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Multi-element Profiles of Sudanese *Acacia senegal* and *Acacia seyal* Gum Arabic Fractions: Implications for Safety and Hydrocolloid Functionality

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ABSTRACT
A well-known hydrocolloid in food and health-related products, gum Arabic (GA) of *Acacia senegal* and *Acacia seyal* has not yet had its elemental profile across solvent-derived fractions resolved (Islam et al., 1997; Prasad et al., 2022). In this experiment, both species of authenticated Sudanese GA were fractionated into aqueous and organic fractions and concentrations of Ca, Mg, Na, K and a wide range of trace elements (Fe, Sr, Si, B, Ba, Cu, Al, Pb, Cd, As, Hg) were determined by ICP-MS (Li et al., 2015; Vanloot et al., 2012). Calcium was the dominant element in all fractions, with particularly high levels in *A. senegal* fractions (Karamalla et al., 1998; Yebeyen et al., 2009). In contrast, Mg, K, Na and a few trace elements showed strong species- and solvent-dependent variation: *A. seyal* fractions were significantly enriched with potassium, whereas *A. senegal* fractions were enriched with Fe, Sr, Si, B, Ba and Cu (Daoub et al., 2018; Hnini et al., 2023). These trends suggest that species-specific environmental and physiological interactions are likely to be the basis of the recorded rheological and interfacial behavioural differences. More importantly, Pb, Cd, As and Hg were not detected in any of the samples, as per the current safety assessment of GA in controlled food, nutraceutical and pharmaceutical applications (Phillips, 2010; Phillips et al., 2008). In general, the findings establish different ionic fingerprints of each *Acacia* species and fraction, which supports the definition of GA as a safe mineral-rich hydrocolloid with its inorganic composition being an inseparable part of its functional activity (Williams and Phillips, 2014; Eghbaljoo et al., 2022).

Keywords: Gum Arabic; *Acacia senegal*; *Acacia seyal*; ICP-MS; multi-element profiling

1. INTRODUCTION
Gum Arabic is a food, beverage, and pharmaceutical ingredient that is used as a stabilizer, emulsifier, and encapsulant (Williams, 2000; Imeson, 2011). It is a dried exudate of *Acacia senegal* and *Acacia seyal*, and one of the foundational ingredients in the food, beverage, and pharmaceutical industry. It is functional due to a complex molecular construction, which is mainly an arabinogalactan-protein network (Akiyama et al., 1984; Mahendran et al., 2008; Nie et al., 2013). In addition to its techno-functional applications, GA is

also known as a dietary fiber and is gaining more status in clinical nutrition, a designation which has been supported by its Generally Recognized as Safe (GRAS) designation, which restricts its contaminants such as heavy metals to strict limits (Phillips, 2010; Phillips et al., 2008; Glicksman, 2021).

Although the organic structure of GA is well studied, little has been done on the inorganic structure. This is also of considerable importance because bound cations can alter the conformation, aggregation, and overall performance of hydrocolloids (Islam et al., 1997; Yadav et al., 2015). The carboxylated groups of the uronic acids of GA are highly attracted to divalent cations like calcium and magnesium, and previous research indicates that GA is mineral-rich in nature (Islam et al., 1997; Daoub et al., 2018). The bulk ash contents, or a limited number of elements in unfractionated gum, are, however, the subject of most published information. Extensive multi-component profiles of separate solvent fractions, which are often employed to adjust the functional characteristics, are uncommon (Sasidharan et al., 2011; Prasad et al., 2022).

This is further complicated by the species of *Acacia. A. senegal* and *A. seyal* have been known to vary in their structural, viscosity, and emulsifying characteristics which are reflected in the industrial work and the local management practices (Karamalla et al., 1998; Daoub et al., 2018; Wekesa et al., 2010). Even though regulation definitions of gum Arabic frequently include both species, increased pressure on clarity of species-level authentication across the value chain is on the rise (Dondain and Phillips, 1999; Kusters et al., 2023). To accomplish this, multi-element signatures may be used as an inorganic fingerprint to supplement structural and rheological data (Vanloot et al., 2012; López-Franco et al., 2021).

