Iranian Journal of Pharmaceutical Sciences
2025: 21 (1): 48- 60
https://journals.sbmu.ac.ir/IJPS

Research Article

OPEN ACCESS

# Assessment of the Phytochemical Profile, Free Radical Scavenging and Antibacterial Effects of *Pilea symmeria* from Mizoram, India

Lalremtluangi Rk[a], Lalthansangi C[a], Esther Lalhmingliani [a*], Mathipi Vabeiryureilai[b]

[a] *Systematics and Toxicology Laboratory, Department of Zoology, Mizoram University, Aizawl 796004, Mizoram, India.*
[b] *Cytogenetics Laboratory, Department of Zoology, Mizoram University, Aizawl 796004, Mizoram, India.*

**Received:** November 7, 2024 **Last Revision:** December 01, 2024 **Accepted:** December 11, 2024 **Available online:** February 03, 2025

**Abstract**

Antibacterial and free radical scavenging properties of *Pilea symmeria*, a traditional medicinal plant from Mizoram, India, have been examined in this study. Chloroform, ethanol, and aqueous were used to extract the plant components. Extracts were phytochemically analyzed qualitatively and quantitatively. The extracted sample were tested for their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3- ethylbenzothiazoline-6-sulfonic acid) ABTS, and superoxide anion ($O2^{\bullet(-)}$) radicals. An antibacterial susceptibility test was performed against the bacterial strain *Escherichia. coli, Bacillus subtilis* and *Klebsiella pneumoniae* using disc diffusion method. The broth microdilution method determined the minimum inhibitory concentration (MIC). Plating samples from a well of MIC and above concentrations on a new agar plate determined minimum bactericidal concentration (MBC). Various phytochemicals, including terpenoids, tannins, flavonoids, cardiac glycosides, steroids, alkaloids, saponins, and phlobatannins, were present in the various extracts of *P. symmeria.* Phytochemical analysis by LC-MS revealed the presence of 34 major compounds having various biological activities. The most potent radical scavenger was ethanol extract, which contains the highest overall phenolic and flavonoid content with the lowest IC50 value. The various extracts also suppressed the tested organisms' growth in a concentration-dependent manner. Therefore, our results suggested that *P. symmeria* extracts contain various phytochemicals with anti-radical and anti-bacterial activities and can potentially develop into novel phytomedicines.

*Keywords*: Minimum inhibitory concentration; Medicinal plant; Minimum bactericidal concentration; Antioxidant; Reactive oxygen species; UV/Vis spectrophotometer.

## 1. Introduction

Unpaired electron compounds or free radicals interact with other molecules to obtain electrons. As the chain reaction proceeds, more free radicals are produced, and normal cell functioning can be severely disrupted [1]. Antioxidants are compounds that can donate electrons to free radicals, thus stabilizing them and making them less reactive [2]. Investigating medicinal plants' antioxidant and antimicrobial activities is crucial because they primarily function based on these capabilities [3]. *P. symmeria* Weddel, locally known as 'Khupnal' in Mizoram, is a shade-loving herbaceous plant found in

*** Corresponding Author**:
**Esther Lalhmingliani**, Systematics and Toxicology Laboratory, Department of Zoology, Mizoram University, Aizawl 796004, Mizoram, India.
E-mail: es_ralte@yahoo.in.

**Cite this article as**: Lalremtluangi RK., Lalthansangi C., Lalhmingliani E., Vabeiryureilai M., Assessment of the Phytochemical Profile, Free Radical Scavenging and Antibacterial Effects of *Pilea symmeria* from Mizoram, India. Iran. J. Pharm. Sci., 2025, 21 (1): 48- 60.

DOI: https://doi.org/10.22037/ijps.v21i1.46680

damp places and belongs to the family Urticaceae. Traditional usage of this plant is mainly for treating wounds [4]. Although locals utilize this plant to treat wounds, no scientific proof supports its effectiveness. Thus, the primary objective of this study is to provide scientific evidence supporting the traditional usage of *P. symmeria*.

