



doi:10.5281/zenodo.18915912

Bactericidal Aromatic Amides in Non-Polar Fractions of Sudanese *Acacia senegal* and *Acacia seyal* Gum Arabic: A Chemoprofile-Linked Comparative Study

Sara Osman*¹ Ahmad Zorin Sahlan² & Mazlyzam Bin Abdul Latif³

Department of Biomedical Sciences, Faculty Health Sciences
National University of Malaysia (UKM), 53100 Kuala Lumpur, Malaysia.

¹Corresponding author: osma.sara20@yahoo.com

²ahmadzorinsahalan@ukm.edu.my

³mazlyzam@ukm.edu.my

ABSTRACT

Gum Arabic (GA) is the exudate of *Acacia senegal* and *Acacia seyal* and is widely used as a safe hydrocolloid and traditional remedy. Its bioactivity is still largely ascribed to its bulk polysaccharide fraction (Goswami & Naik, 2014; Gurib-Fakim, 2006). The contribution of the minor non-polar constituents, and their role in differentiating the two commercial species, remains insufficiently defined. Authenticated Sudanese GA from *A. senegal* and *A. seyal* was therefore fractionated by polarity into n-hexane, ethyl acetate, and aqueous fractions, and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was evaluated by disc diffusion, broth microdilution (MIC/MBC), and time-kill assays (Balouiri et al., 2016). Ultrastructural changes were examined by scanning electron microscopy, and active fractions were characterized by FT-IR and GC-MS (Elnour et al., 2018; López-Franco et al., 2021). Activity was confined to the n-hexane fractions; aqueous and ethyl acetate fractions were inactive up to 400 mg/mL. The *A. senegal* n-hexane fraction was more potent (MIC 100–300 mg/mL; MBC 150–550 mg/mL) than that of *A. seyal* (MIC 150–600 mg/mL; MBC 200–750 mg/mL), with MBC/MIC ratios of 1.25–1.83 indicative of bactericidal action (Cushnie & Lamb, 2011). Time-kill curves showed faster and deeper reductions in viable counts for *A. senegal* n-hexane fractions, particularly against *S. aureus* and *P. aeruginosa*. SEM micrographs showed pronounced envelope damage, including distorted membranes, collapsed cell walls, and loss of intracellular material, supporting a membrane-targeted mechanism. GC-MS further revealed that the n-hexane fraction from *A. senegal* was heavily enriched in a single benzodioxole-type aromatic amide (53.71% relative abundance), whereas *A. seyal* contained this class of compound at lower levels and a higher proportion of phthalate esters. “Together, these data implicate aromatic amide-rich n-hexane (non-polar) fractions, rather than the hydrophilic polysaccharide backbone, as the main contributors to gum Arabic’s antibacterial effect and support *A. senegal* as the preferred source species.

Keywords: Gum Arabic; *Acacia senegal*; aromatic amides; antibacterial activity; non-polar fractions

1. INTRODUCTION

Antimicrobial resistance is considered one of the most critical challenges of modern healthcare, which increases the attempts to find new antibacterial candidates based on natural products with a long history of safe use in humans (Hall et al., 2020; Uddin et al., 2021). Plant exudates occupy a unique niche within this larger context since they offer a combination of chemically rich matrices and a wide history of

traditional utilization (Gurib Fakim, 2006; Mahomoodally, 2013). Gum Arabic, obtained mainly from *Acacia senegal* and *Acacia seyal*, is symbolic of this duality of purpose as industrial hydrocolloid and ethnomedicinal resource.

Technologically, GA functionality is primarily attributed to its arabinogalactan–protein (AGP) complex, a highly branched macromolecule that confers excellent solubility, emulsifying capacity, and film-forming properties that underpins its GRAS E414 status (Islam et al., 1997; Williams & Phillips, 2014). However, ethnobotanical and historical sources point out the application of GA preparations in wound care, gastrointestinal disorders and inflammatory conditions suggesting a wider range of bioactivity than its texturizing application would imply (Bedigian, 2005; Islam et al., 1997). Antioxidant, anti-inflammatory, and nephroprotective effects of GA have since been pharmacologically characterized with clinical assessment of patients with chronic renal disease (Ali et al., 2008; Jaafar, 2019).

In contrast with this expanding evidence for systemic effects, the direct antibacterial properties of GA have been explored less consistently. Many reports have relied on crude aqueous extracts or commercial samples without precise botanical authentication, and they often fail to distinguish between *A. senegal* (“hashab”) and *A. seyal* (“talha”), even though these species differ in gum composition and trade classification (Ashour et al., 2022; Nie et al., 2013). Most GA research has focused on polysaccharide architecture and AGP core models, whereas low-abundance lipophilic components have attracted relatively little attention (Nie et al., 2013; Vanloot et al., 2012). This polysaccharide centric view contrasts with the research on other *Acacia* parts including leaves and bark where phenolics, flavonoids and other secondary metabolites are seen to be central to antibacterial and other bioactivities (Alamgir and Alamgir, 2018; Satti et al., 2018).

