Research Article

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Embedding green synthesized zinc oxide nanoparticles in cotton fabrics and assessment of their antibacterial wound healing and cytotoxic properties: An eco-friendly approach

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Abstract: This study explores the potential of the natural and cost-effective method of wound healing using *Alternanthera sessilis* by an *in vitro* study (using fibroblast L929 cells). Gram-positive bacteria *Staphylococcus aureus* shows a zone of inhibition of 20 mm at 60 µg concentration in the antibiogram profile against the zinc oxide nanoparticles (ZnONPs) wetted in fabrics synthesized from the Amaranthaceae family. Through characterization studies of the AS-ZnONPs, it was found that UV–visible spectra show a peak in the range of 350–460 nm, Fourier transform infrared spectroscopy spectra show a correlation peak in the range of 340–4,500 cm⁻¹, scanning electron microscope with electron diffraction analysis results in a peak in the range of 7.8–9.4, and high-resolution transmission electron microscope, which exposes the morphological character (diamond shape in a black and white background), shows a peak at 200 nm. This work shows that the leaf extract of *A. sessilis* might support the ancient method of wound healing.

Keywords: *Alternanthera sessilis*, ZnONPs, antimicrobial fabrics, wound healing

Abbreviations

- **FT-IR**: Fourier transform infrared spectroscopy
- **HR-TEM**: high-resolution transmission electron microscope
- **MDR**: multidrug resistant
- **MHA**: Muller–Hinton agar
- **MRSA**: methicillin resistant *Staphylococcus aureus*
- **MTT**: yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl))-2,5-diphenyltetrazolium bromide)
- **SEM with EDX**: scanning electron microscope with electron diffraction analysis
- **UV-Vis**: UV-visible
- **XDR**: extreme drug resistant
1 Introduction

The distinctive properties of the nanoparticles and synthesis of innovative materials in nanosize put them at the forefront of science and technology [1]. Nanotechnology involves the manipulation of individual atoms and molecules. A nanoparticle is desirable because of its uniformity, conductivity, and optical property. Excitement was observed in nanotechnology by developing new materials that are applicable in the field of nanomedicine, energy production, and biomaterials. Green nanotechnology provides knowledge about the versatile pharmacological nature of rare plant species [2–4]. Alternatively, nanoparticles play a role as effective pesticides and control plant disease and increase crop production by acting as potent fertilizers. Using nanomembranes helps to remove biological and chemical components from polluted water. Phytonanotechnology is simple, rapid, eco-friendly, and biocompatible. By targeting valence electrons, zinc oxide nanoparticles (ZnONPs) generate reactive oxygen species and bring structural changes to cellular components such as ribosomes, Golgi bodies, bacterial cell membrane, and genetic material [5]. In the transport of drugs, it also serves as a biological carrier. The reduction of metal ions exploits the reduction of phytocompounds due to zero valency of the nanoparticles. It possesses multiple roles in antibacterial activities and in curing ulcers. Zinc oxide nanoparticles prevent bacterial growth in vitro and in vivo, accelerate wound closure, expose biocompatibility, and reduce cytotoxicity. The green synthesis of zinc oxide nanoparticles is cheap and eco-friendly, and zinc oxide nanoparticles are a novel alternative therapeutic agent for wound healing [6]. Alternanthera sessilis is a folklore medicine for treating various ailments. It belongs to Amaranthaceae and is a dwarf copper leaf or sessile joyweed. It is a proanthocyanidin herb. The leaves of A. sessilis contain β-sitosterolpalmalitate, nonacosane, 16-hentriacontane, handianol, and oxalic acid. The presence of α-tocopherol and β-tocopherol shows antimicrobial activity against Trichophyton mentagrophytes and Staphylococcus aureus [7,8]. The root and shoot have antihypertensive properties. The leaves possess medicinal properties to treat bronchitis and diarrhea and help to rejuvenate the skin. It also helps to treat inflamed wounds. It also bears another critical therapeutic role in treating liver disease-related gangrene. Catechin, ethyl gallate, ferulic acid, daidzein, and chlorogenic acid are effective bioactive constituents that act as a precursor in the anti-inflammatory, antimicrobial, and antiwound healing activities. Secondary metabolites or proteins in the plant extract act as a reducing and capping agent, which help to stabilize the formation of nanoparticles.