## 2. Materials and Methods

### *2.1. Plant Collection and Extract Preparations*

*P. symmeria* was obtained from Tanhril, Aizawl district, Mizoram, India, during June-August 2020. Plant identification and authentication were done at the Natural History Museum Mizoram, Mizoram University, with the accession number NHMM-P/000161. The leaves were shade-dried at 25°C and pulverized using an electrical grinder. Powdered leaves (100g) were soaked in solvents (500ml) such as ethanol, chloroform, and aqueous (sequential cold extraction) in continuous agitation for 72 hrs. Upon filtering employing Whatman filter paper No. 1, extracts were evaporated to dryness in a 40ºC oven. The extracted material for each solvent, i.e., *Pilea symmeria* chloroform extract (PSCE), *Pilea symmeria* ethanol extract (PSEE), and *Pilea symmeria* aqueous extract (PSAE), were stored at -20˚C till further usage.

### *2.2. Collection and Preparation of Bacterial Suspension*

*Escherichia coli* (ATCC strain 25922) and *Bacillus subtilis* (ATCC strain 11447) were obtained from the Department of Botany, Mizoram University, Tanhril, Aizawl, and *Klebsiella pneumoniae* (ATCC strain 10031) was obtained from Pachhunga University College, Aizawl. The bacterial isolates were cultured on nutrient agar. Before the antibacterial testing, several bacteria colonies were scooped out from the nutrient agar and mixed with physiological saline (0.9%w/v NaCl) to create a diluted solution. Turbidity was then adjusted to a concentration of 0.5 McFarland standard.

### *2.3. Preliminary Phytochemical Screening*

Standard protocols were used for preliminary phytochemical analysis [5, 6]. Different extracts were evaluated for:

#### *2.3.1. Alkaloids:*

Dragendroff's reagent (0.5ml) was mixed with the extract (0.1g). The presence of a reddish-brown precipitate indicates the presence of alkaloids.

#### *2.3.2. Cardiac glycosides:*

Glacial acetic acid (2ml) with ferric chloride solution (a single drop) and concentrated $H_2SO_4$ (1ml) underlay were mixed with the extract (0.1g). Brown ring development at the interface indicates cardiac glycoside presence.

#### *2.3.3. Saponins:*

3 drops of olive oil were mixed with 0.1 g of extract. After mixing the mixture for a few minutes, a stable emulsion formed, indicating the presence of saponins.

#### *2.3.4. Steroids:*

0.1 g of the extract was dissolved in each solvent. Concentrated $H_2SO_4$ was added in a few drops. Steroid presence is indicated by the bottom layer turning red.

#### *2.3.5. Tannins:*

0.1 g of the extract was dissolved in each solvent. A solution of ferric chloride (0.1%) was added in a few drops. Brownish-green or blue-black color development indicates the presence of tannins.

#### *2.3.6. Terpenoids:*

Each extract (5ml) (0.1 g/ml) was mixed with chloroform (2ml). Concentrated $H_2SO_4$ (3ml) was then carefully added. A reddish-brown color appeared near contact, indicating the presence of terpenoids.

#### *2.3.7. Phlobatannins:*

1% aqueous HCl solution was used to boil 0.1 g of extract. Red precipitate indicates phlobatannins presence.

#### *2.3.8. Flavonoids:*

4 ml of 1N NaOH was mixed with 0.1 g of extract. The appearance of a dark yellow color indicates the presence of alkaloids.

### *2.4. Total Phenolic Content*

Total phenolic content was estimated by applying the standard method [7]. A mixture was prepared by combining Folin-Ciocalteu's reagent (5ml) (diluted tenfold) with varied concentrations (ranging from 0.25-8.0mg/ml) of *P. symmeria* extracts (1ml). Following a 5-minute incubation period, the mixture received an additional sodium carbonate solution (4ml) with a 0.115mg/ml concentration. For 2 hours (in the dark), the mixture was left to incubate at room temperature. Absorbance was then taken at 765nm. The calibration curve was created by combining a methanolic solution containing gallic acid (1mL, 0.25-4.0mg/mL) with the above reagents, and the absorbance was recorded. An experiment was conducted thrice. The total phenolic content was expressed as gallic acid equivalent to mg/g of the dried extract.

### *2.5. Total Flavonoid Content*

Total flavonoid content was estimated by applying the standard method [8]. 0.25 ml of different *P. symmeria* extract concentrations (0.25 to 8.0 mg/ml) were combined with 5% sodium nitrite solution (75µl) and distilled water (1.25ml). After adding aluminum chloride(150µl) with a concentration of 10%(w/v), the solution was left to sit for 5mins. Subsequently, 1M NaOH (0.5ml) was added to the mixture. By adding distilled water, the solution's volume was raised to 2.5ml. The reading at 510nm was taken to determine the absorbance. The standard quercetin solution (0.25ml, 0.25-4.0mg/mL) was mixed with the above reagents to create the calibration curve. The total flavonoid content of *P. symmeria* was expressed as quercetin equivalent to mg/g of the dried extract.

### 2.6. *Liquid Chromatography-Mass Spectrometry* (*LC-MS*) Analysis

LC-HRMS was utilized to identify bioactive secondary compounds in chloroform (PSCE), ethanol (PSEE), and aqueous (PSAE) extracts of *P. symmeria*. An orthogonal ESI source was employed in the analysis, which was carried out with an ACCUCORE high-performance liquid chromatography (HPLC) system linked to a triple quadrupole tandem mass spectrometer. Within the m/z range 150-2000, mass acquisition spectra were acquired. For both ES+ and ES-, the source temperature was adjusted to 120 ºC, while the desolvation temperature was set to 350 ºC. Mobile phase A, including water with "0.1% formic acid, and mobile phase B, containing acetonitrile with 0.1% formic acid eluents combination, was utilized in linear gradient flow. 5µl volume injection" was used. In contrast, the flow rate remained constant at 0.3ml/min during the entire gradient. OpenLynx™ and MassLynx™ Software were used for analyzing the "data.

### *2.7. Assessment of Free Radical Scavenging Activity*

#### *2.7.1. DPPH Radical Scavenging Activity*

DPPH radical scavenging activity was performed following the standard protocol [9]. 500 µl of *P. symmeria* extracts (20-1000 µg/ml) were mixed with 0.1mM DPPH (1ml) in absolute methanol, and the mixture was incubated for 30mins in the dark at room temperature, then absorbance was measured at" 515nm. Methanol was utilized as blank; all reagents except extract samples were taken for control, and the reading was used to calculate the percent inhibition. Ascorbic acid was used instead of the extract for positive control. The scavenging activities were represented as a percentage and computed applying the formula:

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

Here, As represented test sample absorbance, and Ac represented control absorbance.

#### *2.7.2. ABTS Radical Scavenging Activity*

A standard procedure was employed for testing the ABTS radical scavenging activity [10]. ABTS solution (7mM) and potassium persulfate solution (2.45mM) of the same amount were mixed to prepare the stock solution. The solution containing ABTS+ radicals turned dark after being incubated for 12 hours at room temperature in the dark. A freshly prepared ABTS solution was diluted with 50% methanol before each test to acquire an initial absorbance of 0.700 (± 0.02) at 745nm. Then, the ABTS (1.5ml) working standard was mixed with various concentrations (20-1000µg/ml) of extract (150µl). Immediately after the experiment, the reading was taken at 745nm.

A total of 50% methanol was utilized as blank, and for control, all reagents except the extracted sample were taken, and the reading was used to calculate the percent inhibition. Each experiment was repeated thrice. Ascorbic acid was used instead of the extract for positive control. The scavenging activities were represented as a percentage and then computed applying the "formula:

$$\% \text{ Scavenging}=(Ac-As)/Ac\times100$$

Here, Ac represented control absorbance, and As represented test sample absorbance.

### *2.7.3. Superoxide Radical($O_2^{\bullet(-)}$) Scavenging Activity*

Superoxide radical ($O_2^{\bullet(-)}$) scavenging activity" was done following the standard method [11]. NBT (1mg/ml in DMSO) (0.2ml) with different concentrations (20-1000µg/ml) (0.6ml) of extract were mixed. Alkaline DMSO (2ml) was prepared by mixing DMSO (1ml) in 5mM NaOH and was added to the reaction mixture to prepare a final volume of 2.8ml. At 560nm, absorbance was measured. Blank consists of pure DMSO; all reagents except the extract sample were taken for control, and the reading was used to calculate the percent inhibition. Ascorbic acid was used instead of the extract for positive control. The scavenging activities were represented as a percentage and then computed applying the formula:

$$\% \text{ Scavenging}=(As-Ac)/As\times100$$

Here, Ac represented control absorbance, and As represented test sample absorbance.

