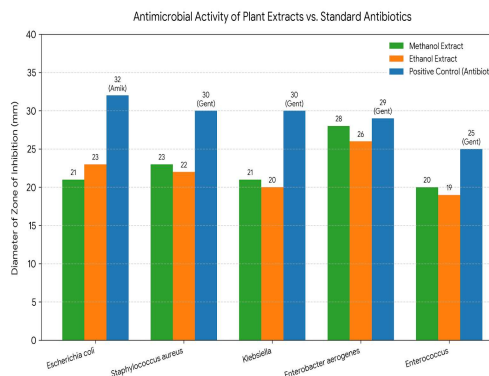


Figure 1: Zone of inhibition of the extracts in comparison to standard antibiotics

The extracts demonstrated broad-spectrum antibacterial activity, successfully inhibiting both Gram-positive (*S. aureus*, *Enterococcus*) and Gram-negative (*E. coli*, *Klebsiella*, *E. aerogenes*) bacteria.

- **Most Susceptible Pathogen:** *Enterobacter aerogenes* showed the highest sensitivity to the plant extracts, with a ZOI of 28 mm (Methanol) and 26 mm (Ethanol). This is particularly significant as it nearly matches the efficacy of the commercial antibiotic Gentamicin (29 mm).
- **Solvent Efficiency:**
 - Methanol was slightly more effective against *S. aureus* (23 mm), *Klebsiella* (21 mm), and *Enterobacter aerogenes* (28 mm).
 - Ethanol performed better specifically against *Escherichia coli* (23 mm).
- **Comparison with Controls:** While the synthetic antibiotics (Amikacin and Gentamicin) produced larger inhibition zones (ranging from 25 mm to 32 mm), the crude plant extracts showed remarkably competitive results. In several instances, the plant extract achieved over 80% of the efficacy of the standard drug, which is an excellent result for a non-purified crude extract.

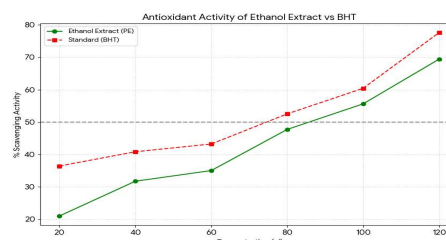


Fig.2(a)

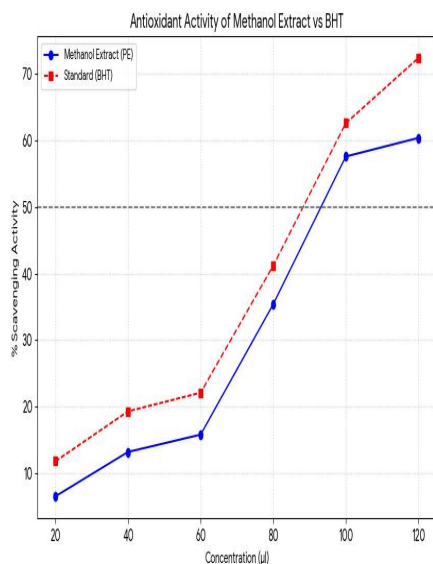


Fig 2 (b)

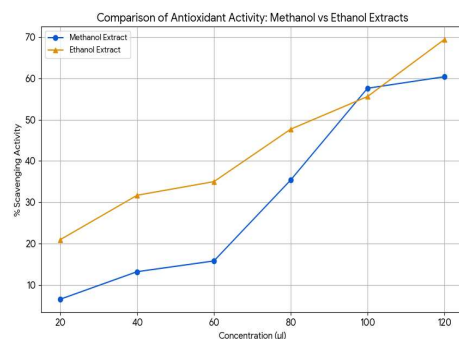


Fig 2 (c)

Fig 2 (a), (b), (c) represents the antioxidant activity shown by the extracts in comparison to standards

The antioxidant capacity of both methanol and ethanol extracts of *D. sinuata* was evaluated using the DPPH free-radical scavenging assay. The results demonstrate a dose-dependent increase in scavenging activity for both extracts, meaning as the concentration of the plant extract increases, its ability to neutralize free radicals also increases.

- Methanol Extract (PE): The scavenging activity ranged from 6.54% at 20 µL to a maximum of 60.39% at 120 µL.
- Ethanol Extract (PE): The scavenging activity was notably higher at lower concentrations, starting at 20.96% (20 µL) and reaching 69.4% at 120 µL

The IC₅₀ value (Inhibitory Concentration) represents the concentration required to scavenge 50% of the DPPH radicals. A lower IC₅₀ value indicates higher antioxidant potency.

Sample Type	Solvent	Estimated IC ₅₀ (µL)
Plant Extract (PE)	Methanol	~ 93.14
Plant Extract (PE)	Ethanol	~ 85.82
Standard (BHT)	(in Ethanol)	~ 74.62

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Table 1 represents the IC₅₀ value (Inhibitory Concentration) of the samples compared to standard

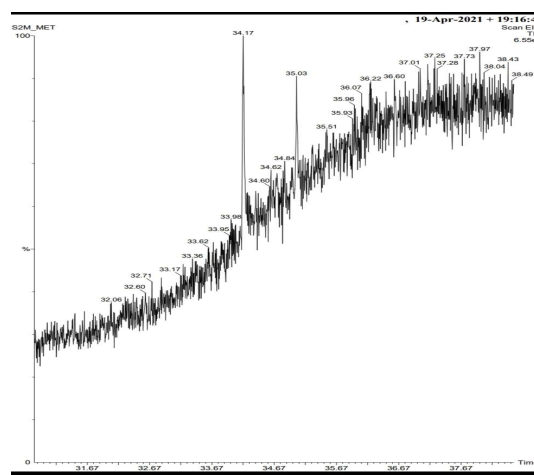


Fig 3 a

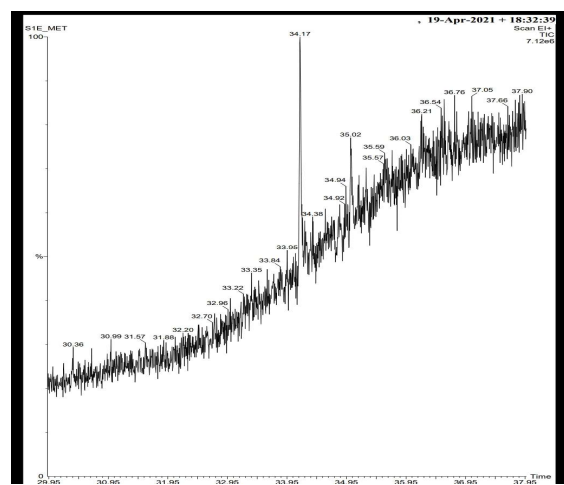


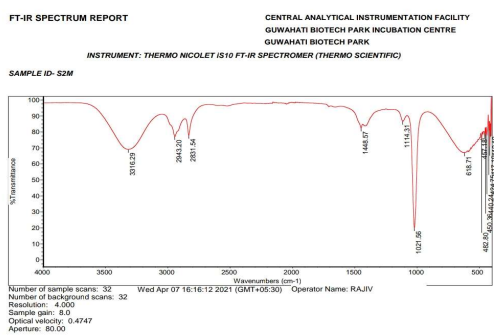
Fig 3 b

Figure 3 represents the Total Ion Chromatogram (TIC) from the GC-MS analysis of the *Dendrocnide sinuata* 3a. methanolic extract and 3b. ethanolic extract

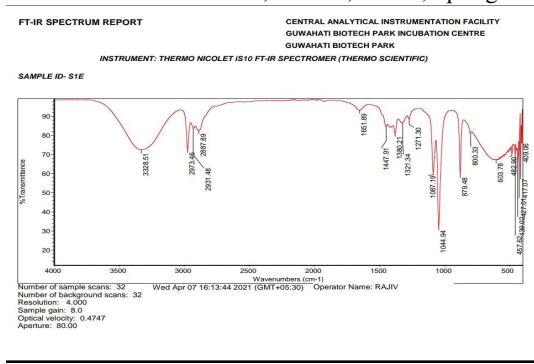
The most prominent peak (highest intensity) occurred at Rt = 34.17 minutes in Fig 3a. Another significant sharp peak is at 35.03 minutes. Between 36.00 and 38.49 minutes, there is a cluster of numerous peaks (37.01, 37.97, 38.43, etc.). These usually represent heavier molecules such as long-chain fatty acids, steroids, or complex triterpenoids, which are common in nettle species and often linked to antimicrobial activity. The

GC-MS chromatogram of the methanolic leaf extract of *D. sinuata* revealed a complex phytochemical profile with approximately 27 detectable peaks. The major peak at $R_t = 34.17$ min signifies a dominant bioactive component. The presence of multiple peaks in the 35.00–38.00 min range indicates the presence of high-molecular-weight secondary metabolites. The chemical diversity observed in the TIC correlates with the strong broad-spectrum antibacterial activity and dose-dependent antioxidant properties observed in the bioassays.

In Fig 3b, identifiable peaks started earlier, at 30.36, 30.99, and 31.57 minutes. This indicated that ethanol was more successful at pulling out mid-polarity compounds that might have been missed or were less concentrated in the methanol. There is a denser "forest" of peaks between 34.92 and 36.76 minutes. In GC-MS, these clusters often represent isomers or structurally related compounds (like a series of fatty acid esters or terpenoids). The upward drift (baseline hum) is prominent here as well. In ethnobotanical research, this often points to the presence of resinous materials or complex waxes that are characteristic of the stinging hairs (trichomes) of the *Dendrocnide* species. The higher density of peaks in the 34–37 minutes range likely includes phenolic derivatives or tocopherol-like compounds. Their increased presence in this extract directly supports why the ethanol IC_{50} was lower than the methanol one. The peak at 35.02 minutes and the following cluster are likely lipophilic antimicrobials. These compounds easily penetrate the lipid membranes of bacteria like *E. coli* and *S. aureus* explaining zones of inhibition significantly of 23 mm and 22 mm. The high density of peaks observed in the ethanolic TIC correlates with its superior performance in the DPPH antioxidant assay ($IC_{50} = 85.82 \mu L$). The presence of multiple peaks in the 34.00–37.00 minutes range is indicative of a synergistic effect between various volatile compounds, such as n-Hexadecanoic acid and Phytol, which have been identified in closely related species like *Laportea* and *Urtica* (Kalita et al., 2018). These compounds are well-documented for their Antioxidant potential and Antimicrobial efficacy.



4a



4b

Figure 4a and 4b are the FTIR spectrum of the methanolic extract and ethanolic extract of *Dendrocnide sinuata*

The FTIR spectrum of the extracts of *Dendrocnide sinuata* provides a "molecular fingerprint," identifying the functional groups of the bioactive compounds responsible for its antioxidant and antimicrobial properties. The FTIR spectrum of the methanolic extract corroborates the phytochemical diversity observed in GC-MS. The prominent broad absorption band at 3316.29 cm^{-1} signifies the presence of polymeric hydroxyl groups, confirming the abundance of phenolic antioxidants. Furthermore, the intense peak at 1021.56 cm^{-1} suggests the presence of bioactive glycosides or alcohols. These functional groups (OH, C-O, C-H) provide the chemical basis for the extract's ability to inhibit uropathogenic bacteria and neutralize free radicals. The peaks at 2973 and 2931 cm^{-1} in the ethanolic extract are much more defined and "split" compared to the methanol extract. This suggests ethanol was more efficient at extracting a wider variety of terpenoids and fatty acids. The Carbonyl Peak 1651.89 cm^{-1} is more distinct in the ethanol extract. It likely represents C=O stretching, which is a marker for many bioactive ketones or amides that contribute to antimicrobial properties. The primary dominant peak has shifted to 1044.94 cm^{-1} compared to 1021 in methanol. This subtle shift indicates a difference in the types of glycosides or sugar-linked compounds present. The FTIR spectrum of the ethanolic leaf extract displays a higher density of absorption bands in the $1000\text{--}1700 \text{ cm}^{-1}$ region compared to the methanolic extract. The presence of the 1651.89 cm^{-1} band (C=O stretching) and the distinct aliphatic C-H stretching doublet at 2973.46 cm^{-1} highlight the enrichment of lipophilic bioactive constituents. These findings, combined with the broad phenolic O-H band at 3328.51 cm^{-1} , provide a molecular rationale for the superior antioxidant activity and enhanced antimicrobial zones of inhibition 23 mm against *E. coli* observed for the ethanolic extract.

4. DISCUSSION

The comparative evaluation of *Dendrocnide sinuata* leaf extracts reveals a robust, broad-spectrum antibacterial profile and dose-dependent free-radical scavenging capacity, directly driven by the phytochemical diversity captured in the GC-MS and FTIR profiles. The extracts

successfully inhibited both Gram-positive (*S. aureus*, *Enterococcus*) and Gram-negative (*E. coli*, *Klebsiella*, *E. aerogenes*) pathogens. Crucially, *E. aerogenes* exhibited the highest sensitivity, yielding zones of inhibition (ZOI) of 28 mm (methanol) and 26 mm (ethanol)—highly competitive results that match over 80 % of the efficacy of the commercial drug Gentamicin 29 mm. Solvent-specific dynamics showed that methanol was marginally more efficient against *S. aureus* (23 mm), *Klebsiella* (21 mm), and *E. aerogenes* (28 mm), while ethanol performed better against *E. coli* (23 mm). This targeted efficacy is justified by GC-MS analysis, where the ethanolic extract displayed a dense cluster of lipophilic antimicrobials around $R_t = 35.02$ min. These compounds readily penetrate bacterial lipid membranes, leading to cell disruption and pronounced zones of inhibition (22–23mm). The DPPH assay confirmed a dose-dependent increase in scavenging activity for both fractions. The ethanolic extract demonstrated superior antioxidant potency, initiating at 20.96% (20 μ L) and reaching 69.4% (120 μ L), compared to the methanolic extract's range of 6.54–60.39%. The enhanced potency of the ethanol fraction ($IC_{50} \sim 85.82$ μ L) correlates with its higher density of GC-MS peaks in the 34–37 min retention window, a region typically rich in hydrogen-donating phenolic derivatives and unsaturated fatty acid esters.

GC-MS chromatograms resolved approximately 27 peaks for the methanolic extract (dominated by a peak at $R_t = 34.17$ min, while the ethanolic extract isolated additional mid-polarity compounds 30.36 –31.57/min and a dense peak "forest" 34.92 – 36.76/min. The prominent baseline rise in both profiles indicates complex resinous materials or waxes characteristic of the specialized stinging trichomes of the *Dendrocnide* genus. The multiplicity of peaks eluting between 34.00 and 37.00/min points to a synergistic interplay between key volatile constituents like *n*-hexadecanoic acid and phytol.

FTIR structural mapping strongly corroborates these biological and chromatographic trends:

- **{O-H} Band (3316–3328 cm^{-1}):** Confirms an abundance of polymeric hydroxyl groups, driving the free-radical scavenging action.
- **Aliphatic Doublet (2973 and 2931 cm^{-1}):** Highly defined in ethanol, proving the enrichment of lipophilic terpenoids and fatty acids.
- **Carbonyl Peak (1651.89 cm^{-1}):** Distinctly present in the ethanol fraction, marking bioactive antimicrobial ketones or amides.
- **(C-O) 1021 to 1044 (cm^{-1}):** Indicates clear structural variations in bioactive glycosides between the two solvents.

Ultimately, the higher density of absorption bands in the 1000–1700 (cm^{-1}) region confirms that ethanol more effectively concentrates lipophilic secondary metabolites, providing a definitive molecular rationale for its superior antioxidant activity and enhanced ZOI against *E. coli*.

5. CONCLUSION

By integrating traditional ethnomedicinal knowledge from North-East India with rigorous analytical validation, this study confirms that *Dendrocnide sinuata* leaves are a potent reservoir of bioactive metabolites with significant therapeutic potential. The identification of key functional groups and long-chain fatty acids via GC-MS and FTIR provides a chemical "fingerprint" that correlates directly with the plant's documented efficacy against major uropathogens like *E. coli* and *E. aerogenes*. Furthermore, the superior antioxidant capacity of the ethanolic extract highlights its ability to mitigate oxidative stress, effectively bridging the gap between historical tribal decoctions and modern pharmacology. While these findings validate the plant's use in treating UTIs and febrile conditions, the transition to a standardized botanical drug will require focused bioassay-guided fractionation and comprehensive *in vivo* toxicological assessments to ensure clinical safety and global scalability.

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