Non-polar metabolites found in small amounts in the gum matrix would also have a similar effect but would be highly diluted in crude preparations. The initial results of this study indicating that the extraction solvent used affects reported activity already indicate that certain fractions, not unfractionated GA, could be causing the reported effects (Abdallah et al., 2023; Nayyef et al., 2023).

Polarity-guided fractionation therefore offers a rational strategy to dissect which parts of the GA matrix harbor antibacterial properties by separating n-hexane (non-polar), ethyl acetate (semi-polar), and aqueous components and comparing them under standardized conditions.

The present work was designed to address these gaps using a comparative, fraction-based approach. Specifically, we aimed (i) to define the antibacterial activity of authenticated Sudanese *A. senegal* and *A. seyal* gum fractions (n-hexane, ethyl acetate, aqueous), (ii) to determine the MIC, MBC, and time-kill kinetics against representative Gram-positive and Gram-negative bacteria, (iii) to visualize ultrastructural changes by SEM, and (iv) to characterize the chemical composition of active non-polar fractions by FT-IR and GC-MS. We hypothesized that non-polar fractions, particularly from *A. senegal*, would display stronger bactericidal activity associated with distinctive aromatic amide signatures and a membrane-directed mode of action.

2. Materials and Methods

2.1 Authentication and collection of plant material

In the Kordofan region of Sudan (11.1993°N, 28.0962°E), gum nodules were collected from *Acacia senegal* and *Acacia seyal* trees in a manner representative of local gum-harvesting practices (Bedigian, 2005). Species identity was confirmed by trained taxonomists, and voucher information was recorded to ensure traceability. Nodules were air dried, cleaned, and ground to coarse powder and stored at 4 °C in airtight containers awaiting extraction.

2.2 Polarity-directed sequential fractionation

Fractionation by polarity was carried out using a sequential liquid-liquid partitioning scheme. In every batch, 50 g of GA powder were weighed in distilled water (1:10 w/v) and stirred until the powder is completely moist. It was then transferred to a separatory funnel and extracted sequentially with n-hexane, ethyl acetate, and n-butanol in equal volumes, based on an overall scheme applied in the natural product fractionation (Mogana et al., 2020). Organic layers were collected, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The remaining aqueous solution was frozen and lyophilized.

The yield of the fractions was determined against the starting gum mass, and all the dried fractions were stored at 4 °C in light-proof containers.

2.3 Strains of bacteria and culture conditions.

The test panel contained *Staphylococcus aureus* (ATCC 9144), *Bacillus subtilis* (laboratory strain), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (laboratory strain). Experiments were preceded by culturing of cultures on Nutrient Agar and subculturing them to broth. Inocula were standardized turbidimetrically to 0.5 McFarland (approximately $1-2 \times 10^8$ CFU/mL) following established procedures (Balouiri et al., 2016).

2.4 Antibacterial susceptibility testing

Antibacterial activity was initially assessed using the disc diffusion method on Mueller–Hinton Agar (MHA). Sterile 6 mm discs were loaded with 20 µL of each fraction at 100–400 mg/mL. After inoculation with standardized bacterial suspensions, plates were incubated at 37 °C for 24 h, and inhibition zones (including disc diameter) were recorded in millimeters. Gentamicin (10 µg) served as positive control, and solvent-only discs served as negative control.

MIC and MBC were determined by broth microdilution in 96-well plates, using CLSI-aligned methodology with minor adaptations (Balouiri et al., 2016). Two-fold serial dilutions of n-hexane fractions in Mueller–Hinton Broth containing 1% DMSO were inoculated at $\sim 5 \times 10^5$ CFU/mL. After incubation at 37 °C for 24 h, the MIC was taken as the lowest concentration that prevented visible turbidity. To determine the MBC, material from wells without apparent growth was streaked onto MHA plates; the smallest concentration producing at most 10 colonies, corresponding to at least a 99.9% drop in viable cells, was recorded as the MBC, and fractions with an MBC/MIC ratio not exceeding 4 were considered bactericidal (Cushnie & Lamb, 2011).

2.5 Time-kill kinetics

Bacterial suspensions adjusted to approximately 10^6 CFU/mL were treated with the n-hexane fractions at concentrations equal to 1× and 2× their MIC values, with untreated cultures used as growth controls. The aliquots were removed at 0, 2, 4, 6, and 24 h, serially diluted, and plated on MHA, and a decrease of at least 3 log₁₀ CFU/mL relative to the starting inoculum was interpreted as evidence of bactericidal activity (Vaou et al., 2021).

2.6 Scanning electron microscopy

Log-phase cultures were treated with the n-hexane fractions at their MBC for 4 h, then harvested and washed. Pellets were fixed in 2.5% glutaraldehyde, dehydrated through a graded ethanol series, dried, sputter-coated with gold, and examined using a Hitachi SU5000 scanning electron microscope operated at 10–15 kV (Tosi et al., 2013; Hnini et al., 2023).

2.7 Chemical profiling

FT-IR spectra for the dried n-hexane fractions were collected in ATR mode between 4000 and 400 cm⁻¹ at 4 cm⁻¹ resolution, focusing on diagnostic signals for carbohydrate backbones and aromatic groups (López-Franco et al., 2021). GC-MS measurements were carried out on a PerkinElmer Clarus 500 instrument fitted with a VF-5MS capillary column under standard conditions for non-polar extracts. Samples in n-hexane were injected in split mode, and oven temperature was programmed from 50 °C (2 min) to 150 °C at 3 °C/min (10 min), then to 300 °C at 10 °C/min (10 min). Helium was the carrier gas and electron ionization was 70 eV. The compounds were tentatively identified using the NIST library and compared with related reports on gum-derived lipophiles (Elnour et al., 2018; Hassani et al., 2020).

2.8 Statistical analysis

All experiments were conducted in triplicate on independently prepared samples, and data are presented as mean and standard deviation. Differences between *A. senegal* and *A. seyal* fractions were interpreted based on consistent trends across the organisms rather than on the hypothesis.

3. Results and Discussions

3.1 Non-polar fractions as the principal antibacterial reservoir

Disc diffusion results revealed that measurable inhibition zones were produced only by the n-hexane fractions of both *A. senegal* and *A. seyal*, whereas aqueous and ethyl acetate fractions were inactive up to 400 mg/mL (Table 3.1). This pattern shifts attention away from the hydrophilic AGP matrix, traditionally

regarded as GA's main functional component (Williams & Phillips, 2014), toward a more restricted pool of non-polar constituents. The inactivity of aqueous fractions helps to rationalize earlier reports of weak or inconsistent antibacterial effects when crude or water extracts of GA were tested without fractionation (Ashour et al., 2022; Abdallah et al., 2023; Nie et al., 2013).

Table 3.1 Disc diffusion activity of polarity-based fractions of *Acacia senegal* and *Acacia seyal* gum Arabic against test bacteria

| Species / Organism | Conc. (mg/mL) | Water | Ethyl acetate | n-Hexane | Gentamicin (10 µg) |
|----------------------------|---------------|-------|---------------|------------|--------------------|
| A. senegal – S. aureus | 400 | – | – | 22.0 ± 0.3 | 28 |
| A. senegal – S. aureus | 200 | – | – | 9.8 ± 0.4 | 26 |
| A. senegal – B. subtilis | 400 | – | – | 25.0 ± 0.7 | 28 |
| A. senegal – E. coli | 400 | – | – | 21.0 ± 0.8 | 24 |
| A. senegal – P. aeruginosa | 400 | – | – | 18.0 ± 0.9 | 28 |
| A. seyal – S. aureus | 400 | – | – | 22.0 ± 0.3 | 28 |
| A. seyal – S. aureus | 200 | – | – | 9.8 ± 0.4 | 26 |
| A. seyal – B. subtilis | 400 | – | – | 25.0 ± 0.7 | 28 |
| A. seyal – E. coli | 400 | – | – | 21.0 ± 0.8 | 24 |
| A. seyal – P. aeruginosa | 400 | – | – | 18.0 ± 0.9 | 28 |

Note. Disc diffusion methodology adapted from Balouiri et al. (2016). Mean inhibition zone in mm ± SD; “–” = no zone at 400 mg/mL

Figure 3.1. Disc diffusion inhibition zones (400 mg/mL n-hexane fractions unless noted) on Mueller-Hinton agar. (A) *A. senegal* vs *S. aureus* (22.0±0.3 mm), *B. subtilis* (25.0±0.7 mm); (B) *A. seyal* vs same (identical zones); (C) 200 mg/mL showing weaker zones (9.8±0.4 mm); (D) Gentamicin control (28 mm). Data as mean ± SD (n=3).



These findings indicate that antibacterial activity is not a diffuse property of the entire gum but is concentrated in the **n-hexane (non-polar) fractions**, in line with general trends for plant-derived membrane-active agents (Gurib-Fakim, 2006; Vaou et al., 2021).

3.2 Quantitative potency differences between *Acacia senegal* and *Acacia seyal*

Broth microdilution assays provided quantitative evidence of a potency hierarchy. For all four test organisms, n-hexane fractions from *A. senegal* achieved both inhibition and killing at lower concentrations than those from *A. seyal* (Table 3.2). For example, *A. senegal* exhibited an MIC/MBC of 250/400 mg/mL against *P. aeruginosa*, whereas *A. seyal* required 600/750 mg/mL under the same conditions.

