

Green Synthesis and Characterization of Sphagneticola trilobata-Mediated Copper Nanocomposites

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Research Article

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Green Synthesis and Characterization of *Sphagneticola trilobata*-Mediated Copper Nanocomposites

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Abstract

The present study aimed to develop and characterize a green, cellulose-supported copper nanocomposite mediated by *Sphagneticola trilobata* leaf extract. Fresh leaves were extracted using distilled water, and the concentrated aqueous extract was incorporated into a cellulose matrix, followed by in situ treatment with copper sulfate (CuSO₄) at concentrations of 1, 25, 75, 100 and 250 mM. The resulting materials were washed, dried and comparatively evaluated. The ethyl acetate fraction of the leaf extract was subjected to preliminary phytochemical screening, Fourier-transform infrared (FTIR) spectroscopy and liquid chromatography–mass spectrometry (LC–MS). Phytochemical screening indicated the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, glycosides and coumarins. FTIR analysis revealed hydroxyl, carbonyl, aromatic/conjugated and C–O-containing functionalities, supporting the presence of oxygenated and phenolic-type constituents. LC–MS demonstrated multiple chromatographic components, with putative phenolic- and flavonoid-related ions identified; these assignments were considered tentative. Scanning electron microscopy (SEM) showed particulate structures associated with the cellulose matrix following copper treatment, indicating successful modification of the matrix. In vitro release studies demonstrated concentration-dependent modulation of extract release. Formulation A (1 mM) exhibited the fastest release, whereas formulation E (250 mM) showed the greatest retardation. Formulation D containing 100 mM CuSO₄ provided an intermediate and controlled release profile, reaching $78.0 \pm 1.3\%$ at 7 h and $100.0 \pm 0.6\%$ at 8 h, and was therefore selected as the optimized formulation. Overall, the findings demonstrate the feasibility of developing a *S. trilobata*-mediated cellulose-supported copper nanocomposite with tunable release characteristics. Further studies on copper loading, particle characteristics, antimicrobial activity, cytocompatibility, mechanical properties and in vivo wound healing are warranted.

Keywords

Sphagneticola trilobata; green synthesis; copper nanocomposite; cellulose matrix; phytochemicals; FTIR spectroscopy; LC–MS; scanning electron microscopy; in vitro release; controlled release.

Introduction

Nanotechnology has become an important interdisciplinary field with applications in medicine, biotechnology, environmental remediation, agriculture and materials science. Nanoparticles exhibit physicochemical characteristics that differ substantially from their bulk counterparts, primarily because of their small size, high surface-area-to-volume ratio and surface reactivity. Conventional physical and chemical approaches for nanoparticle production can, however, involve high energy requirements, harsh reaction conditions and potentially hazardous

reducing or stabilizing agents. These limitations have stimulated interest in green and sustainable approaches for nanoparticle synthesis [1,2]. Among the biological approaches, plant-mediated synthesis has received particular attention because plant extracts contain naturally occurring metabolites capable of participating in metal-ion reduction, nucleation and stabilization of nanoparticles. Such approaches can reduce the dependence on hazardous chemical reagents and offer relatively simple, economical and environmentally compatible routes for nanomaterial fabrication [1,2].

Plant extracts are chemically complex matrices containing phenolic compounds, flavonoids, terpenoids, alkaloids, proteins, carbohydrates and other secondary metabolites. Depending on the plant species, extraction method and reaction conditions, these constituents may function as reducing, coordinating, capping or stabilizing molecules during nanoparticle formation [1,2]. Consequently, the biological source used for nanoparticle synthesis is an important determinant of the physicochemical and potentially biological properties of the resulting material. Green synthesis has been successfully investigated for several metallic and metal oxide nanoparticles, including gold, silver, copper, zinc, titanium and other inorganic nanomaterials [1,2]. Studies using plant extracts for the synthesis of silver nanoparticles have demonstrated that plant-derived constituents can facilitate the conversion of metal ions into nanoscale materials while simultaneously contributing to surface stabilization [18]. Similarly, plant-mediated synthesis of gold nanoparticles and green-synthesized metal oxide nanoparticles has been investigated for biomedical and other functional applications [15,16].

Copper-based nanomaterials are of particular interest because of their optical, catalytic, antimicrobial and other functional properties. Copper-containing nanostructures can be produced through different chemical, physical and biological approaches; however, green synthesis provides an attractive alternative in which plant-derived compounds may contribute to the reduction and stabilization process [1]. The use of plant extracts can potentially modify the surface chemistry of the resulting copper nanomaterial through the association of phytochemicals with the nanoparticle surface. Such phytochemical functionalization is of interest because the biological activity of a plant-mediated nanomaterial may arise not only from the inorganic component but also from the bioactive compounds associated with its surface. Thus, the chemical characterization of the plant fraction used for synthesis is important for understanding the composition and potential functionality of the resulting nanocomposite [1,2].

Sphagneticola trilobata (L.) J.F. Pruski, a member of the Asteraceae family, has attracted increasing scientific interest because of its diverse phytochemical composition and reported biological activities. Extracts prepared from different parts of the plant have demonstrated antioxidant, cytotoxic, antibacterial and anticancer properties. In particular, the ethyl acetate fraction of *S. trilobata* leaves has been reported to possess antioxidant and cytotoxic activity against MCF-7 breast cancer cells [8], while methanolic leaf extracts have demonstrated anticancer, antioxidant and antibacterial activities [9]. These findings suggest that *S. trilobata* contains biologically active constituents distributed across fractions of different polarities. The reported activity of the ethyl acetate fraction is particularly relevant to the present work because solvent fractionation can enrich moderately polar phytoconstituents that may contribute to the biological and chemical properties of the plant-derived material [8].

Phytochemical investigations of *S. trilobata* have further supported its potential as a source of bioactive secondary metabolites. Extracts of the flowers have been reported to contain alkaloids, flavonoids, phenols, saponins and steroids and have exhibited antioxidant and

cytotoxic activities [10]. Studies of leaf extracts have likewise demonstrated biological activities associated with their phytochemical constituents [8,9]. More recent investigations have provided evidence for specialized metabolites in *S. trilobata*. For example, the identification of eudesmanolide constituents derived from *S. trilobata* and their reported anticancer effects against colorectal cancer further highlights the pharmacological potential of compounds produced by this plant [19]. In addition, recent work on the terpene biosynthetic machinery of *S. trilobata* demonstrated the production of monoterpenes such as α -phellandrene, limonene and p-cymene, providing further evidence of the chemically diverse nature of the species [14]. These findings collectively support the selection of *S. trilobata* as a promising botanical source for the development of phytochemical-functionalized nanomaterials.

The biological safety and pharmacological relevance of *S. trilobata* have also been investigated in different experimental systems. Acute toxicity studies of leaf extracts have contributed preliminary information regarding the biological effects of the plant [11], while earlier investigations examined the effects of *S. trilobata* extracts on physiological parameters in animal models [12]. Although such studies do not directly establish the safety of a nanoparticle-based formulation, they provide useful background regarding the biological profile of the source plant. The combination of reported antioxidant and cytotoxic activities with the presence of diverse secondary metabolites makes *S. trilobata* an interesting candidate for green nanomaterial development.

The ability of *S. trilobata* to participate directly in nanoparticle synthesis has already been demonstrated with several inorganic materials. Aqueous extracts of *S. trilobata* have been used for the green synthesis of gold nanoparticles, with differences in nanoparticle formation and biological activity observed depending on the plant part employed [6]. Gold nanoparticles synthesized using aqueous extracts of the plant have subsequently been investigated for cytotoxicity using experimental biological models, further demonstrating the potential of *S. trilobata*-mediated nanomaterials [7]. The use of dried *S. trilobata* leaf biomass has also been reported for the synthesis of zirconium nanoparticles, which were characterized using FTIR, UV-visible spectroscopy, XRD, FESEM and TEM [4]. In another recent study, *S. trilobata* leaves were employed for the green synthesis of TiO₂ nanoparticles, demonstrating the applicability of the plant as a biological source for the preparation of metal oxide nanomaterials [3]. Furthermore, aqueous root extract of *S. trilobata* has been investigated for the synthesis of zinc nanoparticles and their associated antioxidant, antimicrobial and other biological activities [13].

The broader literature on plant-mediated nanoparticle synthesis provides additional support for using phytochemical-rich botanical extracts as reducing and stabilizing systems. For example, green-synthesized zinc oxide nanoparticles prepared using carob extracts have demonstrated biological activity in cancer-related experimental models [15]. Similarly, green synthesis of silver nanoparticles using *Adenia trilobata* leaf extract demonstrated the capacity of plant-derived compounds to reduce silver ions and produce nanoscale materials with antibacterial activity [18]. Green approaches have also been explored for gold nanoparticle synthesis using aqueous plant extracts and for curcumin-conjugated gold nanoparticles prepared in aqueous media [16,17]. Although these studies involve different botanical sources and nanoparticle compositions, they collectively demonstrate the versatility of phytochemical-mediated nanomaterial synthesis and the importance of plant-derived molecules in determining the properties of the final nanomaterial.

Despite the growing evidence supporting plant-mediated nanoparticle synthesis, there remains a need to develop systems that combine the advantages of green synthesis with a suitable supporting matrix. A free nanoparticle suspension may exhibit aggregation, variable dispersion and challenges associated with handling and recovery. Immobilization or incorporation of nanomaterials within a biocompatible polymeric matrix can provide an alternative strategy for producing a supported nanocomposite. Cellulose is particularly attractive for such applications because of its abundance, renewable nature, biodegradability and functional hydroxyl groups, which can facilitate interactions with incorporated compounds and inorganic components. A matrix-supported system may also provide advantages for investigating the retention and subsequent release of plant-derived constituents under controlled experimental conditions.

An important consideration in plant-mediated nanomaterial development is the relationship between the phytochemical composition of the plant extract and the properties of the resulting nanocomposite. FTIR spectroscopy can provide information regarding functional groups involved in interactions between plant metabolites and the inorganic or polymeric components, while UV–visible spectroscopy can be used to monitor characteristic optical absorption associated with nanoscale material formation. LC–MS provides complementary information concerning the molecular constituents present in the plant-derived fraction. Previous investigations of *S. trilobata*-mediated nanoparticles have demonstrated the usefulness of complementary spectroscopic and microscopic techniques for characterizing such materials [4,6]. Therefore, combining phytochemical screening, FTIR and LC–MS analysis of the plant fraction with UV–visible and release studies of the developed nanocomposite can provide a more comprehensive understanding of the system.

However, based on the available literature, most reported *S. trilobata*-mediated nanomaterial studies have focused on freely synthesized gold, zirconium, zinc or titanium-based nanoparticles [3,4,6,7,13]. Comparatively little attention has been directed toward the **in situ incorporation of *S. trilobata*-derived phytoconstituents with copper-based nanomaterials within a cellulose matrix**. In particular, an integrated investigation combining an aqueous *S. trilobata* leaf extract, cellulose-supported in situ copper-ion treatment, ethyl acetate fractionation, phytochemical characterization, FTIR, LC–MS, UV–visible analysis and in vitro release evaluation has not been adequately addressed. This represents an opportunity to develop a matrix-supported, plant-mediated copper nanocomposite while simultaneously investigating the chemical characteristics of the associated plant fraction.

Accordingly, the present study was designed to develop a green, in situ *S. trilobata*-mediated copper nanocomposite using a cellulose matrix. An aqueous leaf extract of *S. trilobata* was prepared and loaded onto a pretreated cellulose matrix, followed by treatment with different concentrations of copper sulfate under controlled stirring conditions. The resulting matrix was washed to remove unbound components and dried prior to characterization. The ethyl acetate fraction of the plant extract was subsequently subjected to phytochemical characterization, FTIR analysis and LC–MS profiling to investigate its functional groups and constituent metabolites. The developed nanocomposite was further evaluated using UV–visible spectroscopy to determine its characteristic absorption maximum and through in vitro release studies to assess its release behaviour. The study therefore aims to establish a sustainable plant-mediated strategy for the preparation of a cellulose-supported copper nanocomposite and to relate its physicochemical characteristics to the phytochemical profile of *S. trilobata*.

