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 diseases [6]. Virgin coconut oil 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 diseases [6]. Virgin coconut oil is very effective as a potential medicine for wound healing, for kidney trouble, for ulcers, and other soft tissue diseases [6]. 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 diseases [6]. Virgin coconut oil is very effective as a potential medicine for wound healing, for kidney trouble, for ulcers, and other soft tissue diseases [6].

Keywords: Cocos nucifera, Tomentum, Antioxidant, Antibacterial, Wound healing.

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of molecular and elemental compositions of unknown organic compounds in a mixture [14]. GC-MS analysis for TE and TM was carried out on the Shimadzu GC-MS (QP2010S). The column used was Rxi-5Sil MS (300 mm×0.2 mm×0.5 µm). Helium was the carrier gas at a flow rate of 1.0 mL/min. The instrument was set to an initial temperature of 80°C and maintained for 2 min. At the end of this period, the oven temperature was raised to 280°C (at 5°C/min) and maintained for 5 min. Injection port temperature was 260°C, the injection volume was 1.0 µL, and the injection flow rate was 3.0 mL/min. The total GC-MS running time was 40.8 min. The phytochemical constituents were identified using the National Institute of Standards and Technology Mass Spectral Database (NIST 11) and WILEY 8 library [15,16].

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 Acquity 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 desolvation temperature. The positive ionization mode was with the energy of 3 kV, with sample cone at 30 V and extraction cone at 1 V, while the negative ionization mode was with the capillary voltage at 2.5 kV, and 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 µg/mL) of the extracts were prepared with Dimethyl sulfoxide and serial two-fold dilutions were made. 50 µL of the test bacteria (1.0×10^7 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]:

\[ \text{DPPH scavenging activity} = \left( \frac{\text{[Control-Test sample]}}{\text{Control}} \right) \times 100 \]

Scratch wound healing assay
Mouse fibroblast cells (L929) were grown in 24-well plates at a density of 1×10^5 cells/mL and cultured to 80% confluency. A small linear scratch was created in the confluent monolayer by gently scraping with a sterile cell scratcher [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 steroids 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), Dodecanolic 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, nematocidal, 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 stigmasteryl (RT 37.2), all of which have been previously reported to possess antinociception and antifouling properties, antimarial, 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 1: Phytochemical profile of Cocos nucifera tomentum extracts

| S. No. | Name of compounds | TE | TM | TA |
|-------|------------------|----|----|----|
| 1     | Alkaloids         | +  | ++ | –  |
|       | a. Mayer’s test   | ++ | ++ | –  |
|       | b. Dragendorff’s test | + | ++ | –  |
|       | c. Wagner’s test  | ++ | ++ | –  |
| 2     | Phenols           | ++ | ++ | –  |
| 3     | Flavonoids        | ++ | ++ | +  |
| 4     | Tannins           | ++ | ++ | +  |
| 5     | Terpenoids        | –  | –  | +  |
| 6     | Sterols           | –  | –  | ++ |

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

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 IC₅₀ 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 at a concentration of 75 µg/mL, TM closed the gap at the 18th h when compared to 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 0th h, non-treated control, TM, TE, and TA at the 18th 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

| Extract/MIC (mg/mL) | *E. coli* ATCC 25922 | *P. aeruginosa* ATCC 27853 | *Proteus vulgaris* NCIM 2027 | *S. aureus* ATCC 25923 |
|---------------------|-----------------------|-----------------------------|-----------------------------|-----------------------|
| TM                  | 2.5                   | 2.5                         | 1.25                        | 5                     |
| TE                  | 5                     | 2.5                         | 2.5                         | 10                    |
| TA                  | 5                     | 2.5                         | 2.5                         | 5                     |
| Reference antibiotic| Nd⁶                   | Nd⁶                         | 0.625⁴                      | 0.625⁴                |

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, alkaloids, 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. Sujitha Kuttimath: 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

