INTRODUCTION

Wounds are breaks in the skin (due to cuts, scrapes, or scratches) or injuries in other body tissues. They often happen due to trauma or due to an accident, but can also be caused due to surgery, or by sutures and stitches. A major problem with wounds is the risk of infections caused by microorganisms. Inhibition or killing of microorganisms is an essential component of the wound healing process and is generally achieved with antimicrobial agents (antibiotics). These can be applied to the wound (topical) or be given systemically (e.g., by oral administration) [1,2].

Cocos nucifera L., commonly known as the Coconut, is a member of the family Arecaceae (Palm). It is highly valued both as a source of food and a source of medicines. The plant is said to have originated from Southeast Asia, or Islands of the Indian and Pacific oceans [3]. India is the third largest coconut producing country [4]. Only recently has modern medical science unlocked the secrets to coconut’s amazing healing powers. It is widely used in Ayurveda for various skin problems and microbial infections [5]. Coconut shell charcoal powder is also very effective as a potential medicine for wound healing, for kidney trouble, for ulcers, and other soft tissue injuries [6]. Virgin coconut oil extract contains various phytochemicals and shows high antioxidant activity [7]. The C. nucifera L. leaf petiole possesses a caducous tomentum abaxially, which appears as a closely matted hair or downy covering. The villagers and farmers of Kerala and Tamil Nadu in India, traditionally apply the tomentum of coconut on cuts and wounds. Such ethnomedicines are widely prevalent and they have been the source for the development of new drugs [8,9].

A survey of literature reveals that so far, no studies have been carried out on the antibacterial activity, antioxidant properties, or wound-healing effects of C. nucifera tomentum and/or its extracts. Thus, the objectives of our present study were to identify and compare the phytochemical constituents, antioxidant activity, antibacterial properties, and in vitro wound healing activity of C. nucifera tomentum extracts.

MATERIALS AND METHODS

Collection of plant materials

Fresh C. nucifera tomentum was collected from Coconut Research Station, Tamil Nadu Agricultural University (Aliyar, Tamil Nadu, India). The tomentum was collected from 4 to 5-year-old coconut trees and was identified and authenticated as C. nucifera L. belonging to family Arecaceae, at the Institute of Forest Genetics and Tree Breeding Coimbatore, Tamil Nadu, India. The herbarium was created and the specimen of leaves with tomentum was deposited at the Institute of Forest Genetics and Tree Breeding Coimbatore, Tamil Nadu, India.

Extraction of plant material

About 50 g of the dried tomentum was continuously extracted with 100% methanol (TM) or 100% ethanol (TE) using Soxhlet apparatus at 60°C up to 12 h [10,11], while aqueous (TA) extraction was carried out by the shaker flask method [12]. The extracts were filtered and concentrated in a rotary evaporator at 35°C–45°C under reduced pressure, to obtain the crude extract. The crude extract was stored in an air-tight container at 4°C and used for further studies.

Preliminary phytochemical screening

The freshly prepared ethanolic, methanolic, and aqueous extracts of C. nucifera tomentum were subjected to qualitative chemical tests (Mayer’s test, Dragendorff’s test, and Wagner’s test). Phenols, flavonoids, tannins, terpenoids, and sterols were assayed by standard methods [13].

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS is used for identification and quantitation of volatile and organic compounds in complex mixtures and for the determination...
Liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-Q-ToF MS) analysis

LC-Q-ToF MS is used to determine the fragmentation and structural information of the known and unknown compounds present in a complex mixture [17]. LC-Q-ToF MS analysis for TA was performed on the Agilent H class (Waters India Pvt. Ltd., Bengaluru, India) ultra-performance LC system connected with Xevo G2 (Waters India Pvt. Ltd., Bengaluru, India) Q-ToF mass spectrometer with LC column BEH C18 column (50 mm×2.1 mm×1.7 µm). The mobile phase was a gradient of water+0.1% formic acid (A), and methanol (B), and the samples were analyzed from 0.1 min to 9.0 min. The flow rate was 0.3 mL/min. Total run time was 9 min. The LC-Q-ToF mass spectrometer analysis was performed with 135°C source temperature and 350°C desolation temperature. The positive ionization mode was with the energy of 3 kV, with sample cone at 30 V and extraction cone at 1 V. The identification of compounds present in the sample was performed by comparison of MS/MS Spectra using the ChemSpider database [18].