Safety and health are also factors that promotes detailed elemental analysis. GA is also becoming a dietary supplement in the treatment of chronic illnesses such as kidney disease and metabolic syndrome, in which chronic consumption is the rule (Ali et al., 2008; Chao et al., 2014; Kaddam et al., 2017; Ali et al., 2020; Zeid and Farajallah, 2018). When dealing with this, it is of utmost importance to make sure there are no trace contaminants. Also, the nutritionally valuable minerals such as calcium, magnesium, and potassium may be included in the extended health profile of GA-containing products (Dauqan and Abdullah, 2013; Yakubu et al., 2021). Several clinical and experimental studies have proposed cardioprotective, antioxidant and metabolic effects with regular GA intake, which should be compensated by strong guarantees of low concentrations of heavy metals (Abd-Allah et al., 2002; Abdelwahed et al., 2011; Jaafar, 2019).

This paper does not examine the organic contents or antibacterial action, but the inorganic makeup of GA (Ashour et al., 2022; Musa et al., 2018). We prepared authenticated Sudanese *A. senegal* and *A. seyal* gums, which are produced in large amounts, as aqueous and organic solvent extracts and determined a broad spectrum of elements by ICP-MS (El Mahi & Magid, 2014; Freudenberger, 1991; Li et al., 2015). We aimed to: (i) determine multi-element specifications of each species and fraction; (ii) establish species-specific ionic patterns, which might be related to functional performance; and (iii) ensure that the materials were safe in terms of heavy metals to be used in food, nutraceutical, and pharmaceutical purposes (Phillips et al., 2008; Eghbaljoo et al., 2022). In this way, we will be able to offer a solid empirical basis of the inorganic aspect of GA, which will be applied to the quality control, species authentication, and the formulation design (Williams and Phillips, 2014; Imam et al., 2013).

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material and Sample

Acacia senegal and *Acacia seyal* gum nodules were gathered at the 2023 harvest at the Kordofan region of Sudan, a region with a long history of management systems and quality production of *Acacia* gum (El Mahi and Magid, 2014; Wekesa et al., 2010). To guarantee purity, only naturally exuded nodules on tree trunks and main branches were collected, which reduced the contamination levels caused by the tree bark and debris, and was in accordance with good practice of premium GA collection (Islam et al., 1997; Yebeyen et al., 2009). Qualified taxonomists authenticated the plant material, voucher data was captured, and the dried nodules were kept in low-temperature airtight containers to maintain their integrity (Mukherjee, 2019; Ashour et al., 2022). They were crushed into granules, then processed to a small particle size for optimal dispersion (Daoub et al., 2018) and stored in closed glass containers to avoid moisture absorption and loss during storage (Cozic et al., 2009).

2.2 Fractionation into Solvent Extracts

The gum was fractionated using a polarity-directed liquid-liquid partitioning technique, which is commonly used in natural product and hydrocolloid research (Sasidharan et al., 2011; Prasad et al., 2022). Ground gum (50 g) from each species was mixed with distilled water at a 1:10 w/v ratio and swirled until thoroughly hydrated (Daoub et al., 2018). The hydrated dispersion was extracted in equal quantities with n-hexane, ethyl acetate, and n-butanol to provide non-polar, intermediate-polarity, and hydrophilic organic fractions, respectively (Abdallah et al., 2023; Bajpai et al., 2021). The organic phase was collected after each extraction stage, dried over anhydrous sodium sulphate, and concentrated under reduced pressure, with the remaining aqueous frozen and lyophilised (Sasidharan et al., 2011; Elnour et al., 2018). Extraction yield was calculated using the initial mass of dry gum for each fraction and species (Prasad et al., 2022). Elemental analysis of all dried fractions was performed in the dark at 40°C to guarantee that compositions did not change (Islam et al., 1997).