S. aureus is a common dangerous Gram-positive coccus-shaped bacterium. It causes pneumonia, heart valve infection, and bone infections. Sneezing enters the bloodstream and causes redness, blisters, and abscesses. S. aureus produces many toxins and causes toxic shock syndrome and food poisoning. Beta-lactam antibiotics show resistance to most strains of S. aureus. This was later entitled methicillin-resistant S. aureus. Antibiotics that show susceptibility to S. aureus are sulfamethoxazole, clindamycin, minocycline, and vancomycin [9]. A revolutionary technique used in cotton fabrics in recent years to expose their active surfaces with antimicrobial characteristics is nano-coating. An in vivo study showed enhanced solubility, efficacy, biocompatibility, and cytotoxic effect of synthesis on fibroblast L929 cells [10]. This cell line derived from the subcutaneous tissue of the mouse shows the features of cancer growth. Fibroblast cells contain all components of proteins, amino glycans, and proteoglycans. The multiple simultaneous roles of fibroblast, which are interwoven in fibrosis, are wound healing, angiogenesis, and inflammation [11]. In this study, we inspect the antibacterial potential and expose the wound healing efficacy of the aqueous extract of A. sessilis leaves mediated zinc oxide nanoparticles toward in the fibroblast L929 cells.

2 Materials and methods

2.1 Plant material collection and extract processing

A cheap, obovate copperleaf, with sessile spike flowers and viridescant perpetual herbs are plucked from the water canals in the surrounded area of Rasipuram [12]. Poststerilization techniques include separation of affected leaves, shadow drying, and pulverization [13]; then, 10 g of fresh leaves was immersed in 200 mL of MilliQ water in a 500 mL Erlenmeyer flask. Then, the solution was boiled at 70°C for 10 min; the obtained solution was filtered using Whatman no. 1 filter paper and used for zinc oxide nanoparticle synthesis.

2.2 Metallic nanoparticle synthesis (zinc)

Zinc acetate is prepared for synthesis by dissolving 1 mM zinc acetate in 50 mL of MilliQ water, followed by stirring continuously for 1 h. Yellow color is obtained at 35°C after adding 20 mL of zinc acetate to 25 mL of the prepared
plant extract solution (A. sessilis leaf extract) slowly [14]. Further purification is done by centrifuging the precipitate or residues several times in deionized water, and at last, calcined white crystals of zinc oxide nanoparticles are obtained. To remove water molecules, the crystals are kept in an oven at 60°C for 2 h. Finally, the obtained pellets are stored in an airtight container for further use.

2.3 Collection of commensals

Sterile and adequately sealed containers are filled with fresh, early morning voided sputum, midstream urine, pus-soaked swab with a rotten egg odor, blood- or mucus-covered stools, and infectious blood samples collected from the private hospitals and laboratories in Madurai by wearing aseptic gloves, a germ-free head cap, and an unsoiled labcoat. The swabs were aseptically handled by placing them in an appropriate medium; in the case of a blood sample, it is laid in EDTA to prevent clotting. Then, complete history of the patients, such as age, sex, and appearance of the sample, was noted clearly on the container using a marker pen [15,16]. To isolate the targeted species and prevent cross contamination, all the commensals were packed in a leak-proof container and placed in ice cubes for transportation.

2.4 Phenotype screening

The commensals are isolated in appropriate media like nutrient agar, McConkey agar, eosin methylene blue agar, and blood agar media in quadrants. The zig-zag method of streaking is performed on the appropriately labeled plate. After 24 h of incubation, a soft raised mucoid, off-white with wintergreen smell colonies is observed on the plate [17,18]. Then, its phenotypic characters are noted by preparing a smear, which is then rinsed with primary stain, mordant, acetone and safranin for a few minutes and, at last, wiped with the help of tissue paper and observed under a microscope to identify whether it is gram-positive or gram-negative isolates. The isolates are further confirmed by rinsing with solvents like methyl red, Kovacs reagent, and glucose broth by showing some color changes, which are collectively known as biochemical tests [19].