Antibacterial activity by microdilution assay

The minimum inhibitory concentrations (MIC) of the extracts against test bacteria were determined using a modified microdilution technique originally described by Eloff [19]. Test solutions (10 mg/mL) of the extracts were prepared with Dimethyl sulfoxide and serial two-fold dilutions were made. 50 µL of the test bacteria (1.0×10⁷ CFU/mL) were grown in tryptone soya broth. The covered microplates were incubated at 37°C for 24 h [20]. To indicate bacterial growth 30 µL of thiazolyl blue tetrazolium bromide dye was added to each well, and plates were further incubated for 30 min at 37°C. Formation of blue color indicated the presence of viable cells [21]. The experiments were carried out in triplicate.

Antioxidant activity

The antioxidant activity of the tomentum extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [22]. Samples and standard (ascorbic acid) were taken in various concentrations, and the tubes were allowed to stand in the dark for 30 min at room temperature. The absorbance of the sample was measured at 517 nm against a blank [23]. The radical scavenging activity was calculated using the following formula [24]:

DPPH scavenging activity % = [(Control–Test sample)/Control]×100

Scratch wound healing assay

Mouse fibroblast cells (L929) were grown in 24-well plates at a density of 1×10⁵ cells/mL and cultured to 80% confluency. A small linear scratch was created in the confluent monolayer by gently scraping with a sterile cell scraper [25]. Cells were thoroughly rinsed with phosphate-buffered saline to remove cellular debris and treated with different concentrations of the methanolic or ethanolic extracts of C. nucifera tomentum. Cell proliferation was monitored at different time points (0 h, 4 h, 18 h, and 24 h), and images of migrated cells were taken at different time points using a digital camera (Nikon, Tokyo, Japan) connected to an inverted phase contrast microscope (Radical Instruments, India).

RESULTS

Preliminary phytochemical screening

The phytochemical analysis of the tomentum of C. nucifera L showed the presence of alkaloids in TE and TM, while it was absent in TA. Tannins, phenols, flavonoids, and sterols were present in varying concentrations in all extracts, as indicated by the intensity of the colored solution and precipitates. Terpenoids were absent in TE and TA but present in TM (Table 1).

GC-MS analysis

GC-MS was carried out to identify the bioactive compounds having long chain hydrocarbons, esters, acids, phenolic compounds, etc. In the present study, more than 15 bioactive compounds were identified in TM and TE. The major constituents of TM are 1-Dodecanol (RT-16.46), Dodecanoc acid methyl ester (RT-17.62), 1- Tetradecanol (RT-21.23) along with some other minor compounds which is proven to have pharmacological activities (Fig. 1). These identified compounds are known to possess antibacterial, anti-inflammatory, anticancer, antifungal, antioxidant, cancer preventive, nematicidal, or hypercholesterolemic properties [12,26,27]. The major constituents of TE were 1,2-benzenedicarboxylic acid, bis (2-methyl propyl) ester (RT-16.19), E-15-heptadecenal (RT-14.94), and stigmastanol (RT-37.20), all of which have been previously reported to possess antimicrobial and antifouling properties, antimalarial, antioxidant, hypoglycemic, thyroid inhibiting, anticancer, antiarthritic, or anti-inflammatory activities (Fig. 2) [28-34].

LC-Q-ToF MS analysis

The total ion chromatogram of TA is represented in Figs. 3 and 4. Although several bioactive compounds have been identified, further studies are needed to get complete profiling of components present in the sample [35,36].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of compounds</th>
<th>TE</th>
<th>TM</th>
<th>TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoids</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Sterols</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ : High, ++ : Moderately present, + : Weakly present, – : Absent

Table 1: Phytochemical profile of Cocos nucifera tomentum extracts

Fig. 1: Gas chromatography and mass spectroscopy chromatogram of TM
The MIC of tomentum extracts
The MIC – determined as the lowest concentration of the crude extract that showed no microbial growth – was carried out against three Gram-negative and one Gram-positive bacteria using various extracts of *C. nucifera* (Table 2). The MIC of TM extract against *Escherichia coli* was 2.5 mg/mL, while the MICs for TE and TA were 5 mg/mL. In the case of *Pseudomonas aeruginosa*, all the three extracts showed similar MICs (2.5 mg/mL). The MICs of gentamicin was not determined against *E. coli* and *P. aeruginosa*. The MIC value against *Proteus vulgaris* of TM was 1.25 mg/mL, while the other two extracts showed a MIC of 2.5 mg/mL. Against *Staphylococcus aureus*, TM and TA had the highest MIC of 5 mg/mL, while for TE it was 10 mg/mL. Chloramphenicol showed 0.625 mg/mL MIC against *P. vulgaris* and *S. aureus*. The different susceptibilities of the various organisms to different concentrations of extracts were noted. It was observed that *P. vulgaris* and *E. coli* were more sensitive to TM.