Table 3. 2 MIC, MBC, and MBC/MIC ratios of n-hexane fractions of *Acacia senegal* *Acacia seyal* against test bacteria

| Species / Bacterium | MIC (mg/mL) | MBC (mg/mL) | MBC/MIC ratio | Classification |
|--|-------------|-------------|---------------|----------------|
| <i>A. senegal</i> – <i>B. subtilis</i> | 100 | 150 | 1.50 | Bactericidal |
| <i>A. senegal</i> – <i>S. aureus</i> | 150 | 200 | 1.33 | Bactericidal |
| <i>A. senegal</i> – <i>E. coli</i> | 300 | 550 | 1.83 | Bactericidal |
| <i>A. senegal</i> – <i>P. aeruginosa</i> | 250 | 400 | 1.60 | Bactericidal |
| <i>A. seyal</i> – <i>B. subtilis</i> | 150 | 200 | 1.33 | Bactericidal |
| <i>A. seyal</i> – <i>S. aureus</i> | 200 | 250 | 1.25 | Bactericidal |
| <i>A. seyal</i> – <i>E. coli</i> | 400 | 650 | 1.63 | Bactericidal |
| <i>A. seyal</i> – <i>P. aeruginosa</i> | 600 | 750 | 1.25 | Bactericidal |

Note. Bactericidal classification based on $MBC/MIC \leq 4$ (Cushnie & Lamb, 2011).

All MBC/MIC ratios ranged from 1.25 to 1.83, indicating a bactericidal rather than bacteriostatic effect according to standard criteria (Cushnie & Lamb, 2011). The general pattern of higher susceptibility among Gram-positive bacteria compared to Gram-negative ones aligns with the protective role of the Gram-negative outer membrane against hydrophobic agents (Vaou et al., 2021).

3.3 Time–kill kinetics and the tempo of bactericidal action

Time–kill data complemented MIC/MBC values by revealing the speed of bactericidal activity. At twice the MIC, the *A. senegal* n-hexane fraction produced a rapid drop in *S. aureus* viability, exceeding a 3- \log_{10} decrease within 4 h, whereas the *A. seyal* fraction only reached a comparable reduction after roughly 6 h.

Figure 3.2a. Time-kill kinetics of *S. aureus* (ATCC 9144) with n-hexane fractions at 2× MIC: *A. senegal* (300 µg/mL), *A. seyal* (400 µg/mL), untreated control. Mean log₁₀ CFU/mL ± SD (n=3) over 24 h. *A. senegal* achieved >3-log reduction by 4 h.

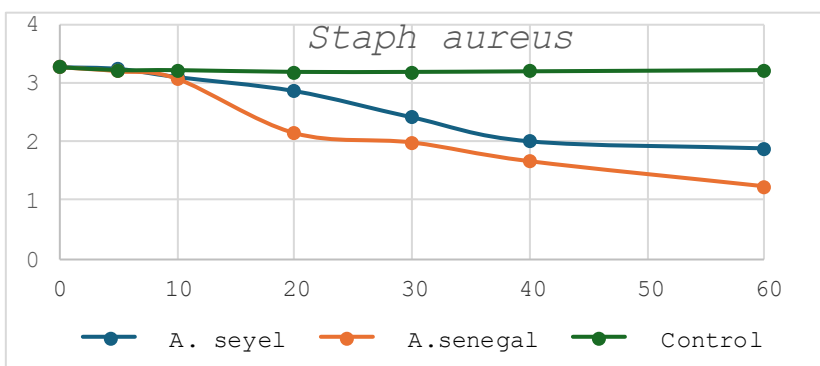
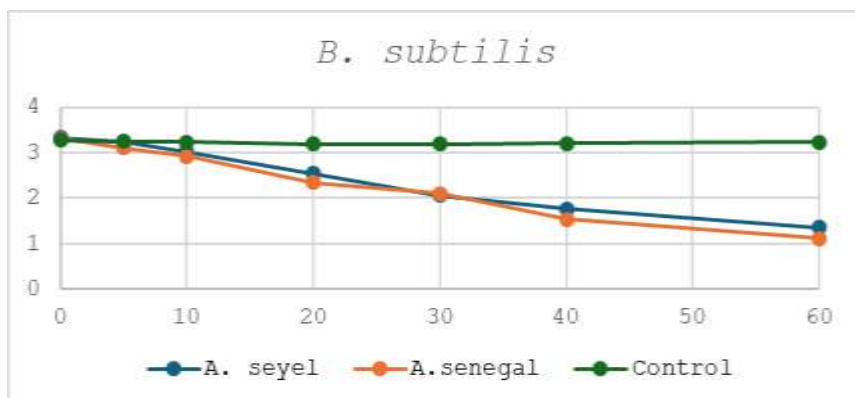


Figure 3.2b. Time-kill kinetics of *B. subtilis* with n-hexane fractions at 2× MIC: *A. senegal* (200 µg/mL), *A. seyal* (300 µg/mL), control. Mean log₁₀ CFU/mL ± SD (n=3).



Across the remaining test bacteria, kill curves followed the same pattern, with *A. senegal* preparations consistently driving earlier and steeper CFU declines than those from *A. seyal*. Such kinetics suggest that *A. senegal*-derived material may be better suited to uses where antimicrobial contact is brief, including hard-surface treatments or short-application topical products (Hall et al., 2020; Vaou et al., 2021).

