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# Anti-pathogenic, anti-diabetic, anti-inflammatory, antioxidant, and wound healing efficacy of *Datura metel* L. leaves

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**Abstract**  *Datura metel* L. is an important medicinal plant of Solanaceae family which has extensive pharmacological properties. The present investigation was aimed to identify the presence of phytoconstituents and assess *in vitro* antibacterial, anti-biofilm, anti-diabetic, anti-inflammatory, antioxidant, cytotoxicity, and wound healing efficacy of *D. metel* leaves extract. Among different solvent extracts, methanolic extract showed higher amount of phenolic (124.61 ± 0.68 mg GAE/g), alkaloid (88.77 ± 1.01 mg AE/g), flavonoids (42.24 ± 0.18 mg QE/g), and tannins contents (38.72 ± 0.51 mg GAE/g). The extract exhibited not only significantly ($P < 0.05$) different antibacterial activities against pathogens tested but also showed maximum biofilm inhibition of 94, 88, and 92% against *B. subtilis*, MRSA, and *E. coli*, respectively. Anti-diabetic assay depicted 22.55 ± 0.62–79.41 ± 1.13% and 24.31 ± 1.47–72.59 ± 0.22% of α-amylase and α-glucosidase inhibition abilities of methanolic extract, respectively at varied concentrations. The methanolic extract showed potential anti-inflammatory effect ($P < 0.05$) by showing 28.11 ± 0.13, 34.94 ± 1.11, 55.73 ± 0.42, 73.28 ± 0.72, and 92.62 ± 1.33% of inhibition of protein denaturation at different concentrations with an $IC_{50}$ value of 52.45 μg/mL. The extract revealed significant ($P < 0.05$) rate of ABTS scavenging, DPPH degradation, and reducing power assay in a concentration dependent manner. The cytotoxicity assay was demonstrated on L929 mouse fibroblast cell line and found > 90% of cell viability in the presence of methanolic extract, thereby indicating its non-toxicity effect. Wound healing assay indicated that methanolic extract at 50 μg/mL closed 100% of wound gap after 24 h with high rate of migration and proliferation. Furthermore, GC–MS chromatogram revealed the presence of several components in methanolic extract, including neophytadiene, hexadecanoic acid, and hentriacontane as principal phytoconstituents. In conclusion, methanolic extract of *D. metel* leaves could be used as potent therapeutic agent not only for treating metabolic diseases but also superficial chronic diabetic wounds.

## 1. Introduction

The practice of herbal plants and its medicinal applications varies geographically, as they are influenced by several factors viz. abiotic parameters, culture, history, personal attitudes, and philosophy (Fitzgerald et al., 2020; Faheem et al., 2022). About 80% of the world population is dependent on herbal medicines for good health maintenance. Plants contain a number of chemical compounds that can be used to cure chronic and infectious diseases (Ingle et al., 2017; Chakraborty et al., 2022; Ahmed et al., 2022). Plant metabolites such as alkaloids, tannins, and flavonoids display microbial defence characteristics, while phenolic compounds have been associated with antioxidant activities due to their free radical scavenging features. All plant parts, *i.e.*, leaves, flowers, roots, stems, barks, rhizomes, bulbs, and/or seeds are frequently used to prepare herbal products (Matotoka and Masoko, 2018; Barbabosa-Pliego et al., 2022).

Wound healing is a dynamic, regulated, and highly complex process that is typically classified into acute and chronic. Acute wounds are surgical or traumatic injury wounds occurring in healthy people that generally heal without any difficulties. Chronic wounds are generally non-healing or take prolonged time to heal, namely pressure sores, leg ulcers, and diabetic foot ulcers, which largely do not occur in healthy people and they are clinically associated with old age, obesity, and diabetes (Han and Ceilley, 2017). Some common phytoconstituents including 13-docosenamide, (Z)-, quercetin, phytol, 1-hexadecanol, and 1-hexadecene exhibited antimicrobial, anti-inflammatory, and antioxidant effects which are required attributes for acute and chronic wound healing process (Beldal et al., 2017; Kabir et al., 2016).

A diabetic patient who has hypoglycemia will be most likely exposed to neuropathy and microvascular complication, thus the development of neuropathy may increase the risk of diabetic foot ulcers due to enlarged shearing forces and pressure load (Mariam et al., 2017). The bacteriology of diabetic foot ulcers is extremely complicated and they are frequently infected with Gram-positive and Gram-negative bacterial pathogens (Banu et al., 2015). Hence, diabetic foot ulcers require broad-spectrum antimicrobial drugs with considerable anti-diabetic and wound healing efficacies to avoid major and minor amputations. Many herbal plant formulations whether monoherbal or polyherbal with potential antimicrobial and antioxidant properties have been studied to hasten the wound healing process (Nagoba and Davane, 2019). For instance, the monoherbal *Aloe vera* is a first-line therapy for surgical wounds, ulcers, and burn wounds as it exhibits stronger antimicrobial activity due to the presence of acemannan, saponins, and phytol bioactive compounds. Polyherbal root extracts formulation of *Rehmannia glutinosa* and *Astragalus propinquus* were reported to be effective for diabetic ulcer treatment (Shedoeva et al., 2019).

*Datura metel* L. (Family: Solanaceae) is considered one of the most important medicinal plants due to its well-known antimicrobial and anti-inflammatory properties. It contains several bioactive phytoconstituents such as alkaloids, triterpenoids, flavonoids, steroids, tannins, and saponins to treat human diseases like asthma, bronchitis, diabetes, heart diseases, skin disorders, fever, diarrhea, epilepsy (Tijani et al., 2015; Dhawan and Gupta, 2017), and psychological problems (Tijani et al., 2015). The Ayurvedic preparations of *D. metel* and its phytoconstituents are widely used to treat hair fall, skin disorders, hysteria, and convulsions in India. Traditionally, it was also used to treat burns and wounds. The seeds of *D. metel* have been found to be effective in treating bleeding disorders. In Africa and Indo-China, the powdered seeds or leaves are often mixed with cannabis and smoked to relieve rheumatism and asthma (Dhawan and Gupta, 2017). *D. metel* leaves contain hyoscyamine and atropine, which is immensely useful for mind-altering drug development (Tijani et al., 2015).

In view of the enormous benefits of *D. metel*, the present study was intended not only to determine the phytochemical composition (qualitative and quantitative) of different solvent extracts of *D. metel* leaves but also assess crucial *in vitro* biological activities (antimicrobial activ-

ity, antibiofilm, anti-diabetic, anti-inflammatory, antioxidant, cytotoxicity, and wound healing efficacy) of potential solvent extract for suggesting its therapeutic roles.

## 2. Materials and methods

### 2.1. Plant collection and identification

Leaves of *D. metel* were collected in the autumn season from Bharathiar University premises, Coimbatore, India. The plant specimen was identified at the Botanical Survey of India, Coimbatore, India by referring to the deposited plant specimen (BSI/SRC/5/23/2017/Tech./1780).

### 2.2. Extract preparation

Fresh harvested leaves were washed with water and kept in shade to dry for 5 days, and ground into a fine powder. Powdered leaves (30 g/300 mL) were subjected to the successive extraction at 40 °C for 6–8 h with petroleum ether, chloroform, ethyl acetate, and methanol by using soxhlet apparatus. To extract compounds (polar and non polar), distilled water was used for maceration and subsequently extracted with different solvents (Senguttuvan et al., 2014). Following the extraction, the crude extracts were kept at 60 °C in a water bath for evaporating excess solvent. For further analysis, the extracted samples were stored in a tight container.

### 2.3. Screening of phytochemicals

The phytochemical components in *D. metel* leaves extracts were determined using both qualitative and quantitative techniques. By using standard methodology, the extracts were analyzed to ascertain the presence of flavonoids, alkaloids, saponins, tannins, phenols, proteins, cardiac glycosides, terpenoids, and carbohydrates (Prabhavathi et al., 2016).

### 2.4. Quantitative estimation of phytochemicals

Total contents of alkaloid, flavonoids, tannin, and phenols in the extracts were estimated using the methodology of Prasathkumar et al. (2021).

### 2.5. In vitro antibacterial activity

Based on the preliminary screening of phytochemical characterization, major phytochemicals were found in the methanolic extract. Hence, methanolic extract of *D. metel* leaves was utilized subsequently for further experiments.

Anti-bacterial activity of the methanolic extract was investigated against *Bacillus subtilis*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa* using agar well diffusion method (Kanmani and Lim, 2013). These human pathogens were obtained from Department of Microbial Biotechnology, Bharathiar University, Coimbatore, India. Bacteria were sub-cultured at 37 °C in Mueller-Hinton agar slants. After 16 h, the bacterial cultures were centrifuged at 6000 rpm for 15 min and then dissolved in sterile water. Mueller-Hinton agar medium was transferred into sterilized Petri dishes and set it aside to cool.

The bacterial cultures were swabbed onto the plates and agar wells were prepared. Then, varied concentrations (20, 50, and 80 μL) of methanolic extract were poured into the respective wells, allowed to incubate at 37 °C, and the zone of inhibition was recorded after 24 h of incubation.

### 2.6. In vitro anti-biofilm activity

Bacterial biofilm inhibitory activity of methanolic extract was investigated against *B. subtilis*, MRSA, and *E. coli* ($OD_{545} = 0.2$). Bacteria were treated with two different doses (50 and 100 μg/mL) of methanolic extract in a 24-well titer plate. Afterwards, a clean glass slide was placed separately on top of each well. The plate (control and treated samples) was allowed to incubate at 37 °C for 3 days. Then, the dunked glass slides were gently lifted and washed thrice with sterile distilled water, followed by staining with 1 mL of crystal violet solution (0.5%) for 20 min. To remove the stain, slides were washed with distilled water and the inhibition of biofilm formation was observed under light microscope (Optika, X-LED 8 W). The absorbance was measured at 570 nm using a UV– Vis spectrophotometer (JASCO UV VIs model v − 750) to determine the quantitative measurement of anti-biofilm activity (Al Mahmud et al., 2016).

### 2.7. In vitro anti-diabetic activities

Alpha (α)-amylase inhibitory effect of methanolic extract was quantified based on the previous protocol (Wan et al., 2013) with minor modifications. In brief, 20 to 100 μg/mL of methanolic extract and Acarbose (standard) were diluted with α-amylase (10 μL) and allowed to incubate for 5 min at 37 °C. To produce a total volume of 2 mL, 0.2% starch solution was mixed into the solution and allowed to incubate for 5 min at 37 °C. Further, dinitrosalicylic acid (1 mL) reagent was added and placed in a water bath for 5 min. Later, 6 mL of deionized water was mixed with the reaction solution. The absorbance was recorded by UV–Vis spectrophotometer (JASCO UV VIs model v − 750) at 540 nm.

Alpha (α)-glucosidase inhibitory effect was measured based on the previous study (Wan et al., 2013) with slight modifications. In brief, 20–100 μg/mL of methanolic extract and Acarbose were mixed with 75 μL of α-glucosidase and allowed to incubate at 37 °C for 15 min. After incubation, 20 μL of 5 mM para-nitrophenyl-α-D-glucopyranoside in 0.1 M potassium phosphate buffer was added to start the reaction. The reaction was stopped by adding 80 μL of 0.2 M $Na_2CO_3$ prepared in 0.1 M potassium phosphate buffer (pH 6.8) after incubation. The absorbance of α-glucosidase inhibition activity was recorded using UV–Vis spectrophotometer (JASCO UV VIs model v − 750) at 405 nm. α-amylase and α-glucosidase inhibition (%) were calculated as per the equation mentioned below:

$$\text{Inhibition } (\%) = Ac - As/Ac \times 100$$

where, Ac is the absorbance of control and As is the absorbance of methanolic extract.

## 2.8. In vitro anti-inflammatory activity

The protein denaturation inhibition was assessed based on the previous methodology of Gunathilake et al. (2018) with minor changes. In brief, the reaction mixture contained methanolic extract (20–100 µg/mL), 1% of bovine albumin, and phosphate-buffered saline or PBS (4.78 mL, pH 6.4). The mixture was allowed to incubate for 20 min at 37 °C and further transferred to water bath for 4 min at 70 °C. The turbidity of the reaction mixture was recorded at 660 nm after cooling using PBS as a control and aspirin as a reference. Finally, inhibition of protein denaturation was calculated as per the equation mentioned below:

$$\text{Protein denaturation inhibition } (\%) = 100 \times (1 - A2/A1)$$

where, A2 is the absorbance of methanolic extract and A1 is the absorbance of control.

## 2.9. In vitro antioxidant activities

### 2.9.1. ABTS assay
The antioxidant activity of methanolic extract was performed based on ABTS scavenging method (Ismail et al., 2017). In short, ABTS diammonium salt (2.0 mM) and potassium persulfate (3.5 mM) were dissolved in distilled water and incubated under dark condition for 16 h. Then, the various concentrations of methanolic extract (100–500 µg/mL) were dissolved in $ABTS^+$ solution (290 µL) and kept under incubation for 10 min. After that, the absorbance of reaction mixture was observed at 750 nm, using ascorbic acid as a standard. ABTS scavenging was estimated according to the equation shown below:

$$\text{ABTS scavenging } (\%) = Ac - As/Ac \times 100$$

where, Ac is the absorbance of control and As is the absorbance of methanolic extract.

### 2.9.2. DPPH assay
The antioxidant activity of methanolic extract was performed based on DPPH scavenging method (Tshibangu et al., 2017) with slight modifications. In short, 1 mL of various concentrations of methanolic extracts (100–500 µg/mL) was dissolved in 5.0 mL of DPPH (0.1 mM) solution and the samples were kept under dark condition for 30 min. The absorbance of the sample was measured at 517 nm and ascorbic acid was employed as a standard. DPPH radical scavenging activity was calculated by the equation given below:

$$\text{DPPH scavenging } (\%) = Ac - As/Ac \times 100$$

where, Ac is the absorbance of control and As is the absorbance of methanolic extract.

### 2.9.3. Reducing power assay
The reducing power assay of the methanolic extract was carried out according to Forootanfar et al. (2014). Based on the assay, the antioxidant activity was measured by the reduction of ferric ions ($Fe^{3+}$ to $Fe^{2+}$). Different concentrations of methanolic extracts (100 to 500 µg/mL) were dissolved with 0.2 M sodium phosphate buffer (0.5 mL, pH 6.6) and 30 mM potassium ferricyanide (0.5 mL). The reaction mixture
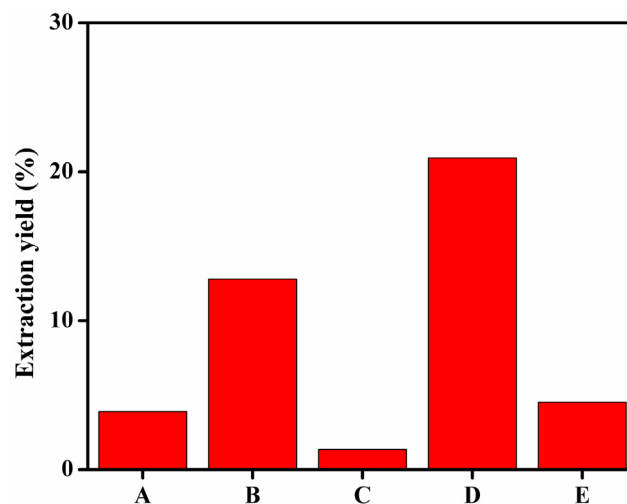


**Fig. 1** Total yield (%) of *D. metel* leaves extracts using different solvents. A) Petroleum ether, B) chloroform, C) ethyl acetate, D) methanol, and E) distilled water. Methanol showed highest yield of extract as compared to the other solvents.

was then allowed to centrifuge at 100 rpm for 20 min at 50 °C. Further, 2.0 mL of trichloroacetic acid (TCA, 0.6 M) was dissolved into the above mixture and again centrifuged at 3000 rpm for 10 min. 0.5 mL of supernatant was taken and diluted with deionized water and $FeCl_3$ solution (6 mM). Then the absorbance was recorded at 700 nm. The highest absorbance indicated the highest reducing power. Ascorbic acid was used as a standard.

## 2.10. In vitro cytotoxicity analysis

### 2.10.1. Cell line
Mouse fibroblast cell line (L929 cells) was obtained from the National Centre for Cell Science (NCCS) in Pune, India. The cell line was fed with 1% antibiotic–antimycotic and 10% fetal bovine serum (FBS) combination. It was then allowed to incubate at 37 °C in a $CO_2$ incubator (5% $CO_2$, 20% $O_2$) in MEM medium with high glucose.

### 2.10.2. MTT assay
The MTT assay was performed to investigate the cytotoxic effect of methanolic extract on L929 mouse fibroblast cell lines. Briefly, for cell adhesion, a density of $2 \times 10^4$ cells were seeded in a 96-well plate with MEM culture medium and 10% FBS was added and allowed to grow overnight. Later, the medium was replaced with fresh MEM medium (200 µL) and treated with 5–100 µg/mL of methanolic extracts and incubated for 24 h. From the MTT stock solution (5 mg/mL prepared in PBS), 50 µL was taken and poured into each well and incubated for 2–4 h in a $CO_2$ incubator at 37 °C. After incubation, MTT-containing medium was discarded and replaced with 200 µL of DMSO. The samples were incubated for 20 min under shaking condition and the absorbance was recorded at 570 nm using ELISA reader (Biotech USA, microplate reader Elx 800) (Prasathkumar et al., 2021). The cell viability (%) was calculated by the equation given below.

**Table 1** Qualitative phytochemical analysis of *D. metel* leaves extracts.

| Tests | Solvent extracts | | | | |
|---|---|---|---|---|---|
| | Petroleum ether | Chloroform | Ethyl acetate | Methanol | Distilled water |
| Flavonoids | + | + | + + | + | + |
| Alkaloids | + | − | − | + + | + |
| Saponins | − | + + | + | + | − |
| Tannins | − | + | + | + | + |
| Phenols | − | + + | + | + + | − |
| Proteins | + | + | − | − | − |
| Glycosides | − | − | − | + | − |
| Terpenoids | − | − | + + | + | − |
| Carbohydrates | − | − | + | − | − |

Note. -. Absent, +. Present, + +. Highly present.

**Table 2** Quantitative phytochemicals analysis of *D. metel* leaves extracts.

| Tests | Extracts | | | | |
|---|---|---|---|---|---|
| | Petroleum ether | Chloroform | Ethyl acetate | Methanol | Distilled water |
| TAC (mg AE/g) | $30.93 \pm 0.21^d$ | $36.78 \pm 0.06^c$ | $46.32 \pm 0.11^b$ | $88.77 \pm 1.01^a$ | $31.39 \pm 0.14^d$ |
| TFC (mg QE/g) | $34.59 \pm 0.24^d$ | $45.50 \pm 0.23^b$ | $118.09 \pm 0.37^a$ | $42.24 \pm 0.18^c$ | $31.47 \pm 0.08^e$ |
| TPC (mg GAE/g) | $35.31 \pm 0.15^d$ | $86.22 \pm 0.09^b$ | $55.54 \pm 0.19^c$ | $124.61 \pm 0.68^a$ | $34.43 \pm 0.06^d$ |
| TTC (mg GAE/g) | $31.29 \pm 0.47^d$ | $35.35 \pm 0.16^b$ | $33.31 \pm 0.26^c$ | $38.72 \pm 0.51^a$ | $31.02 \pm 0.11^d$ |

Note. [abcd]Different letters in each row indicate that values are significantly different ($P < 0.05$). Each value was expressed as mean $\pm$ SD; n = 3 independent experiments.

**Table 3** Antibacterial activity (mm) of methanolic extract of *D. metel* leaves against bacterial pathogens.

| Pathogens | Extract and antibiotic | | | |
|---|---|---|---|---|
| | 80 μg/mL | 50 μg/mL | 20 μg/mL | Vancomycin (30 μg) |
| *B. subtilis* | $9.33 \pm 0.34^b$ | $6.66 \pm 0.21^b$ | $4.33 \pm 0.17^b$ | $13.33 \pm 0.65^a$ |
| MRSA | $12.33 \pm 0.42^a$ | $5.33 \pm 0.14^c$ | $3.66 \pm 0.12^c$ | $8.33 \pm 0.42^d$ |
| *E. coli* | $12.66 \pm 0.13^a$ | $8.33 \pm 1.21^a$ | $5.33 \pm 0.52^a$ | $11.66 \pm 0.65^b$ |
| *P. aeruginosa* | $8.33 \pm 0.36^c$ | $5.33 \pm 0.44^c$ | $3.66 \pm 0.26^c$ | $9.33 \pm 0.73^c$ |

Note. [abcd]Different letters in each column indicate that values are significantly different ($P < 0.05$). Values are expressed as the mean $\pm$ SD; n = 3 independent experiments.

$$\text{Cell viability} (\%) = Ac - As/Ac \times 100$$

where, Ac is the absorbance of control and As is the absorbance of methanolic extract.

### 2.10.3. Trypan blue assay

For cell adhesion, a density of $5 \times 10^5$ cells were seeded in a six-well plate with MEM culture medium and 10% FBS. After overnight incubation, the medium was then discarded and normal MEM medium was added. Then, the cells were treated with 5–100 μg/mL of methanolic extracts and allowed to incubate for 24 h. Followed by incubation, the medium was removed and 0.4% of trypan blue stain was added into each well (Prasathkumar et al., 2021). The cell viability was observed using an inverted microscopy (Radical, RTC-7).

### 2.11. In vitro wound healing (scratch assay)

The density of $2 \times 10^5$ cells were cultured in a 24-well plate and grown to 80% confluency. At this point, on the surface of the monolayer, a tiny linear wound was observed by gently scraping with a sterile tip. To remove debris, the cells were washed with PBS and then treated with different concentrations of methanolic extract (5, 25, and 50 μg/mL) for 24 h (Prasathkumar et al., 2021). The proliferation of cells was noted, and the contraction of wound was observed under inverted phase-contrast microscopy (Radical, RTC-7).

### 2.12. Cells proliferation assay

The attachment and migration of L929 mouse fibroblasts in methanolic extracts treated cells were assessed using fluorescent microscope (EVOS M7000) (Jiji et al., 2019). After 8, 16, and 24 h of incubation, the cell culture medium was discarded and sterile PBS was added to the specimens. Further, the specimens were stained with 5 μL of Acridine orange (AO)/ ethidium bromide (EtBr) (1 mg/mL prepared in PBS) and the proliferation was observed immediately under the fluorescent microscope. Acridine orange penetrates inside the cell
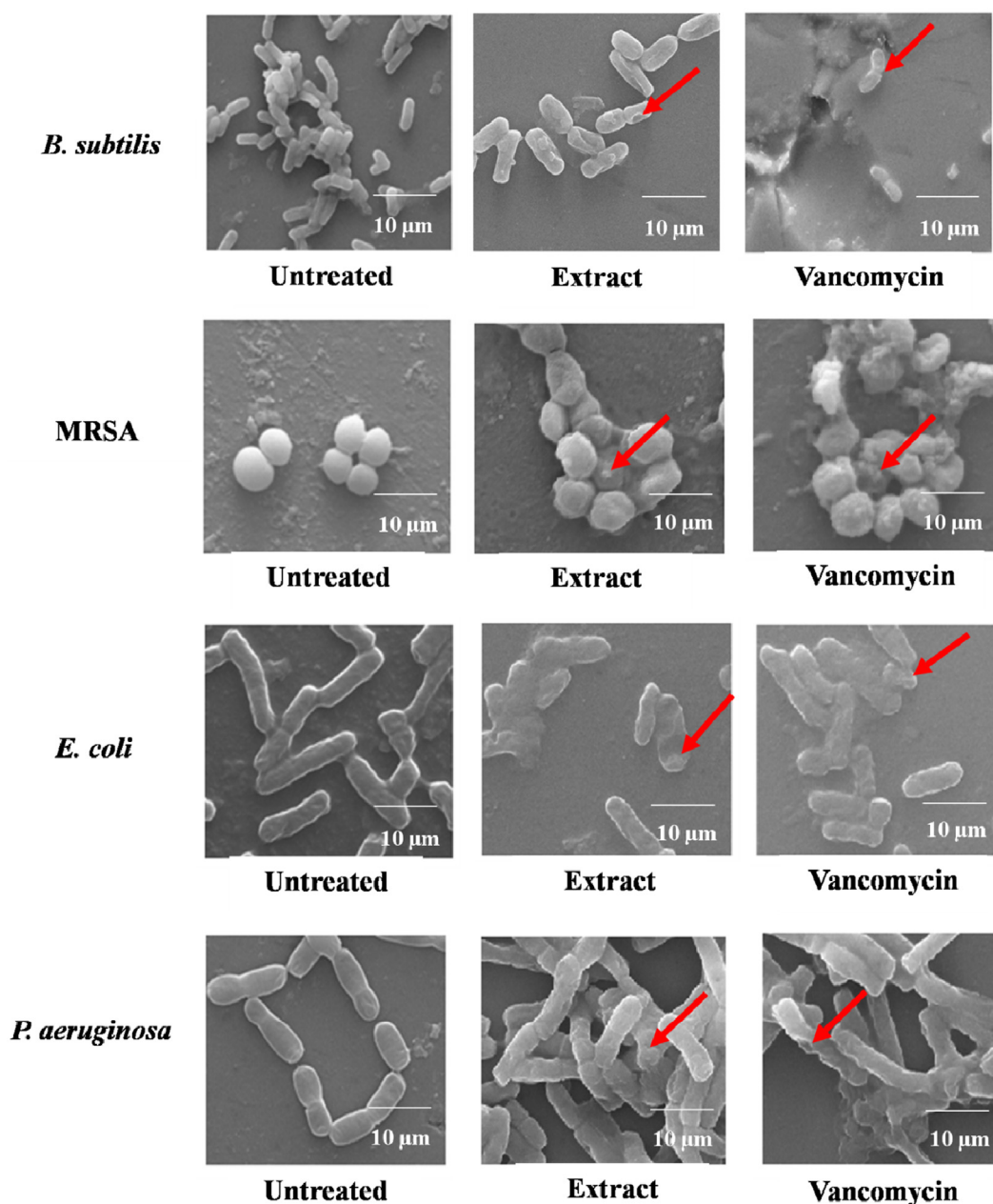
**Fig. 2** SEM analysis of untreated and treated [methanolic extract (50 μg/mL) and vancomycin (30 μg)] bacterial pathogens. Arrows indicate the destruction of bacterial cell wall due to the inhibitory effects of methanolic extract and vancomycin.

without causing cell damage and binds to the DNA inside the nucleus to produce green color fluorescence. Similarly, ethidium bromide (EtBr) enters *via* the damaged cell membrane and binds with fragmented nucleic acid to form red color fluorescence of the dead cells.

### 2.13. GC–MS analysis

Thermo Scientific™ GC–MS was used to determine the presence of varied phytoconstituents in the methanolic extract. The analysis was carried out in Elite–MS fused silica column with a helium transporter gas (steady flow rate of 1 mL/min). The injector temperature was kept constant at 260 °C. The spectra of the methanolic extract components were compared with standard components contained in the GC–MS NIST (2008) library.

### 2.14. Statistical analysis

All the experiments were conducted in triplicate and data were given as mean $\pm$ standard deviation (mean $\pm$ SD). The statistical analysis was done in SPSS version 12.0 using one-way ANOVA. When the $P$-value was $< 0.05$, the differences between all experimental groups were considered as significant.

## 3. Results and discussion

### 3.1. Extraction

Among the solvents tested, methanol showed maximum extraction yield (20.93%), followed by chloroform (12.8%), distilled water (4.53%), petroleum ether (3.9%), and ethyl acetate (1.36%) (Fig. 1).

### 3.2. Qualitative and quantitative screening of phytochemicals

The qualitative screening showed the presence of distinct bioactive phytochemicals including flavonoids, alkaloids, saponins, tannins, phenols, proteins, cardiac glycosides, terpenoids, and carbohydrates in different solvent extracts (Table 1). However, the methanolic extract accounted for the presence of maximum phytoconstituents. Ethyl acetate and chloroform extraction reported 5 to 6 types of the major phytocompounds. The petroleum ether and aqueous extracts showed relatively lesser phytochemicals as compared to other solvents.

Similarly, among different solvent extracts, methanolic extract showed higher amount of phytochemicals. In methanolic extracts, total phenol, alkaloid, flavonoid, and tannin content were estimated as $124.61 \pm 0.68$ mg GAE/g, $88.77 \pm 1.01$ mg AE/g, $42.24 \pm 0.18$ mg QE/g, and $38.72 \pm 0.51$ mg GAE/g, respectively (Table 2). The relevance of natural phyto-
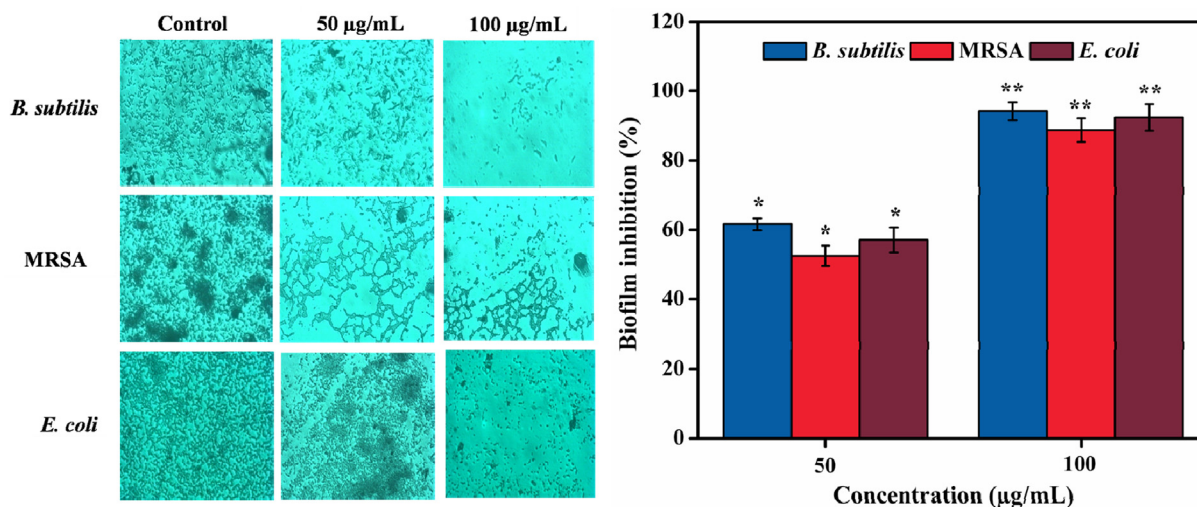


**Fig. 3** Anti-biofilm effect of methanolic leaves extract against *B. subtilis*, MRSA, and *E. coli* on cover glass. The cover glass was not attached with biofilm in the presence of methanolic extract when compared with untreated control group. Values are expressed as the mean $\pm$ SD. Statistical significance was considered at $P < 0.05$ in all cases. **denotes $P < 0.01$ and *denotes as $P < 0.05$.
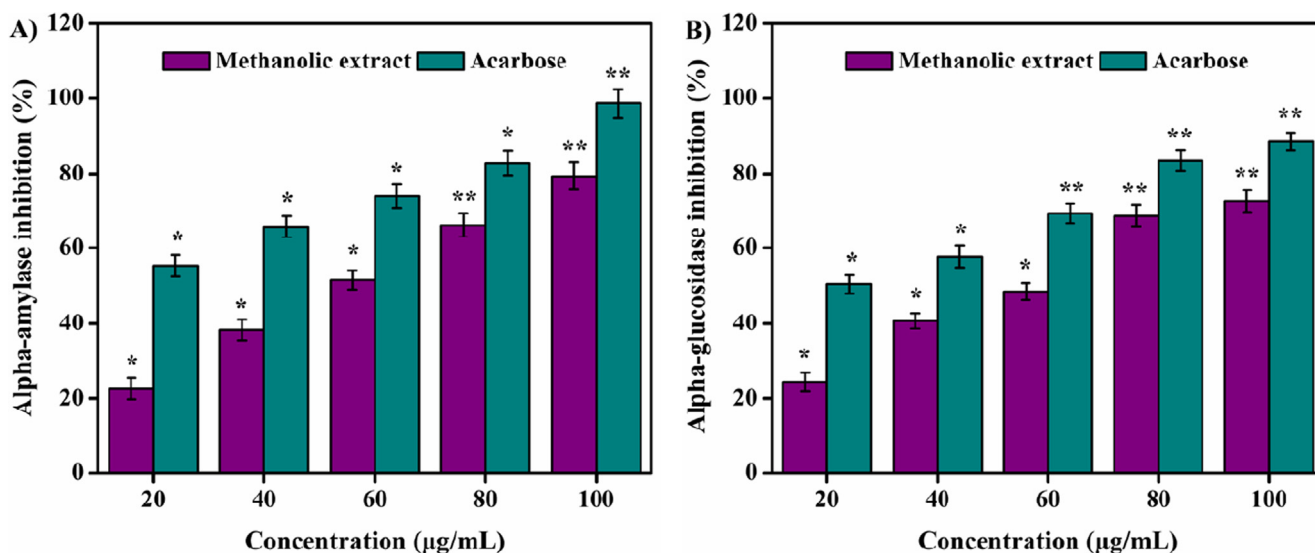


**Fig. 4** (A) a-amylase and (B) a-glucosidase inhibition activities of methanolic extract. Values are expressed as the mean $\pm$ SD. Statistical significance was considered at $P < 0.05$ in all cases. **denotes $P < 0.01$ and *denotes as $P < 0.05$.

constituents for the treatment of several illnesses is widely documented. Hence, the screening of phytochemicals is valued in the field of phytomedicine, and the identification of bioactive compounds may subsequently have a benefit in drug discovery and development (Farooq et al., 2022). Major secondary metabolites like flavonoids are of low molecular weight which possesses antibacterial, antioxidant, and anti-aging activities. Flavonoids can also inhibit carcinogenesis at various stages and can be used as potential chemotherapeutic agents for cancer treatment. Tannins are water-soluble polyphenols which exhibit antimicrobial activity (Abraham et al., 2018). Plant-based secondary metabolites including flavonoids, phenols, tannins, essential oils, and saponins are found to have free radical scavenging activity and protect from pathogen infections due to their antimicrobial action (Prabu et al., 2018; Eftekhari et al., 2021).

### 3.3. In vitro antibacterial activity

The methanolic extract showed significantly different antibacterial properties against all the tested bacterial pathogens. However, maximum activity was observed against *E. coli* with zone of inhibition of $5.33 \pm 0.52$, $8.33 \pm 1.21$, $12.66 \pm 0.13$ mm at 20–80 µg/mL of methanolic extract, respectively. Vancomycin (30 µg) showed zone of inhibition of $13.33 \pm 0.65$, $8.33 \pm 0.42$, $11.66 \pm 0.65$, and $9.33 \pm 0.73$ mm against *B. subtilis*, MRSA, *E. coli*, and *P. aeruginosa*, respectively (Table 3). Furthermore, the bacterial surface was examined by SEM before and after methanolic extract treatment. The morphological changes observed in the bacterial cell wall by untreated (control) and treated bacteria are illustrated in Fig. 2.

The antibacterial activity of methanolic extracts of *Punica granatum*, *Syzygium cumini*, *Delonix elata*, *Digera muricata*, *Jatropha gasipifed*, *Maerua oblongifolia*, *Pterocarpus santalinus*, *Gyrocaspus americana*, *Acalipha alinifolia*, *Hygrophilia auriculata*, and *Euphorbia heterophilla* leaves against multidrug-resistant bacterial pathogens (*S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *E. coli*) have been reported in the past (Pallavali et al., 2019). Among all the plants studied, *P. granatum* and *S. cumini* extracts only showed potential antibacterial activities against all the tested bacterial strains. Similarly, the ethanolic extract of *P. granatum* peels inhibited *P. aeruginosa*, *E. coli*, *S. typhi*, *B. cereus*, and *S. aureus* (Mostafa et al., 2018). Antibacterial activities of seeds extracts of *Portulaca oleracea*, *Ocimum basilicum*, *Brassica juncea*, *Phyllanthus emblica*, and *Lycium shawii* were determined against both *S. aureus* and MRSA. *P. emblica* and *L. shawii* inhibited both *S. aureus* and MRSA strains. The seed extracts of *P. emblica* and *L. shawii* were reported to be rich sources of alkaloids, flavonoids, tannins, and phenolic contents (Tayel et al., 2018).

### 3.4. In vitro anti-biofilm activity

Two different concentrations of methanolic extract (50 and 100 µg/mL) significantly ($P < 0.05$) reduced the biofilm formation against all of the tested pathogens when compared to the control group. The bacterial cells incubated with methanolic extract were found to be detached from the surface of the slide and dispersed. On the other hand, the untreated cells adhered and proliferated on the surface of the slide (Fig. 3). Maximum

inhibition effects of methanolic extract were found at 100 µg/mL which showed $94.14 \pm 0.27$, $88.73 \pm 1.51$, and $92.38 \pm 1.81\%$ inhibition of biofilm against *B. subtilis*, MRSA, and *E. coli*, respectively. Similarly, the lower concentration of methanolic extract (50 µg/mL) exhibited $61.66 \pm 0.36$, $52.59 \pm 0.24$, and $57.12 \pm 0.14\%$ inhibition of biofilm against *B. subtilis*, MRSA, and *E. coli*, respectively (Fig. 3). Based on prior reports, biofilm formation inhibitory activity exhibited by methanolic extracts was due to the presence of the bioactive compounds such as lucenin 2, 1-hexadecanol, 1-tetradecanol, and quercetin (Swamy et al., 2017), as suggested by our findings too. The anti-biofilm potential of methanolic extracted of *D. metel* leaves against *B. subtilis*, MRSA, and *E. coli* suggested its role in the mitigation of wound infections (Banu et al., 2015). Alam et al. (2020) reported 60% biofilm inhibition of *P. aeruginosa* by treating with the aqueous extract of *Clematis viticella*. Similarly, the biofilm formation of MRSA was inhibited by polyphenolic extracts of *Opuntia ficus-indica* effectively (Blando et al., 2019). Ethyl acetate extract of *Frangula alnus* bark prevented the biofilm formation ability of clinically isolated *S. aureus* due to the presence of important phytoconstituents, particularly phenolic acids (vanillic acid, ferulic acid, and gallic acid) and flavonoids (vitexin, diosmetin, and rutin) (Ðukanović et al., 2020).

### 3.5. In vitro anti-diabetic activities

The inhibition of both α-amylase and α-glucosidase are vital for the regulation of hyperglycemia as well as type 2 diabetes mellitus treatment (Esther Lydia et al., 2019; Monica et al., 2022). The concentration-dependent α-amylase and α-glucosidase inhibitory potentials of methanolic extract are illustrated in Fig. 4. At lower concentration of the methanolic extract, the enzyme inhibition was found to be minimal and the increase in inhibition was observed when the methanolic extract concentrations were increased linearly. Results showed
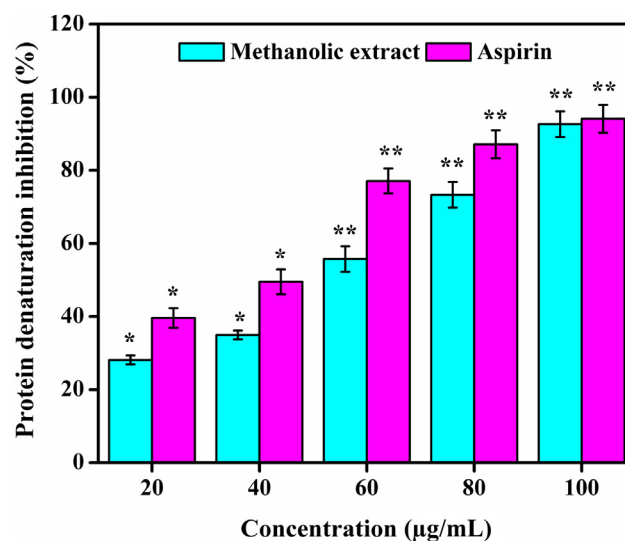


Fig. 5 Anti-inflammatory effect of methanolic extract. Values are expressed as the mean $\pm$ SD. Statistical significance was considered at $P < 0.05$ in all cases. **denotes $P < 0.01$ and *denotes as $P < 0.05$.

$22.55 \pm 0.62$–$79.41 \pm 1.13\%$ of α-amylase inhibition and $24.31 \pm 1.47$–$72.59 \pm 0.22\%$ of α-glucosidase inhibition at 20–100 µg/mL concentrations of the extract. The $IC_{50}$ values of the extract were estimated as 53.14 and 69.7 µg/mL for α-amylase and α-glucosidase, respectively. Previously, α-amylase and α-glucosidase inhibitory efficacy of acetone, ethanol, and aqueous extracts of *Morinda lucida* leaves with lower $IC_{50}$ values were reported (Kazeem et al., 2013). Similarly, methanolic extract of *Carica papaya* seeds showed an $IC_{50}$ value of 94.63 and 79.35 mg/mL towards α-amylase and α-glucosidase inhibition, respectively (Agada et al., 2020).

### 3.6. In vitro anti-inflammatory activity

Typically, tissue injury results in protein denaturation of the intercellular substances or protein constituents of cells. Protein denaturation has been associated with the rate of the inflammatory response and an indicator for the development of different inflammatory diseases including arthritis. Thus, the ability of a material to inhibit the protein denaturation process

denotes significant anti-inflammatory activity (Osman et al., 2016). In this study, methanolic extract revealed significant ($P < 0.05$) anti-inflammatory effect in a concentration dependent manner. The extract showed $28.11 \pm 0.13$, $34.94 \pm 1.11$, $55.73 \pm 0.42$, $73.28 \pm 0.72$, and $92.62 \pm 1.33\%$ of inhibition of protein denaturation at varied concentrations. $IC_{50}$ value of protein denaturation was estimated as 52.45 µg/mL (Fig. 5). In a previous study, methanolic extract of *Sesbania grandiflora*, *Cassia auriculata*, *Olax zeylanica*, *Passiflora edulis*, *Gymnema lactiferum*, and *Centella asiatica* leaves depicted protein denaturation inhibition in the range of 36–75% at distinct concentrations (Gunathilake et al., 2018). Moreover, the ethanolic extract of *Mikania scandens* stem and leaves at various concentrations showed anti-inflammatory activities (Banerjee et al., 2014).

### 3.7. In vitro antioxidant activities

Natural antioxidants are able to inhibit or reduce the oxidative damage that occurs due to reactive oxygen species (ROS) or
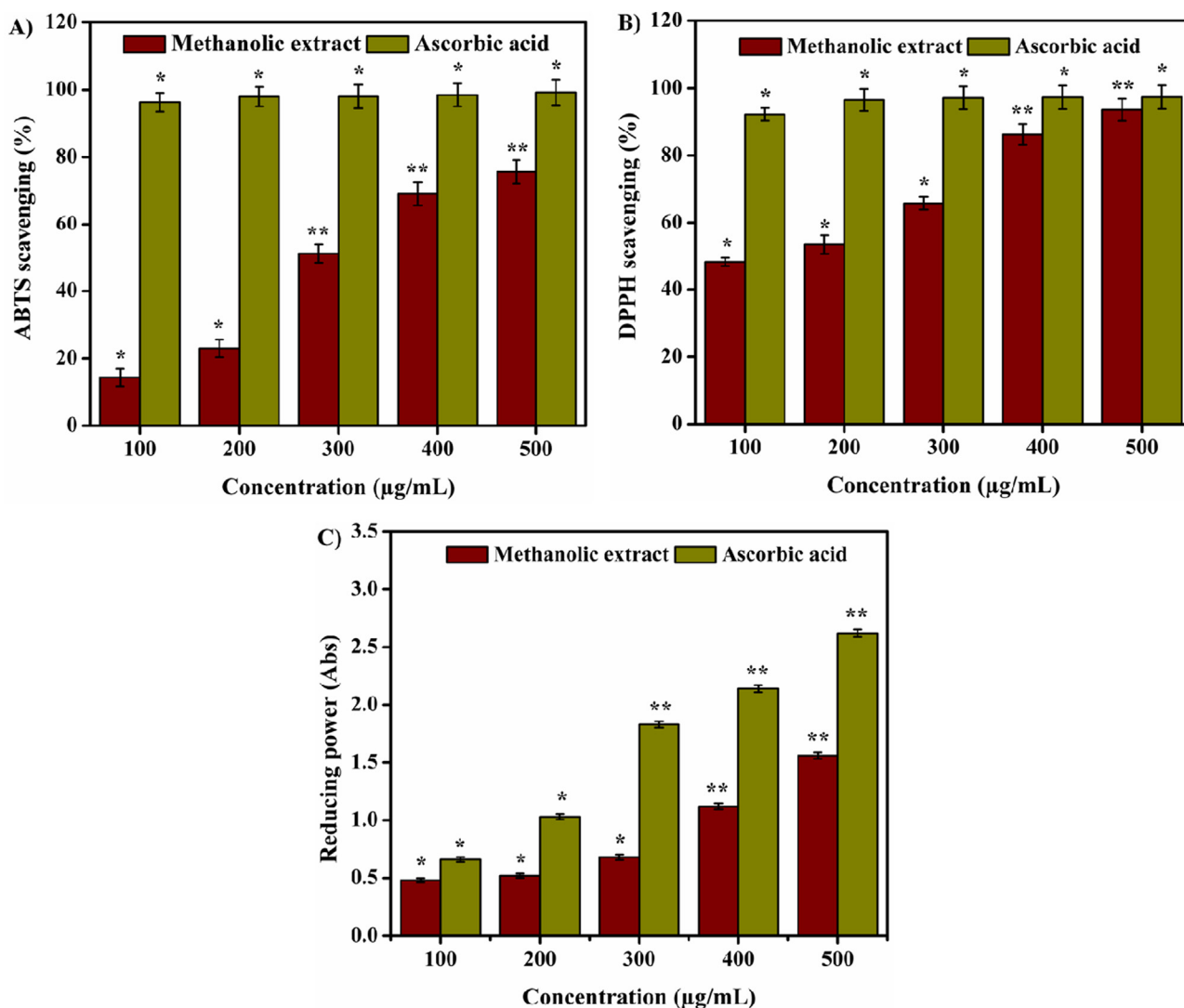


**Fig. 6** (A) ABTS radical scavenging, (B) DPPH radical scavenging, and (C) reducing power activities of methanolic extract. Values are expressed as the mean ± SD. Statistical significance was considered at $P < 0.05$ in all cases. **denotes $P < 0.01$ and *denotes as $P < 0.05$.

atmospheric oxygen. They play a major role in maintaining good health condition and are known to reduce risks associated with chronic diseases (Saraswathi et al., 2021). Fruits, vegetables, leaves, and whole grains are considered major sources of antioxidants. Antioxidants obtained from plants such as carotenes, phenolic acids, phytoestrogens, vitamin C, and vitamin E have the potential to reduce disease risk (Naji et al., 2020). Various methods of antioxidant assays such as ABTS, DPPH, and reducing power were developed to measure the free radical scavenging properties to compare and grade natural antioxidants present in food and beverages for thera-peutic applications (Schaich et al., 2015). Fig. 6 shows the significant antioxidant activity of methanolic extract of *D. metel* leaves, as estimated by ABTS, DPPH, and reducing power assays. As per ABTS assay, the antioxidant activity was observed from $14.32 \pm 0.24$ to $75.61 \pm 0.31\%$ in a concentration dependent manner with an $IC_{50}$ value of 293.47 µg/mL. The DPPH assay showed radical scavenging of $48.16 \pm 0.43$ to $93.55 \pm 0.62\%$ at different concentrations with an $IC_{50}$ value of 146.53 µg/mL. Likewise, the reducing power assay showed potential free radical scavenging activity by reducing $Fe^{3+}$ to $Fe^{2+}$ in a concentration dependent manner.
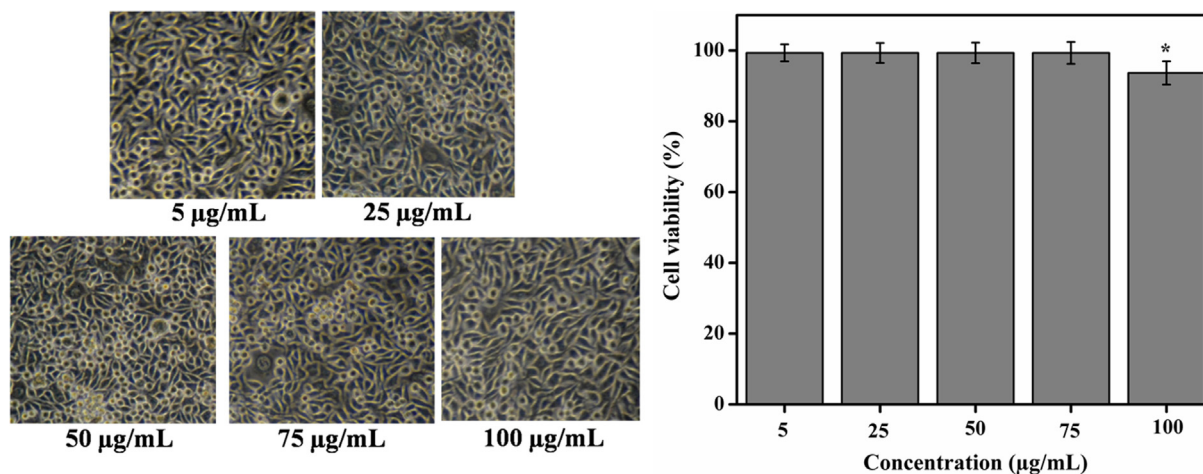


**Fig. 7**  Cytotoxicity effect (morphology and viability) of different concentrations of methanolic extract on L929 mouse fibroblast cell lines. Results showed $99.33 \pm 0.26\%$ cell viability at all concentrations of the extract (5–75 µg/mL) while 100 µg/mL of concentration showed $93.66 \pm 1.14\%$ cell viability. Values are expressed as the mean $\pm$ SD.
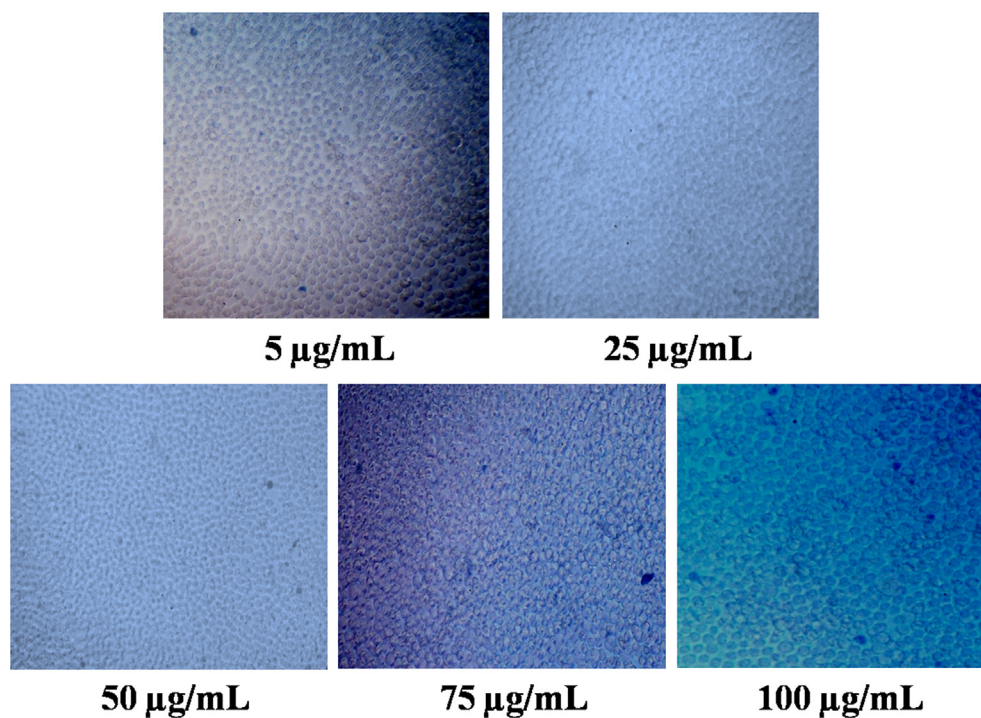


**Fig. 8**  Cytotoxicity assessment of methanolic extract in L929 cell line by trypan blue assay. The extract at different concentrations (5–100 µg/mL) showed no significant toxicity on L929 cell lines.

Herbal plants are rich in natural antioxidants and phenolic compounds, and are used to prevent the oxidative stress by free radical scavenging activity (Murali et al., 2020; Mayasankaravalli et al., 2020). It is presumed that the major phytochemicals, namely phenol and flavonoids are responsible for the enhanced antioxidant activity as reported by other studies. Phenolic compounds are widely utilized as potential antioxidants for various medical applications and are also
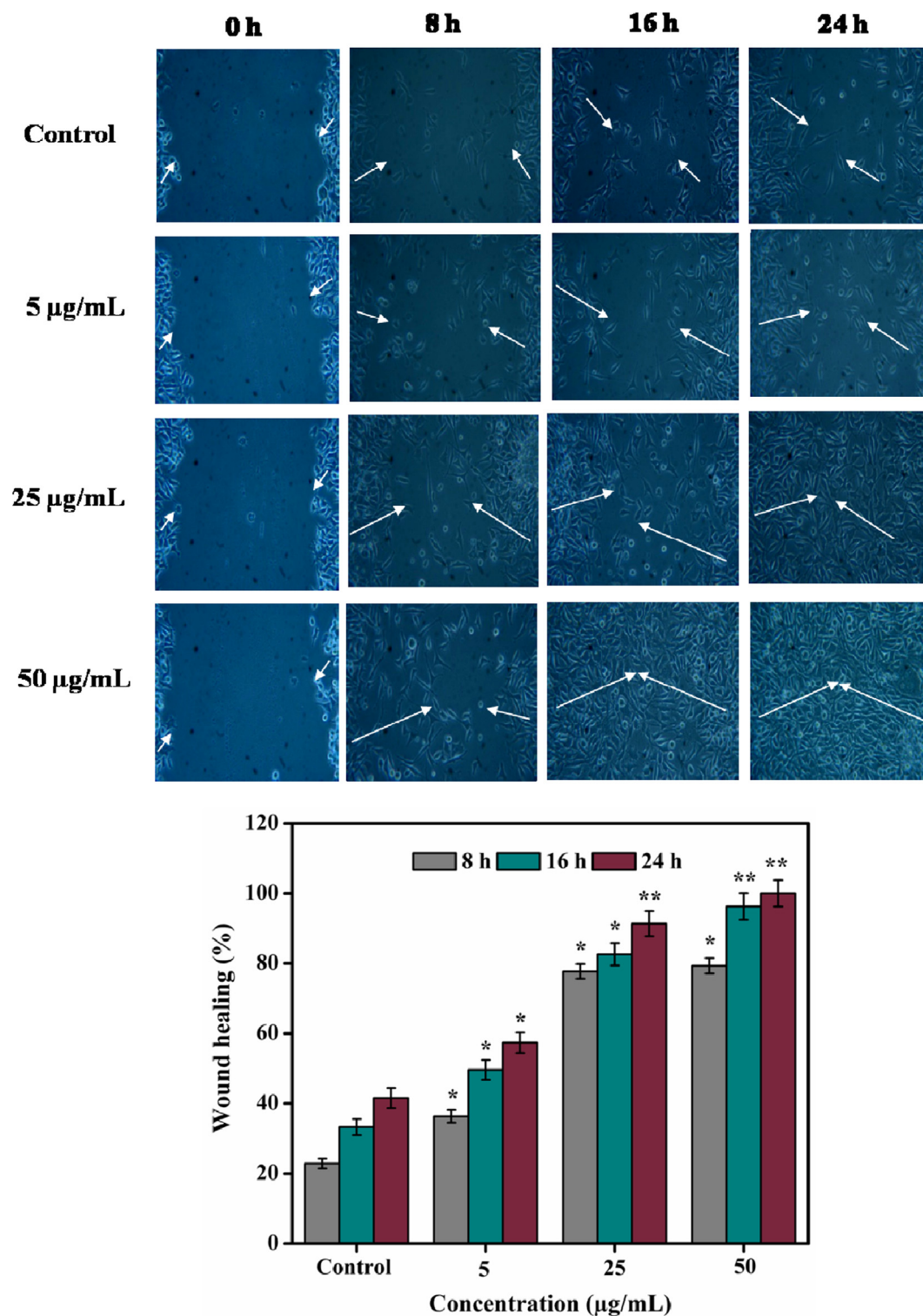


**Fig. 9** Healing of scratch wounds at different time intervals using three different concentrations (5, 25, and 50 μg/mL) of methanolic extract. White arrow indicates rate of wound closure. Values are expressed as the mean ± SD. Statistical significance was considered at $P < 0.05$ in all cases. **denotes $P < 0.01$ and *denotes as $P < 0.05$.

gaining interest in human health. The aerial parts of *Stachys molea* extracts showed free radical scavenging activity (Elfalleh et al., 2019). *Aerva lanata* exhibited significant rate of DPPH scavenging (Al-Ansari et al., 2019). Similarly, acetone, ethanol, and methanol extract of *Dendranthema morifolium* flower depicted significant antioxidant effects due to the substantial amount of phenolic contents (Ma et al., 2020). *In vitro* ferrous ion chelating, DPPH radical, and nitric oxide radical scavenging assays also showed potential antioxidant properties of *Pimenta dioica* ethanolic leaves extract.

### 3.8. Cell cytotoxicity by MTT and trypan blue assays

The cytotoxicity of methanolic extract of *D. metel* on L929 mouse fibroblast cell line is presented in Fig. 7. Results showed that 99.33 ± 0.26% of cell viability were recorded at 5–75 μg/mL, while the cell viability was reduced to 93.66 ± 1.14% at 100 μg/mL. Fig. 8 shows the trypan blue assay of live and dead cells. Results revealed that methanolic extract had no signifi-

cant cytotoxic effect on L929 cell line. Previous report demonstrated no toxic effect of aqueous and ethanolic extracts of *Gynura divaricata* against L929 cell lines, suggesting the expediency of *G. divaricata* extract in drug development (Poeaim et al., 2008). Similarly, the cytotoxicity of methanolic extract of *Aristolochia saccata* leaves was investigated on L929 mouse fibroblasts which showed > 97% of cell viability (Bolla et al., 2019). In contrary, the aqueous extract of *Sargassum illicifolium* revealed a significant reduction (< 50%) in the viability of L929 cells (Premarathna et al., 2019).

### 3.9. In vitro wound healing and cells proliferation

Several existing *in vitro* and *in vivo* protocols are developed to evaluate the efficacy of medically important novel drugs and plant extracts (compounds) for wound healing. Among them, fibroblast cells migration/proliferation scratch assay is a promising technique. Scratch assay indicates the primary understanding of wound healing trait of plant extracts (com-
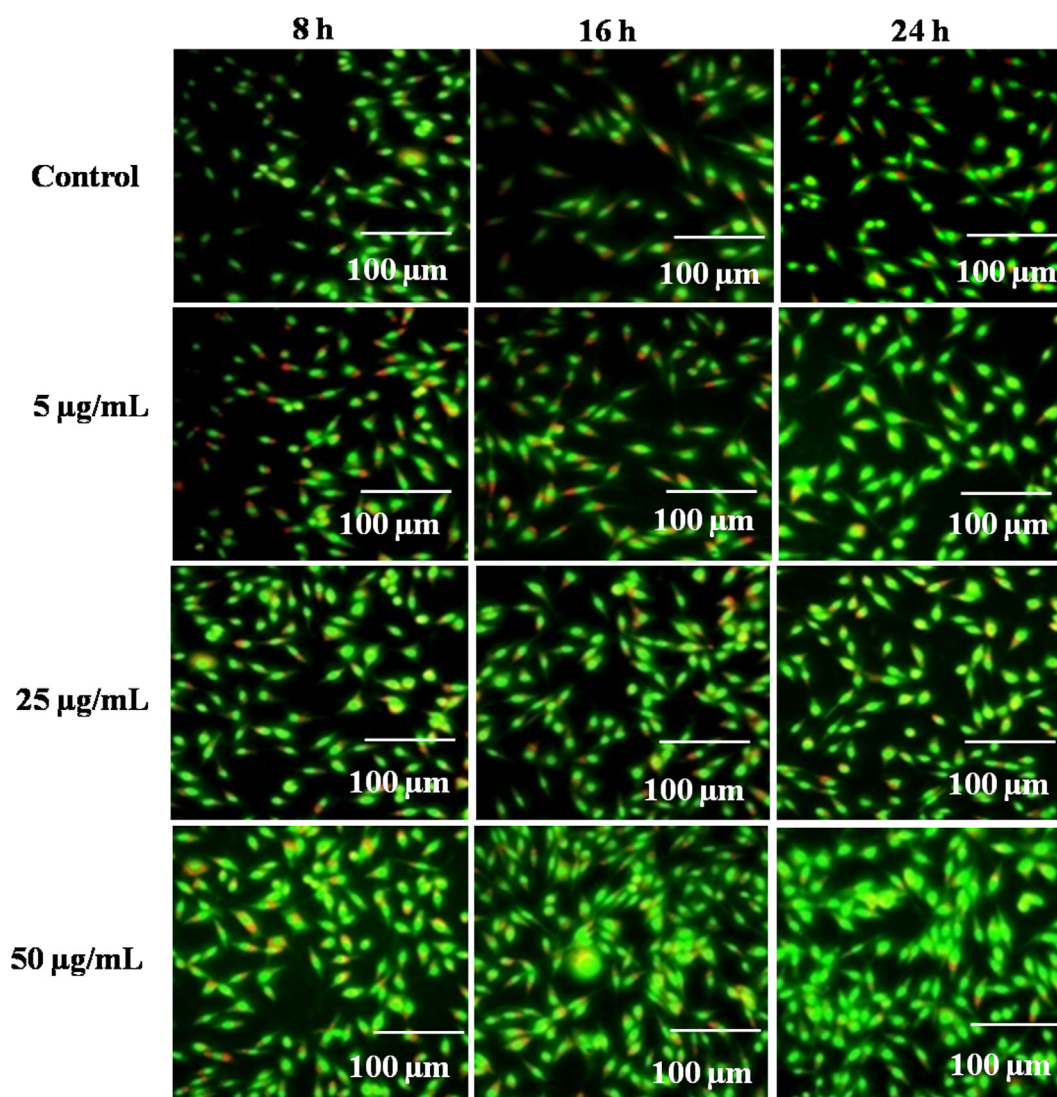


**Fig. 10** Fluorescent proliferation images of control and methanolic extract (5, 25, and 50 μg/mL) at 8, 16, and 24 h in L929 mouse fibroblast cells.

pounds) through stimulating the cells migration/proliferation (Bolla et al., 2019). In the wounded surface of cell monolayer followed by scratching, the stimulating collective cells migration/proliferation towards wound contraction of the resultant gap was observed which is required for tissue regeneration (Javer et al., 2020). In this investigation, the wound healing potency of the methanolic extract (5, 25, and 50 μg/mL) of *D. metel* leaves was estimated against L929 mouse fibroblasts for 24 h. Results indicated that methanolic extract at 5 μg/mL closed 36.33 ± 0.52, 49.61 ± 0.27, and 57.36 ± 1.89% of wound gap at 8, 16, and 24 h, respectively. The wound closure was even better at 25 μg/mL (77.72 ± 1.19–91.33 ± 0.28%) and 50 μg/mL (79.31 ± 1.55 – 100%) for the same period. On the other hand, the control group showed only 22.83 ± 2.14–41.55 ± 1.62% of wound closure (Fig. 9). *In vitro* wound healing activity of *A. saccata* methanolic leaves extract was evaluated in L929 cells and showed 34, 70, and 93.5% of wound closure at 12, 24, and 48 h, respectively (Bolla et al., 2019). Likewise, the chloroform and methanolic extracts of *Boerhavia diffusa* leaves were tested against HaCaT cell line. The chloroform and methanolic extracts showed 60 and 90% of wound healing, respectively after 60 h (Juneja et al., 2020).

The migration of fibroblast cells plays a crucial role in the initial stage of wound healing process. It also promotes the synthesis of new extracellular matrix and thick actin myofibroblasts (Dutta et al., 2020). In this study, the cell proliferation potency of methanolic extract was observed under fluorescent microscopy. The cell density of the methanolic extract was observed considerably higher than the untreated cells (Fig. 10). In an earlier report, the wound healing potential of three different seaweed extracts (*Sargassum illicifolium*: S.i-SW23, S.i-SW12, and S.i-SW13) was demonstrated using L929 cells (Premarathna et al., 2019). Results showed that S.i-SW23 had a significant proliferative and migratory effect with a wound closure rate of 97.8 ± 0.05% as compared to the control (46.1 ± 0.54%) for 24 h.

### 3.10. GC–MS analysis

GC–MS analysis of the methanolic extract of *D. metel* leaves is presented in Fig. 11. The presence of the bioactive phytoconstituents, peak area (%), retention time, molecular formula, and molecular weight are shown in Table 4. The major bioactive compounds identified were (–)-Loliolide, neophytadiene, hexadecanoic acid, phytol, 13-docosenamide, (Z)- and hentriacontane. (–)-Loliolide is a commonly available monoterpenoid lactone and it was reported to exhibit significant biological properties including antibacterial, antifungal, antioxidant, and anticancer efficacies (Grabarczyk et al., 2015). Further, it has been used as alternative medicine for diabetes and depression (Dias et al., 2020). Neophytadiene has been reported to reveal antimicrobial, analgesic, antipyretic, antioxidant, and anti-inflammatory properties. It is also used to treat skin diseases, rheumatism, and headache (Raman et al., 2012;
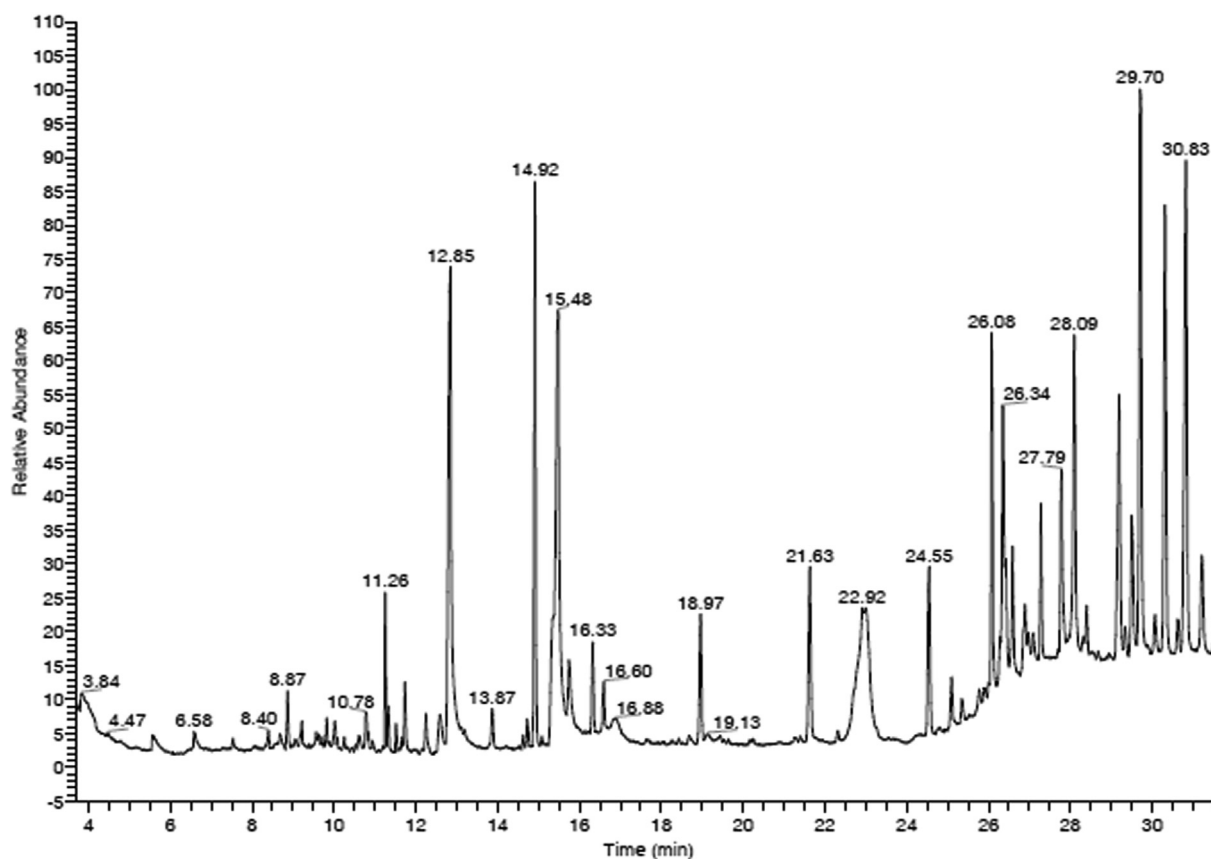


**Fig. 11** GC–MS chromatogram of methanolic extract. Each peak shows the relative abundance and area of specific compound in the methanolic extract.

**Table 4** Different bioactive compounds present in the methanolic extract.

| S.No | Compounds | Area (%) | Retention Time | Molecular Formula | Molecular Weight |
|---|---|---|---|---|---|
| 1 | N-(1-hydroxy-4-oxo-1-pheny l-decahydro-pyrido[1,2-a] azep in-3-yl)carbamic acid, benzyl ester | 0.84 | 3.84 | $C_{24}H_{28}N_2O_4$ | 408 |
| 2 | 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-tri methyl- | 0.67 | 8.87 | $C_{11}H_{16}O_2$ | 180 |
| 3 | (-)-Loliolide | 0.95 | 10.78 | $C_{11}H_{16}O_3$ | 196 |
| 4 | Neophytadiene | 1.72 | 11.26 | $C_{20}H_{38}$ | 278 |
| 5 | Hexadecanoic acid | 9.23 | 12.85 | $C_{16}H_{32}O_2$ | 256 |
| 6 | Silicone oil | 0.62 | 13.87 | NA | 0 |
| 7 | Phytol | 5.94 | 14.92 | $C_{20}H_{40}O$ | 296 |
| 8 | 9,12,15-octadecatrienoic acid, (Z,Z,Z)- | 10.40 | 15.48 | $C_{18}H_{30}O_2$ | 278 |
| 9 | Silicone oil | 1.11 | 16.33 | NA | 0 |
| 10 | Heptasiloxane, hexadecamethyl- | 1.77 | 18.97 | $C_{16}H_{48}O_6Si_7$ | 532 |
| 11 | Silicone oil | 2.83 | 21.63 | NA | 0 |
| 12 | 1,5-dimethyl-6-(1,5-dimethyl hexyl)-15,16-epoxy-18-oxatet racyclo[9.6.1.0(2,10)0.0(5,9)]o ctdecane-13-one | 6.97 | 22.92 | $C_{28}H_{46}O_2$ | 414 |
| 13 | Silicone oil | 2.63 | 24.55 | NA | 0 |
| 14 | 13-docosenamide, (Z)- | 4.00 | 26.08 | $C_{22}H_{43}NO$ | 337 |
| 15 | Silicone oil | 4.99 | 26.34 | NA | 0 |
| 16 | Silicone oil | 2.66 | 27.79 | NA | 0 |
| 17 | Methyl 6-(chloromethyl)-4-(3,4-dimet hoxy-2-(phenylmethoxy)-phe nyl)-3-methyl-2-pyridinecarb oxylate | 4.67 | 28.09 | $C_{24}H_{24}ClNO_5$ | 441 |
| 18 | Hentriacontane | 8.23 | 29.70 | $C_{31}H_{64}$ | 436 |
| 19 | 5-amino-2-phenyl-4-(p-meth oxyphenyl)-4-[4′-(N,N-dimet hylamino)phenyl]-7-(pyrrolidi n-1′-yl)-1,6-naphthyridine-8-c arbonitrile | 8.35 | 30.83 | $C_{26}H_{23}N_5O$ | 421 |

NA-Not available.

Swamy et al., 2017). Phytol is a potent compound to inhibit *S. aureus*. Additionally, it possesses antinociceptive and antioxidant properties (Santos et al., 2013). Hexadecanoic acid is a well-known anti-inflammatory saturated fatty acid that exhibits antifungal and antibacterial capacity (Aparna et al., 2012). 13-docosenamide, (Z)- has significant antiandrogenic, anti-ulcerogenic, anticancer, antimicrobial, anti-inflammatory, antioxidant, lubricant, nematicide, and hypocholesterolemic activities (Beldal et al., 2017). Hentriacontane stimulated the expression of inflammatory mediators such as TNF-α, iNOS, COX-2, PGE(2), and IL-6 and it has been recommended for development of novel drugs to treat inflammatory diseases (Kim et al., 2011).

## 4. Conclusions

This study reports *in vitro* antibacterial, anti-biofilm, anti-diabetic, anti-inflammatory, and antioxidant activities of methanolic extract of *D. metel* leaves. Most importantly, the potential wound healing efficiency of methanolic extract was observed at 50 μg/mL and that was further complemented with no cytotoxicity on L929 mouse fibroblasts for 24 h. The collective biological properties of *D. metel* leaves extract could be useful in treating not only metabolic diseases but also superficial mycoses or chronic diabetic wounds.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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