Reference antibiotics
Chloramphenicol for Gram-positive bacteria and gentamicin for Gram-negative bacteria were used.

Antioxidant activity
Comparison of the antioxidant activity of the tomentum extracts and ascorbic acid by DPPH method is shown in Fig. 5. The various extracts TM, TE, and TA exhibited significant dose-dependent inhibition of DPPH activity. Among these, TM showed the highest potential to scavenge DPPH when compared to TE and TA. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm which is induced by antioxidants [37]. At 400 µg/mL concentration, the percentage of inhibition of TM, TE, and TA is 49%, 15.8%, and 38%, respectively, when compared to ascorbic acid as the standard (39%). The IC\textsubscript{50} value of TM was 400 µg/mL, TA was 800 µg/mL, and the TA was 1000 µg/mL. In our study, the highest antioxidant activity was found in TM, followed by TA and TE.

Scratch assay
Proper healing of wounds is necessary for the restoration of the skin [5]. For our in vitro wound-healing assay, the L929 mouse fibroblast cell line was used for the surrogate “Scratch assay.” A small linear scratch was created in a confluent monolayer of L929 cells by scraping with a sterile cell scraper. An image analyzer was then used to calculate the time required to close the gap at different concentrations of extracts. The time taken to close the gap using various extracts was plotted and compared with untreated cells (Fig. 6). The results showed that at a concentration of 75 µg/mL, TM closed the gap in the scratch at the 18\textsuperscript{th} h when compared the TE, TA, and control (Fig. 7). In the present study, formulation of at the concentration of 50 µg/mL and 75 µg/mL of TM showed significant mobilization of L929 cells and closed the gap, when compared to the control without the addition of formulation. Photographs indicating comparative cell migration in normal control at the 0\textsuperscript{th} h, non-treated control, TM, TE, and TA at the 18\textsuperscript{th} h time intervals.

**DISCUSSION**
*C. nucifera* is a well-known plant, and its parts (including the milk and oil) are used both as a food, as well as in herbal medicines [38]. Earlier studies have reported the antipyretic, wound healing activity, and anti-hypertensive effects of other parts of *C. nucifera* [39,40].

### Table 2: MICs of TM, TE, and TA extract of tomentum by microdilution method

<table>
<thead>
<tr>
<th>Extract/MIC (mg/mL)</th>
<th><em>E. coli</em> ATCC 25922</th>
<th><em>P. aeruginosa</em> ATCC 27853</th>
<th><em>Proteus vulgaris</em> NCM 2027</th>
<th><em>S. aureus</em> ATCC 25923</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td>2.5</td>
<td>2.5</td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td>TE</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>TA</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Reference antibiotic</td>
<td>Nd\textsuperscript{d}</td>
<td>Nd\textsuperscript{d}</td>
<td>0.625\textsuperscript{a}</td>
<td>0.625\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Nd: Not determined, G: Gentamicin, C: Chloramphenicol, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. aureus*: *Staphylococcus aureus*, MICs: Minimum inhibitory concentrations
The presence of phenolic compounds such as sterols and terpenoids contributes to the antioxidant properties of the tomentum [41]. GC-MS is the best technique to identify the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, alkaloïds, steroids, amino acid, and nitro compounds [33]. In the present study, all the compounds identified are known to possess biological activities. However, for the LC-Q-ToF MS analysis, further studies are needed to obtain the complete profile of compounds.

Our MIC studies show that test organisms are more sensitive to methanolic extracts of the coconut tomentum and that this antibacterial activity could very well be due to the phytoconstituents present (such as the phenolic compounds) [42-44]. It has earlier been reported that C. nucifera oil with silver sulfadiazine is useful in treating burns and wounds [45]. However, our present study is the first to demonstrate the wound healing effect of the hitherto un-studied C. nucifera tomentum. The wound healing property of tomentum may be due to either a rapid cell proliferation stimulated by growth-promoting phytochemicals and antioxidants present, or by prevention of infection due to antimicrobial activity.

CONCLUSION
This work is the first report to identify compounds from the extracts of C. nucifera tomentum, through qualitative and quantitative phytochemical analyses. This study also tested the antioxidant, antibacterial, and in vitro wound healing activities of alcoholic and aqueous extracts of C. nucifera tomentum. The methanolic extract of the coconut tomentum has significant antimicrobial, antioxidant, and wound healing properties. This study reveals the potential source of useful drugs from C. nucifera tomentum.

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AUTHORS' CONTRIBUTIONS

Haritha KH: All fieldwork, laboratory experiments, and preparation of documents. Sajitha Kutinath: Supporting laboratory experiments. Ram Rammohan: Guide, Experimental designs, troubleshooting, and preparation of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES