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# Phytochemical and Trace Metal Analysis of *Agave* Leaves Used in Cosmetics from KwaZulu-Natal, South Africa

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The global preference for natural, bioactive cosmetics has increased interest in plant-based ingredients rich in phytochemicals and essential trace minerals. This study examined the phytochemical composition and trace metal content of *Agave angustifolia* (AA) and *Agave sisalana* (AS) leaves from KwaZulu-Natal, South Africa, to evaluate their potential as raw materials for cosmetics. Methanolic leaf extracts were analysed both qualitatively and quantitatively for phytochemical components using UV-visible spectrophotometry, while functional groups were characterised by Fourier-transform infrared spectroscopy (Agilent Cary 630 FTIR). Trace metal levels were measured with inductively coupled plasma mass spectrometry (Perkin-Elmer NexION® 350 X ICP- MS). Qualitative screening confirmed the presence of key phytochemical classes, including flavonoids, saponins, tannins and phenolic compounds. Quantitative tests showed high levels of phenolics (AA: 771 ppm; AS: 691 ppm), flavonoids (AA: 1280 ppm; AS: 1895 ppm) and tannins (AA: 1378 ppm; AS: 1963 ppm), indicating strong antioxidant potential. Low ascorbic acid levels (0.09-0.13 ppm) suggest limited vitamin C contribution. The high phenolic and flavonoid contents imply the extracts possess significant antioxidant, anti-inflammatory and antimicrobial properties, making them suitable for formulations targeting photoageing, irritation and acne-prone skin. ICP-MS analysis identified eleven trace elements (As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Se, Zn), including essential micronutrients (Zn, Cu and Se), which support collagen synthesis, wound healing and antioxidant defence. The analysis also identified skin sensitizers such as Co and Cr, along with toxic elements like Cd and As, though their levels remained within regulatory limits. However, Pb and Ni were found to slightly exceed the permissible limits set by the European Union and Health Canada. These findings highlight the phytochemical richness and mineral diversity of *A. angustifolia* and *A. sisalana*, supporting their potential as multifunctional bioactive ingredients in natural cosmetic formulations, provided they are carefully sourced and refined under safety standards.

**Keywords: *Agave* leaves, Phytochemicals, Trace metals, Natural cosmetics.**

## INTRODUCTION

The global cosmetic industry is undergoing a significant transformation, driven by increasing consumer demand for safer, eco-friendly and naturally derived products [1,2]. At the heart of this change is an increasing interest in plant-based ingredients, especially phytochemicals, which are naturally occurring bioactive compounds in plants that offer various therapeutic benefits for skin health. These include antioxidant, anti-inflammatory, antimicrobial and anti-ageing properties [3]. In addition to phytochemicals, essential and trace metals found in plants also promote skin health by supporting enzymatic processes, boosting collagen production and preserving the structure of dermal tissues. However, it is crucial to carefully monitor the accumulation of heavy metals to ensure safety, as high levels can pose toxicity risks [4]. Among the many botanical species studied for their cosmetic potential, *Agave* species are among the most promising due to their unique phytochemical profiles, ecological resilience and ability to adapt to diverse environments [2]. Native to arid and semi-arid regions of North America, these succulents, belonging to the genus *Agave* in the family Agavaceae, are now found worldwide, including in China, Brazil, Mexico, Tanzania and large parts of Southern Africa [5-8]. Their capacity to thrive in nutrient-poor soils and withstand prolonged droughts makes them valuable for sustainable agriculture and bioresource industries.

Traditionally, various *Agave* species have been used in ethnomedicine to treat ailments and skin conditions related to microbial infections, inflammation and oxidative stress, conditions often linked to free radical damage to the skin and hair, which contribute to signs of ageing, such as fine lines, wrinkles and dull skin [9]. Their therapeutic effects are attributed to a range of bioactive constituents, including steroidal saponins, flavonoids, phenolic acids and inulin-type fructans, all of which show potential for use in dermatological formulations due to their antioxidant, anti-inflammatory and antimicrobial properties [10]. Although *A. angustifolia* (Fig. 1a) and *A. sisalana* (Fig. 1b) are primarily cultivated for sisal fibre used in ropes and textiles, their potential as cosmetic ingredients remains underexplored. Preliminary research suggests that extracts from these species contain phytoconstituents that can enhance the skin barrier, reduce inflammation and neutralise oxidative stress, features particularly relevant for anti-ageing and skin-protective formulations [11].

Despite their traditional and industrial importance, scientific research on *Agave* species found in South Africa, particularly in KwaZulu-Natal, where these plants are ubiquitous, remains limited. Given the region's favourable agro-climatic conditions and rich biodiversity, it is crucial to carry out the comprehensive assessments of these species' phytochemical and metal compositions. This research is essential not only for unlocking their cosmetic potential but also for ensuring product safety, efficacy and regulatory compliance according to international standards.

Mtunzi *et al.* [12] examined large traditional medicinal and edible plant species from KwaZulu-Natal, reporting that various plants contained both beneficial essential elements (*e.g.*, zinc, copper, magnesium, calcium) and potentially harmful heavy metals (*e.g.*, lead, cadmium), although most remained within safe limits. Moreover, a recent study by Zondo [13] found that toxic metals exceeded safe thresholds in some edible plant parts. Other studies highlight the need for thorough screening of these *Agave* species before utilisation in the cosmetic production [14]. Hence, the present study carried out qualitative screening and quantitative evaluation of methanolic leaf extracts from *A. angustifolia* (AA) and *A. sisalana* (AS) to determine their phytochemical composition and potential for use in natural cosmetics. In addition, metal content was analysed using ICP-MS [15] to provide a comprehensive understanding of these plant-based ingredients with respect to safety, efficacy and biodiversity.

## EXPERIMENTAL

Methanol, water, sodium hydroxide, glacial acetic acid, ferric chloride, chloroform, sulfuric acid, acetic anhydride, hydrochloric acid, thiourea, Folin-Cinocalteu reagent, sodium carbonate, aluminium chloride, sodium nitrite, metaphosphoric acid, 2,4-dinitrophenylhydrazine (DNPH) reagent, nitric acid, rutin, quercetin, ascorbic acid, tannic acid and gallic acid were analytical grade chemicals purchased from Sigma-Aldrich, South Africa and MINEMA Chemicals Pty. Ltd., South Africa.

**Sample collection and preparation:** Mature and healthy leaves of *A. angustifolia* (AA) and *A. sisalana* (AS) were collected from Stanger 29º20′17.5″S 31º17′21.3″E and Ingwavuma 27º08′20.2″S 32º01′06.3″E in KwaZulu-Natal province, South Africa. The samples were washed under running tap water to remove dust and soil particles, then rinsed with distilled water. The clean leaves were chopped into small pieces and dried at ambient temperature in an efficient fume hood for two weeks to preserve heat-sensitive phytoconstituents. The dried material (50 g) was then ground into a fine powder using a laboratory grinder and stored in airtight containers for phytochemical extraction processes. The remaining dried samples were kept for metal evaluation.