2.5 Bactericidal action

A well is created in the Muller–Hinton agar plate using a sterile cork borer of 6 mm, and the isolates were spread uniformly using a sterile cotton swab tip and incubated in an inverted position [20,21]. Forceps are used to remove the plugs in an aseptic manner. After emptying the crude zinc oxide nanoparticles in various concentrations (20, 40, and 60 µg) using a micropipette into each well, and without any disturbances, the plates are placed in an upright manner in an incubator.

2.6 Cotton sensitivity test

Cotton and mixed cotton cloth are cut into small pieces and soaked in the crude zinc oxide nanoparticles later on they are aseptically placed in the freshly prepared Muller–Hinton agar plate in which the isolates are spread evenly [22,23]. A plate labeled as control contains a piece of cotton and mixed cotton cloth that is free from nanoparticle extract.

2.7 Characterization study of zinc oxide nanoparticles coated on cotton

2.7.1 UV-Vis spectra

UV-Vis spectroscopy is a soft, analytical technique to identify the organic and inorganic compounds and also measure the absorbed light and express the derivatives of the solid or liquid nanomaterial in the form of wavelengths.

2.7.2 FT-IR

Fourier transform infrared (FT-IR) spectroscopy is a valuable tool that converts the detector output to an interpretable spectrum. It identifies the atom and bond arrangements within the organic molecule. Through the frequency and software in the spectroscopy, it is easy to identify the molecular and functional groups in the sample [24]. It helps to easily characterize the stability and variability of the various antioxidant properties in the sample.

2.7.3 SEM with EDAX

Scanning electron microscopy (SEM) describes the qualitative and quantitative analyses of the specimen with a peak magnification. Then, the samples in SEM are prepared by cutting them into thin slices using a microtome
and rubbed on a coarse surface and cleansed using organic solvents [25]. Energy-dispersive X-ray spectrometers focus on the specimen by an array of electrons, and the X-ray signals fall on the specimen and obtain the composition of the elements present in the sample [26].

### 2.7.4 HR-TEM

The sample for a high-resolution transmission electron microscope (HR-TEM) is made into thin sections using a microtome and mounted on the slide, and the crystallographic structure of the specimen is obtained against the black and white surrounding [26].

### 2.7.5 Enrichment of collected cell lines

The cell lines were procured from the cell repository of the National Centre for Cell Sciences (NCCS), Pune, India. These were maintained in a humidified environment with 5% CO$_2$ at 37°C and cultured in Dulbecco’s modified Eagle’s medium (DMEM), which was enhanced with 10% fetal bovine serum (FBS). Penicillin (100 U·mL$^{-1}$) and streptomycin (100 μg·mL$^{-1}$) acted as bactericidal agents.

### 2.7.6 L929 assay

Dulbecco’s modified Eagle’s medium consists of L929 cell lines and model nanoparticles (AS-ZnONPs) whose viability is branded after 24 h of incubation. Then, the reductase in cell lines reduces MTT (yellow 3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) to formazan crystals by developing a purple color product; these crystals show absorbance at 570 nm in a microtitre plate after dissolving them in dimethylsulfoxide (DMSO) and are statistically analyzed.

### 2.7.7 Cell migration assay

About $1 \times 10^5$ cells·mL$^{-1}$ are appropriately cultured at 37°C for a day in 24 wells. Then, a straight-line scratch is made using the tip of the micropipette (200 μL). Debris cells are washed clean two times using PBS. Then, the cells are mingled with different concentrations of AS-ZnONPs (5/15 μg) and maintained in the cell incubator for 5% CO$_2$ at 37°C for 1.5 days (36 h). By using a fluorescent and electron microscope, the images of cells mingled with zinc oxide nanoparticles are captured at different time intervals and processed using the software.

### 3 Results and discussion

#### 3.1 Metallic nanoparticle synthesis

After taking the dry weight of the leaf powder, they undergo heating with solid magnetic stirring for 1h with zinc acetate, and bronze yellow solution at 35°C is obtained after treating with zinc acetate and stirring slowly [27]. Further purification is done, and the crystals of zinc oxide nanoparticles are obtained after calcination at 250°C for 4–5 h, in which the *A. sessilis* extract is added dropwise. The nontoxic nature and the presence of the biomolecules in the plant extract play an efficient role in reducing metals and act as a capping agent in the synthesis of zinc oxide nanoparticles, which is clearly explained and shown in Figure 1.

#### 3.2 Isolated microbes

From different private hospitals and laboratories in Madurai, nearly 25 samples were isolated from the other age groups of patients in various sectors of the hospitals. About 7% of isolated strains from males in the dental ward, CCU, general ward, and ICU are Gram positive and Gram negative. The other 7% of strains isolated from females in the labor ward, CCU, ICU, and general ward are only Gram negative. The rest 6% of isolated strains from infants and children of ICU, CCU, general ward, and SOT are both Gram positive and Gram negative, and 1% are other commensals. A detailed explanation is given in Figure 2. Biochemical tests, which are other confirmatory tests for Gram-positive and Gram-negative organisms, show the positive sign for their respective isolates [28].

#### 3.3 Bactericidal action

Among the isolated strains, the Gram-positive *S. aureus* shows an elevated zone of inhibition of 20 ± 0.577 mm at 60 μg concentration against the AS-ZnONPs. Gram negative *E. coli* (19 ± 0.577 mm), *Klebsiella pneumoniae* (18 ± 1.732 mm), *Klebsiella oxytoca* (17 ± 2.309 mm), and *Acinetobacter baumannii* (12 ± 1.732 mm) show less susceptibility than the Gram-positive organism. Vancomycin antibiotic showed a resistance and sensitivity pattern. A similar report on Lebbeck stem extract reveals that the Gram-positive bacteria *S. aureus* also shows a zone of inhibition of around 21 mm at 60 μg concentration, as presented in Table 1 and Figure 3a.
3.4 Cotton sensitivity test

Small square-shaped cotton fabric is wetted with zinc oxide nanoparticles [29]. *S. aureus* shows a minimal zone of inhibition of 18 ± 1.732 mm when compared with *E. coli*, *K. pneumonia*, *K. oxytoca*, and *A. baumanii*, which show minimal zone of inhibition of 18 ± 1.732, 16 ± 1.732, 15 ± 1.155, and 14 ± 2.309 mm, respectively, in cotton fabric than in mixed cotton fabric. The results are briefly explained in Table 2 and Figure 3b.

3.5 Characterization study of zinc oxide nanoparticles coated on cotton

3.5.1 UV-Vis spectra

In UV-Vis spectra, the wavelength of the AS-ZnONPs [30] is observed in the range of 350–460 nm, which is shown clearly in Figure 4a.

3.5.2 FT-IR

The presence of alkyl groups, ketones, flavonoids, terpenoids, and oxygen atoms is recorded in the peak lengths of 700, 1,200, 2,000, 2,900, and 3,500 cm⁻¹, respectively;
As-ZnONPs the potential characteristic narrow bands absorbed which represents its corresponding functional groups. The peak range is represented by the reduction of the ions by identifying their peaks with phyto compound in the aqueous extract of Acorus calamus rhizome of 500–3,554 cm\(^{-1}\) [19] aqueous extract of Limonia acidissima represents the peak range of 500–4,500 cm\(^{-1}\) [31] aqueous and methanol leaf extract of the Azadirachta indica represents its wave number in the range of 1,200–2,500 cm\(^{-1}\) for aqueous and in the case of ethanol extract, the slight changes observed in the shifting of the oxygen atoms from 1,550–1,700 cm\(^{-1}\) in FT-IR work a transmission peak is observed in the range of 340–4,700 cm\(^{-1}\). This bandwidth in AS-ZnONPs reveals the phytochemistry and surface chemistry of the nanoparticle, as shown in Figure 4b.

### 3.5.3 SEM with EDAX

AS-ZnONP-embedded cotton fabrics are viewed under SEM and energy-dispersive X-ray (EDX) spectroscopy with a width of 9.54 mm, and the images are obtained at a magnification of 433\(\times\) and a scan speed of 4, as shown in Figure 4c [32]. The carbon weight is 47%, the oxygen and potassium weight is 44%, and the zinc weight is 8.1%, and energy-dispersive X-ray (EDX) spectra show a peak in the range of 7.8–9.4. The metal’s reflection and atomic mass are also shown in Figure 4d.

### Table 1: Antimicrobial activity of synthesized AS-ZnONPs and antibiotics against the isolates

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>20 µg</th>
<th>40 µg</th>
<th>60 µg</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>16 ± 1.732</td>
<td>17 ± 2.309</td>
<td>18 ± 1.732</td>
<td>22 ± 2.887</td>
</tr>
<tr>
<td>S. aureus</td>
<td>17 ± 2.309</td>
<td>18 ± 1.732</td>
<td>20 ± 0.577</td>
<td>7 ± 0.000</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>17 ± 2.309</td>
<td>19 ± 0.577</td>
<td>19 ± 0.577</td>
<td>18 ± 1.732</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>15 ± 1.155</td>
<td>16 ± 1.732</td>
<td>18 ± 1.732</td>
<td>07 ± 0.000</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>13 ± 1.155</td>
<td>15 ± 1.155</td>
<td>17 ± 2.309</td>
<td>17 ± 2.309</td>
</tr>
<tr>
<td>A. baumanii</td>
<td>11 ± 0.577</td>
<td>11 ± 0.577</td>
<td>12 ± 1.732</td>
<td>7 ± 0.000</td>
</tr>
</tbody>
</table>

### Table 2: Antimicrobial sensitivity test of AS-ZnONPs coated cotton fabrics against the isolates

<table>
<thead>
<tr>
<th>S. no</th>
<th>Name of the organism</th>
<th>Mixed cotton fabric (ZOI)</th>
<th>Cotton fabric (ZOI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>15 ± 1.155</td>
<td>18 ± 1.132</td>
</tr>
<tr>
<td>2</td>
<td>S. aureus</td>
<td>14 ± 2.309</td>
<td>18 ± 1.732</td>
</tr>
<tr>
<td>3</td>
<td>P. aeruginosa</td>
<td>15 ± 1.155</td>
<td>18 ± 1.732</td>
</tr>
<tr>
<td>4</td>
<td>K. pneumoniae</td>
<td>15 ± 1.155</td>
<td>16 ± 1.732</td>
</tr>
<tr>
<td>5</td>
<td>K. oxytoca</td>
<td>14 ± 2.309</td>
<td>15 ± 1.155</td>
</tr>
<tr>
<td>6</td>
<td>A. baumanii</td>
<td>13 ± 1.155</td>
<td>14 ± 2.309</td>
</tr>
</tbody>
</table>

Figure 3: (a) Antimicrobial activity of synthesized AS-ZnONPs against the isolates. (b) Antimicrobial sensitivity test of AS-ZnONPs coated fabrics against the isolates.
3.5.4 HR-TEM

A high-resolution transmission electron microscope (HR-TEM) investigates the 200-nm diamond-shaped zinc oxide nanoparticle embedded in the cotton fabric, and the image is taken by an LCD camera is given in Figure 5.

3.5.5 Obtained biopsy vs AS-ZnONPs

The chemically extracted and sediment AS-ZnONPs were probed for their efficacy via the cells in connective tissue producing collagen and proteins. The specimens were soiled with AS-ZnONPs for 24 h at 27°C. Diapedising and crowding of histological and cytological ingredients toward the lesion floor denote the progressive prognosis, and healing toward keloid-free scar formation, which indicates this mechanism due to the adjunction of AS-ZnONPs (Figure 6a and b). The description and explanatory details of the histological practical performed with 2.5–10 µg of adjuncting AS-ZnONPs evidenced the cytological diapedesis, resulting in their massive accumulation (proliferation), which denotes wound healing than the control, as shown in Figure 7 and a detailed explanation of the efficacy of the synthesized AS-ZnONPs toward biopsy (L929 cell lines) and the elevated concentrations of AS-ZnONPs in the wound healing assay is also schematically represented in Figure 7a.

4 Discussion

The replacement of hormonal, gonadal, insane, and dependency faults is of broad therapeutic value. The therapeutic advantages of A. sessilis an all-seasonal habit and environment are gained by their pheno and geno personalities. As a result, the osmoregulation is fully satisfied with the provision of expected occurrences together with metals' invisible substances in extrinsic and intrinsic ways. Ionic exchanges are also crucial for precipitation because of this [22]. These micro and ultra-micro status is an important structural...
appearance innovating gifts for our beneficial expectations accession of the barrier boundaries by these invisible substances is the main criteria. This phenomenon values the products. This behavior triggers the inventors to concentrate on furthermore inventions. Avalanche, spread, accession, invasion, osmosis, bulging, bursting,

Figure 5: High-resolution transmission electron microscope of synthesized AS-ZnONPs.

Figure 6: (a) Morphological changes in control and AS-ZnONPs treated Normal fibroblast L929 cells for 24 h. (a) Photomicrograph (20×) represents the morphology of AS-ZnONPs treated with L929 cells. (b) AS-ZnONPs treatment did not show any toxicity and cell changes to the L929 cells, assay. Various concentrations of synthesized zinc oxide nanoparticles and their mode of healing efficacy against human fibroblast (L929 cell line) are graphically explained.
atomic and ionic permeability, and invasion and elimination are all regulatory occurrences that end up resulting in the expectational target in the animalcules \[16\]. This process is processed by beneficial efforts emitting phytochemical botanical floral metallic (zinc) invisible micro and ultra-micro pharmacotherapies. Inventors materialize their healing efficacy through very many inventions. It is proved that in vitro diapedesis is not only progressive criteria but adjunction with the AS-ZnONPs metallic products evidence as to the rear repairing of the lesions through practical done in biopsy obtained. Updated based on their appropriate beneficial efforts thus their economic importance is probed. Accuracy, abundance, and abrupt availability is confirmed as artificial plant production provides discomfort. The same when an appeal to naturalists is very well economical and eco-friend and hazard-free expel [33]. The natural primary and secondary metabolites adjoin with innovative invisible substances to enhance the healing characteristics. The proliferative healthy cells imitate crowding. Dryness of the floor of the lesion indicates healing. Diabetes is directly proportional to progressive prognosis our Phyto therapeutic nanoparticles encourage and promote the above mechanism. This renders the fast healing. The obtained benefit is also cost-free, with hazard-free retiring in the health institutions restricted.

5 Conclusion

Wound healing naturally delays if it is not followed up carefully. Caretaking includes cleanliness and application of soothing curatives since epic era methods of soiling and covering the lesion with layers of healers. Later, the expectational emulsion contains ingredients that suppress the offensive secretions from the wet wound field and dry the wound, thus enhancing the repairing. This phenomenon is updated as the pharma preparations with
botanical floral phytochemicals and metallic ultra-micronanoparticles repair the expectations before time, resulting in fruit fullness. Through an in vitro human fibroblast cell line study, which reflects the efficacy of the fabricated As–zinc oxide nanoparticle synthesis, it is proved that AS-ZnONP is a worthwhile nano wound healer and can fight and win the battle against any chronic ulcers and gangrenes caused by multidrug-resistant bacteria and extreme drug-resistant bacterial strains in the medical field (XDR).

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Author contributions: Nivedhitha Kabeerdass, Selvankumar Thangasamy, and Karthikeyan Murugesan: conceptualization, investigation, supervision, formal analysis, methodology, validation, writing – original draft, and visualization; Natarajan Arumugam and Abdulrahman I. Almansour: investigation, methodology, validation, and writing – review and editing; Raju Suresh Kumar: project administration, visualization, and writing – review and editing; Plalanivel Velmurugan and Vinayagam Mohanavel: formal analysis, validation, and writing – review and editing; Selvaraj Vijayanand and Thajuddin Nooruddin: formal analysis, validation, resources, and writing – review and editing; Subpiramaniyan Sivakumar and Maghimaa Mathanmohun: data curation, formal analysis, validation, resources, writing – original draft: data curation, formal analysis, validation, resources, and writing – review and editing.

Conflict of interest: One of the authors (Plalanivel Velmurugan) is a Guest Editor of the Green Processing and Synthesis’ Special Issue “Use of magnetic resonance in profiling bioactive metabolites and its applications” in which this article is published.

Data availability statement: The data used to support the findings of this study are included in the article. Should further data or information be required, these are available from the corresponding authors upon request.

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