2.3 Elemental Analysis by ICP-MS

The inductively coupled plasma mass spectrometry (ICP-MS) was used to determine elemental composition, which is a technique used widely to multi-element profile plant-derived materials and food hydrocolloids (Li et al., 2015; Vanloot et al., 2012). Each of the dried fractions was weighed in the amount of approximately 0.5 g into digestion vessels where they were reacted with concentrated nitric acid and hydrogen peroxide (Islam et al., 1997). The digestion was done under controlled heating until clear solutions appeared, and then samples were cooled and diluted to volume using ultrapure water (Li et al., 2015). The samples were analysed using an Agilent 7900 ICP-MS in helium collision mode to reduce polyatomic interferences (Vanloot et al., 2012). Calibration curves were constructed using multi-element standards with expected concentration ranges, and certified reference materials were included in each batch to demonstrate accuracy and precision (Li et al., 2015; Mukherjee, 2019). The cations elements examined included elements of nutritional and technological significance, as well as key hazardous metals, such as Ca, Mg, Na, K, Fe, Sr, Si, B, Ba, Cu, Al, Pb, Cd, As, and Hg. Results are presented in mean and standard deviation in ppm (mg/kg) of three independent digestions per fraction (Phillips et al., 2008). Pb, Cd, As, and Hg were not detected below 0.1 ppm; the value below the limit was taken as not detected (Li et al., 2015).

2.4 Data Analysis

Each ICP-MS assay was performed three times on different aliquots that had been digested separately (Li et al., 2015). Elemental concentrations and patterns between the species and fractions were analyzed using descriptive statistics and compared means (Daoub et al., 2018). Since the work is exploratory and the number of fractions is relatively small, it focused on reproducible trends and species-specific signatures as opposed to large-scale hypothesis testing, focusing on macro-element balances, trace-element fingerprints, and the behaviour of regulated heavy metals (Islam et al., 1997; Phillips et al., 2008).

3. RESULTS

3.1 Extraction Yields and Fraction Distribution

Yields were used to describe how the gum mass was distributed among the different solvent fractions. The largest portion of the gum mass was in the aqueous fraction, which confirms the highly hydrophilic and polysaccharide-containing nature of GA (Daoub et al., 2018; Prasad et al., 2022). The aqueous fraction of *A. senegal* represented about 78% of the initial dry weight, and the n-butanol, ethyl acetate and n-hexane fractions were obtained in very low yields. *A. seyal* also exhibited a similar pattern (Islam et al., 1997). The non-polar (n- hexane) fractions of the two species constituted less than 2 percent of the original gum mass, which shows that lipophilic constituents comprise only a small part of the total matrix (Bajpai et al., 2021). This mode of extraction corresponds to the perception of GA as a mostly polysaccharidic hydrocolloid, which still has low concentrations of related non-polysaccharide and mineral substances (Mahendran et al., 2008; Musa et al., 2018)

3.2 Solvent Fractions Macro-Element Profiles

The analysis of ICP-MS revealed that the main macro-elements in all fractions were calcium and magnesium, but their specific concentrations depended on species and solvent (Islam et al., 1997; Daoub et al., 2018). The highest concentration of calcium was found in *A. senegal* n-hexane (7373 ppm), then *A. senegal* ethanol and *A. seyal* ethanol and n-hexane fractions, which is in line with the high affinity of Ca to the uronic acid residues of GA (Yadav et al., 2015). The distribution of magnesium was more variable, ranging from about 703 to 1342 ppm, with *A. seyal* n-hexane having the highest Mg content and *A. senegal* ethanol and aqueous fractions taking intermediate positions (Daoub et al., 2018). There was stronger species dependence in alkali metals. The distributions of potassium and sodium were not as homogeneous as that of Ca and Mg and the distribution patterns of these elements displayed distinct ionic differences between *A. senegal* and *A. seyal* (Karamalla et al., 1998; Yebeyen et al., 2009). Specifically, the *A. seyal* n-hexane fraction had significantly higher K (2750.98 ppm) than the *A. senegal* n-hexane fraction (457.50 ppm), whereas Na was not detected in *A. seyal* n-hexane but was approximately 177.61 ppm and 250.25 ppm in *A. senegal* ethanol and n-hexane, respectively (Islam et al., 1997).

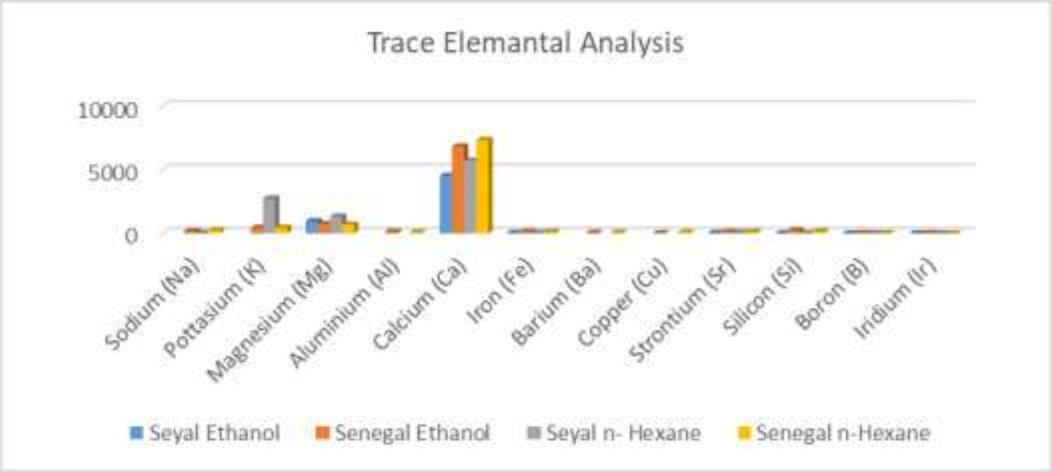
Table 1. Selected macro-elements (ppm) in ethanol and n-hexane fractions of *A. senegal* and *A. seyal* GA

Element	***A. seyal* Ethanol**	***A. seyal* n-Hexane**	***A. senegal* Ethanol**	***A. senegal* n-Hexane**
Ca	4559.10	5733.76	6861.19	7373.00
Mg	977.06	1342.52	754.23	703.50
K	ND	2750.98	453.98	457.50
Na	ND	41.34	177.61	250.25

Note: ND = not detected (< 0.1 ppm) (Islam et al., 1997).

These macro-element patterns suggest that *A. seyal* non-polar fractions are especially enriched in K, whereas *A. senegal* fractions carry higher Ca and Na, which may contribute to species-dependent differences in viscosity, gelation, and interfacial behavior observed in applied systems (Daoub et al., 2018; Karam & Gaiani, 2016; Schmitt et al., 2015).

Figure 1. Macro- and trace-element profiles of ethanol and n-hexane fractions of *Acacia seyal* and *Acacia senegal* gum Arabic. Elemental concentrations (ppm) were determined by ICP-MS and are shown for Na, K, Mg, Ca, Fe, Ba, Cu, Sr, Si, B, and Ir for each fraction (n = 3 digestions per fraction; mean values)



3.3 Trace-element Signatures and Species Differences

Trace-element profiles further distinguished the two gums. *Acacia senegal* fractions had higher amounts of Fe, Sr, Si, B, Al, Ba, and Cu than *A. seyal*, but both species had low Ir levels, which likely reflected regional geochemistry and Acacia's ability to access deeper soil strata (Hnini et al., 2023; Freudenberger, 1991). These species-specific ionic signatures support previous findings that *A. senegal* and *A. seyal* differ in rheology, emulsifying capacity, and structural features, implying that they should not be treated as fully interchangeable (Daoub et al., 2018; Williams & Phillips, 2014; Yakubu et al., 2021).

Table 2. Example trace-element trends (ppm) in n-hexane fractions

Element	***A. seyal* n-Hexane**	***A. senegal* n-Hexane**
Fe	lower	Higher
Sr	lower	Higher
Si	lower	Higher
B	lower	Higher
Ba	lower	Higher
Cu	lower	Higher

Note: Values follow the direction reported by the ICP-MS data; full numeric tables are provided elsewhere (Islam et al., 1997; Daoub et al., 2018).

Such trace-element differences may affect colour stability, redox behaviour, and interactions with other formulation components, adding an inorganic dimension to GA species selection and quality control (Eghbaljoo et al., 2022; López-Franco et al., 2021).

3.4 Heavy Metals and Safety-Related Assessment

GA is used widely as a fibre supplement and as an excipient; therefore, safety considerations are key to this work as suggested by Phillips et al., (2008) and Dauqan & Abdullah, (2013). The concentrations of Pb, Cd, As, and Hg remained below the technique detection limit of 0.1 ppm in all fractions and both species, hence reported as not detected (ND) in line with Li et al., (2015). This further explains that the Kordofan is free of any heavy metal contamination and is safe to use (El Mahi & Magid, 2014). These findings are consistent with the regulatory history of GA, which has long been recognised as a food additive and dietary fibre under international standards (Dondain & Phillips, 1999; Phillips, 2010). They also support clinical studies revealing excellent safety profiles when GA is administered for extended periods of time in patients with chronic kidney disease, metabolic risk, or sickle-cell anaemia (Ali et al., 2008; Chao et al., 2014; Kaddam et al., 2017; Jaafar, 2019). Importantly, the heavy-metal data indicate that fractionation into aqueous and organic phases does not selectively concentrate Pb, Cd, As, or Hg in any fraction at levels of concern, making risk assessment easier for GA-based products that rely on specific fractions or modified forms (Ma et al., 2015; Reis et al., 2006).

4. DISCUSSION

4.1 Elemental Profiles in the Context of GA Structure and Function

The preponderance of Ca and Mg across fractions is fully compatible with GA's uronic acid-rich backbone and high ability to bind divalent cations (Islam et al., 1997; Yadav et al., 2015). These ions can affect chain conformation, inter-chain associations and interfacial behaviour and their elevated levels in *A. senegal* and *A. seyal* contribute to the strong emulsifying and film forming activity of GA in drinks, flavour encapsulates and foams (Williams, 2000; Buffo and Reineccius, 2017; Schmitt et al., 2015). The fact that the concentration of Ca in even non-polar fractions remains high suggests that a fraction of the metal load is firmly fixed to the polysaccharide-protein matrix, or is co-extracted, and not present only in the form of loosely bound surface salts (Daoub et al., 2018; Vanloot et al., 2012).

The different distributions of K and Na provide richness to the picture The *A. seyal* GA exhibited increased levels of K in the n-hexane extracts, which can be attributed to more potassium-rich ionic environment that could be related to the variations in solution behaviour and ionic-strength-sensitive properties, such as viscosity or droplet stabilisation, in certain formulations (Karamalla et al., 1998; Yakubu et al., 2021).In contrast, the increased Na levels in *A. senegal* ethanol and n-hexane fractions may lead to subtle changes in how this gum interacts with electrolytes in complex food or pharmaceutical matrices (Imeson, 2011; Eghbaljoo et al., 2022). Such macro-element balances are rarely published directly, but they can have practical implications when GA is combined with other charged biopolymers, surfactants, or mineral additives (Xu et al., 2019; Hu et al., 2016).

4.2 Species-Specific Ionic Signatures and Quality Implications

The trace-element data support the assumption that *A. senegal* and *A. seyal* are distinctively different raw materials and therefore should not be viewed as a single generic gum, as observed by Daoub et al. (2018) and Williams & Phillips (2014). In our samples, the *A. senegal* fractions were comparatively more in Fe, Sr, Si, B, Ba, and Cu. This gives the species a distinctive geochemical “signature” that may reflect species-specific differences in rooting depth, soil properties, and preferred micro-habitats described in earlier ecological studies (Hnini et al., 2023; Freudenberger, 1991). These inorganic fingerprints line up with previously reported differences in viscosity, emulsifying behaviour, and molecular structure, and together they offer an additional way to distinguish the two species in mixed supply chains (Karamalla et al., 1998; Yebeyen et al., 2009; López-Franco et al., 2021).

From quality-control standpoint, the multi-element profiles could in future be fed into the chemometric models to help with authentication and setting specifications, as suggested by Mukherjee (2019) and Kusters et al. (2023). Ratios that combine Ca, K, and selected trace elements may have promise as practical markers of botanical identity, especially in contexts where contracts or standards call specifically for *A. senegal* GA (Dondain & Phillips, 1999; Yakubu et al., 2021). This is important particularly in high-value uses, like beverages, emulsions, nano-delivery systems, or biomedical products that rely on GA as a biocompatible carrier, where tight control of rheology, flavour protection, and encapsulation performance is crucial (Imam et al., 2013; Hassani et al., 2020; Ahmed et al., 2023).