3.4 SEM evidence for membrane-directed mechanisms

The ultrastructural evidence indicated a severe damage to the level of cell envelope. The untreated *B. subtilis* and *E. coli* in SEM images had an identical morphology of regular rods with smooth and continuous surfaces, but cells subjected to n-hexane fractions at the MBC had a distorted morphology and surface defect. In *B. subtilis* the *A. senegal* fraction produced many smaller visibly buckled cells with irregular cell boundaries and decreased internal contrast whereas the *A. seyal* fraction produced such abnormalities in a lower percentage and with less severe deformation. Both fractions led to the formation of rough and uneven surfaces containing blister-like protrusions in the outer-membrane in *E. coli*, a morphology linked to the damage of the outer-membrane by lipophilic phytochemicals (Cushnie & Lamb, 2011; Mogana et al., 2020). The combination of these characteristics enables a membrane-disruptive,

bactericidal mechanism of action of the components of the n-hexane (non-polar) fractions. SEM micrographs (Figures 3.3–3.5) confirmed this damage pattern, showing untreated *B. subtilis* with smooth rod morphology alongside treated cells exhibiting membrane blebbing, cell wall collapse, and loss of intracellular material after 4 h exposure at MBC concentrations.

Figure 3.3. SEM of untreated control *B. subtilis* (laboratory strain) showing regular rods with smooth, continuous surfaces (bar = 1 μm ; 10,000 \times magnification)

Figure 3.4. SEM of *B. subtilis* treated 4 h with *A. senegal* n-hexane fraction at MBC (150 $\mu\text{g}/\text{mL}$). (a) Normal rods; (b) buckled cells with irregular boundaries, surface defects, and lost internal contrast (arrows; bar = 1 μm ; 10,000 \times).

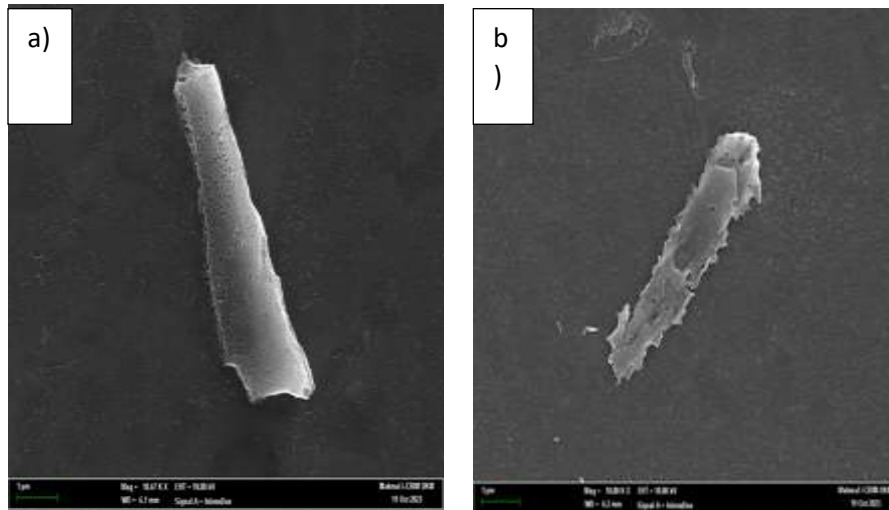


Figure 3.5. SEM of *B. subtilis* treated 4 h with *A. seyal* n-hexane fraction at MBC (200 $\mu\text{g}/\text{mL}$). (a) Fewer affected cells; (b) milder membrane blebbing vs *A. senegal* treatment (bar = 1 μm ; 10,000 \times)

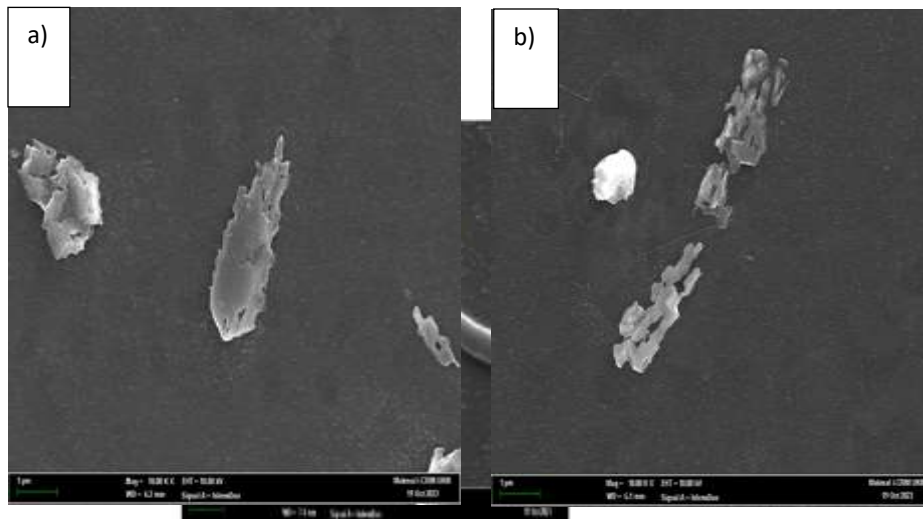
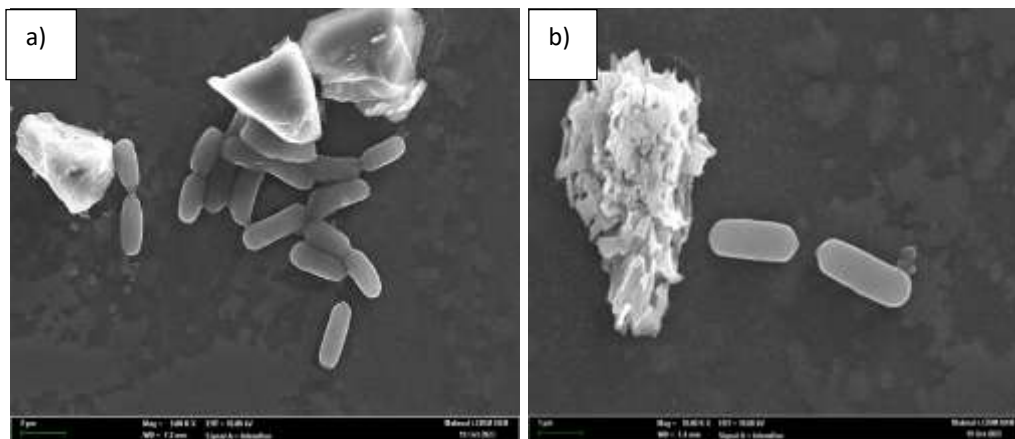