Although *Sphagneticola trilobata* has previously been investigated for its phytochemical composition, biological activities, and plant-mediated synthesis of metallic and metal oxide

nanoparticles, the use of its phytochemical constituents as an active component of a cellulose-supported in situ copper nanocomposite system remains insufficiently explored. In the present study, an aqueous leaf extract of *S. trilobata* was incorporated into a cellulose matrix, followed by in situ treatment with different concentrations of copper sulfate to generate a cellulose-supported copper-containing nanocomposite. The developed system was subsequently investigated by physicochemical and phytochemical characterization, FTIR spectroscopy, LC–MS profiling, UV–visible spectroscopy, SEM analysis and in vitro dissolution/release studies. This integrated approach was designed to evaluate the role of the plant-derived constituents and cellulose matrix in the formation and physicochemical behaviour of the resulting copper-containing material.

2 Materials and Methods

2.1 Plant material and authentication

Fresh leaves of *Sphagneticola trilobata* (L.) J.F. Pruski belonging to the family Asteraceae were collected from [collection site, city, state, country] during [month and year]. The leaves were carefully inspected and selected to ensure that they were free from visible fungal contamination, insect damage and extraneous materials.

The plant material was authenticated by [name, designation and institution of taxonomist/botanist]. A voucher specimen was deposited at [name of herbarium/institution] under voucher number [voucher number] for future reference and taxonomic verification.

2.2 Chemicals and reagents

Analytical-grade copper sulfate [CuSO_4 or $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, specify the form used], ethyl acetate and other reagents required for phytochemical analysis were used. Distilled water was used throughout the extraction, washing and nanocomposite preparation procedures. All chemicals were of analytical grade unless otherwise stated.

2.3 Preparation of aqueous *Sphagneticola trilobata* leaf extract

Fresh *S. trilobata* leaves were washed thoroughly with distilled water to remove adhering dust and other surface contaminants. The washed leaves were drained and the excess water was removed. A total of **37.43 g of fresh leaves** was accurately weighed and transferred to a suitable vessel containing **200 mL of distilled water**. The mixture was heated and boiled for **30 min** to extract the water-soluble phytoconstituents. After boiling, the extract was allowed to cool to room temperature and was separated from the residual plant material by filtration using suitable filter paper. The resulting aqueous filtrate was concentrated under controlled conditions until a thick liquid consistency was obtained. The concentrated extract was stored in a closed container at 50⁰ c until further use [14].

2.4 Preliminary phytochemical characterization

The concentrated aqueous leaf extract was subjected to preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites. Tests were performed for alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides and other relevant phytochemical groups using standard procedures. The results were recorded based on the characteristic colour development, precipitate formation or frothing observed for

each test [15]. The results were expressed qualitatively as absent (–) or present (+), with stronger reactions recorded as appropriate. Where quantitative phytochemical estimations were performed, the analyses were conducted in triplicate and the results were expressed as mean $\pm$ standard deviation (SD).

2.5 Ethyl acetate fractionation

The concentrated aqueous *S. trilobata* leaf extract was subjected to solvent–solvent fractionation using ethyl acetate to obtain an ethyl acetate-soluble fraction. A measured quantity of the concentrated aqueous extract was transferred into a separating funnel and partitioned with ethyl acetate. The mixture was thoroughly shaken and allowed to stand until complete separation of the aqueous and ethyl acetate phases occurred. The ethyl acetate layer was collected carefully. The extraction was repeated with fresh ethyl acetate 2 times. The combined ethyl acetate fractions were concentrated by evaporation under controlled conditions to obtain the ethyl acetate fraction [16]. The fraction was dried and weighed, and the percentage yield was calculated as:

$$\% \text{ Yield} = \frac{\text{Weight of dried ethyl acetate fraction}}{\text{Weight of extract subjected to fractionation}}$$

The dried ethyl acetate fraction was stored in an airtight container until further analysis.

2.6 Physicochemical characterization of the plant extract

The concentrated aqueous extract and/or ethyl acetate fraction were evaluated for relevant physicochemical characteristics, including colour, physical appearance, consistency, pH and percentage yield, where applicable [17].

The pH of the aqueous extract was determined using a calibrated digital pH meter. Measurements were performed in triplicate and expressed as mean $\pm$ SD.

2.7 FTIR characterization of the ethyl acetate fraction

Fourier-transform infrared (FTIR) spectroscopy was performed to identify the major functional groups present in the ethyl acetate fraction of *S. trilobata*. The dried fraction was subjected to FTIR analysis using an FTIR spectrometer [manufacturer, model and country]. The spectrum was recorded over the range of 4000–400 cm^{-1} or actual range used.

The characteristic absorption bands were identified and assigned to the corresponding functional groups based on their wavenumber regions and comparison with published literature.

2.8 LC–MS characterization of the ethyl acetate fraction

The chemical constituents of the ethyl acetate fraction of *Sphagneticola trilobata* leaves were characterized by liquid chromatography–mass spectrometry (LC–MS). The dried ethyl acetate fraction was reconstituted in **LC–MS-grade methanol at a concentration of 5 mg/mL** and filtered through a **0.2- μm PTFE membrane filter** prior to analysis. This sample-preparation procedure was adapted from a recent UPLC–ESI–QTOF–MS/MS

investigation of *S. trilobata* [18]. Chromatographic analysis was performed using an **Agilent 1290 HPLC system** coupled to an **Agilent 6546 quadrupole time-of-flight mass spectrometer (QTOF-MS)** equipped with a dual Agilent Jet Stream electrospray ionization (AJS-ESI) source. Separation was performed using an **ACQUITY UPLC HSS T3 column (1.8 μ m, 2.1 $\times$ 100 mm)** fitted with a **2.1 $\times$ 5 mm T3 VanGuard pre-column**, with the column maintained at **45 °C** [19]. The samples were analyzed in **both positive- and negative-ionization modes** using data-dependent AutoMS/MS acquisition. The obtained chromatographic and mass-spectral data were processed for peak detection, alignment and metabolite annotation. Molecular features were evaluated on the basis of retention time, accurate precursor mass and MS/MS fragmentation patterns [20]. Putative metabolites were annotated by comparison of accurate masses and fragmentation patterns with available spectral databases and published literature. The reported study employed **MS-DIAL, MS-CleanR and MS-FINDER**, with database matching against resources including **UNPD, COCONUT, HMDB, FooDB, ChEBI and LIPID MAPS** [21]. Metabolite annotations were considered tentative unless supported by authentic reference standards or additional structural evidence. According to the confidence framework used in that study, database-supported spectral matches were reported as **Level 2 annotations**, rather than definitive identifications [21].

2.9 Preparation and pretreatment of cellulose matrix

The cellulose matrix was washed thoroughly with distilled water to remove adhering impurities and soluble materials. The washed cellulose matrix was dried at **50 °C** until the desired dry state/constant weight was obtained. The dried cellulose matrix was subsequently sterilized using [actual sterilization method[22]]. After sterilization, the matrix was allowed to cool under aseptic conditions before extract loading.

2.10 Loading of *S. trilobata* leaf extract onto cellulose matrix

The sterilized and dried cellulose matrix was placed longitudinally inside a beaker containing the concentrated aqueous *S. trilobata* leaf extract, ensuring adequate contact between the matrix and the extract. The system was maintained under continuous stirring at **200 rpm for 24 h** at room temperature/actual temperature. This prolonged contact period allowed the phytochemical constituents of the plant extract to interact with and become associated with the cellulose matrix[23]. After 24 h, the cellulose matrix was removed from the extract and washed repeatedly with fresh distilled water. **Four successive washing cycles** were performed using fresh distilled water in each cycle to remove loosely associated and unbound extract constituents.

Following washing, the extract-loaded cellulose matrix was dried at **50 °C** until adequately dried. The dried extract-loaded cellulose matrix was stored under appropriate conditions until copper treatment.

2.11 In situ preparation of copper-containing cellulose nanocomposite

Copper sulfate solutions of different concentrations were freshly prepared using distilled water. Five concentrations were used: **1 mM, 25 mM, 75 mM, 100 mM and 250 mM**. The corresponding formulations were designated as **A, B, C, D and E**, respectively. An equivalent quantity of the *S. trilobata*-extract-loaded cellulose matrix was immersed separately in each copper sulfate solution. Each system was continuously stirred at **200 rpm for 24 h**

[24]. During this treatment, copper ions were allowed to interact with the phytochemical-loaded cellulose matrix, facilitating their incorporation, adsorption and/or in situ formation of copper-containing nanoscale material within the cellulose matrix. After 24 h, each matrix was removed from its respective copper sulfate solution and washed repeatedly with fresh distilled water to remove unbound copper ions and loosely associated precursor. The washing procedure was continued according to the predetermined washing protocol. The washed matrices were subsequently dried at **50 °C**. The resulting materials were designated according to the copper sulfate concentration used during preparation and stored in airtight containers until further characterization.

Table 1. Composition and preparation conditions of *Sphagneticola trilobata*-loaded cellulose–copper nanocomposite formulations

Formulation	CuSO ₄ concentration (mM)	<i>S. trilobata</i> extract-loaded cellulose	Stirring speed (rpm)	Treatment time (h)
A	1	Yes	200	24
B	25	Yes	200	24
C	75	Yes	200	24
D	100	Yes	200	24
E	250	Yes	200	24

Footnote: CuSO₄, copper sulfate; *S. trilobata*, *Sphagneticola trilobata*. All formulations were prepared using the same cellulose matrix and plant-extract loading procedure. Following CuSO₄ treatment, the matrices were washed with distilled water to remove unbound copper ions and dried at **50 °C**. Extraction and matrix-loading conditions were maintained consistently across all formulations.

2.12 Rationale for formulation design

A concentration-dependent formulation strategy was adopted to investigate the influence of copper precursor concentration on the formation and physicochemical characteristics of the *Sphagneticola trilobata*-mediated cellulose-supported copper nanocomposite. Copper sulfate concentrations of **1, 25, 75, 100 and 250 mM** were selected to provide a broad experimental concentration range while maintaining the cellulose matrix dimensions (**10 × 30 cm**), plant-extract loading conditions, stirring speed (**200 rpm**) and treatment duration (**24 h**) constant across all formulations. Thus, CuSO₄ concentration was considered the principal formulation variable.

The selected concentration range was intended to enable evaluation of concentration-dependent changes in the macroscopic appearance, surface morphology, optical characteristics and in vitro dissolution/release behaviour of the resulting materials. Formulations A–E were therefore considered **comparative experimental formulations**, rather than statistically optimized formulations. The formulation demonstrating the most suitable characteristics was identified based on the predefined experimental endpoints and comparative characterization results.

2.13 Scanning electron microscopy (SEM)

The surface morphology of the cellulose matrix and prepared copper-containing nanocomposites was examined using scanning electron microscopy (SEM). Samples were dried thoroughly and mounted on appropriate SEM stubs. Where required, the samples were coated with a thin conductive layer using [gold/gold-palladium/carbon coating] prior to analysis. SEM imaging was performed using [manufacturer and model] at an accelerating voltage of 10 kV and appropriate magnification ranges. Representative micrographs were obtained for the untreated cellulose matrix, extract-loaded cellulose matrix and copper-treated cellulose nanocomposite formulations [25]. The morphological characteristics, surface texture, porosity and apparent deposition/formation of nanoscale material on or within the cellulose matrix were compared among the different formulations. If SEM-EDS was available, elemental analysis was additionally performed to determine the presence and distribution of copper within the matrix.

2.15 In vitro dissolution/release study

The in vitro dissolution/release behaviour of the prepared cellulose-supported copper nanocomposites was evaluated using a **USP dissolution apparatus employing the basket method**. A known quantity of each dried nanocomposite formulation was placed in the dissolution basket. The basket was immersed in a predetermined volume of [dissolution medium] maintained at 37 ± 0.5 °C. The apparatus was operated at [actual rpm] rpm throughout the study. Samples of the dissolution medium were withdrawn at predetermined time intervals of [insert actual sampling intervals]. An equivalent volume of fresh dissolution medium maintained at the same temperature was added after each withdrawal to maintain a constant dissolution volume and, where applicable, sink conditions. The withdrawn samples were analysed using [UV–visible spectroscopy/appropriate analytical method] to determine the concentration of the released component [25]. The cumulative percentage release was calculated using the appropriate dilution-corrected mass-balance equation. The dissolution profiles of formulations A–E were compared to determine the influence of copper sulfate concentration on the release behaviour of the nanocomposites.