4.3 Safety Perspective: Heavy Metals and Regulatory Expectations

The uniform non-detection of Pb, Cd, As, and Hg across all fractions and both species is a central outcome of this work, directly addressing regulatory and clinical concerns associated with chronic exposure to trace contaminants (Phillips et al., 2008; Dondain & Phillips, 1999). These elements are among the most tightly controlled toxicants in international standards for food additives and excipients, and detection limits below 0.1 ppm place the Sudanese GA well within typical maximum allowance thresholds (Phillips, 2010; Li et al., 2015). For products in which GA is used as a carrier, stabilizer, or fiber supplement over prolonged periods, such as in dialysis patients or individuals at cardiometabolic risk, this finding reinforces its suitability as a low-risk component (Ali et al., 2008; Chao et al., 2014; Jarrar et al., 2021).

Analyses of Pb, Cd, As, and Hg in *Acacia senegal* (Kordofan) and *Acacia seyal* gums indicate that sequential extraction with water, ethanol, and n-hexane does not lead to a detectable accumulation of these metals in any single phase (Ma et al., 2015; Reis et al., 2006). This homogeneous distribution is particularly relevant for advanced applications, such as nano-enabled systems and GA-based composites, which may utilize specific gum fractions or chemically modified structures. While these findings do not replace the need for batch-specific quality control, they suggest that gums from these sources can meet stringent heavy-metal limits without the necessity for fraction-targeted purification.

4.4 Limitations and Future Directions

Several limitations warrant mention. First, samples were drawn from a single production region and harvest season, so the reported elemental profiles may not capture the full range of geochemical variability across the broader gum belt of Africa (Bedigian, 2005; Makonda, 2003). Second, only selected fractions were analyzed, and the specific binding sites or molecular compartments associated with each element remain to be defined (Nie et al., 2013; Vanloot et al., 2012). Finally, the present work focused on composition rather than on direct correlations with rheological or interfacial measurements, which would be needed to quantify how differences in ionic profiles translate into functional outcomes (Daoub et al., 2018; Eghbaljoo et al., 2022).

Future research should therefore extend multi-element profiling to GA from other countries, ecological zones, and management systems, and combine ICP-MS with structural and rheological analyses to link specific ionic patterns to measurable changes in viscosity, emulsification, and film-forming behavior (Kusters et al., 2023; Williams & Phillips, 2014). It will also be useful to explore how GA's mineral profile interacts with other bioactive ingredients, including plant extracts, nanoparticles, and conventional drugs, in the context of modern delivery systems and antimicrobial strategies (Bilal et al., 2017; Chang et al., 2022; Şen Karaman et al., 2020).

5. CONCLUSIONS

This study provides a detailed multi-element characterization of solvent fractions from Sudanese *Acacia senegal* and *Acacia seyal* gum Arabic and demonstrates that both gums are mineral-rich yet free from detectable heavy-metal contamination under the conditions examined, and in line with Islam et al., (1997) and Li et al., (2015). Calcium and magnesium dominated the elemental profiles, while potassium, sodium, and several trace elements showed clear species- and solvent-dependent variations that extend earlier descriptions of GA ionic composition and highlight distinct inorganic environments around each hydrocolloid matrix (Karamalla et al., 1998; Daoub et al., 2018; Yebeyen et al., 2009).

The non-detection of Pb, Cd, As, and Hg (<0.1 ppm) in every fraction and for both species reinforces the favourable safety profile that underpins GA's acceptance as a food additive, dietary fiber, and pharmaceutical excipient, particularly in applications involving long-term intake (Phillips et al., 2008; Zeid & Farajallah, 2018). At the same time, the species-specific macro- and trace-element signatures observed here support the view that *A. senegal* and *A. seyal* gums are not interchangeable and that their inorganic compositions may contribute to differences in rheology, interfacial behavior, and overall functional performance in complex formulations (Williams & Phillips, 2014; Yakubu et al., 2021).

In conclusion, these findings position Sudanese GA as a safe, mineral-rich hydrocolloid whose fraction- and species-dependent elemental patterns can be exploited to refine quality specifications, support botanical authentication, and guide formulation choices in food, nutraceutical, and pharmaceutical applications (Prasad et al., 2022; Eghbaljoo et al., 2022). Integrating systematic multi-element profiling and heavy-metal screening into routine quality control thus offers a practical route to strengthening both the safety documentation and the functional predictability of GA-based products along evolving value chains (Dondain & Phillips, 1999; Kusters et al., 2023).

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