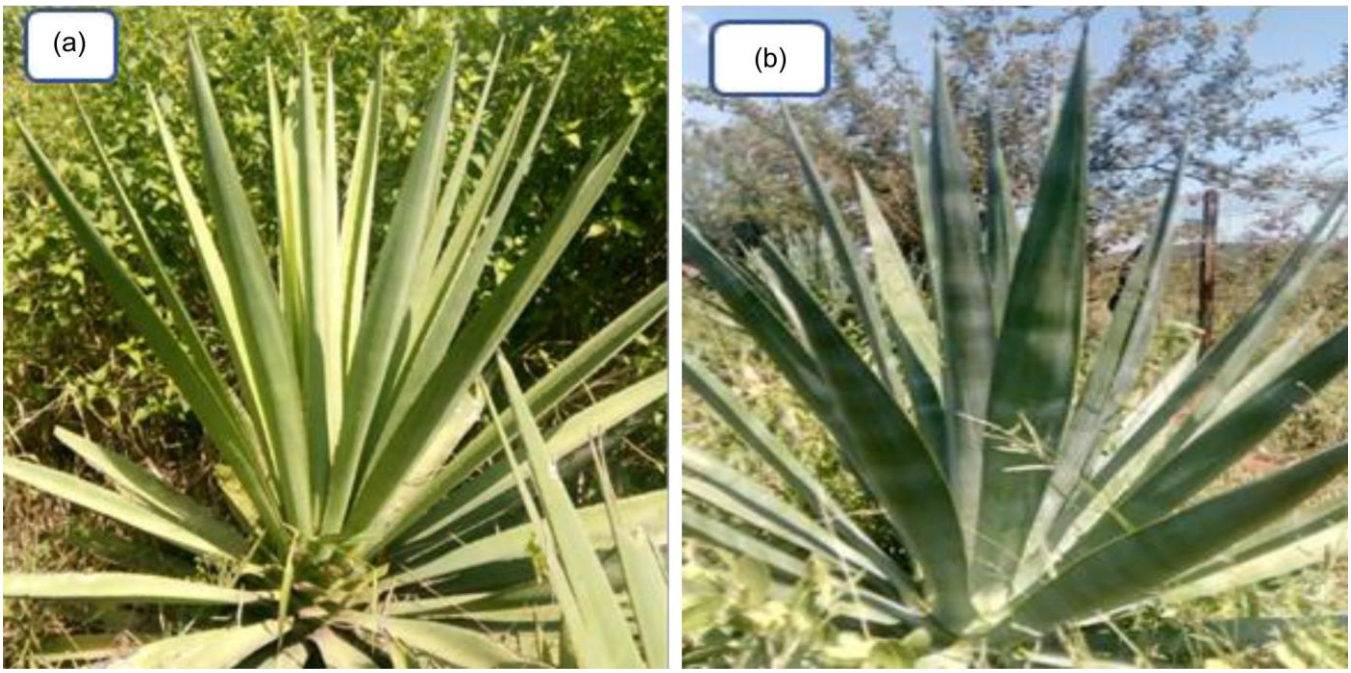


Fig. 1. *Agave angustifolia* plant (a), *Agave sisalana* plant (b)

Approximately 50 g of each powdered sample was subjected to maceration at ambient temperature in 500 mL of 80% methanol for 72 h, with occasional agitation to ensure thorough solvent penetration. Methanol was chosen for its polarity and effectiveness in extracting a wide range of phytochemicals, including flavonoids, saponins and phenolics [16]. After maceration, the extracts were filtered through Whatman No. 1 filter paper. The filtrates were then concentrated using a rotary evaporator under reduced pressure at 40 ºC to remove excess solvent. The resulting crude extracts were stored in a fridge at 4 ºC until analysis.

**Qualitative phytochemical screening:** Standard protocols outlined by Balamurugan *et al.* [17] were employed to detect major classes of phytochemicals. Each test was conducted in triplicate for reproducibility.

**Test for flavonoids (NaOH test):** An intense yellow colour was produced when 2 mL of 2.0% NaOH solution was added to 1 mL of aqueous *Agave* leaf extract. This colour changed to colourless when 2 drops of acetic acid were added, indicating the presence of flavonoids.

**Test for tannins:** *Agave* extract (0.5 mL) and water (10 mL) were mixed in a test tube. After boiling and filtering the mixture, a few drops of 0.1% $FeCl_3$ were added and gently shaken. The development of a blue-black colouration in the mixture confirms the presence of tannins.

**Test for phenols ($FeCl_3$ test):** About 5 drops of 5% $FeCl_3$ were added to 1 mL of plant extract, a yellow-green fluorescence was observed, indicating the presence of resorcinol.

**Test for terpenoids (Salkowski test):** About 2 mL of plant extract was dissolved in 2 mL of chloroform in a clean test tube. Subsequently, 2 mL of conc. $H_2SO_4$ was carefully added along the sides of the test tube to form a distinct layer beneath the chloroform. The test tubes were then observed for the formation of a colour interface. A reddish-brown at the junction of the two layers was taken as a positive indication of the presence of terpenoid compounds.

**Test for saponins:** To 1 mL of *Agave* extract, 3 mL of distilled water and 5 drops of olive oil were added. Then, the mixture was shaken vigorously for about 1-2 min. A persistent foam was observed, which confirmed the presence of saponins in the plant extract.

**Test for steroids (Liebermann-Burchard test):** To a 1 mL of *Agave* chloroform extract, 2 mL of acetic anhydride was added, followed by 2 mL of 0.5 mL of conc. $H_2SO_4$. The reaction mixture changed from violet to green, indicating the presence of steroids.

**Test for glycoside (Keller-Killian test):** Glacial acetic acid (2 mL) and 5 drops of 2.0% $FeCl_3$ were added to 5 mL of aqueous *Agave* extract and the mixture was shaken well. To the mixture, 1 mL of conc. $H_2SO_4$ was added and a brownish ring was observed between the layers, which suggests the presence of cardiac steroidal glycosides.

**Test for alkaloid (Wagner's test):** To a 2 mL of aqueous *Agave* extract was added 1.5% HCl, followed by the addition of a few drops of Wagner's reagent. The appearance of a brownish precipitate confirmed the existence of alkaloids in the sample.

**Quantitative phytochemical analysis:** Quantitative phytochemical analysis using UV/Vis spectroscopy (Genesys 10S) was performed to measure the levels of key bioactive constituents *viz.* total phenolics, total flavonoids, total tannins and ascorbic acid (vitamin C) in the methanolic leaf extracts of AA and AS. These secondary metabolites are known to significantly contribute to the antioxidant, anti-inflammatory and skin-rejuvenating properties relevant to cosmetic and dermatological uses [3,18].

**Total phenolic content (TPC):** The total phenolic content of each extract was determined using the Folin-Ciocalteu reagent assay, as described by Ainsworth & Gillespie [19], with minor modifications. A 0.5 mL of extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 min, then 2 mL of 7.5% $Na_2CO_3$ was added. The reaction mixture was kept in the dark for about 30 min at room temperature. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer.

**Total tannin content (TTC):** The total tannin content was determined using tannic acid as a standard, following the method described by Ainsworth & Gillespie [19], with slight modifications. A stock solution was prepared by weighing 50 mg of tannic acid into a 50 mL volumetric flask and diluting to volume with methanol. From this, a working standard solution of 50 µg/mL was prepared by diluting 5 mL of the stock solution to 100 mL with distilled water. For calibration, aliquots of 1, 2, 3, 4 and 5 mL of the working standard solution were transferred into separate 50 mL volumetric flasks. To each flask, 1 mL of 5% $Na_2CO_3$ and 0.5 mL of 1 N Folin-Ciocalteu reagent were added and the mixture was thoroughly mixed. The mixtures were incubated for 30 min at room temperature and absorbance was measured at 700 nm against a blank (deionised water) using a UV-Vis spectrophotometer.

**Total flavonoid content (TFC):** The flavonoid concentration was assessed using the $AlCl_3$ colorimetric method described by Chang *et al.* [20]. About 1 mL of plant extract was mixed with 0.3 mL of 5% $NaNO_2$, followed by 0.3 mL of 10% $AlCl_3$ after 5 min. After an additional 6 min, 2 mL of 1 M NaOH was added to the mixture and the volume was adjusted to 10 mL with distilled water. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer.

**Total ascorbic acid (TAA):** Total ascorbic acid in AA and AS methanolic leaf extracts was measured using a UV-Vis spectrophotometric method. About 1 g of dried extract was mixed with 25 mL of 5% metaphosphoric acid, filtered and treated with bromine water in acetic acid to oxidise ascorbic acid to dehydroascorbic acid. Thiourea was added to neutralise excess bromine, followed by 1 mL of DNPH reagent. The mixture was incubated at 37 ºC for 3 h. After adding 5 mL of 85% $H_2SO_4$, the absorbance was measured at 521 nm [21].

**Microwave digestion method for ICP-MS trace metal analysis:** Dried and chopped leaf samples of AA and AS (0.5 g each) were independently digested in triplicate by soaking in 20 mL of freshly prepared aqua regia (5 mL $HNO_3$ + 15 mL HCl) and processed using an Anton Paar Multiwave PRO microwave digestion system in accordance with established plant matrix protocols [22]. Post-digestion, each sample was diluted to 100 mL with distilled water, filtered through Whatman No. 42 paper and refrigerated until analysis. Elemental quantification for trace metal contents of the samples was performed using a Perkin-Elmer NexION® 350X ICP-MS.

## RESULTS AND DISCUSSION

**Determination of trace elements:** Table-1 presents the concentrations of trace elements (in ppm) detected in the leaf tissues of AA and AS following microwave-assisted acid digestion and ICP-MS analysis. The elements analysed include both essential micronutrients (*e.g.*, Zn, Cu, Se) and potentially toxic metals (*e.g.*, Pb, Cd, As, Cr), providing insight into the safety and suitability of the *Agave* species for cosmetic applications.

### Comparative analysis of trace elements: Significance to cosmetic formulation

**Toxic elements and safety implications:** Toxic trace metals, including As, Cd and Pb, were found at higher concentrations in sample AA compared to sample AS. Specifically, Pb levels measured 21.33 ppm in AA and 13.6 ppm in AS samples. Both values surpass the typical limits recommended for raw plant materials used in cosmetics [1]. Prolonged exposure to lead can cause dermal toxicity and systemic effects such as neurotoxicity and kidney damage [23]. Arsenic and cadmium are classified as Group 1 carcinogens by the International Agency for Research on Cancer [24]. In AA sample, As was present at 0.291 ppm and Cd at 0.134 ppm. In contrast, AS showed lower levels, for example, 0.088 ppm for As and 0.038 ppm for Cd. These findings suggest that AS may pose a lower toxicological risk and is likely to be more compliant with international cosmetic safety standards. This assertion is supported by the recommendations of Health Canada (2012), which proposes acceptable limits of ≤ 3 ppm for both arsenic and cadmium [25].

**Beneficial trace elements:** Essential elements, including Zn, Cu and Se, known for their biological and dermatological significance, were present in both AA and AS. Zn, slightly higher in AS (0.27 ppm) than in AA (0.25 ppm), contributes to skin barrier repair, inflammation regulation and wound healing [26]. Cu, at 0.088 ppm in AA and 0.063 ppm in AS, is crucial for enzymatic processes involving collagen and elastin synthesis, making it advantageous in anti-ageing formulations [27]. Se was detected in trace amounts (0.0004-0.0005 ppm) in both plants; its antioxidant properties aid in protecting keratinocytes from oxidative damage, thereby maintaining skin structure and function [28].

**Allergenic and industrial trace elements:** Elements such as Ni, Co and Cr, though not severely regulated in most countries and regions, are known sensitisers and may trigger allergic contact dermatitis [28]. Nickel concentrations were 5.24 ppm in AA and 3.23 ppm in AS, both surpassing the typical thresholds for cosmetic products. Cobalt level is also slightly elevated in AA relative to AS. The presence of these metals necessitates caution when formulating products for sensitive or allergic skin types and may require the application of purification or chelation techniques during extraction processes.

Thus, compared to AA, AS shows a more desirable trace element composition for the cosmetic applications. Its lower content of harmful and allergenic metals supports its use as a safer ingredient, especially in products intended for prolonged skin contact. Incorporating regular heavy metal analysis [30] can further ensure product safety and compliance.

**Metals in cosmetics: Functions and threshold limits:** Table-2 summarises the functions, skin effects and typical regulatory threshold limits of selected metals relevant to cosmetic formulations. The table distinguishes between essential metals intentionally included for their dermatological benefits and those present as trace impurities, highlighting applicable international safety limits.

**Toxic metals in cosmetics: Health effects, threshold limits and safety concerns:** Table-3 summarises toxic metals that are generally of concern in cosmetics, their effects on human health and regulatory threshold limits. These metals are not intentionally added to cosmetic products but may be present as contaminants or impurities in raw materials, such as *Agave* leaf extracts.

**Qualitative phytochemical analysis:** Qualitative screening of methanolic leaf extracts from AA and AS confirmed the presence of diverse secondary metabolite with recognised dermatological benefits (Table-4). Both species tested positive for flavonoids, phenolics, saponins, tannins, steroids, glycolsides, alkaloids and terpenoids, albeit with minor differences in abundance. Flavonoids were abundant in both species, providing antioxidant and anti-inflammatory properties that help prevent oxidative stress, soothe the sensitive skin and delay signs of ageing [27,38]. Phenolic compounds were especially prominent in AA, indicating strong antioxidative capabilities and functions that support the skin barrier. Saponins, found in moderate amounts in both extracts, are valued for their

TABLE-1
LIST OF TRACE ELEMENTS PRESENT IN *Agave angustifolia* (AA) AND *Agave sisalana* (AS) LEAVES

| Elements | Concentration (ppm) | | Relative standard deviation RSD% | | Standard deviation SD (ppm) | |
|---|---|---|---|---|---|---|
| | AA | AS | AA | AS | AA | AS |
| As | 0.291 | 0.088 | 2.1 | 2.4 | 0.006 | 0.002 |
| Cd | 0.134 | 0.038 | 0.9 | 2.4 | 0.0012 | 0.0009 |
| Co | 0.0103 | 0.0058 | 4.0 | 3.5 | 0.0004 | 0.0002 |
| Cr | 0.027 | 0.029 | 1.2 | 0.3 | 0.0003 | 0.000087 |
| Cu | 0.088 | 0.063 | 0.3 | 0.3 | 0.00026 | 0.000189 |
| Mo | 0.127 | 0.134 | 0.5 | 0.1 | 0.0006 | 0.00013 |
| Ni | 5.24 | 3.23 | 2.0 | 2.1 | 0.105 | 0.069 |
| Pb | 21.33 | 13.6 | 0.2 | 0.6 | 0.043 | 0.082 |
| Sb | 0.044 | 0.045 | 1.7 | 1.9 | 0.00075 | 0.00086 |
| Se | 0.0004 | 0.0005 | 10.4 | 9.0 | 0.000046 | 0.000045 |
| Zn | 0.250 | 0.27 | 0.8 | 0.5 | 0.002 | 0.0014 |

TABLE-2
LIST OF METALS IN COSMETICS: FUNCTIONS AND THRESHOLD LIMITS

| Metal | Function/effect on skin | Typical threshold limit in cosmetics/remarks | Ref. |
|---|---|---|---|
| Zn | Antimicrobial, anti-inflammatory, regulates sebum, promotes healing, UV protection | Not limited (commonly used as ZnO/$TiO_2$ in sunscreens); ZnO: up to 25% (FDA, EU) | [4] |
| Cu | Supports collagen/elastin synthesis, wound healing, antioxidant, antimicrobial | ~20 ppm (as impurity, not intentionally added) | [4,31] |
| Mg | Enhances hydration, skin barrier, anti-inflammatory | Not specifically restricted (low toxicity, widely used) | [32,33] |
| Ca | Supports skin renewal, keratinocyte function, barrier maintenance | Not restricted (considered safe at normal concentrations) | [4,33] |
| Se | Antioxidant, anti-ageing, protects against UV damage | ≤ 0.01 ppm (EU); restricted due to potential toxicity | [31,34] |
| Fe | Supports skin metabolism, essential for oxygen transport (excess may cause oxidation) | ≤ 50 ppm (as contaminant) | [4,30] |
| Mn | Antioxidant enzyme cofactor, wound healing support | ≤ 10 ppm (as impurity) | [4,31] |
| Si | Collagen synthesis, skin elasticity, strengthens nails and hair | Not restricted (commonly used in silicones like dimethicone) | [35] |

TABLE-3
LIST OF TOXIC METALS IN COSMETICS AND THEIR HEALTH EFFECTS, THRESHOLD LIMITS AND SAFETY CONCERNS

| Metal | Health effects/concerns | Regulatory threshold limit (cosmetics) | Ref. |
|---|---|---|---|
| Pb | Neurotoxicity, developmental toxicity, renal damage, reproductive toxicity | ≤ 10 ppm (Canada); ≤ 2 ppm (Germany); Must be technically unavoidable (EU) | [25,30,31] |
| As | Carcinogenic, skin irritation, endocrine disruption, hepatotoxicity | ≤ 3 ppm (Canada); ≤ 0.5 ppm (Germany); Not allowed as ingredient (EU) | [25,31] |
| Cd | Nephrotoxicity, carcinogenicity, bone demineralisation, skin absorption risk | ≤ 3 ppm (Canada); ≤ 0.1 ppm (Germany); Not permitted (EU) | [30,31] |
| Hg | Neurotoxicity, immunotoxicity, skin rashes, kidney damage | ≤ 1 ppm (as impurity only); Allowed at 0.007% in eye-area products (USA) | [36,37] |
| Ni | Allergen, causes contact dermatitis, especially in sensitive individuals | ≤ 1 ppm (as impurity); must be technically unavoidable (EU) | [31] |
| Cr | Allergenic, irritant and potentially carcinogenic in hexavalent form ($Cr^{6+}$) | ≤ 1 ppm (as impurity); not intentionally added (EU) | [30,31] |