Figure 3.6 a and b SEM of *Escherichia coli* (ATCC 25922). (a) Untreated control with smooth rod morphology; (b) cells after 4 h exposure to the *A. senegal* n-hexane fraction at its MBC, showing rough, blistered surfaces and envelope collapse (bar = 1 μm ; 10,000 \times)



3.5 Aromatic amide chemoprofiles and species-specific activity

GC-MS profiling of the n-hexane fractions revealed distinct aromatic amide signatures for *A. senegal* and *A. seyal* (Table 3.3). The *A. senegal* fraction was dominated by a benzodioxole-type aromatic amide (53.71% relative area), whereas *A. seyal* contained a different benzamide derivative at only 20.29% relative area and higher levels of diethyl phthalate and related esters. FT-IR analysis (Figures 3.7–3.8) revealed diagnostic amide (1700 cm^{-1} C=O, 3300 cm^{-1} N-H) and aromatic ($1500\text{-}1600\text{ cm}^{-1}$ C=C) peaks that supported these GC-MS identified chemoprofiles in Table 3.3.

Figure 3.7. FT-IR spectrum (ATR mode, $4000\text{-}400\text{ cm}^{-1}$, 4 cm^{-1} resolution) of *A. senegal* n-hexane fraction. Key peaks: 3300 cm^{-1} (N-H stretch), 1700 cm^{-1} (amide C=O), $1500\text{-}1600\text{ cm}^{-1}$ (aromatic C=C).

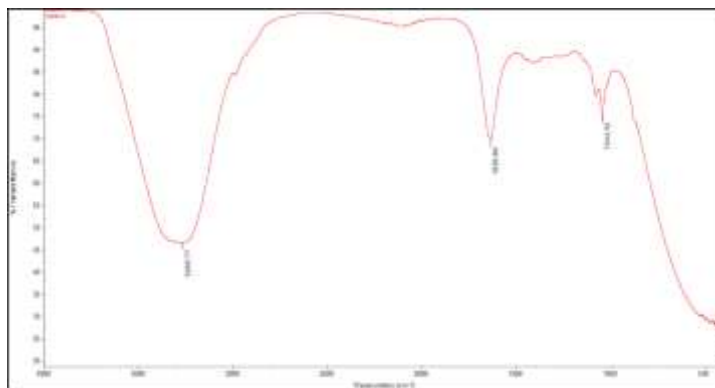
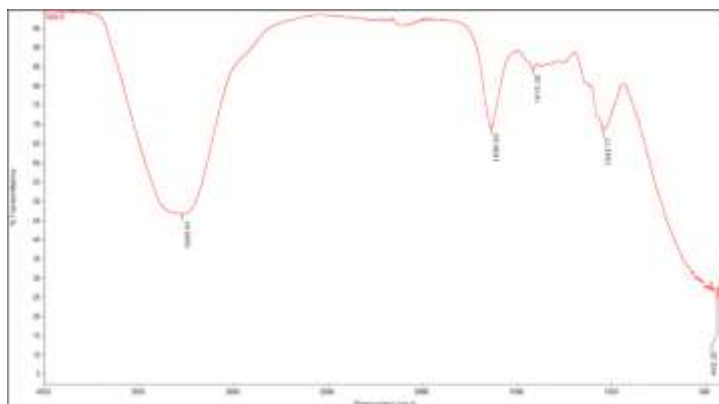


Figure 3.8 FT-IR spectrum of *A. seyal* n-hexane fraction under identical conditions, showing weaker amide signals.Table 3.3 Major constituents identified by GC–MS in n-hexane fractions of *Acacia senegal* and *Acacia seyal* gum Arabic

| Species | Peak | Compound name | Formula | MW | Rt (min) | Area (%) |
|-------------------|------|--|---|-----|----------|----------|
| <i>A. senegal</i> | 1 | Tetrahydrobenzofuran-3-carboxylic acid naphthalen-1-ylamide | C ₂₀ H ₁₉ NO ₂ | 305 | 14.09 | 1.42 |
| <i>A. senegal</i> | 6 | Benzodioxole-5-carboxylic acid (2-cyano-4,5-diethoxyphenyl)amide | C ₁₉ H ₁₈ N ₂ O ₅ | 354 | 16.53 | 53.71 |
| <i>A. senegal</i> | 7 | Phthalic acid, butyl hexyl ester | C ₁₈ H ₂₆ O ₄ | 306 | 16.68 | 14.76 |
| <i>A. senegal</i> | 8 | 1,2-Benzenedicarboxylic acid, diethyl ester | C ₁₂ H ₁₄ O ₄ | 222 | 16.66 | 20.54 |
| <i>A. seyal</i> | 1 | Benzamide, N-(2-cyano-4,5-diethoxyphenyl)-2-ethoxy- | C ₂₀ H ₂₂ N ₂ O ₄ | 354 | 16.20 | 20.29 |
| <i>A. seyal</i> | 3 | 1,2-Benzenedicarboxylic acid, diethyl ester | C ₁₂ H ₁₄ O ₄ | 222 | 16.54 | 48.47 |
| <i>A. seyal</i> | 6 | o-Benzenedicarboxylic acid | C ₁₂ H ₁₄ O ₄ | 222 | 16.68 | 13.63 |

Note. Compound identification based on NIST library matching and comparison with related reports on gum-derived lipophilic fractions (Elnour et al., 2018; Hassani et al., 2020).

Aromatic amides are widely recognized as privileged scaffolds with the potential for membrane-interacting and multifunctional antibacterial effects (Cushnie et al., 2020). The strong enrichment of a single benzodioxole-type aromatic amide in *A. senegal* is therefore highly compatible with its superior bactericidal potency and faster kill kinetics. Conversely, the more diluted aromatic amide content and increased presence of phthalate esters in *A. seyal* provide a plausible explanation for its weaker activity. From a chemotaxonomic and practical perspective, these distinct chemoprofiles offer a

functional fingerprint that can guide the selection of GA sources for antimicrobial applications, supporting preferential use of authenticated *A. senegal* gums (Nie et al., 2013).

4. CONCLUSION AND PERSPECTIVES

The present study shows that the direct antibacterial activity of Sudanese GA is not an inherent property of its polysaccharide matrix but is associated with n-hexane, aromatic amide-rich (non-polar) fractions embedded within the gum. Within this framework, *Acacia senegal* emerges as a more promising source than *A. seyal*, yielding n-hexane fractions that are consistently more potent and faster acting against representative Gram-positive and Gram-negative bacteria.

When MIC/MBC data, time-kill kinetics, SEM imaging, and GC–MS profiling are considered together, a coherent picture emerges in which a dominant benzodioxole-type aromatic amide in the *A. senegal* fraction plays a central role in membrane-targeted bactericidal activity (Cushnie & Lamb, 2011; Cushnie et al., 2020). At the same time, the fractionation approach preserves the broader GA matrix, suggesting that these non-polar constituents might be exploited either as isolated agents or as part of structured “active carrier” systems that use the polysaccharide scaffold as a delivery or stabilizing medium (Williams & Phillips, 2014).

Further studies are needed to isolate and characterize the major aromatic amide to prove its contribution and facilitate the study of structure-activity relationships, evaluate cytotoxicity and selectivity indices in mammalian models, and determine performance in application matrices that may be realistic in food coatings, wound dressings, or oral care formulations, where GA is already a proven functional ingredient (Goswami and Naik, 2014; Prasad et al., 2022). This work sheds light on a well-known natural product in a more detailed way through clarifying the fraction and species-specific nature of GA antibacterial activity and positions *A. senegal* GA as a rational starting material for developing next-generation plant-derived antibacterial interventions.

REFERENCES

- Ali, A. A., Ali, K. E., Fadlalla, A. E., & Khalid, K. E. (2008). The effects of gum Arabic oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. *Natural Product Research*, 22(1), 12–21.
- Alamgir, A., & Alamgir, A. (2018). Biotechnology, in vitro production of natural bioactive compounds, herbal preparation, and disease management (treatment and prevention). In A. Alamgir (Ed.), *Therapeutic use of medicinal plants and their extracts: Phytochemistry and bioactive compounds* (Vol. 2, pp. 585–664). Springer.
- Ashour, M. A., Fatima, W., Imran, M., Ghoneim, M. M., Alshehri, S., & Shakeel, F. (2022). A review on the main phytoconstituents, traditional uses, inventions, and patent literature of gum Arabic emphasizing *Acacia seyal*. *Molecules*, 27(4), 1171.
- Abdallah, E. M., Alhatlani, B. Y., De Paula Menezes, R., & Martins, C. H. G. (2023). Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, 12(17), 3077.
- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.
- Baien, S. H., Seele, J., Henneck, T., Freibrodt, C., Szura, G., Moubasher, H., Nau, R., Brogden, G., Mörgelin, M., & Singh, M. (2020). Antimicrobial and immunomodulatory effect of gum Arabic on human and bovine granulocytes against *Staphylococcus aureus* and *Escherichia coli*. *Frontiers in Immunology*, 10, 3119.
- Bedigian, D. (2005). *Acacia senegal* and the gum Arabic trade: Monograph and annotated bibliography. *Economic Botany*, 59(4), 405–406.
- Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38(2), 99–107.
- Cushnie, T. P. T., Cushnie, B., & Lamb, A. J. (2020). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 56(3), 106118.
- Elnour, A., Mirghani, M., Musa, K., Kabbashi, N., & Alam, M. (2018). Challenges of extraction techniques of natural antioxidants and their potential application opportunities as anti-cancer agents. *Health Science Journal*, 12(5), 596.

- Goswami, S., & Naik, S. (2014). Natural gums and its pharmaceutical application. *Journal of Scientific and Innovative Research*, 3(1), 112–121.
- Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27(1), 1–93.
- Hall, C. W., Mah, T.-F., & Pang, C. M. (2020). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, 44(1), 1–28.
- Hassani, A., Azarian, M. M. S., Ibrahim, W. N., & Hussain, S. A. (2020). Preparation, characterization and therapeutic properties of gum Arabic-stabilized gallic acid nanoparticles. *Scientific Reports*, 10(1), 17808.
- Hnini, M., Taha, K., & Aurag, J. (2023). Botany, associated microbiota, traditional medicinal uses, and phytochemistry of *Vachellia tortilis* subsp. *raddiana* (Savi): A systematic review. *Journal of Agriculture and Food Research*, 12, 100566.
- Islam, A., Phillips, G. O., Slijvo, A., Snowden, M. J., & Williams, P. A. (1997). A review of recent developments on the regulatory, structural and functional aspects of gum Arabic. *Food Hydrocolloids*, 11(4), 493–505.
- Jaafar, N. S. (2019). Clinical effects of Arabic gum (*Acacia*): A mini review. *Iraqi Journal of Pharmaceutical Sciences*, 28(2), 9–16.
- López-Franco, Y., Higuera-Ciapara, I., Lizardi-Mendoza, J., Wang, W., & Goycoolea, F. M. (2021). Other exudates: Tragacanth, karaya, mesquite gum, and larchwood arabinogalactan. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of hydrocolloids* (3rd ed., pp. 673–727). Woodhead Publishing.
- Mahomoodally, M. F. (2013). Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2013, 617459.
- Mogana, R., Adhikari, A., Tzar, M., Ramliza, R., & Wiart, C. (2020). Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. *BMC Complementary Medicine and Therapies*, 20, Article 32. (or page range as in your source)
- Nayyef, S. H., Yaseen, L. Q., & Wahab, M. H. A. (2023). In vitro determination effectiveness of *Acacia senegal* and juice of *Actinidia deliciosa* with estimate synergistic effect towards some pathogenic bacteria of human. *Journal for Research in Applied Sciences and Biotechnology*, 2(5), 22–28.
- Nie, S. P., Wang, C., Cui, S. W., Wang, Q., Xie, M. Y., & Phillips, G. O. (2013). A further amendment to the classical core structure of gum Arabic (*Acacia senegal*). *Food Hydrocolloids*, 31(1), 42–48.
- Prasad, N., Thombare, N., Sharma, S., & Kumar, S. (2022). Gum Arabic—A versatile natural gum: A review on production, processing, properties and applications. *Industrial Crops and Products*, 187, 115304.
- Satti, S. M., Shah, A. A., Marsh, T. L., & Auras, R. (2018). Biodegradation of poly(lactic acid) in soil microcosms at ambient temperature: Evaluation of natural attenuation, bio-augmentation and bio-stimulation. *Journal of Polymers and the Environment*, 26, 3848–3857.
- Tosi, G., Bortot, B., Ruozi, B., Dolcetta, D., Vandelli, M. A., Forni, F., & Severini, G. (2013). Potential use of polymeric nanoparticles for drug delivery across the blood–brain barrier. *Current Medicinal Chemistry*, 20(17), 2212–2225.
- Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, M. K. H., Gajdács, M., & Sahibzada, M. U. K. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health*, 14(12), 1750–1766.
- Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041.
- Vanloot, P., Dupont, A. L., Dupont, S., & Bardet, M. (2012). Characterization of *Acacia* gums by size exclusion chromatography and FTIR spectroscopy. *Carbohydrate Polymers*, 87(2), 1376–1383.
- Williams, P. A., & Phillips, G. O. (2014). Gum Arabic: Industrial applications and future perspectives. *Food Hydrocolloids*, 42, 249–256.