1. Bowler PG, Duering BL, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14:244-69.
2. Kumar B, Vidyakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing: exploring medicinal plants of India. J Ethnopharmacol 2007;114:103-13.
3. Lima EB, Sousa CN, Meneses LN, Ximenes NC, Santos Júnior MA, Vascconcelos GS, et al. Cocos nucifera (L.) (Arecales): A phytochemical and pharmacological review. Braz J Med Biol Res 2014;48:953-64.
4. DebMandal M, Mandal S. Coconut (Cocos nucifera L.; Arecales): In health promotion and disease prevention. Asian Pac J Trop Med 2011;4:24-71.
5. Srivastava P, Durgaprasad S. Burn wound healing property of Cocos nucifera: A review. Indian J Pharmcol 2008;40:144-6.
6. Lenza G, Odem MM, Esther C. Powdered coconut shell charcoal: A potential alternative medicine for some identified ailments in soft tissues. An Interdisciplinary research. Int J Adv Res IT Eng 2013;2:61-9.
7. Zunairah A, Mohamad RS, Rosnani H. Evaluation of wound closure activity of Cocos nucifera oil on scratched monolayer of human dermal fibroblasts. Chem Eng Trans 2017;56:1657-62.
8. Latheef AK, Smitha PK, Remashree AB. Ethnomedicine used for treating cuts and wounds by the tribes of Attappady, Kerala. Int J Herb Med 2014;2:1-8.
9. Thomas B, Arumugam R, Veerasamy A, Ramanourthy S. Ethnomedicinal plants used for the treatment of cuts and wounds by Kuruma tribes, Wayanadu districts of Kerala, India. Asian Pac J Trop Biomed 2014;4:5889-91.
10. Devendra G, Ganesan S. Phytochemical analysis of leaf extract of plant Costus spicatus By GC-MS method. J Drug Deliv Ther 2015;5:24-6.
11. Jayaveera KN, Yogandhan Reddy K, Govindarajula Y, Kumanan R. Phytochemical screenings, antibacterial activity and physico chemical constants of ethanolic extract of Euphorbia thymifolia. Int J Pharm Pharm Sci 2010;3:81.
12. Handa SS, Khamaja SP, Longa G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Italy. United Nations: Industrial Development Organization and the International Centre for Science and High Technology; 2008.
13. Harbone JB. Phytochemical Methods. London: Chapman & Hall; 1999. p. 60-6.
14. Lakshmi Hima Bindu MR, Parameswari AS, Gopinath C. A review on GC-MS and method development and validation. Int J Pharm Qual Assur 2013;4:42-51.
15. National Institute of Standards and Technology. NIST Standard Reference Database 1A. NIST Mass Spectral Search Program Version 2.0g. Gaithersburg, MD: National Institute of Standards and Technology; 2011.
16. Willey/NIST. The Willey/NBS Registry of Mass Spectral Data. Mass Spectral Library version 2.0. 8th ed. New York: J. Willey and Sons, Inc., NIST/EPA/NIH; 2005.
17. Ferrer I, Thurman ME. Liquid Chromatography/time of flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants. Trends Anal Chem 2003;22:750-6.
18. Chemspider. Chem Foyal Society of Chemistry. Cambridge. Available from: http://www.chemspider.com. [Last accessed on 2018 Jun 20].
19. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 1998;64:711-3.
20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disc Susceptibility Tests. Approved Standard CLSI Document. M100 S26. 27th ed. Clinical and Laboratory Standards Institute; 2017.
21. Balouiri M, Sadiki M, Ibnoussa SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
22. Braca A, De Tommasi N, Di Bari L, Puzza C, Politi M, Morelli I, et al. Antimicrobial principles from Bauhinia tarpontens. J Nat Prod 2001;64:892-5.
23. Udaya Prakash NK, Bhuvaneswari S, Sriprithi N, Premalea L, Bhagya R, Radhika B, et al. Antioxidant activity of common plants of Northern Tamil Nadu, India. Int J Pharm Pharm Sci 2014;6:128-32.
24. Shahibana R, Elia B, Noviani A. Anti-oxidant activities of fractions from ethyl acetate extracts of farnicia fruticosa lauterb leaves. Int J Appl Pharm 2018;10:44-50.
25. Liang CC, Park AY, Guan JI. In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc 2007;2:326-33.
26. Yogeswari S, Ramalakshmi S, Neelavathy R, Muthumary J. Identification and comparative studies of different volatile fractions from Monochaetia kansensis By GC-MS. Global J Pharm 2012;6:65-71.
27. Kalaivani KM, Jomalgadda B, Arookiasamy S. GC-MS analysis of chlorofrom extract of Croton bongolaudianum. Int J Pharm Bio Sci 2013;6:4137.
28. Sermakkan M, Thanganpandan V. GC-MS analysis of Cassia Italic leaf methanol extract. Asian J Pharm Clin Res 2012;5:90-4.
29. Thomas E, Aneesh TP, Thomas DG, Ananthand R. GC-MS analysis of phytochemical compounds present in the rhizomes of Nervilia aragouana gaup. Asian J Pharm Clin Res 2013;6:68-74.
30. Dandekar R, Bagade B, Bhaskar VH. GC-MS analysis of phytoconstituents in alcohol extract of Epiphyllum oxypetalum leaves. J Pharmacoogn Phytother 2015;4:149-54.
31. Patel J, Reddy V, Kumar Satyara GS, Bajari B. Gas chromatography and mass spectroscopy analysis of bioactive components on the leaf extract of Terminalia coriaceae: A potential folkloric medicinal plant. Int J Green Pharm 2017;11:S140.
32. Parasaruman S, Ravendran R, Madhava Rao C. GC-MS analysis of leaf extracts of Cleistanthus collinus roxb: Euphorbiaceae. Int J Pharm Sci 2009;1:284-6.
33. Jeneecis A, Mohan VR. GC-MS analysis of bioactive compounds on the stem extract of Bacopopsis nervosa decne. Ex.Moe (Periploceaceae). Asian J Pharm Clin Res 2013;6:129-33.
34. Duke JA. Duke’s Phytochemical and Ethnobotanical Databases. 12.6 Beta. Vol. 9. Beltsville, Md.: ARS/USDA; 2018.
35. Pubchem from NCBI. Available from: http://www.pubchem.ncbi.nlm.nih.gov. [Last accessed on 2018 Jun 20].
36. Narendhrakannan RT, Jethnankaraj GN, Caroline A, Lincy S, Saj M, Durai D. Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian Pac J Trop Biomed 2012;2:1245-53.
37. Patel J, Reddy V, Kumar Satyara GS, Bajari B. GC-MS analysis of leaf extracts of Cleistanthus collinus roxb: Euphorbiaceae. Int J Pharm Res Anal 2011;1:21-5.
38. Alansis AD, Calzada F, Cervantes JA, Torres J, Ceballos GM. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 2005;100:153-7.
39. Alleyne T, Roache S, Thomas C, Shirley A. The control of hypertension by use of coconut water and Mauby: Two tropical food drinks. West Indian Med J 2005;54:3-8.
40. Zakaria ZA, Reezal I, Mat Jais SY, Saik Ho, Kumar PK, Sastry GV. Screening of wound healing activity of bark of Averratius moluccanus L. Int J Pharm Res Anal 2011;1:21-5.
41. Alanis AD, Calzada F, Cervantes JA, Torres J, Ceballos GM. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 2005;100:153-7.
42. Abbey C, Serwaa A, Ageypong DN, Mensah KB, Ayanda PG, et al. Antimicrobial, antioxidant and wound healing properties of Kigelia africana (Lam) beneth and Strophanthus hispidus jacq. Adv Pharm Sci Tech 2013;2013;1-10.
43. Cyri MB, Pai V, Shantammam M, Jose M. Anti-microbial properties of coconut husk aqueous extract on carcinogenic bacteria. Arch Med Res 2013;1:126-30.
44. Silahlji L, Perrnata YM, De Lux Putra E. Antimicrobial Activity of hydrolyzed virginate coconut oil. Asian J Pharm Clin Res 2014;7:90-4.
45. Abbas AA, Assikong EB, Martins A, Peter U, Keneth TT. Antibacterial activity of coconut oil and its derivative (Lauric acid) on some selected clinical isolates. Int J Med Sci Clin Invest 2017;4:3173-7.