TABLE-4
QUALITATIVE PHYTOCHEMICAL SCREENING DATA OF AA AND AS EXTRACTS

| Phytochemical class | Test applied | *Agave angustifolia* | *Agave sisalana* |
|---|---|---|---|
| Alkaloids | Wagner's reagent | ++ | +++ |
| Flavonoids | Alkaline reagent test | +++ | +++ |
| Tannins | Ferric chloride test | ++ | ++ |
| Saponins | Frothing test | ++ | ++ |
| Terpenoids | Salkowski test | ++ | + |
| Steroids | Liebermann-Burchard test | ++ | ++ |
| Phenols | Ferric chloride test | +++ | ++ |
| Glycosides | Keller-Killiani test | ++ | ++ |

Intensity: +++ = abundant, ++ = present, + = trace.

natural cleansing, emulsifying and gentle antimicrobial effects, making them ideal for formulations aimed at sensitive or acne-prone skin [39]. AS showed a higher alkaloid content, indicating enhanced antimicrobial and wound-healing potential, suitable for blemish-prone or inflamed skin [40]. Terpenoids were more prominent in AA and are linked to regenerative, anti-inflammatory and aromatic properties [41]. Steroids and glycosides were present in both species and contribute to anti-inflammatory effects, hydration and barrier reinforcement [1].

**Quantitative phytochemical analysis:** UV/Vis spectrophotometric quantification further confirmed the presence and levels of key biologically active compounds in both species (Table-5). AA methanol extract contained higher total phenolics (771.00 ppm), while AS showed higher concentrations of tannins (1963.15 ppm) and flavonoids/quercetin (1895.86 ppm). Ascorbic acid was found in small amounts in both species, but it is known to contribute to a synergistic antioxidant effect [42]. The calibration curves for the TPC, TTC, TFC and TAA are shown in Fig. 2.

The phytochemical contents of AA and AS methanolic extracts are presented in Table-5. All analyses were performed in triplicate and results are expressed as mean ± standard deviation (SD). Statistical comparisons between AA and AS methanolic extracts were carried out using an unpaired student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

Both extracts contained appreciable levels of phenolics, tannins and flavonoids; however, significant differences were observed between the two species. AA exhibited a significantly higher total phenolic content compared to AS ($p < 0.05$). In contrast, AS showed significantly higher concentrations of total tannins and total flavonoids ($p < 0.05$). The ascorbic acid content of AS was also significantly greater than that of AA ($p < 0.05$). The higher phenolic content in AA indicates

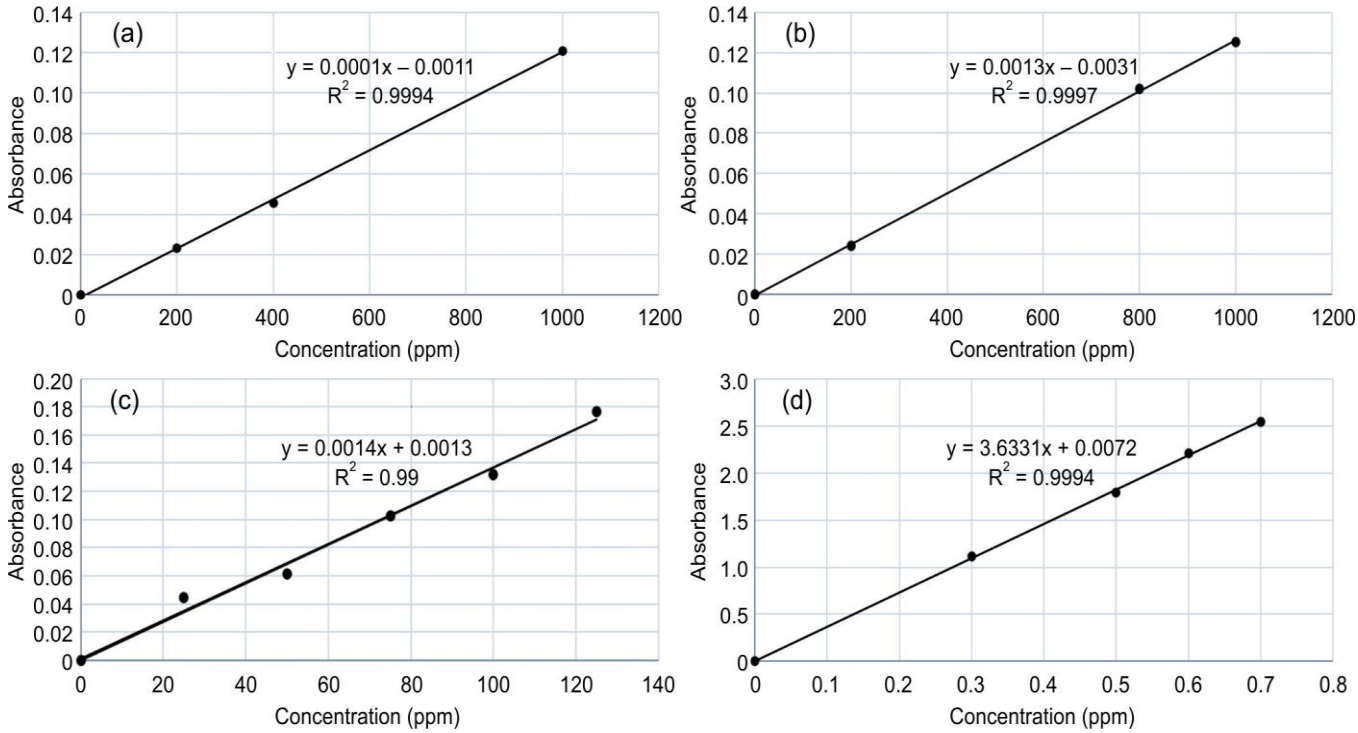


Fig. 2. Calibration curve of (a) total phenolic content, (b) total tannin content, (c) total flavonoid and (d) total ascorbic acid content

TABLE-5
LIST OF PHYTOCHEMICAL CONTENTS IN
HE METHANOLIC EXTRACTS OF AA AND AS

| Phytochemical assay | AA (ppm) | AS (ppm) |
|---|---|---|
| Total phenolics (TPC) | 771.00 ± 18.42[a] | 691.00 ± 15.36[b] |
| Total tannins (TTC) | 1378.15 ± 32.10[b] | 1963.15 ± 41.88[a] |
| Total flavonoids (TFC) | 1280.50 ± 29.75[b] | 1895.86 ± 37.62[a] |
| Ascorbic acid (TAA) | 0.09 ± 0.01[b] | 0.13 ± 0.01[a] |

Values are expressed as mean ± standard deviation (SD) of three independent determinations (n = 3).
Values in the same row followed by different superscript letters ([a],[b]) differ significantly ($p < 0.05$).

a higher antioxidant capacity, making it suitable for formulations targeting oxidative stress, photoaging and environmental damage [43,44]. Similarly, increased tannins and quercetin levels in AS offer strong astringent, anti-inflammatory and antimicrobial effects, ideal for acne-prone, sensitive or irritated skin [45].

**FTIR studies:** Table-6 shows a FTIR spectral data of AA and AS. The overlaid spectra highlight similar and distinct functional group absorptions, including broad O–H stretching (~3400-3200 $cm^{-1}$), aliphatic C–H stretching (~2920-2850 $cm^{-1}$), carbonyl C=O vibrations (~1730-1650 $cm^{-1}$), aromatic/olefinic C=C bands (~1600-1500 $cm^{-1}$) and C–O/C–O–C

TABLE-6
LIST OF KEY FTIR PEAKS IDENTIFIED IN THE METHANOLIC LEAVES EXTRACTS of *Agave angustifolia* (AA) AND *Agave sisalana* (AS)

| Wavenumber ($cm^{-1}$) | | Assignment & possible phytochemicals | | Commonality |
|---|---|---|---|---|
| AA | AS | *Agave angustifolia* (AA) | *Agave sisalana* (AS) | |
| 3440.3 | 3272.6 | O–H stretching (alcohols, phenols) | O–H stretching (phenolics, polysaccharides) | Similar |
| 3272.6 | 3026.6 | N–H/O–H stretching | =C–H stretching (alkenes) | Different |
| 3015.4 | 2970.7 | =C–H stretching (alkenes) | C–H stretching (alkanes) | Different |
| 2970.7 | 2918.5 | C–H stretching (alkanes) | $CH_2$ stretching (lipids) | Different |
| 1736.9 | 2847.7 | C=O stretching (esters, triglycerides) | $CH_2$ symmetric stretching | Different |
| 1621.9 | 1736.9 | C=C/amide I | C=O stretching (esters, triglycerides) | Different |
| 1533.4 | 1589.4 | Amide II/N–H bend | Aromatic C=C/amide II | Different |
| 1435.0 | 1533.4 | $CH_2$ scissoring (lipids) | Amide II/N–H bend | Different |
| 1367.9 | 1423.8 | $CH_3$ bending | $CH_2$ scissoring (lipids) | Different |
| 1215.1 | 1367.9 | C–O stretching (esters, polysaccharides) | $CH_3$ bending | Different |
| 1036.2 | 1215.1 | C–O stretching (glycosides) | C–O stretching (esters, polysaccharides) | Similar |
| 902.0 | 1148.0 | =C–H bending | C–O–C stretching (polysaccharides) | Different |
| 838.7 | 1028.7 | Aromatic C–H bending | C–O stretching (fructans) | Different |
| 775.3 | 898.3 | Vinyl C–H bending | β-glycosidic linkage (saponins) | Different |
| | 720.1 | | $(CH_2)_n$ rocking (long-chain lipids) | – |
| | 667.5 | | Alkene C–H bending | – |

stretching in the fingerprint region (~1200-1000 $cm^{-1}$). The variations in band intensity and position reflect differences in phytochemical composition between AA and AS.

**Comparative FTIR spectra of AA and AS leaf extracts with cosmetic relevance:** The overlaid spectra reveal both shared and distinct functional group absorptions, including broad O–H stretching (~3400-3200 $cm^{-1}$), aliphatic C–H stretching (~2920-2850 $cm^{-1}$), carbonyl C=O vibrations (~1730-1650 $cm^{-1}$), aromatic or olefinic C=C bands (~1600-1500 $cm^{-1}$) and C–O/C–O–C stretching within the fingerprint region (~1200-1000 $cm^{-1}$). Differences in peak intensity and position reflect variations in the phytochemical composition between AA and AS (Fig. 3).

The broad O–H band is the characteristic of phenolics, flavonoids and polysaccharides, indicating strong hydrogen-bonding capacity and potential humectant, antioxidant and soothing properties relevant to skin hydration and barrier support. Aliphatic C–H bands suggest the presence of lipidic and waxy constituents that contribute to emollience and skin-conditioning effects. The carbonyl absorptions are associated with esters, fatty acids and amide-containing compounds, supporting roles in skin repair, mild exfoliation and formulation stability. Aromatic C=C vibrations indicate phenolic structures linked to antioxidant and anti-inflammatory activity, while the fingerprint region confirms alcohols, glycosides and polysaccharides that impart moisturising and film-forming functionality.

Phytochemical profiling supports the FTIR findings, showing both species to be rich in secondary metabolites. AS contained higher levels of flavonoids (1895.86 ppm) and tannins (1963.15 ppm) than AA (1280.50 and 1378.15 ppm, respectively), whereas AA exhibited a higher total phenolic content (771.00 ppm *vs.* 691.00 ppm). Accordingly, AA demonstrates stronger phenolic-associated antioxidant potential, while AS shows greater contributions from lipids and saponins, suggesting enhanced surfactant and emollient functionality for cosmetic formulations [1-4].

## Comparative evaluation of AA and AS relative to their effect in cosmetics

**Role and benefits of phytochemicals in cosmetics:** As consumer awareness of the potential adverse effects of synthetic ingredients grows, the demand for natural, multifunctional and eco-friendly cosmetic products has increased. This shift aligns with the principles of clean and green beauty, which emphasise safety, sustainability and effectiveness through the use of plant-based ingredients [2,3]. Among these, both medicinal and non-conventional plants are being re-evaluated for their cosmetic potential, especially for their rich phytochemical profiles and therapeutic skin benefits [46]. *Agave* species, particularly AA and AS, are traditionally recognised for their fibre production; however, they also produce a variety of secondary metabolites with potential cosmetic uses. These xerophytic plants are found across different regions in South Africa, including KwaZulu-Natal, where local soil and climate conditions may affect their phytochemical and mineral content. Phytochemicals such as flavonoids, saponins, tannins and phenolic acids are commonly found in *Agave* and have been shown to support skin health by neutralising free radicals, enhancing skin barrier function, reducing inflammation and promoting collagen synthesis [3,39]. These properties are vital for creating anti-ageing, soothing and skin-repairing formulations.

The diverse and synergistic phytochemical composition of AA and AS supports their use as multifunctional botanical ingredients in cosmetics [47]. Their natural bioactives, particularly flavonoids, phenolics, tannins, saponins and mucilage like polysaccharides, offer dermatological benefits, including:

**(a): Antioxidant and anti-ageing:** Flavonoids and phenolics reduce oxidative stress and support collagen synthesis, ideal for serums and day creams [43].

**(b): Cleansing and astringency:** Saponins and tannins regulate sebum, tighten pores and combat microbial growth in cleansers, toners and scalp treatments [48].

**(c): Moisturising and skin barrier support:** Polysaccharides and glycosides improve hydration, elasticity and barrier function in masks and moisturisers [49].

**(d): Soothing and post-sun care:** Quercetin and phenolic acids help alleviate UV-induced irritation and redness in after sun gels and soothing formulations [50].

Furthermore, their solubility in both aqueous and alcohol-based systems provides versatility for various formulations, including creams, gels, emulsions and sprays. Their botanical origin aligns with the growing clean beauty trend, reflecting consumer demand for sustainable, plant-based skincare [51]. Both AA and AS display rich phytochemical profiles with promising uses in cosmetic science. While AA excels in antioxidant activity due to its higher phenolic content, AS provides stronger anti-inflammatory and astringent effects owing to its high content of tannins and flavonoids. These distinct

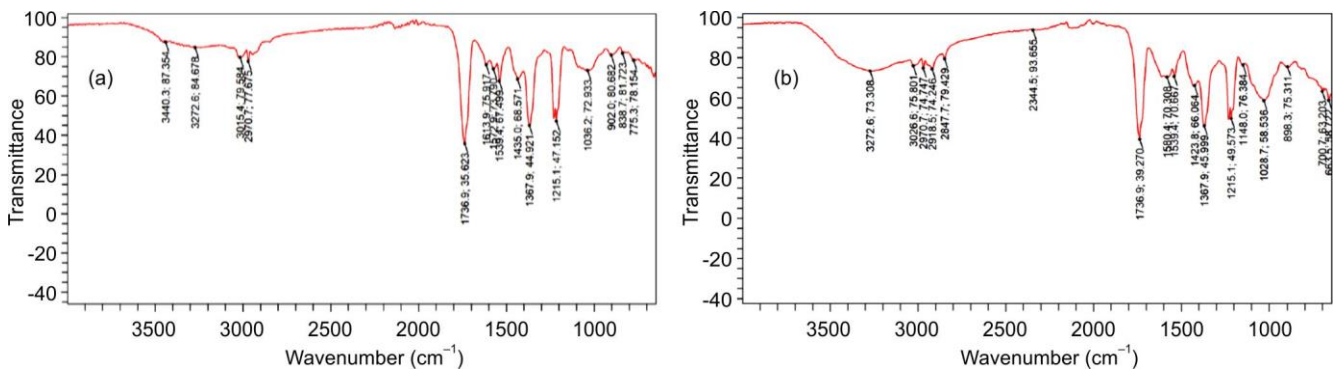


Fig. 3. Comparative FTIR spectra of the methanolic leaves extracts of (a) *Agave angustifolia* (AA) and *Agave sisalana* (AS)

yet complementary profiles allow for targeted formulation strategies in skincare and haircare.