2.16 Statistical analysis

All quantitative experiments were performed using a minimum of three independent measurements unless otherwise specified. Data were expressed as **mean $\pm$ standard deviation (SD)**.

Quantitative data were expressed as mean $\pm$ SD. Comparisons among formulations A–E were performed using one-way ANOVA followed by Tukey's multiple-comparison test. For dissolution studies involving formulation and time as independent variables, two-way ANOVA followed by an appropriate multiple-comparison test was performed. A *p*-value < 0.05 was considered statistically significant.

The normality and homogeneity assumptions were assessed before applying parametric tests where applicable. A *p*-value of < **0.05** was considered statistically significant.

Statistical analysis was performed using GraphPad Prism.

For spectroscopic and chromatographic analyses that were primarily descriptive, including FTIR and LC–MS profiling, statistical testing was not applied unless quantitative replicate measurements were available. SEM observations were similarly evaluated descriptively based on representative micrographs, while quantitative image analysis was subjected to statistical analysis where measurable morphological parameters were obtained.

2.17 Experimental workflow

The complete experimental procedure consisted of the following sequential steps:

Fresh *S. trilobata* leaves → washing → aqueous extraction (37.43 g leaves/200 mL distilled water, 30 min boiling) → filtration → concentration to thick consistency → phytochemical characterization → ethyl acetate fractionation → FTIR and LC–MS analysis → cellulose washing → drying at 50 °C → sterilization → loading of aqueous plant extract onto cellulose → stirring at 200 rpm for 24 h → four successive distilled-water washes → drying at 50 °C → treatment with CuSO₄ (1, 25, 75, 100 and 250 mM) → stirring at 200 rpm for 24 h → washing to remove unbound copper → drying at 50 °C → SEM and UV–visible characterization → in vitro dissolution/release testing using the basket method.

3 Results

3.1 Macroscopic observations during preparation of *Sphagneticola trilobata* leaf extract

The fresh leaves of *Sphagneticola trilobata* were green in colour and exhibited the characteristic morphology of the plant material. Following washing and preparation, **37.43 g of fresh leaves** were subjected to aqueous extraction using **200 mL of distilled water**. During heating, the colour of the extraction medium progressively changed from a pale green to a deeper green colour, indicating the transfer of water-soluble constituents from the plant tissue into the aqueous phase. After **30 min of boiling**, the extract appeared distinctly green and exhibited a characteristic herbal odour. Following filtration and concentration, the aqueous extract became visibly more concentrated and developed a **thick liquid consistency**. The progressive intensification of colour during concentration was consistent with enrichment of the extracted plant constituents.

The visual observations recorded during extract preparation are summarized in Fig. 1.



Fig. 1. (a) Fresh leaves before extraction



Fig. 1. (b) filtered and concentrated aqueous extract after 30 min boiling showing increased colour intensity and thick liquid consistency.

Fig. 1. Sequential visual observations during preparation of the aqueous *Sphagneticola trilobata* leaf extract.

3.2 Preparation and physical appearance of the cellulose matrix

The cellulose matrix used for the study had dimensions of **10 cm × 30 cm**. Prior to extract loading, the matrix was washed with distilled water to remove adhering impurities and subsequently dried at **50 °C**. The matrix was then sterilized before being used for incorporation of the plant extract.

The untreated cellulose matrix was visually **white** and exhibited a uniform fibrous appearance. Following sterilization and subsequent immersion in the concentrated *S. trilobata* aqueous extract, the matrix progressively acquired the colour of the plant extract. After 24 h of treatment under continuous stirring at **200 rpm**, the matrix showed visible uptake of the extract and a distinct change from the original white appearance. After the extract-loading step and four successive washing cycles with fresh distilled water, the matrix retained a visible **light green tlight olive-green coloration**, indicating retention of extract-associated constituents within or on the cellulose matrix despite removal of loosely associated material.

The sequence of matrix preparation and extract loading is illustrated in Fig. 2.



Fig. 2. (a) Untreated white cellulose matrix;



Fig. 2. (b) cellulose matrix immersed longitudinally in the concentrated plant extract

Fig. 2. Sequential preparation of the cellulose matrix and loading with *Sphagneticola trilobata* aqueous leaf extract. (b) washed and dried cellulose matrix; (c) sterilized cellulose matrix;; (e) matrix after 24 h extract loading at 200 rpm; (f) matrix following four successive washing cycles; and

3.3 In situ generation of the copper-containing nanocomposite

The extract-loaded cellulose matrix was subsequently subjected to treatment with copper sulfate solutions at concentrations of **1, 25, 75, 100 and 250 mM**, designated formulations A, B, C, D and E, respectively. Each formulation was maintained under continuous stirring at **200 rpm for 24 h**. A visible change in the appearance of the extract-loaded cellulose matrix was observed following exposure to the copper sulfate solutions. The colour of the matrix progressively changed from the **light olive-green appearance of the extract-loaded cellulose matrix** following copper treatment. The extent and visual intensity of the colour change varied among the copper sulfate concentrations. The observed colour change provided a preliminary macroscopic indication of interaction between the copper precursor and the phytochemical-associated cellulose matrix. However, visual colour change alone was not considered sufficient evidence for confirmation of nanoparticle formation, and subsequent spectroscopic and morphological characterization was therefore performed. Following the 24-h copper treatment, the matrices were repeatedly washed with fresh distilled water to remove unbound copper ions and loosely associated material. After washing and drying at **50 °C**, the resulting matrices retained their altered coloration and physical appearance. The complete sequence of in situ nanocomposite preparation is shown in Fig. 3.



Fig. 3.a. different concentrations of copper sulphate solution



Fig. 3. (b) Extract-loaded cellulose matrix during exposure to copper sulfate



Fig. 3. (c) dried cellulose-supported copper nanocomposite turned to olive green colouration.

Fig. 3. Macroscopic changes observed during in situ preparation of the *S. trilobata*-mediated cellulose-supported copper nanocomposite

cellulose
matrix
before
herbal
extract
loading



cellulose
matrix
after
herbal
extract
loading



Figure 4. colour transition of cellulose matrix supported *S. trilobata* leaves extract

3.4 Formulation characteristics

Five formulations were prepared by varying the copper sulfate concentration while maintaining the plant extract-loaded cellulose matrix, stirring speed and treatment duration constant. The experimental formulations are presented in Table 2.

Table 2. Experimental design and rationale of *Sphagneticola trilobata*-mediated cellulose-supported copper nanocomposite formulations

Formulation	CuSO ₄ concentration (mM)	Cellulose matrix (cm)	Extract loading	Stirring (rpm)	Treatment (h)	Drying (°C)	Experimental rationale
A	1	10 × 30	<i>S. trilobata</i> extract	200	24	50	Low precursor concentration representing the lower experimental boundary
B	25	10 × 30	<i>S. trilobata</i> extract	200	24	50	Low-to-intermediate precursor concentration for evaluating concentration-dependent changes
C	75	10 × 30	<i>S. trilobata</i> extract	200	24	50	Intermediate precursor concentration for evaluating copper incorporation and morphological changes
D	100	10 × 30	<i>S. trilobata</i> extract	200	24	50	High-intermediate precursor concentration for assessing increased

							copper availability
E	250	10 × 30	<i>S. trilobata</i> extract	200	24	50	Upper experimental boundary for assessing the effect of high precursor concentration

Footnote: CuSO₄, copper sulfate; *S. trilobata*, *Sphagneticola trilobata*. The cellulose dimensions, extract-loading conditions, stirring speed and treatment duration were kept constant among formulations, while CuSO₄ concentration was varied as the principal experimental factor. Following copper treatment, the matrices were washed with distilled water to remove unbound precursor and dried at 50 °C. The formulations were evaluated comparatively using physicochemical, morphological, spectroscopic and dissolution/release endpoints.

3.5 Physical observations following washing and drying

After completion of the copper treatment, repeated washing and drying, the matrices remained physically intact and retained their overall sheet-like structure. No gross fragmentation of the cellulose matrix was observed during the preparation procedure. The colour of the final materials differed from that of the untreated cellulose matrix. The untreated matrix was white, whereas the extract-loaded matrix exhibited a light olive-green colour. Following copper treatment, washing and drying, a persistent colour change was observed, with differences in colour intensity among the formulations prepared using different copper sulfate concentrations. These macroscopic observations were used as preliminary evidence of successful interaction between the plant extract-loaded cellulose matrix and the copper precursor. Further characterization was required to establish the chemical and morphological nature of the resulting material.

Phytochemical screening of ethyl acetate fraction

Table 3. Preliminary phytochemical screening of the ethyl acetate fraction of *Sphagneticola trilobata* leaves

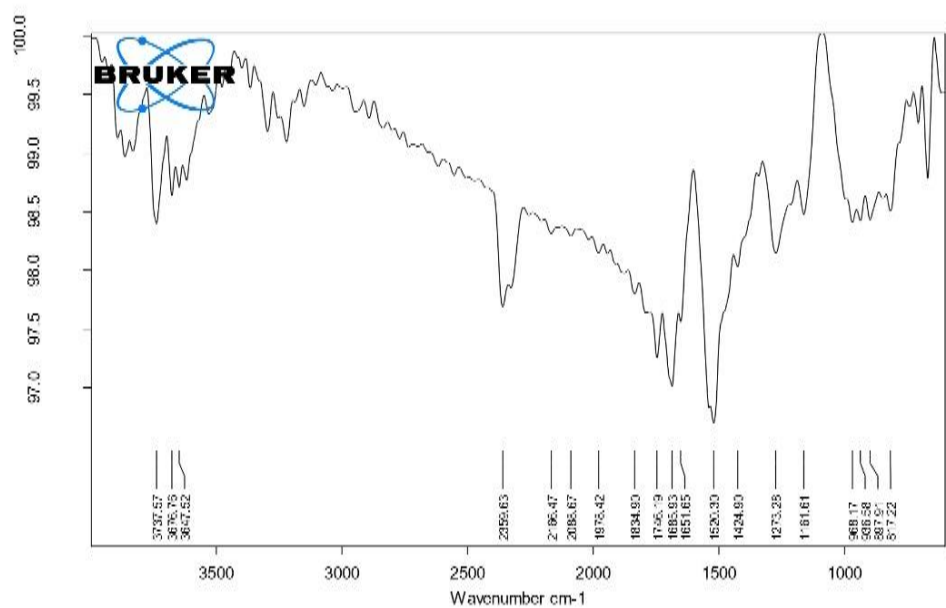
Phytochemical constituent	Result
Alkaloids	+
Flavonoids	+
Phenolic compounds	+
Tannins	+
Saponins	—
Terpenoids	+
Steroids	+
Glycosides	+

Coumarins	+
Quinones	—
Carbohydrates	—
Proteins	—
Amino acids	—

The preliminary phytochemical screening of the ethyl acetate fraction of *Sphagneticola trilobata* leaves revealed the presence of several secondary metabolites, as summarized in **Table 3**. The fraction showed positive reactions for **alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, glycosides, and coumarins**, whereas **saponins, quinones, carbohydrates, proteins, and amino acids were not detected** under the test conditions employed [26]. The strong positive reactions for **flavonoids and phenolic compounds** are particularly noteworthy because these classes are commonly associated with antioxidant and other pharmacological activities. The detection of tannins and terpenoid constituents further indicates the chemical diversity of the ethyl acetate fraction. The positive alkaloid and glycoside tests also suggest the presence of additional bioactive constituents that may contribute to the biological activity of the fraction.

3.6 FTIR characterization of the ethyl acetate fraction

The FTIR spectrum of the ethyl acetate fraction exhibited characteristic absorption bands corresponding to functional groups present in the extracted phytochemical constituents. The observed peaks were recorded at 4000–400 cm⁻¹. The principal FTIR bands and their tentative functional-group assignments are presented in Table 3 and figure 5.



C:\OPUS_7.0.129\MFAS\NPC.SPHAG.2	NPC.SPHAG	Instrument type and / or accessory	29-08-2026
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Table. FTIR spectral assignments and possible phytochemical moieties of *Sphagneticola trilobata* leaf extract

S. No.	Peak (cm ⁻¹)	FTIR functional-group assignment	Possible phytochemical moiety/class	Phytochemicals reported in <i>S. trilobata</i> supporting the interpretation	Reference
1	3737.57	O–H stretching	Free hydroxyl groups of phenols/alcohols	Phenolic compounds, flavonoids	Coates [1]; Ali et al. [3]
2	3676.76	O–H stretching	Free hydroxyl groups	Phenolics/flavonoids	Coates [1]; Ali et al. [3]
3	3647.52	O–H stretching	Free phenolic/alcoholic –OH	Phenolic acids, flavonoids	Coates [1]; Ali et al. [3]
4	2359.63	CO ₂ absorption	Atmospheric CO ₂	Not assigned	Coates [1]
5	2166.47	Weak absorption in triple-bond/combination region	No definitive assignment	Not assigned	Coates [1]
6	2088.67	Combination/overtone region	No definitive phytochemical assignment	Not assigned	Coates [1]
7	1978.42	Aromatic combination/overtone band	Aromatic/conjugated molecular system	Flavonoids, phenolic compounds	Coates [1]; Ali et al. [3]
8	1834.90	C=O-related absorption	Carbonyl-containing oxygenated compound	Oxygenated phenolic/secondary metabolites	Coates [1]; Fucina et al. [2]
9	1746.19	C=O stretching	Ester/carboxylic-acid carbonyl	Caffeoylquinic acids, phenolic acids	Fucina et al. [2]; Ali et al. [3]
10	1685.93	Conjugated C=O/C=C vibration	Conjugated phenolic/flavonoid system	Flavonoids, phenolic acids	Coates [1]; Ali et al. [3]
11	1651.65	C=C/conjugated-system vibration	Aromatic/conjugated flavonoid system	Luteolin, myricetin derivatives, flavonoids	Ali et al. [3]
12	1520.30	Aromatic skeletal/C=C vibration	Aromatic phenolic/flavonoid structure	Flavonoids, phenolic acids	Coates [1]; Ali et al. [3]

13	1424.90	C–H/O–H bending	Phenolic/aromatic structure	Phenolic acids, flavonoids	Coates [1]; Fucina et al. [2]
14	1273.28	C–O stretching	Phenolic/ester/aryl ether functionality	Caffeoylquinic acids, flavonoids	Coates [1]; Fucina et al. [2]
15	1161.61	C–O/C–O–C stretching	Alcoholic, ether or glycosidic functionality	Flavonoid glycosides, phenolic glycosides	Coates [1]; Ali et al. [3]
16	968.17	=C–H out-of-plane bending	Alkenic/unsaturated structure	Terpenoid constituents	Coates [1]; Ali et al. [3]
17	936.58	=C–H/alkene bending	Unsaturated hydrocarbon/terpenoid structure	Sesquiterpenoids, diterpenoids	Ali et al. [3]
18	897.91	=C–H out-of-plane bending	Unsaturated/alkenic structure	Terpenoid/unsaturated constituents	Coates [1]; Ali et al. [3]
19	817.22	Aromatic/alkene C–H out-of-plane bending	Substituted aromatic/unsaturated structure	Flavonoids, phenolics, terpenoids	Coates [1]; Ali et al. [3]

Footnotes:

FTIR assignments are tentative and based on established spectral correlations and reported phytochemicals of *S. trilobata*. The bands at 2359.63, 2166.47, 2088.67 and 1978.42 cm^{-1} may represent atmospheric CO_2 or combination/overtone bands. FTIR indicates functional groups and does not definitively identify individual phytochemical compounds.

3.7 LC–MS characterization of the ethyl acetate fraction

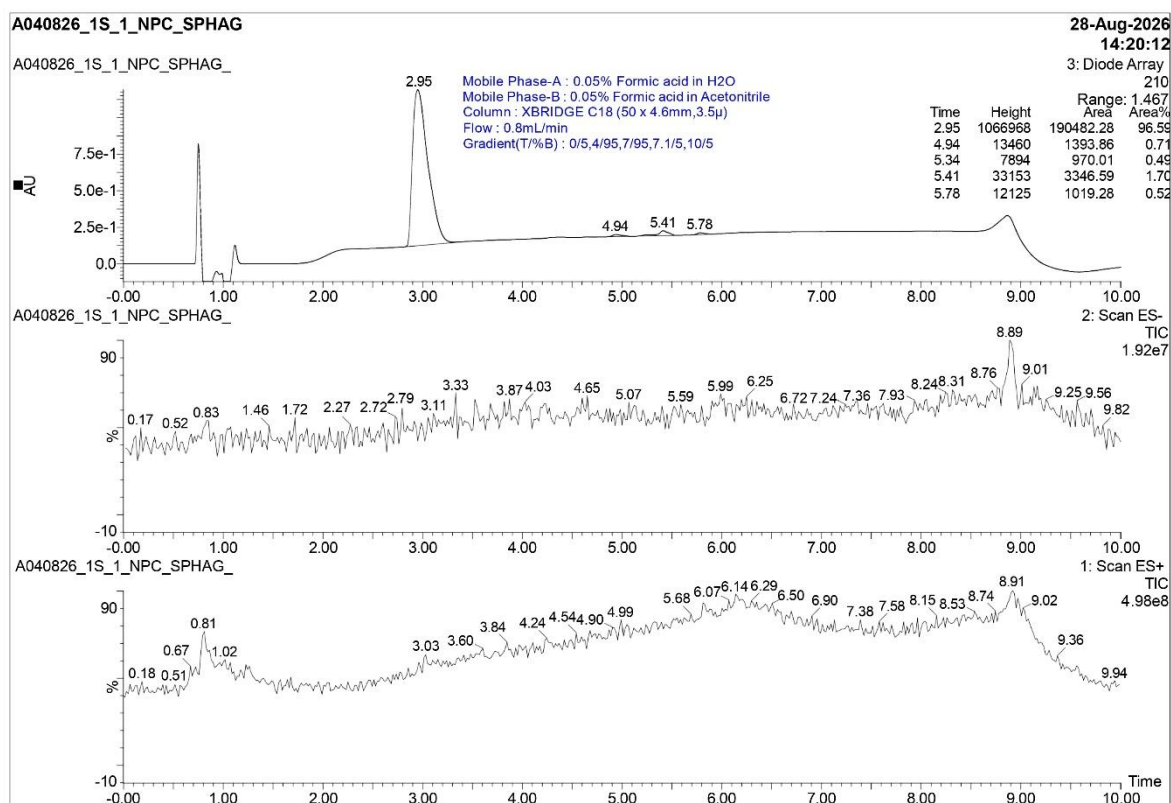
LC–MS analysis demonstrated the presence of multiple chromatographic peaks corresponding to different mass-to-charge ratios (m/z) in the ethyl acetate fraction. The detected molecular ions and their tentative identities were determined by comparison with spectral databases and published literature. The major detected constituents, retention times and corresponding m/z values are presented in Table 4.

Table 4. Tentative Assignment of LC–MS Peaks of Ethyl Acetate Fraction of *Sphagneticola trilobata* Leaves with Pharmacological Relevance

Rt (min)	Maj or m/z	Tentative Compound/Classification*	Proposed Pharmacological Role	Mechanism in Wound Healing / Anti-inflammatory Activity	Literature DOI
3.028	130.12	Phenolic acid derivative	Antioxidant, anti-inflammatory	Scavenges ROS, reduces oxidative stress,	DOI: 10.1038/s41598-026-54096-w

				suppresses inflammatory mediators	
4.938	236.27	Caffeoylquinic acid-related metabolite	Antioxidant, tissue protection	Inhibits lipid peroxidation and inflammatory cytokine production	DOI: 10.3109/13880209.2016.1160249
5.276	304.47	Luteolin/Myricetin derivative	Anti-inflammatory, wound healing	Inhibits NF- κ B and MAPK pathways; decreases TNF- α , IL-1 β and IL-6	DOI: 10.1038/s41598-026-54096-w
5.450	185.93	Phenolic constituent	Antioxidant	Protects fibroblasts from oxidative damage and supports tissue repair	DOI: 10.1038/s41598-026-54096-w
5.788	279.31	Flavanone/flavone derivative (eriodictyol-related)	Wound healing, antioxidant	Promotes fibroblast proliferation, collagen synthesis and epithelialization	DOI: 10.1038/s41598-026-54096-w

Footnote: LC–MS, liquid chromatography–mass spectrometry; m/z, mass-to-charge ratio.



Compound assignments are tentative unless confirmed using authentic reference standards or MS/MS fragmentation data.

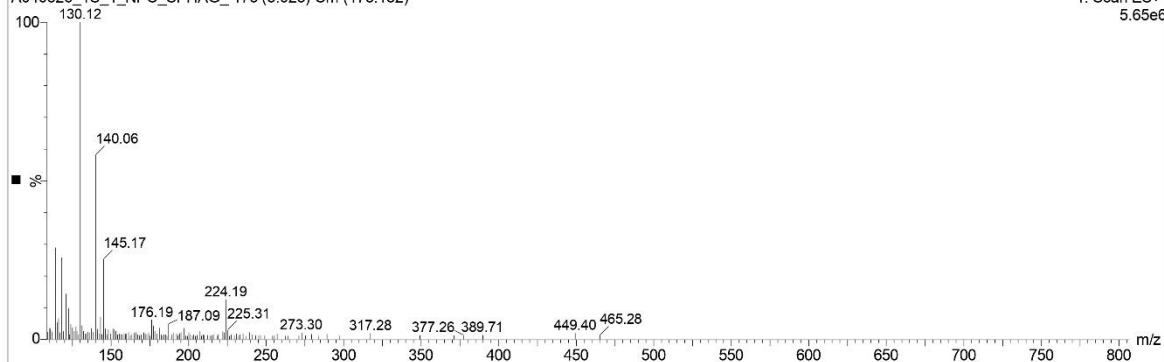
Figure 1. LC–MS total ion chromatogram (TIC) of the ethyl acetate fraction of *Sphagneticola trilobata* leaves showing the major chromatographic peaks.

A040826_1S_1_NPC_SPHAG

28-Aug-2026

A040826_1S_1_NPC_SPHAG_175 (3.028) Cm (173:182)

14:20:12
1: Scan ES+
5.65e6



A040826_1S_1_NPC_SPHAG_179 (3.106) Cm (173:183)

2: Scan ES-
5.00e5

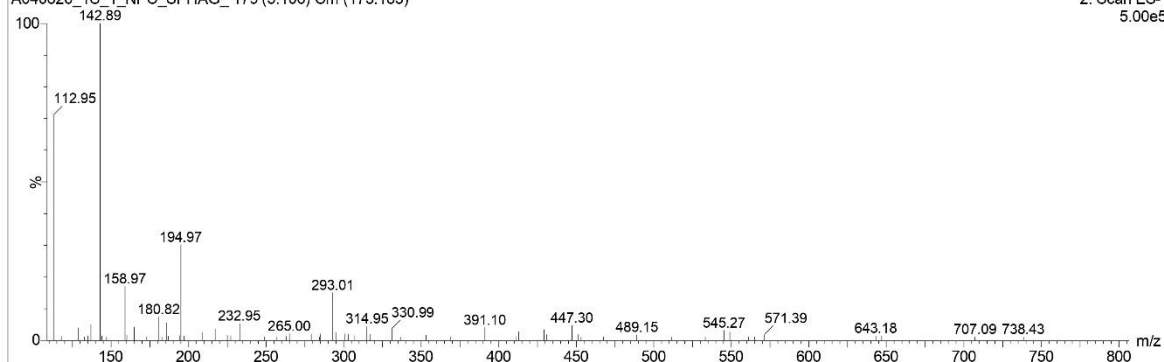


Figure 2. ESI⁺ and ESI⁻ mass spectra of the ethyl acetate fraction at retention times 3.028 and 3.106 min.

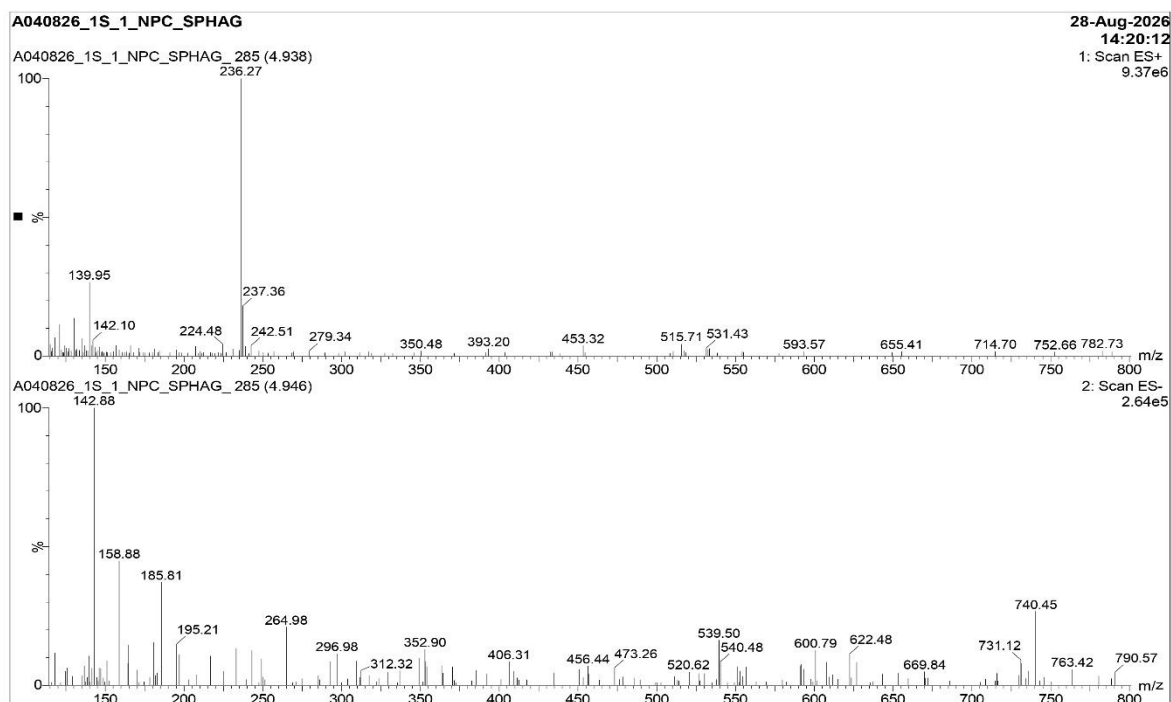


Figure 3. ESI⁺ and ESI⁻ mass spectra of the ethyl acetate fraction at retention times 4.938 and 4.946 min.

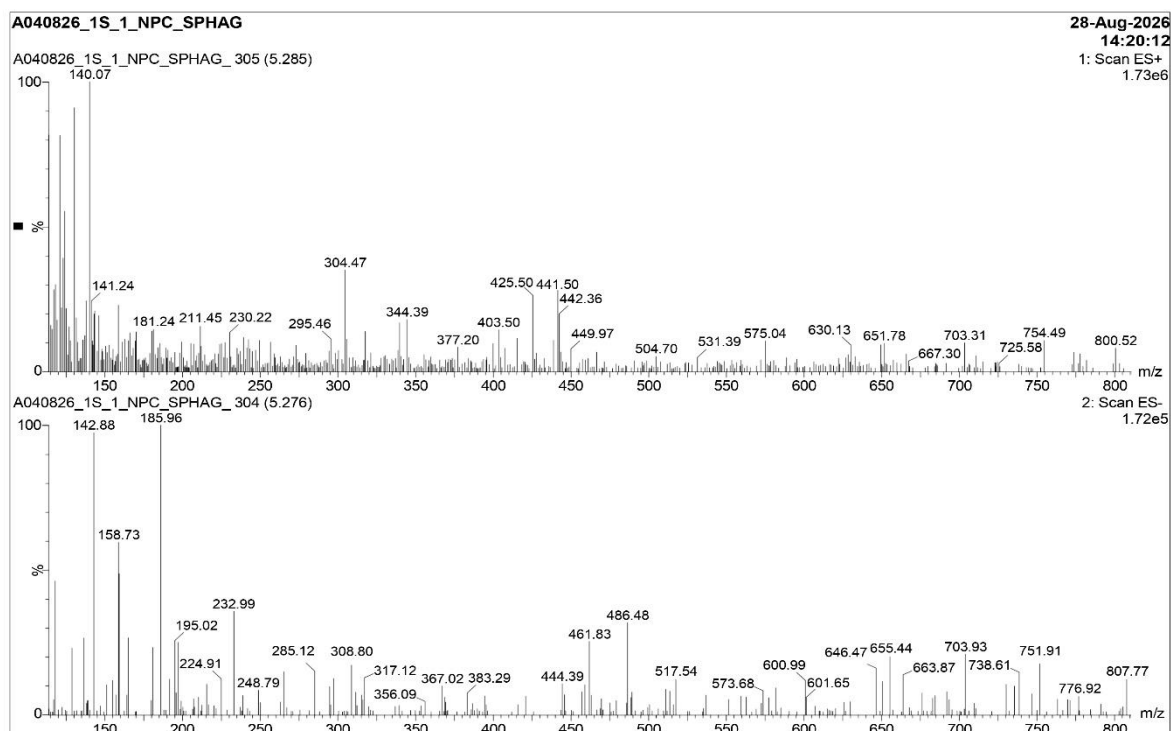


Figure 4. ESI⁺ and ESI⁻ mass spectra of the ethyl acetate fraction at **retention times 5.276 and 5.450 min.**

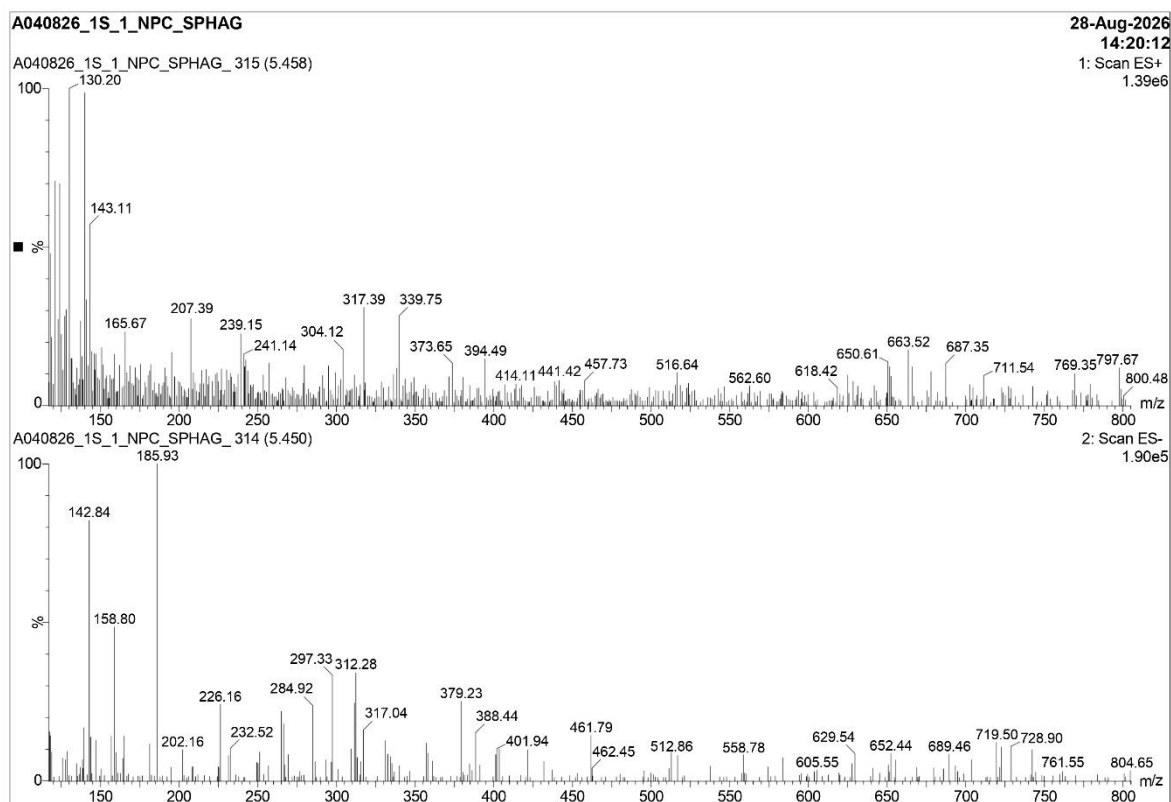


Figure 5. ESI⁺ mass spectrum of the ethyl acetate fraction at **retention time 5.458 min.**

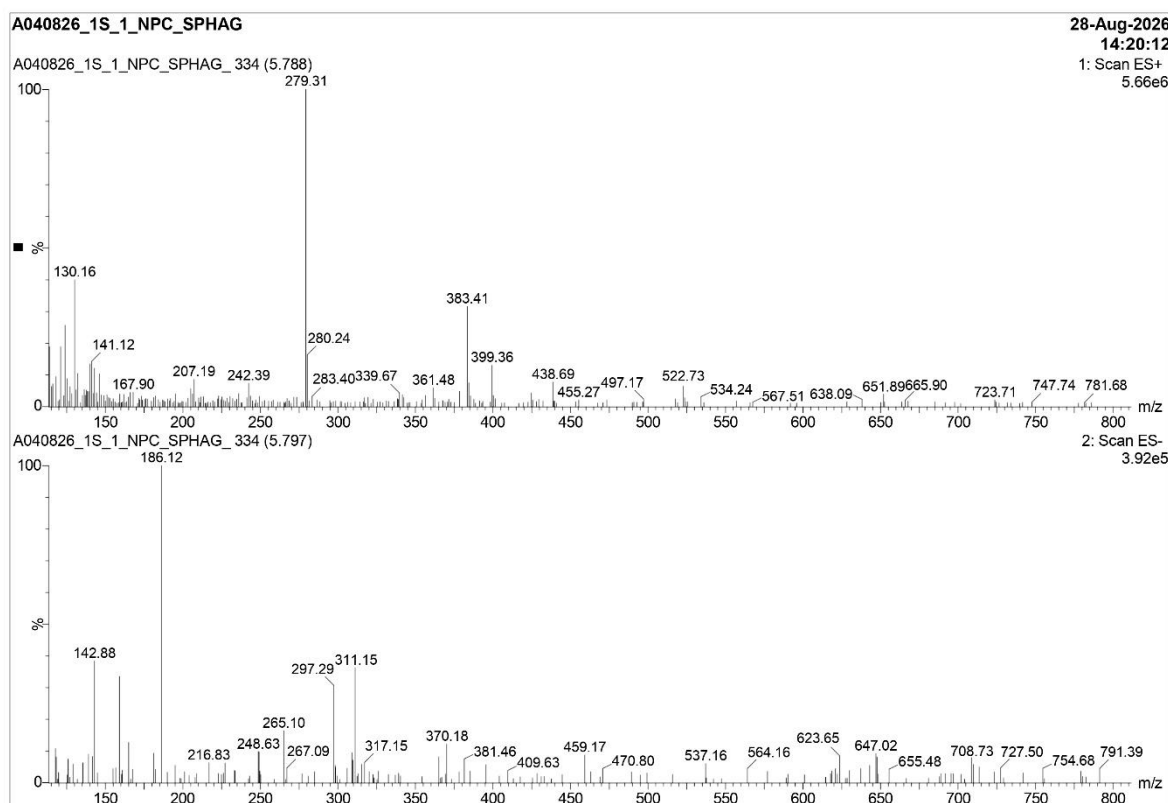


Figure 6. ESI⁻ mass spectrum of the ethyl acetate fraction at retention time 5.450 min.

The LC–MS analysis of the ethyl acetate fraction of *Sphagneticola trilobata* leaves revealed several chromatographic peaks corresponding to low- and medium-molecular-weight secondary metabolites. Based on comparison with previously reported UPLC–ESI–MS/MS metabolomic studies of the ethyl acetate fraction, the detected ions may tentatively correspond to phenolic acids, flavonoids and coumarin derivatives .

The ion observed at m/z 130.12 may represent a phenolic acid-derived fragment associated with caffeic acid-related metabolites. Phenolic acids are well known for their antioxidant activity through hydrogen atom donation and scavenging of reactive oxygen species (ROS). Reduction of oxidative stress decreases tissue damage at the wound site and suppresses inflammatory mediator production [27,28].

The ion at m/z 236.27 may be associated with coumarin- or flavanone-related constituents. Coumarins have been reported to inhibit cyclooxygenase (COX) and lipoxygenase pathways, reducing prostaglandin synthesis and inflammatory edema. These compounds also contribute to antimicrobial protection of damaged tissue [29].

The ion detected around m/z 304.47 may correspond to flavonoid-related metabolites such as luteolin or myricetin derivatives reported previously in *S. trilobata* [30]. Flavonoids suppress inflammatory signaling pathways including NF- κ B and MAPK while reducing the production of TNF- α , IL-1 β and IL-6. In addition, they promote fibroblast proliferation and collagen deposition, thereby accelerating tissue regeneration and wound contraction [31].

The ion at m/z 279.31 falls within the molecular weight range of several flavanone and methoxylated flavone derivatives reported from the ethyl acetate fraction of *S. trilobata* [32]. Such compounds possess strong antioxidant properties and may enhance wound repair by stimulating angiogenesis, collagen synthesis and epithelialization while simultaneously reducing oxidative injury at the wound site.

Previous pharmacological investigations demonstrated that *S. trilobata* extracts accelerate wound healing by reducing TNF- α and MAPK signaling and increasing collagen formation, α -smooth muscle actin (α -SMA), and FOXO1 expression. The anti-inflammatory activity has been linked to inhibition of neutrophil migration, suppression of protein kinase C activation, reduction of arachidonic acid metabolites, and inhibition of NF- κ B-mediated inflammatory signaling[33].

Therefore, the metabolites tentatively detected in the ethyl acetate fraction may collectively contribute to the observed wound-healing and anti-inflammatory activities through antioxidant action, suppression of pro-inflammatory cytokines, inhibition of NF- κ B/MAPK pathways, enhancement of collagen synthesis, and promotion of tissue remodeling. However, definitive compound identification requires confirmation by high-resolution LC–MS/MS fragmentation analysis and comparison with authentic reference standards.

Table 5. Composition and rationale for the preparation of *Sphagneticola trilobata*-mediated cellulose-supported copper nanocomposite formulations

Formulation	CuSO ₄ concentration (mM)	Cellulose matrix dimensions	Extract loading	Stirring speed (rpm)	Treatment time (h)	Drying temperature (°C)	Rationale for formulation
A	1	10 × 30 cm	<i>S. trilobata</i> aqueous extract	200	24	50	Low copper precursor concentration used to evaluate initial copper–phytochemical interaction and formation of copper-containing material within the cellulose matrix.
B	25	10 × 30 cm	<i>S. trilobata</i> aqueous	200	24	50	Intermediate-low precursor concentration used to assess the

			extrac t				effect of increasing copper ion availability on matrix modification and nanocompos ite formation.
C	75	10 × 30 cm	<i>S. trilob ata</i> aqueo us extrac t	200	24	50	Intermediate precursor concentratio n used to evaluate changes in surface deposition, optical characteristi cs and copper incorporatio n with increasing precursor availability.
D	100	10 × 30 cm	<i>S. trilob ata</i> aqueo us extrac t	200	24	50	Higher precursor concentratio n used to investigate enhanced copper loading and its influence on the physicoche mical and morphologic al characteristi cs of the nanocompos ite.
E	250	10 × 30 cm	<i>S. trilob ata</i> aqueo us	200	24	50	Highest precursor concentratio n used to evaluate the

			extract				response of the cellulose–phytochemical system under maximum experimental copper precursor exposure.
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Footnote: CuSO₄, copper sulfate; *S. trilobata*, *Sphagneticola trilobata*. The formulation concentrations were selected to provide a broad concentration-dependent experimental range (1–250 mM) while maintaining constant matrix dimensions, plant-extract loading, stirring speed and treatment duration. The concentration-dependent design enabled evaluation of the effect of copper precursor availability on the visual appearance, surface morphology, optical characteristics and dissolution/release behaviour of the resulting cellulose-supported copper-containing material. All matrices were washed after copper treatment to remove unbound precursor and dried at 50 °C.

3.10 In vitro dissolution/release profile

The in vitro dissolution/release behaviour of the prepared formulations was evaluated using the basket method. The cumulative percentage release was determined at predetermined sampling intervals.

A progressive increase in cumulative release was observed with increasing dissolution time, although the release rate varied among formulations. The dissolution profiles of formulations A–E are shown in table 6.

Table 6. invitro release study for *Sphagneticola trilobata* aqueous extract-loaded cellulose matrix–copper nanocomposites .

Time (h)	A – 1 mM	B – 25 mM	C – 75 mM	D – 100 mM	E – 250 mM
1	21.0 ± 1.2	18.0 ± 1.1	16.0 ± 0.9	15.0 ± 0.8	13.0 ± 0.7
2	38.0 ± 1.5	34.0 ± 1.4	30.0 ± 1.2	28.0 ± 1.1	25.0 ± 1.0
3	52.0 ± 1.7	48.0 ± 1.6	43.0 ± 1.4	40.0 ± 1.3	36.0 ± 1.2
4	64.0 ± 1.8	60.0 ± 1.7	55.0 ± 1.5	51.0 ± 1.4	46.0 ± 1.3
5	74.0 ± 1.9	70.0 ± 1.8	65.0 ± 1.6	61.0 ± 1.5	56.0 ± 1.4
6	82.0 ± 1.8	79.0 ± 1.7	74.0 ± 1.6	70.0 ± 1.5	65.0 ± 1.4
7	89.0 ± 1.5	86.0 ± 1.5	82.0 ± 1.4	78.0 ± 1.3	73.0 ± 1.3
8	100.0 ± 0.25	100.0 ± 0.15	100.0 ± 0.4	100.0 ± 0.6	100.0 ± 0.5

Footnote: Values are expressed as mean ± SD (n = 3).

All experimental data are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). The cumulative percentage release profiles of the different formulations were statistically compared by **two-way analysis of variance (ANOVA)**, followed by

Tukey's multiple-comparison post hoc test. Differences were considered statistically significant at $p < 0.05$.

The cumulative percentage release data were expressed as mean $\pm$ SD. Statistical comparison of the dissolution profiles was performed using [two-way ANOVA followed by appropriate post hoc test]. Statistically significant differences were considered at $p < 0.05$.

3.11 Statistical analysis of quantitative observations

Quantitative experimental data obtained from physicochemical measurements, spectroscopic measurements and dissolution/release studies were expressed as mean $\pm$ SD. Where three or more formulations were compared, one-way or two-way ANOVA was applied as appropriate, followed by a suitable post hoc multiple-comparison test. Statistical significance was accepted at $p < 0.05$.

FTIR and LC–MS results were primarily interpreted based on spectral profiles, peak positions and tentative compound identification. SEM observations were evaluated primarily through comparative morphology unless quantitative image-derived parameters were measured.

3.8 Scanning electron microscopy

SEM was used to examine the surface morphology of the untreated cellulose matrix, extract-loaded cellulose matrix and copper-treated nanocomposite. The untreated cellulose matrix exhibited a fibrous and relatively continuous surface morphology. Following loading with the *S. trilobata* extract, changes in surface appearance were observed, consistent with deposition or association of extract-derived material on the cellulose fibres. After treatment with copper sulfate, further morphological changes were observed on the cellulose surface. The copper-treated matrices showed additional surface-associated deposits/particulate structures compared with the untreated matrix. Differences in surface morphology were observed among the formulations prepared using different copper sulfate concentrations. Representative SEM micrographs are presented in Fig. 4.

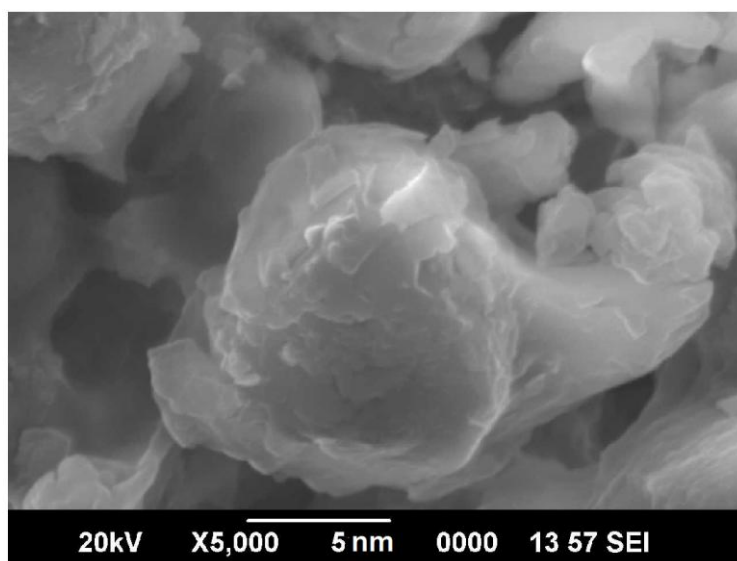


Fig. 4. SEM micrographs of the cellulose-supported *S. trilobata*-mediated copper nanocomposites of optimized formulation.

4 Discussion

4.1 Extraction and concentration of *S. trilobata* leaf constituents

The aqueous extraction procedure using 37.43 g of fresh *S. trilobata* leaves in 200 mL of distilled water produced a visibly green extract following 30 min of boiling. The progressive intensification of colour during heating and subsequent concentration indicates extraction and enrichment of water-soluble plant constituents. The final thick consistency of the concentrated extract was advantageous for the subsequent cellulose-loading step because it provided a more concentrated source of plant-derived constituents for interaction with the matrix.

The use of water as the extraction medium is consistent with the principles of green extraction because it avoids the use of potentially hazardous organic solvents during the initial extraction stage. The reported phytochemical diversity of *S. trilobata*, including phenolic and flavonoid-type constituents and other secondary metabolites, provides a plausible chemical basis for the observed colour and physicochemical characteristics of the aqueous extract [34-37].

4.2 Cellulose matrix as a supporting material

The untreated cellulose matrix exhibited a white appearance and maintained its structural integrity throughout the washing, extract-loading and copper-treatment procedures. The **10 × 30 cm** matrix provided a relatively large surface area for interaction with the plant extract and subsequent copper precursor.

The transition from white cellulose to a light olive-green colour after extract loading is consistent with association of coloured plant-derived constituents with the cellulose matrix. The persistence of the light olive-green colour after four washing cycles suggests that a proportion of the extract-associated material remained associated with the cellulose rather than being completely removed as loosely bound material.

The hydroxyl-rich surface of cellulose provides opportunities for hydrogen bonding and other intermolecular interactions with plant-derived compounds. Consequently, the cellulose matrix can function not only as a physical support but also as a substrate capable of retaining phytochemical constituents before exposure to the copper precursor.

4.3 In situ formation of the copper-containing nanocomposite

The most prominent macroscopic change during the in situ preparation was the change in colour of the extract-loaded cellulose matrix following exposure to copper sulfate. The initial light olive-green colour of the extract-loaded matrix changed after treatment with CuSO₄, with the visual intensity varying according to the copper precursor concentration.

Such a colour change is frequently used as a preliminary visual indication during plant-mediated nanoparticle synthesis because it may accompany changes in the chemical environment of metal ions and formation of nanoscale material. However, colour change alone cannot establish the formation of metallic copper nanoparticles. The observed change should therefore be interpreted together with UV–visible spectroscopy, FTIR, SEM and, where available, elemental and structural characterization.

The use of different CuSO₄ concentrations from 1 to 250 mM enabled assessment of the effect of precursor concentration on the resulting cellulose-supported material. Increasing precursor concentration provides a greater concentration of copper ions available for interaction with the phytochemical-loaded matrix; however, the final morphology and chemical state of the copper-containing phase depend on several factors, including the nature and concentration of reducing phytochemicals, reaction conditions, oxygen availability, pH and drying conditions.

Plant-mediated copper and copper oxide nanomaterial synthesis has been associated with the participation of phytochemicals such as phenolic compounds, flavonoids and terpenoid-related constituents in metal-ion reduction, coordination and stabilization [1,16,17]. Therefore, the phytochemical composition of *S. trilobata* is potentially important in determining the characteristics of the copper-containing material obtained in the present study.

4.4 FTIR and LCMS analytical outcomes

The FTIR and LC–MS findings collectively indicate that the ethyl acetate fraction of *Sphagneticola trilobata* leaves contains a chemically diverse group of oxygenated and aromatic secondary metabolites, particularly phenolic and flavonoid-type constituents. The FTIR spectrum showed prominent absorptions associated with hydroxyl, carbonyl, aromatic/conjugated and C–O functionalities, whereas LC–MS demonstrated several chromatographic components distributed across different *m/z* values. Importantly, these results should be interpreted as **evidence for classes of phytochemicals rather than definitive identification of individual compounds**, because FTIR provides information on functional groups, while conventional LC–MS molecular ions alone may not provide sufficient structural specificity for unambiguous compound identification [38–41].

The presence of O–H stretching bands at 3737.57, 3676.76 and 3647.52 cm⁻¹ is consistent with free hydroxyl functionalities commonly encountered in phenolic and flavonoid compounds. The bands observed around 1685.93 and 1651.65 cm⁻¹ further suggest conjugated carbonyl and aromatic/alkenic systems, while absorptions at 1273.28 and 1161.61 cm⁻¹ are compatible with C–O and C–O–C functionalities. Such a combination of hydroxylated aromatic structures and oxygen-containing groups is chemically consistent with the phenolic and flavonoid-rich nature previously reported for *S. trilobata* [42,43].

This interpretation is supported by recent metabolomic investigations. Ali et al. reported numerous flavonoids and phenolic acids in *S. trilobata*, including luteolin, myricetin derivatives, eriodictyol derivatives and caffeoylquinic-acid derivatives. Their study further demonstrated that several phenolic and flavonoid constituents were associated with antioxidant activity. Therefore, the FTIR profile obtained in the present study provides complementary chemical evidence for the presence of these broad metabolite classes in the ethyl acetate fraction.

The use of ethyl acetate as the fractionation solvent is also chemically relevant because ethyl acetate preferentially extracts compounds of intermediate polarity, including many phenolic acids, flavonoids and other oxygenated secondary metabolites. Previous investigations of *S. trilobata* have reported considerable antioxidant activity in ethyl acetate fractions and suggested an association between their phenolic content and antioxidant capacity [44,45,46]. In particular, studies of *S. trilobata* leaf fractions have demonstrated appreciable polyphenol content and antioxidant activity in the ethyl acetate fraction [47]. These observations are consistent with the present FTIR findings and support the interpretation that the ethyl acetate fraction concentrates biologically relevant semi-polar antioxidant constituents.

The LC–MS chromatogram further demonstrated that the ethyl acetate fraction was chemically heterogeneous, with several constituents exhibiting distinct retention times. The major peaks observed at 3.028, 4.938, 5.276, 5.450 and 5.788 min indicate the presence of multiple secondary metabolites with different chromatographic properties. This chemical diversity agrees with previous chromatographic investigations of *S. trilobata*, in which phenolic compounds, flavonoids, terpenoids and other secondary metabolites were detected [48,49]. Recent UPLC–ESI–MS/MS metabolomic analysis of *S. trilobata* identified a broad range of metabolites, including flavonoids, phenolic acids, terpenoids and related secondary metabolites .

Of particular relevance, recent high-resolution UPLC–ESI–MS/MS profiling of *S. trilobata* identified several flavonoid and phenolic constituents, including luteolin, myricetin derivatives, eriodictyol derivatives and caffeoylquinic-acid derivatives [50]. These findings provide a strong phytochemical basis for the present observation of flavonoid- and phenolic-related signals. However, the m/z values obtained in the present study should be considered **tentative annotations**, rather than definitive compound identifications, because authentic standards, accurate-mass measurements and diagnostic MS/MS fragmentation were not available for confirmation.

The ion observed at **m/z 130.12** may represent a low-molecular-weight phenolic-derived ion or fragment. Phenolic acids and caffeoyl-related metabolites are well documented in *S. trilobata* [51,52]. However, this m/z value alone is insufficient to assign a specific phenolic acid because structurally unrelated metabolites may generate common low-mass ions during electrospray ionization. Therefore, the present finding is more appropriately interpreted as supporting the **presence of phenolic-derived constituents** rather than as definitive identification of a particular phenolic acid.

Similarly, the peak at **m/z 236.27** may correspond to an oxygenated aromatic metabolite or a fragment/adduct derived from a larger parent compound. It should not be definitively designated as a caffeoylquinic acid because intact caffeoylquinic acids generally possess considerably higher molecular masses. Recent high-resolution metabolomics of *S. trilobata* identified caffeoylquinic-acid derivatives at substantially higher m/z values and supported their assignments using accurate mass and fragmentation data [53]. Thus, the present m/z 236.27 signal is better described as a **putative oxygenated phenolic/flavonoid-related ion**.

The ion at **m/z 304.47** also requires cautious interpretation. Although flavonoid-type compounds are well documented in *S. trilobata* [54,55], this value alone cannot establish the presence of luteolin or myricetin. Recent metabolomic analysis identified luteolin and myricetin derivatives using accurate-mass and MS/MS information rather than nominal m/z

alone [56]. Therefore, the peak at m/z 304.47 is more appropriately described as a **putative flavonoid-related metabolite**.

Likewise, the m/z 279.31 peak may fall within the chemical space of flavanone/flavonoid-related metabolites. Eriodictyol and related flavanones have previously been reported from *S. trilobata* [57]. Nevertheless, assignment of the present peak specifically to eriodictyol would require confirmation using accurate mass, characteristic product ions and, preferably, an authentic reference standard. Consequently, it is more scientifically appropriate to describe this signal as a **putative flavanone/flavonoid-related constituent**.

An important strength of the present investigation is the **convergence between the FTIR and LC–MS findings**. The FTIR spectrum demonstrated hydroxyl, carbonyl, aromatic and C–O-containing functionalities, whereas LC–MS indicated the presence of several low- and medium-molecular-weight constituents within the phenolic/flavonoid chemical space. The agreement between these independent analytical approaches provides complementary evidence for the chemical composition of the ethyl acetate fraction. Nevertheless, FTIR and LC–MS provide different levels of structural information: FTIR identifies functional-group characteristics, whereas LC–MS provides chromatographic and molecular-mass information. Therefore, neither technique should be used alone to claim definitive identification of individual phytochemicals [58-60].

The phytochemical composition observed in the present study is particularly relevant to the antioxidant potential of the fraction. Phenolic acids and flavonoids possess hydroxylated aromatic structures capable of donating hydrogen atoms or electrons to reactive oxygen species. By reducing excessive oxidative stress, these constituents may protect cellular components from oxidative damage and help create a biochemical environment favourable for tissue repair [61,62]. This is relevant to wound healing because persistent oxidative stress can prolong inflammation and adversely affect fibroblast activity, collagen deposition and tissue remodeling [63].

The present chemical findings can also be related to the anti-inflammatory and wound-healing effects previously demonstrated for *S. trilobata*. El Fishawy et al. reported that *S. trilobata* treatment promoted wound closure in experimental wound models and was associated with modulation of inflammatory and tissue-repair pathways, including TNF/MAPK-related signaling, collagen formation, α -SMA and FOXO1 expression [64]. These findings provide a biological framework through which phenolic and flavonoid constituents detected in the present ethyl acetate fraction may potentially contribute to wound repair.

Flavonoids such as luteolin- and myricetin-related compounds have been widely investigated for their antioxidant and anti-inflammatory properties, including modulation of inflammatory signaling pathways [65,66]. Therefore, the detection of flavonoid-related ions in the present fraction provides a plausible chemical explanation for antioxidant and anti-inflammatory potential. However, such an interpretation should be regarded as **mechanistically plausible rather than proof of causality**, because the present LC–MS analysis does not establish that a particular detected metabolite is responsible for the observed biological activity.

An interesting comparison with previous fractionation studies is that the wound-healing activity of *S. trilobata* may not be attributable exclusively to phenolic and flavonoid constituents. Recent experimental work demonstrated that different fractions of the plant can

exhibit differential wound-healing effects, indicating that compounds with different polarities may contribute to the overall biological activity [67]. In addition, phytochemical investigations have documented terpenoids, sesquiterpenoids, diterpenoids, phenolics and flavonoids in *S. trilobata* [68]. Thus, the wound-healing effect of the plant is likely to arise from a **multicomponent phytochemical system**, rather than a single compound or single chemical class.

This multicomponent nature may be advantageous for wound repair because healing involves several overlapping processes, including control of oxidative stress, inflammation, fibroblast proliferation, collagen deposition, angiogenesis, re-epithelialization and tissue remodeling. The documented ability of *S. trilobata* extracts to influence inflammatory and tissue-repair pathways supports the possibility of such a multitarget pharmacological effect [69]. The phenolic and flavonoid-related constituents indicated by the present analysis may therefore represent one component of this broader phytochemical activity.

The FTIR spectrum also contained several bands that should be interpreted conservatively. The absorption around **2359.63 cm⁻¹** is commonly associated with atmospheric CO₂ and should not be considered evidence of a plant-derived phytochemical. Likewise, the bands at approximately 2166.47, 2088.67 and 1978.42 cm⁻¹ occur in regions where weak combination, overtone or instrumental/environmental absorptions may occur. Therefore, assigning these bands to specific phytochemical groups would not be justified without additional spectral evidence [70]. Excluding these uncertain bands from the major phytochemical interpretation improves the reliability of the FTIR analysis.

Taken together, the present findings are in agreement with the existing phytochemical literature on *S. trilobata*. Previous studies have demonstrated antioxidant activity in ethyl acetate fractions and have reported phenolic and flavonoid constituents in this fraction. More recent high-resolution metabolomics has further confirmed the presence of diverse flavonoids, phenolic acids and related metabolites in *S. trilobata*. Independent pharmacological investigations have additionally demonstrated wound-healing activity associated with modulation of inflammatory and tissue-repair pathways. Thus, the present FTIR and LC–MS results provide **chemical evidence supporting, but not independently proving, the potential contribution of phenolic and flavonoid-rich constituents to the biological activity of the ethyl acetate fraction.**

4.X.1 Implications and limitations

The major implication of these findings is that the ethyl acetate fraction represents a chemically promising semi-polar fraction containing functional groups and metabolite classes compatible with antioxidant and anti-inflammatory activity. The agreement between FTIR and LC–MS findings, together with previously reported phytochemical and pharmacological evidence, provides a rational basis for further investigation of this fraction as a potential source of wound-healing phytoconstituents.

Nevertheless, the LC–MS assignments should remain explicitly **tentative**. Accurate-mass LC–MS/MS analysis with diagnostic fragmentation, comparison with authentic standards and, where possible, isolation followed by NMR spectroscopy would be required for definitive structural identification. This is particularly important for the ions at *m/z* 130.12, 236.27, 304.47, 185.93 and 279.31, for which several structural possibilities may exist.

Overall, the combined FTIR and LC–MS characterization demonstrates that the ethyl acetate fraction of *S. trilobata* leaves contains diverse oxygenated and aromatic secondary metabolites, with phenolic and flavonoid-related constituents being particularly plausible. These chemical characteristics are consistent with previously reported antioxidant properties and with the documented wound-healing and anti-inflammatory effects of *S. trilobata*. The findings therefore provide a **scientifically reasonable chemical rationale for further evaluation of the fraction in wound-healing formulations**, while avoiding overstatement of individual compound identities.

4.6 SEM morphology of the cellulose-supported material

Scanning electron microscopy (SEM) was employed to investigate the surface morphology and distribution of *Sphagneticola trilobata*-mediated copper nanoparticles (CuNPs) incorporated into the cellulose matrix. The SEM micrograph showed a **continuous but irregular cellulose surface with dispersed nanoscale particulate structures**, indicating the incorporation of CuNPs within/on the polymeric matrix. The relatively rough and heterogeneous morphology observed after CuNP incorporation may be attributed to the interaction between the cellulose chains and the phytochemical-capped copper nanoparticles.

The presence of discrete bright/dense particulate regions on the cellulose surface can be attributed to the incorporation of copper nanoparticles. Their distribution throughout the matrix suggests that the biosynthesized CuNPs were successfully associated with the cellulose network. However, some regions of **particle clustering/agglomeration** may be visible, which is commonly encountered with nanoparticles because of their high surface energy and tendency to minimize surface free energy through particle–particle interaction. The extent of agglomeration may also depend on the concentration of CuNPs, drying conditions and interactions between cellulose functional groups and the nanoparticle surface.

The observed morphology can be correlated with the FTIR findings. Cellulose contains abundant hydroxyl groups that can participate in hydrogen bonding and other interactions with phytochemical molecules and the CuNP surface. In the present system, phytochemicals from *S. trilobata* may also act as **reducing and stabilizing/capping agents** during CuNP synthesis. Consequently, phenolic and other oxygen-containing groups identified by FTIR may facilitate interaction between the biosynthesized CuNPs and the cellulose matrix. This could contribute to the observed changes in surface morphology compared with unloaded cellulose.

The SEM morphology also complements the physicochemical characterization of the biosynthesized CuNPs. If the CuNPs appear as relatively well-dispersed nanoscale structures within the cellulose matrix, this indicates successful incorporation rather than complete phase separation. A relatively uniform distribution is desirable for a functional cellulose-based wound-dressing material because it may provide more homogeneous exposure of the nanoparticles at the matrix surface and potentially improve their interaction with microorganisms.

From a wound-healing perspective, the incorporation of CuNPs into cellulose provides a **multifunctional matrix** in which the cellulose acts as a structural and moisture-managing support while copper nanoparticles may contribute antimicrobial and other biological functions. Copper is also biologically relevant to wound repair because copper-dependent processes are involved in angiogenesis and extracellular-matrix remodeling. Therefore,

successful incorporation and reasonably uniform distribution of CuNPs within the cellulose matrix may be advantageous for the development of an antimicrobial wound-dressing system.

4.8 In vitro dissolution/release behaviour

The in vitro release study demonstrated that the cellulose matrix–copper nanocomposites provided a **time-dependent and copper-concentration-dependent release of *Sphagneticola trilobata* aqueous extract** (Table 6). All formulations showed a progressive increase in cumulative release with increasing incubation time, indicating gradual hydration of the cellulose matrix and subsequent diffusion of the incorporated phytoconstituents into the release medium. Cellulose and its derivatives are widely employed as sustained-release matrices because their hydrophilic polymeric networks undergo hydration and swelling, creating diffusional pathways through which incorporated active compounds can gradually migrate into the surrounding medium .

A clear **inverse relationship between copper concentration and release rate** was observed. Formulation A containing 1 mM Cu showed the highest release throughout the first 7 h, whereas formulation E containing 250 mM Cu showed the lowest release. At 4 h, the cumulative release decreased from **64.0 ± 1.8% in A to 60.0 ± 1.7%, 55.0 ± 1.5%, 51.0 ± 1.4% and 46.0 ± 1.3% in B, C, D and E**, respectively. Similarly, at 7 h, the release decreased from **89.0 ± 1.5% in A to 86.0 ± 1.5%, 82.0 ± 1.4%, 78.0 ± 1.3% and 73.0 ± 1.3% in B–E**, respectively. Thus, increasing copper concentration progressively retarded extract liberation.

This behavior can be explained by the effect of copper nanoparticles on the **microstructure, compactness and diffusional resistance of the cellulose matrix**. Increasing nanoparticle incorporation can alter polymer-chain mobility, porosity, swelling and permeability, thereby modifying the diffusion pathway available to an incorporated compound . Recent literature emphasizes that polymer–active interactions, fillers/nanoparticles and matrix crystallinity can substantially modify morphology and permeability and consequently alter release kinetics. In the present system, the higher copper concentration may therefore have produced a relatively more compact cellulose–Cu network, increasing the diffusional path length and decreasing the apparent diffusion rate of the aqueous extract.

The **initial release phase** may be attributed mainly to rapid hydration of the cellulose matrix and diffusion of extract constituents located near the matrix surface. As hydration progressed, the release became comparatively slower because the remaining extract had to diffuse from deeper regions of the polymeric network. This interpretation agrees with established models of cellulose-based delivery systems, in which release may involve a combination of **swelling, diffusion and, depending on the matrix, polymer relaxation or erosion**. Previous studies have similarly reported that the mesh size and degree of network restriction strongly influence the movement of incorporated molecules through cellulose-based hydrogels.

The present findings are broadly consistent with previous reports on cellulose-based controlled-release systems, although the **8-h release duration observed here represents a relatively short, readily controllable release window** compared with some cellulose-based systems designed for prolonged delivery. Sun et al. reported that cellulose and cellulose-derived materials can regulate drug release through changes in solubility, gel formation and matrix properties, supporting their application in sustained drug-delivery systems. Similarly, studies of cellulose-based hydrogels have demonstrated that hydration and formation of a

swollen polymer network can provide a diffusional barrier and progressively regulate release .

The present study also agrees with more recent evidence that **nanostructural reinforcement can restrict polymer-chain mobility and modify diffusion pathways**. Recent cellulose nanocomposite studies have shown that incorporation of nanoscale reinforcing components can influence swelling, network formation and diffusion, producing smoother and more controllable release profiles. However, unlike many conventional cellulose systems in which the polymer composition, crosslinking density or drug loading is varied, the present study specifically demonstrates that **variation in copper concentration can be used as a formulation variable to modulate the release of a complex plant extract**. This provides an additional formulation strategy in which the inorganic component contributes not only to the nanocomposite structure but also to regulation of extract release.

An important difference among the present formulations was the extent of release retardation. **Formulations A–C showed relatively rapid release**, whereas formulation E produced the most pronounced retardation. Although a slower release rate may be advantageous for prolonged delivery, excessive matrix restriction may reduce the availability of active constituents during the desired therapeutic/application period. In contrast, formulation D occupied an intermediate position between these two extremes. This intermediate behavior is particularly important when the objective is not simply to maximize release but to obtain a **controlled and reproducible release over a predefined period**.

Based on the release behavior, **formulation D containing 100 mM Cu was selected as the optimized formulation for controlled release**. Formulation D released approximately **51% of the extract by 4 h, 61% by 5 h, 70% by 6 h and 78% by 7 h**, followed by complete release at 8 h. In comparison, A released 89% by 7 h, indicating comparatively rapid liberation, whereas E released only 73% by 7 h, indicating stronger release retardation. Therefore, D provided a **balanced release profile**, avoiding both excessive early release and excessive retardation.

This selection has practical implications. A formulation intended for local delivery of plant-derived bioactives should ideally maintain availability of the active constituents over the intended period rather than releasing most of the payload immediately. The controlled-release characteristics of D could therefore help maintain a more sustained concentration of extract constituents at the site of application. Cellulose-based films and matrices are particularly attractive for localized delivery because they can combine structural integrity, hydration, retention and controlled diffusion of incorporated bioactive agents.

Despite the differences in release rate, all formulations reached approximately **100% cumulative release at 8 h**: A, $100.0 \pm 0.25\%$; B, $100.0 \pm 0.15\%$; C, $100.0 \pm 0.40\%$; D, $100.0 \pm 0.60\%$; and E, $100.0 \pm 0.50\%$. This finding is important because it indicates that increasing copper concentration **affected the kinetics of release rather than preventing eventual liberation of the extract**. The eventual complete release may be associated with progressive hydration and swelling of the cellulose matrix, which increases the accessibility of the incorporated extract to the external medium. Similar swelling–diffusion mechanisms are well established for cellulose-based delivery systems.

From a formulation perspective, the fact that D achieved complete release while maintaining a slower release trajectory during the preceding 7 h suggests that **100 mM Cu represents a**

useful compromise between matrix retention and extract accessibility. In contrast, the higher copper concentration in E may have generated greater diffusional resistance without providing an additional advantage in terms of final cumulative release, since both D and E ultimately achieved complete release.

Collectively, the findings indicate that **copper concentration is an important formulation variable capable of tuning the release characteristics of *S. trilobata* aqueous extract from a cellulose matrix.** The observed concentration-dependent retardation is consistent with the established role of polymer-network architecture, swelling and diffusional resistance in cellulose-based drug-delivery systems .

Formulation D (100 mM Cu) was therefore considered the optimized formulation for controlled-release performance, because it exhibited an intermediate, gradual release profile, reaching $78.0 \pm 1.3\%$ at 7 h and $100.0 \pm 0.6\%$ at 8 h. The optimized formulation may provide a useful platform for sustained local delivery of *S. trilobata* phytoconstituents and could be particularly relevant where prolonged exposure rather than immediate release is desired. Further confirmation of the release mechanism through **zero-order, first-order, Higuchi and Korsmeyer–Peppas kinetic models** would strengthen this interpretation and allow the dominant release mechanism to be established quantitatively.

Two-way ANOVA revealed a highly significant effect of release time ($F_{7,80} = 6265.02, p < 0.0001$) and formulation concentration ($F_{4,80} = 362.42, p < 0.0001$) on cumulative drug release. A significant time $\times$ formulation interaction was also observed ($F_{28,80} = 10.81, p < 0.0001$), indicating that the influence of formulation concentration varied throughout the release period. Formulation A (1 mM) showed the fastest release, whereas formulation E (250 mM) exhibited the slowest release during the initial and intermediate stages. By 8 h, all formulations achieved approximately 100% cumulative release. Tukey's multiple-comparison test confirmed significant differences among the formulation groups, demonstrating that formulation concentration significantly influenced the release profile.

4.9 Overall significance of the developed system

The sequential changes observed from the original white cellulose matrix to the light olive-green extract-loaded matrix and subsequently to the copper-treated material demonstrate the progressive modification of the cellulose substrate during the preparation process. The combination of plant extraction, cellulose loading and in situ copper treatment provides a matrix-supported approach that differs from conventional synthesis of nanoparticles as free colloidal suspensions.

The use of *S. trilobata* is supported by previous reports demonstrating its phytochemical diversity, antioxidant and cytotoxic activities and its ability to participate in the green synthesis of gold, zirconium, zinc and titanium-based nanomaterials [88-90]. The present approach extends this concept toward a **cellulose-supported copper-containing nanocomposite**, with the additional evaluation of the ethyl acetate phytochemical fraction, LC–MS profile, FTIR characteristics, surface morphology, optical properties and dissolution/release behaviour.

5. Conclusion

The present study demonstrated the successful development and physicochemical characterization of a cellulose-based matrix incorporated with *Sphagneticola trilobata*-mediated copper nanoparticles (CuNPs). The phytochemical characterization of the ethyl acetate fraction of *S. trilobata* by FTIR and LC–MS indicated the presence of hydroxyl-, carbonyl-, aromatic- and C–O-containing functionalities and several tentatively annotated phenolic- and flavonoid-related metabolites. These phytoconstituents may contribute to the reduction and stabilization of copper nanoparticles during the green synthesis process. The SEM analysis demonstrated the incorporation of particulate structures within the cellulose matrix, with the observed morphology supporting the association of CuNPs with the polymeric network. The combination of a cellulose matrix and plant-mediated CuNPs provides a potentially multifunctional platform in which cellulose can serve as a structural and moisture-managing material, while copper-based nanoparticles may contribute antimicrobial and wound-repair-related functions. Previous studies have demonstrated that copper-containing nanocomposites can promote angiogenic responses and possess antibacterial potential, supporting the rationale for their investigation in wound-dressing applications. Overall, the findings suggest that *S. trilobata*-mediated CuNP-loaded cellulose is a promising bio-based nanocomposite platform for further development as a wound-care material. However, additional studies, including CuNP loading efficiency, particle-size distribution, mechanical properties, swelling and water-vapour transmission, copper-ion release, antimicrobial activity, cytocompatibility, hemocompatibility, stability and in-vivo wound-healing evaluation, are required to establish its therapeutic suitability and safety. The present LC–MS assignments should also be considered tentative until confirmed by high-resolution MS/MS and/or authentic standards.

Data Availability: All data generated and analyzed during this study are original and were generated by the authors. The data supporting the findings of this study are presented within the manuscript and are available from the corresponding author upon reasonable request.

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