**Benefits of trace metals in cosmetics:** Several trace metals present in both AA and AS are essential for skin repair, hair growth and cellular metabolism. Both agave species contain essential trace elements, including Zn, Cu and Se, which are known to support skin health. Zinc, detected in comparable amounts in both AA (0.250 ppm) and AS (0.270 ppm), is a critical element in skin barrier repair, anti-inflammatory response and hair follicle health [26]. Zinc's role in enzyme function and tissue regeneration makes it a desirable component in anti-acne and scalp soothing products [26]. Copper, slightly higher in AA (0.088 ppm) than in AS (0.063 ppm), plays a role in collagen cross-linking, angiogenesis and melanin synthesis, which are essential processes for maintaining skin elasticity and hair pigmentation [52,53]. Molybdenum, detected at near-equal levels (AA: 0.127 ppm; AS: 0.134 ppm), is a cofactor in various oxidative enzymatic processes. Though less explored in dermocosmetics, its presence supports cellular detoxification mechanisms [54]. Selenium, present in trace amounts in both AA and AS (0.0004-0.0005 ppm), is important for antioxidant defence systems, protecting the skin from oxidative and UV damage through enzymes such as glutathione peroxidase [28].

Table-7 summarises the trace elements found in AA and AS leaves, emphasising their roles in skin and hair health. It also describes their potential cosmetic uses based on known bioactivities.

**Trace metal composition of AA and AS: Implications for cosmetic:** Besides beneficial metals, plants can also accumulate toxic heavy metals such as Pb, Cd, As and Hg, especially when grown in contaminated soils. These elements pose significant health risks, as they can be absorbed through the skin; therefore, they are strictly regulated in cosmetic products [30]. Their presence emphasises the importance of rigorous screening and quality control of plant-based ingredients.

Measurement of the samples using ICP-MS showed higher levels of toxic metals (As, Cd and Pb) in AA compared to AS. Notably, Pb concentrations in AA (21.33 ppm) and AS (13.6 ppm) exceeded the permissible limits recommended for plant-based raw materials used in cosmetics [1]. Arsenic and cadmium, both classified as Group 1 carcinogens by the International Agency for Research on Cancer [24], were also higher in AA (0.291 and 0.134 ppm, respectively) than in AS (0.088 and 0.038 ppm). These results show that AS poses a lower toxicological risk and may be more suitable for formulations designed for extended skin contact.

Some trace metals, though essential in minute amounts, may pose allergenic risks in sensitive individuals (Table-2). Allergenic elements such as Ni, Co and Cr were also detected. Nickel, detected at significantly higher levels in AA (5.24 ppm) than in AS (3.23 ppm), is a common contact allergen that may cause dermatitis, especially in leave-on formulations [29]. This level exceeds acceptable limits (≤ 1 ppm) set by regulatory agencies for cosmetic ingredients [31]. Cobalt (Co) and chromium (Cr) are recognised for their allergenic and sensitising properties, particularly under conditions of chronic or cumulative exposure. While total Cr levels appear similar between the species (AA: 0.027 ppm; AS: 0.029 ppm), the toxicological impact depends greatly on its oxidation state, with hexavalent chromium [Cr(VI)] being significantly more toxic and allergenic than trivalent chromium [Cr(III)] [57]. Similarly, cobalt is a well-documented sensitiser associated with allergic contact dermatitis and occupational asthma [58,59]. These findings emphasise the importance of thorough metal screening and considering metal removal strategies in formulations aimed at sensitive or allergy-prone consumers.

**Comparative suitability, safety and efficacy of AA and AS in cosmetic applications:** In the comparison of AS and AA, AS demonstrates a more favourable elemental profile, featuring lower concentrations of toxic and sensitising metals while still providing essential trace elements beneficial for skin health. From the perspective of cosmetic formulation, AS is deemed more suitable for several reasons: it contains lower levels of toxic metals such as Pb, As and Cd; it has comparable or slightly elevated levels of beneficial trace elements like Zn, Se and Mo and displays a lower allergenic potential, making it more appropriate for sensitive skin and daily-use products. While AA does have higher concentrations of certain beneficial elements, such as Cu, the increased presence of toxic heavy metals necessitates additional purification or regulatory scrutiny before it can be safely utilised in cosmetics [60]. The trace metal composition of AS indicates that it is not only safer but also more advantageous for inclusion in cosmetic formulations focused on skin rejuvenation, anti-ageing and scalp nourishment.

**Cosmetics formulation applications of *A. angustifolia*:** *A. angustifolia* is notable for its rich phenolic content and strong antioxidant properties, making it a valuable ingredient in cosmetic formulations as described below [38,39,47].

**Anti-ageing emulsions (O/W creams and lotions):** The higher total phenolic content and strong antioxidant activity of AA make it well-suited for anti-ageing and dermocosmetic

TABLE-7
LIST OF TRACE ELEMENTS PRESENT IN THE METHANOLIC LEAVES EXTRACTS OF AA AND AS: BENEFITS AND APPLICATIONS IN COSMETICS

| Trace elements | Function on skin | Functions on hair | Potential cosmetic applications | Ref. |
|---|---|---|---|---|
| Zn | Regulates oil production, accelerates healing, anti-inflammatory | Promotes hair growth, prevents dandruff | Acne creams, healing serums, scalp treatments | [26] |
| Cu | Stimulates collagen and elastin, maintains skin firmness | Involved in melanin production, supports hair pigmentation | Anti-ageing creams, pigmentation balancers | [52] |
| Se | Antioxidant defence: protects against UV and pollution damage | Maintains healthy scalp, reduces oxidative stress | Antioxidant-rich serums, scalp health products | [55] |
| Fe | Enhances tissue oxygenation, supports skin vitality | Prevents hair thinning, supports follicle health | Revitalising creams, hair-strengthening serums | [56] |

emulsions aimed at reducing oxidative stress, improving skin tone and supporting barrier repair.

**Leave-on serums and ampoules:** The flavonoid and phenolic profile of AA supports its use in dilute aqueous or hydro-alcoholic serums where antioxidant protection, calming effects and protection against environmental aggressors are required without heavy occlusion.

**Soothing gels:** AA is suitable for gel-based formulations (*e.g.* carbomer or cellulose gels) intended for sensitive or irritated skin, such as after-sun, post-peel or post-shave products, due to its anti-inflammatory and free-radical-scavenging potential.

**Barrier-repair and sensitive-skin products:** AA can be incorporated into creams or emulsions targeting compromised skin barriers, including products for mature or reactive skin, where antioxidant and calming efficacy are prioritised over surfactant performance.

**Cosmetics formulation applications of *A. sisalana*:** *A. sisalana* offers a rich profile of flavonoids, tannins and saponins for cosmetics formulation [61,62].

**Cleansers and wash-off formulations:** Higher tannin and flavonoid content in AS, along with FTIR indications of saponins and lipid components, make it particularly suitable for facial cleansers, body washes and shampoos, where mild natural surfactant activity and antimicrobial benefits are advantageous.

**Emollient creams and body butters:** The stronger aliphatic C–H bands in AS support its use in richer emulsions and body care products, where enhanced skin-conditioning, softness and occlusivity are desired.

**Foaming gels and micellar waters:** AS can be effectively incorporated into gel-based or low-foaming formulations, contributing mild cleansing action, antioxidant protection and improved skin feel without harsh surfactants.

**Scalp and hair-care formulations:** The combination of flavonoids, tannins and saponins supports the use of AS in the scalp tonics, hair cleansers and conditioning treatments, where sebum regulation, antimicrobial activity and mild cleansing are valuable.

**Comparative formulation guidance:** Table-8 presents a comparative summary of the suitability of AA and AS extracts for various cosmetic formulation applications, highlighting key characteristics and potential benefits of each extract in the cosmetic industry.

*A. angustifolia* is best positioned for leave-on, antioxidant-focused dermocosmetic formulations, while AS demonstrates superior performance in cleansing, emollient and multifunctional wash-off systems. Their complementary profiles support both single-extract targeting and dual-extract formulation strategies in the development of natural cosmetics.

**Cosmetic formulation implications and potential challenges:** Although the methanolic extracts of AA *and* AS demonstrate promising phytochemical profiles for cosmetic applications, several formulation challenges must be considered. Trace metal analysis indicates that purification or selective extraction steps may be required to ensure compliance with cosmetic heavy metal limits, particularly for leave-on products where dermal exposure is prolonged. Techniques such as activated carbon treatment, chelation, or membrane filtration may be necessary to reduce metal residues without compromising bioactive integrity [63].

The natural pigmentation of agave extracts, arising from polyphenols and tannins, may impart yellow to brown colour, potentially limiting their use in transparent or lightly coloured formulations such as gels and serums. Colour stabilisation strategies, including controlled extract concentration, antioxidant co-formulants or encapsulation, may therefore be required. In addition, the presence of saponins and other secondary metabolites, particularly in AS, may influence foaming behaviour and emulsion stability, necessitating formulation optimisation to prevent excessive foaming or phase separation [64].

Odour is another consideration, as plant-derived extracts can exhibit characteristic earthy or vegetal notes that may affect consumer acceptability. Deodorisation steps, fragrance masking, or microencapsulation can be employed to mitigate sensory impact. Furthermore, the relatively high phenolic and tannin content may increase the risk of protein interaction or skin staining at elevated concentrations, highlighting the need for careful dose optimisation and compatibility testing [65].

Generally, while AA and AS extracts offer significant functional benefits, their successful incorporation into cosmetic formulations depends on appropriate purification, sensory optimisation and stability-focused formulation strategies.

**Study limitations:** The present study provides the preliminary insight into the phytochemical composition and trace metal content of *A. angustifolia* and *A. sisalana* methanolic leaf extracts with potential cosmetic relevance. However, individual bioactive compounds were not identified or quantified using chromatographic techniques such as HPLC or GC-MS and no chromatograms were generated, limiting compound-level resolution and structural confirmation. The antioxidant and antimicrobial activities were inferred from phytochemical concentrations that had previously been validated through standard *in vitro* assays, such as DPPH, FRAP, broth microdilution and cytotoxicity tests. Furthermore, the

TABLE-8
COMPARATIVE SUITABILITY FEATURES OF AA AND AS METHANOLIC EXTRACTS FOR COSMETIC FORMULATION APPLICATIONS

| Formulation type | Key cosmetic function | AA | AS |
|---|---|---|---|
| Anti-ageing creams and serums | Antioxidant activity, skin barrier support, elasticity enhancement | +++ | + |
| Sensitive-skin and calming gels | Anti-inflammatory, soothing and hydration support | +++ | + |
| Cleansers and wash-off products | Mild cleansing and impurity removal | + | +++ |
| Emollient body products | Occlusive moisturisation and skin softening | + | +++ |
| Hair and scalp care | Scalp hydration and hair conditioning | + | ++ |

+++ high suitability (strong functional performance and formulation versatility); ++ good suitability (moderate functional performance); + suitable (limited functional contribution)

behaviour, bioavailability and potential leaching of trace metals in finished cosmetic formulations were also not assessed, as these parameters are formulation-dependent and influenced by matrix composition, pH and processing conditions. These limitations support the need for chromatographic profiling, further bioactivity testing (*e.g.*, anticancer test) and formulation-based safety evaluations in future studies.

## Conclusion

Methanolic extract of *Agave angustifolia* (AA) and *Agave sisalana* (AS) leaves show significant potential as bioactive ingredients in skin and hair care products. Both species are rich in flavonoids, saponins and phenolics, conferring antioxidant, antimicrobial and anti-inflammatory properties that support skin regeneration and anti-ageing. Essential trace metals, including Zn, Cu and Se, were detected, contributing to the enzymatic activity, collagen synthesis and protection against oxidative stress. Most toxic metals were within safe limits and no mercury was detected; however, Pb and Ni exceeded permissible levels in some regions, highlighting the need for purification or formulation control. The study is limited by the absence of chromatographic identification of individual compounds, direct antioxidant activity assays and evaluation of metal leaching in formulations, restricting definitive conclusions regarding bioactivity and safety in finished products. Nevertheless, the combined presence of bioactive phytochemicals and essential trace elements supports the multifunctional potential of AA and AS in natural cosmetics. Future work should focus on compound-level profiling (HPLC/GC-MS) and formulation-based safety and stability studies to fully exploit these agave species in cosmetic applications.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

## DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

## REFERENCES

1. Z.D. Draelos, *Dermatol. Clin.*, **30**, 85 (2012); https://doi.org/10.1016/j.clindermatol.2011.08.018
2. H. López-Salazar, M.L. Arenas-Ocampo, B.H. Camacho-Díaz, F. Rodríguez-González and S.V. Ávila-Reyes, *BioResources*, **20**, 8149 (2025); https://doi.org/10.15376/biores.20.3.Lopez-Salazar
3. P.K. Mukherjee, N. Maity, N.K. Nema and B.K. Sarkar, *Phytomedicine*, **19**, 64 (2011); https://doi.org/10.1016/j.phymed.2011.10.003
4. K. Poonia, G.P. Thami, M. Bhalla, S. Jaiswal and J. Sandhu, *J. Cosmet. Dermatol.*, **18**, 401 (2019); https://doi.org/10.1111/jocd.12559
5. X. Hu, Y. Li, S. Tan, L. Chen, D.S. Mkapa, C. Lin, Q. Liu, G. Jin, T. Chen, X. Qin, K. Yi and X. Huang, *Agronomy*, **15**, 722 (2025); https://doi.org/10.3390/agronomy15030722
6. F.T. Raya, L.M. de Carvalho, J. José, L.P. da Cruz, R.L. Almeida, H.A.A. Delevatti, N.M. Silveira, S.F. da Silva, M.D. Pissolato, A.B. de Oliveira, W.J.V. dos Reis, L.G.F. de Abreu, J. Gutiérrez, M.F. Carazzolle, A.C.F. Soares, J. Nieto Sotelo, R.V. Ribeiro and G.A.G. Pereira, *Front. Chem. Eng.*, **3**, 1218668 (2023); https://doi.org/10.3389/fceng.2023.1218668
7. W. Crawford, D.K.Y. Tan and F. Van Ogtrop, *Front. Chem. Eng.*, **4**, 1039675 (2022); https://doi.org/10.3389/fceng.2022.1039675
8. J.A. Honorato-Salazar, J. Aburto and M.A. Amezcua-Allieri, *Sustainability*, **13**, 12263 (2021); https://doi.org/10.3390/su132112263
9. M. Bermúdez-Bazán, G.A. Castillo-Herrera, J.E. Urias-Silvas, A. Escobedo-Reyes and M. Estarrón-Espinosa, *Molecules*, **26**, 6789 (2021); https://doi.org/10.3390/molecules26226789
10. E. Ramírez-Moreno, R. Salmerón-Torres, L.A. Ochoa-Martínez, E. Pérez-Carrillo and A. Quintero-Ramos, *Foods*, **2**, 44 (2023); https://doi.org/10.3390/foods2030044
11. A. Andrade-Cetto and M. Heinrich, *J. Ethnopharmacol.*, **99**, 325 (2005); https://doi.org/10.1016/j.jep.2005.04.019
12. F. Mtunzi, E. Muleya, J. Modise, A. Sipamla and E. Dikio, *Pak. J. Nutr.*, **11**, 757 (2012).
13. S.G. Zondo, *Environ. Monit. Assess.*, **196**, 752 (2024); https://doi.org/10.1007/s10661-024-12920-8
14. A.C. Dweck, Formulating with Botanical Extracts, In: Cosmetic Science and Technology: Theoretical Principles and Applications, Amsterdam, Netherlands: Elsevier, pp. 595-618 (2009).
15. PerkinElmer Inc., NexION® 350X ICP-MS: The Complete Solution for Elemental Analysis. Waltham, MA: PerkinElmer Inc. (2013).
16. S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram and L. Yogalatha, *Afr. J. Tradit. Complement. Altern. Med.*, **8**, 1 (2011).
17. V. Balamurugan, S. Fatima and S. Velurajan, *Int. J. Adv. Res. Innov. Ideas Educ.*, **5**, 236 (2019); https://doi.org/10.6084/m9.figshare.12559683.v1
18. M.-J. In, E.J. Kim and D.C. Kim, *J. Appl. Biol. Chem.*, **61**, 105 (2018); https://doi.org/10.3839/jabc.2018.017
19. E.A. Ainsworth and K.M. Gillespie, *Nat. Protoc.*, **2**, 875 (2007); https://doi.org/10.1038/nprot.2007.102
20. C. Chang, M. Yang, H. Wen and J. Chern, *Yao Wu Shi Pin Fen Xi*, **10**, 178 (2020); https://doi.org/10.38212/2224-6614.2748
21. A.P. Desai and S. Desai, *Int. J. Environ. Sci Nat. Resour.*, **21**, 556056 (2019).
22. Anton Paar, Multiwave PRO: High-Performance Microwave Digestion System, Operating Manual, Anton Paar GmbH (2020)
23. U.S. Food and Drug Administration (FDA), Guidance for Industry: Lead in Cosmetic Lip Products and Externally Applied Cosmetics. Silver Spring (MD): FDA (2022).
24. International Agency for Research on Cancer (IARC), Arsenic, Metals, Fibres and Dusts. IARC Monogr Eval Carcinog Risks Hum. Vol. 100C. Lyon: WHO (2012).
25. Health Canada, Draft Guidance on Heavy Metal Impurities in Cosmetics, Health Canada (2012).
26. Y. Ogawa and M. Kinoshita, *Arch. Biochem. Biophys.*, **611**, 108031 (2020); https://doi.org/10.1016/j.abb.2020.108031
27. G. Borkow, *Curr. Chem. Biol.*, **8**, 89 (2015); https://doi.org/10.2174/2212796809666150227223857
28. A. Pappas, *Dermatoendocrinol*, **1**, 72 (2009); https://doi.org/10.4161/derm.1.2.7811
29. G.M. Recer, T.B. Johnson and A.K. Gleason, *Regul. Toxicol. Pharmacol.*, **36**, 122 (2002); https://doi.org/10.1006/rtph.2002.1569
30. J.A. Araya, R.L. Carneiro, C. Arévalo, J. Freer and R.P. Castillo, *Microchem. J.*, **134**, 91 (2017); https://doi.org/10.1016/j.microc.2017.05.019

31. Scientific Committee on Consumer Safety (SCCS), Guidance on the Safety Assessment of Impurities in Cosmetic Ingredients, European Commission (2021).
32. R.A. Bojar and K.T. Holland, *World J. Microbiol. Biotechnol.*, **20**, 491 (2004); https://doi.org/10.1023/B:WIBI.0000040406.62329.fd
33. P.M. Elias, S.K. Ahn, B.E. Brown, D. Crumrine and K.R. Feingold, *Prog. Lipid Res.*, **41**, 207 (2002); https://doi.org/10.1016/S0163-7827(01)00020-6
34. E. Dumont, F. Vanhaecke and R. Cornelis, *Anal. Bioanal. Chem.*, **385**, 1304 (2006); https://doi.org/10.1007/s00216-006-0529-8
35. L.A. de Araújo, F. Addor and P.M.B.G. Maia Campos, *An. Bras. Dermatol.*, **91**, 331 (2016); https://doi.org/10.1590/abd1806-4841.20163986
36. U.S. Food and Drug Administration (FDA), Guidance for Industry: Safety of Nanomaterials in Cosmetic Products. U.S. Department of Health and Human Services (2020); Available: https://www.fda.gov/
37. Anton Paar GmbH, Multiwave PRO: High-Performance Microwave Digestion System – Operating Manual, Anton Paar GmbH (2020).
38. A. Roy and N. Bharadvaja, in eds.: V.K. Gupta and N. Bharadvaja, Role of Flavonoids in Cosmetic Formulations., In: Phytochemistry: Therapeutic uses and Pharmacological Properties, Cham: Springer; pp. 97–112 (2020); https://doi.org/10.1007/978-981-13-7248-3_6
39. K.H. Kim, R. Tsao, R. Yang and S.W. Cui, *Food Chem.*, **95**, 466 (2006); https://doi.org/10.1016/j.foodchem.2005.01.032
40. M.W. Akram, M.M.U. Hoque, M.S. Miah, M.A. Shahid, M.F. Hossain and S.H. Mahmud, *Heliyon*, **9**, e17961 (2023); https://doi.org/10.1016/j.heliyon.2023.e17961
41. F. Nazzaro, F. Fratianni, R. Coppola and V.D. Feo, *Pharmaceuticals*, **10**, 86 (2017); https://doi.org/10.3390/ph10040086
42. J.M. Pullar, A.C. Carr and M.C.M. Vissers, *Nutrients*, **9**, 866 (2017); https://doi.org/10.3390/nu9080866
43. A. Scalbert, I.T. Johnson and M. Saltmarsh, *Am. J. Clin. Nutr.*, **81(Suppl)**, 215S (2005); https://doi.org/10.1093/ajcn/81.1.215S
44. P. Soundararajan and J.S. Kim, *Phytochem. Rev.*, **17**, 1119 (2018); https://doi.org/10.1007/s11101-018-9565-5
45. Y. Li, J. Yao, C. Han, J. Yang, M.T. Chaudhry, S. Wang, H. Liu and Y. Yin, *Nutrients*, **8**, 167 (2016); https://doi.org/10.3390/nu8030167
46. M. Michalak, *Int. J. Mol. Sci.*, **23**, 585 (2022); https://doi.org/10.3390/ijms23020585
47. N.G. Mkhize, M.C. Achilonu, I.T. Manduna, X.V. Ngubane and S.M. Nkosi, *J. Med. Plants Econ. Dev.*, **8**, a263 (2024); https://doi.org/10.4102/jomped.v8i1.263
48. N. Takano, Y. Inokuchi and M. Kurachi, *Yakugaku Zasshi*, **131**, 581 (2011); https://doi.org/10.1248/yakushi.131.581
49. M. Michalak, *Int. J. Mol. Sci.*, **24**, 15444 (2023); https://doi.org/10.3390/ijms242015444
50. N. Saewan and A. Jimtaisong, *J. Cosmet. Dermatol.*, **14**, 47 (2015); https://doi.org/10.1111/jocd.12123
51. M. Tomas, D. Günal-Köroğlu, S. Kamiloglu, T. Ozdal and E. Capanoglu, *Immun. Ageing*, **22**, 5 (2025); https://doi.org/10.1186/s12979-025-00498-9
52. M.C. Linder, *Int. J. Mol. Sci.*, **21**, 4932 (2020); https://doi.org/10.3390/ijms21144932
53. W. Diao, P. Li, X. Jiang, J. Zhou and S. Yang, *Wound Repair Regen.*, **32**, 314 (2024); https://doi.org/10.1111/wrr.13122
54. U.S. National Research Council, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Manganese, Iron, Molybdenum, Nickel, Silicon, Vanadium and Zinc, National Academies Press: Washington, DC (2001).
55. M.P. Rayman, *Lancet*, **379**, 1256 (2012); https://doi.org/10.1016/S0140-6736(11)61452-9
56. H.M. Almohanna, A.A. Ahmed, J.P. Tsatalis and A. Tosti, *Dermatol. Ther.*, **9**, 51 (2019); https://doi.org/10.1007/s13555-018-0278-6
57. S. Garg, Y.B. Tripathi, V. Pandey and R.K. Chaturvedi, *Int. J. Environ. Res.*, **8**, 627 (2014); https://doi.org/10.22059/ijer.2014.792
58. A. Julander, M. Hindsén, L. Skare and C. Lidén, *Contact Dermat.*, **60**, 165 (2009); https://doi.org/10.1111/j.1600-0536.2008.01497.x
59. R. Lauwerys and D. Lison, *Sci. Total Environ.*, **150**, 1 (1994); https://doi.org/10.1016/0048-9697(94)90125-2
60. European Commission, Commission Regulation (EC) No. 1223/2009 on Cosmetic Products, Official Journal of the European Union (2009).
61. G.D. Mazo, J.A. Fracasso, L.T. da Costa, V. Farias Ximenes, N.A. Zoppe, A.M. Viel, L.P. Guarnier, B.D. Silva, L.V. de Almeida and L. dos Santos, *Cosmetics*, **11**, 104 (2024); https://doi.org/10.3390/cosmetics11030104
62. L.T. da Costa, J.A. Fracasso, R.P. Gonçalves, W.R. Martins, F.A. Oliveira, E.B. Coelho, G.O. Barbosa, N.A. Zoppe, K.A. Miyashiro, J.P. Gomes, B.D. Silva, D.B. Barbosa, V.F. Ximenes, P.O. Neto and L. Santos, *Cosmetics*, **12**, 259 (2025); https://doi.org/10.3390/cosmetics12060259
63. D.A. Basketter, G. Angelini, A. Ingber, P.S. Kern and T. Menné, *Contact Dermat.*, **49**, 1 (2003); https://doi.org/10.1111/j.0105-1873.2003.00149.x
64. T. Bujak, M. Zagórska-Dziok, A. Ziemlewska, K. Lal, Z. Nizioł-Łukaszewska, T. Wasilewski and Z. Hordyjewicz-Baran, *Molecules*, **27**, 922 (2022); https://doi.org/10.3390/molecules27030922
65. A.C. Dweck, *Int. J. Cosmet. Sci.*, **24**, 287 (2002); https://doi.org/10.1046/j.1467-2494.2002.00148.x