### *2.8. In Vitro Assessment of the Antimicrobial Activity of P. symmeria*

The disc diffusion method was employed to study *P. symmeria* antibacterial activity in vitro. [12]. Sterile paper discs (6mm diameter) impregnated with extracted samples at the desired concentration were placed on the surface of the agar medium inoculated with the target organisms. 5% DMSO was used for the negative control, while a standard antibiotic disc containing streptomycin (25µg) was used as a positive control. Plates were left for incubation at 37°C for 24 hours. The diameter of the zone of inhibition was then "measured.

### 2.9. *Determination of Minimum Inhibitory Concentration (MIC)*

MIC was determined" employing broth microdilution method with few minor modifications [13, 14]. A twofold dilution of the extract was mixed with broth medium in a 96-well microtiter plate. The starting concentration is 5 mg/ml for chloroform and ethanol extracts and 20 mg/ml for aqueous extract. Each well is then inoculated with the microbial inoculum. Positive control included inoculated broth medium without the test sample, while negative control contained non-inoculated DMSO-supplemented media. Plates were incubated for 24 hours at 37˚C. After incubation, each well received 0.5% TTC dye (20µl) and was incubated again for 30 minutes at room temperature. The red color signifies bacterial growth, while the original color indicates inhibition of bacterial growth by the extract. MIC was determined to be the extract's lowest concentration, displaying no observable growth.

### *2.10. Determination of Minimum Bactericidal Concentration (MBC)*

MBC was determined following the standard method with a slight modification [14]. 20 µl of the sample was taken from each well with a concentration of MIC and above the MIC and spread onto nutrient agar fresh plates. The plates were then incubated for 24 hours at 37˚C. MBC was considered the plant extract's lowest dose and exhibited no bacterial growth on the agar plates.

### *2.11. Statistical Analysis*

One-way ANOVA and Tukey multiple comparisons of means were used for the statistical analysis, which was performed employing SPSS software (online). The mean ± SEM (n=3) expressed the values. GraphPad Prism software (online) was used to plot the percent inhibition of free radicals versus the log dosages to determine the IC50 values. p value<0.05 was considered statistically significant.

## 3. Results and Discussion

### *3.1. Phytochemical Analysis*

**Table 1** displays various phytochemicals in ethanol, chloroform, and aqueous extracts of *P. symmeria*.

### *3.2. Total Phenolic Content*

The various extracts of *P. symmeria* show an increase in phenolic content with increasing concentrations ($F_{2,6}$= 1497.68 (at 8 mg/ml); $p< 0.001$). Ethanol extract exhibited the highest phenolic content (750.89 ± 11.20), followed by aqueous extract (576.08 ± 4.89) and chloroform extract (200.36 ± 2.85). All values were expressed as mg gallic acid equivalent/g dry extract (**Fig.1A**).

**Table 1.** Phytochemical analysis of leaf extracts of *P. symmeria*.

| Phytochemicals | Colour indication | PSEE | PSCE | PSAE |
|---|---|---|---|---|
| Alkaloids | Reddish brown precipitate | + | _ | _ |
| Tannins | Brownish green or blue-black color | + | _ | _ |
| Flavonoids | Colorless | + | + | + |
| Terpenoids | Reddish brown | + | + | + |
| Saponins | Whitish emulsion | + | + | + |
| Steroids | Red color | _ | + | + |
| Cardiac glycosides | Brown ring | + | + | _ |
| Phlobatannins | Red precipitate | _ | _ | _ |

The symbols (+) and (-) denote the presence and absence of phytochemicals, respectively. PSEE= *P. symmeria* ethanol extract, PSCE= *P. symmeria* chloroform extract, PSAE= *P. symmeria* aqueous extract.

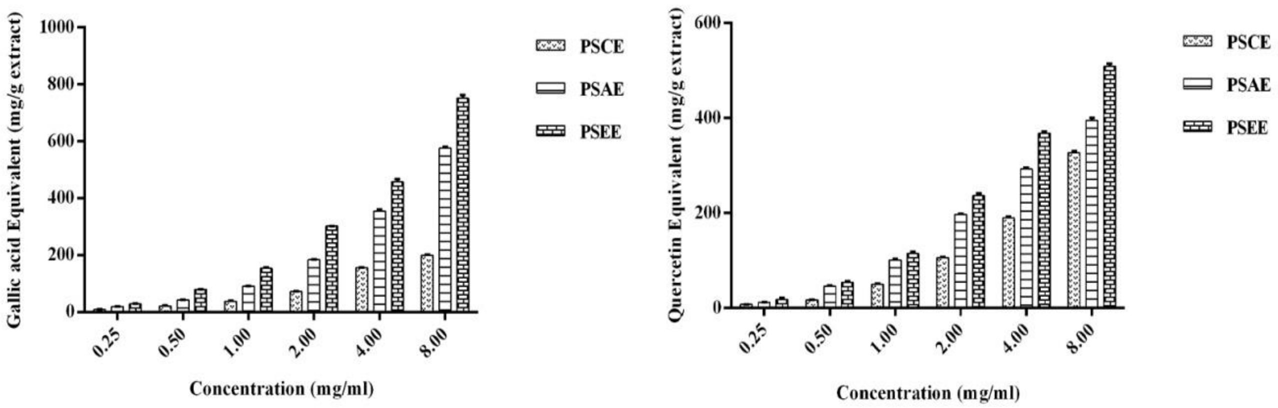


**Figure 1.** A) Total Phenolic Content B) Total Flavonoid Content of the various extracts of *P. symmeria*. PSEE= *P. symmeria* ethanol extract, PSCE= *P. symmeria* chloroform extract, PSAE= *P. symmeria* aqueous extract.

### *3.3. Total Flavonoid Content*

Ethanol, aqueous, and chloroform extracts' flavonoid content also increases significantly with an increase in concentration ($F_{2,6}$ =387.77 (at 8 mg/ml); $p< 0.001$). The ethanol extract has a higher concentration of flavonoid content (509.20 ± 5.23) compared to that of aqueous (395.10 ± 5.01) and chloroform (327.38 ± 3.57) extracts. All values were expressed as mg quercetin equivalent/g dry extract (**Fig. 1B**).

### *3.4. Liquid Chromatography-Mass Spectrometry* (*LC-MS*) Analysis

The results of the LC-MS analysis revealed that 34 major phytochemicals were present in the various extracts of *P. symmeria*. **Table 2-4** shows each compound's peak area, retention time, molecular formula, molecular weight, and biological activity.

### *3.5. DPPH Radical Scavenging Activity*

*P. symmeria* extracts exhibit significant dose-dependent inhibition of DPPH, with ethanol (194.86 ± 3.87 µg/ml) being the most effective due to its lower $IC_{50}$ value, followed by aqueous (266.66 ± 1.97 µg/ml) and chloroform (377.06 ± 3.09 µg/ml) extracts. $IC_{50}$ for the positive control, ascorbic acid is 33.85±0.34µg/ml. Maximum scavenging activity for ethanol, aqueous, and chloroform extracts was documented at 400µg/ml, 500µg/ml, and 800µg/ml, respectively (**Fig. 2**).

**Table 2.** Compounds identified in PSEE by LC-MS.

| Compound | Peak area | Retention time | Molecular formula | Molecular weight (Da) | Biological activity |
|---|---|---|---|---|---|
| (-)-Vestitone | 1.63E+07 | 22.2899666 | $C_{16}H_{14}O_5$ | 286.28 | Antimicrobial activity [15] |
| Apigenin | 4950586 | 23.9276009 | $C_{15}H_{10}O_5$ | 270.24 | Anti-inflammatory, antioxidant, antibacterial, and wound-healing activities [16-18] |
| Barbinine | 468326.8 | 35.1232491 | $C_{36}H_{46}N_2O_{10}$ | 666.8 | Antimicrobial, antitumor and anti-inflammatory activities [19] |
| Biochanin A | 1194671 | 20.2746162 | $C_{16}H_{12}O_5$ | 284.26 | Anti-inflammatory, anticancer, and antidiabetic activities [20, 21] |
| Biotin | 1025798 | 28.1809158 | $C_{10}H_{16}N_2O_3S$ | 244.31 | Wound healing activity [22] |
| Calafatimine | 1.55E+07 | 34.4692841 | $C_{38}H_{40}N_2O_7$ | 636.7 | Antimicrobial and antiparasitic activities [23, 24]) |
| Cassaine | 16704869 | 32.8654671 | $C_{24}H_{39}NO_4$ | 405.6 | Anti-inflammatory and antiangiogenic activities [25, 26] |
| Cernuine | 326512.3 | 31.5576496 | $C_{16}H_{26}N_2O$ | 262.39 | Anti-inflammatory, antibacterial, and anticancer activities [27] |
| Dihomo-gamma-linolenate | 178202.8 | 34.314251 | $C_{20}H_{34}O_2$ | 306.5 | Anti-inflammatory activity [28] |
| Isobrucein A | 3223732 | 21.9123001 | $C_{26}H_{34}O_{11}$ | 522.5 | Anticancer activity [30] |
| Ketoprofen | 3552923 | 19.9645672 | $C_{16}H_{14}O_3$ | 254.28 | Anti-inflammatory activity [31] |
| Metocurine | 201488.9 | 34.1930504 | $C_{40}H_{48}N_2O_6^{+2}$ | 652.8 | Unknown |
| Pheophorbide a a | 1227189.615 | 37.2061653 | $C_{35}H_{36}N_4O_5$ | 592.7 | Anti-inflammatory activity [32] |
| Pimelea factor P2 | 117718.8 | 30.5062676 | $C_{37}H_{50}O_9$ | 638.8 | Anti-inflammatory, antitumor and antibacterial activities [33] |
| Rescinnamine | 11342853.9 | 36.5860825 | $C_{35}H_{42}N_2O_9$ | 634.7 | Antihypotensive activity [34] |
| Reserpine | 1081749.525 | 31.5238495 | $C_{33}H_{40}N_2O_9$ | 608.7 | For the treatment of hypertension, cardiovascular disease, and nervous disorder [35] |
| Rhodoxanthn | 98000.48 | 37.6036148 | $C_{40}H_{50}O_2$ | 562.8 | Antioxidant activity [36] |
| Salidroside | 5232889.85 | 26.9745503 | $C_{14}H_{20}O_7$ | 300.3 | Wound healing activity [37] |
| Secologanate | 129908.1 | 17.4841995 | $C_{16}H_{22}O_{10}$ | 374.34 | Unknown |
| Soyasapogenol B 3-O-D-glucuronide | 308863.5121 | 36.6396675 | $C_{36}H_{58}O_9$ | 634.8 | Antimicrobial, antimutagenic, anticarcinogenic, hepato- and cardiovascular-protective activities, anti-inflammatory. [38] |
| Squalene | 212612.7 | 32.5554161 | $C_{30}H_{50}$ | 410.7 | Anti-inflammatory and wound healing activities [39] |
| taxifolin | 86618.78 | 27.7158508 | $C_{15}H_{12}O_7$ | 304.25 | Antimicrobial, anticancer, anti-inflammatory, antioxidant, and antitumor activities and for cardiovascular treatment and liver disorders. [40, 41] |
| Tingenone | 163893.4 | 30.9713841 | $C_{28}H_{36}O_3$ | 420.6 | Analgesic and anti-inflammatory activities [42] |
| Zeaxanthin | 441835.9 | 33.4179344 | $C_{40}H_{56}O_2$ | 568.9 | Antioxidant activity [43] |

**Table 3.** Compounds identified in PSCE by LC-MS.

| Compound | Peak area | Retention time | Molecular formula | Molecular weight (Da) | Biological activity |
|---|---|---|---|---|---|
| (-)-Vestitone | 4.18E+07 | 22.2899666 | $C_{16}H_{14}O_5$ | 286.28 | Antimicrobial activity [15] |
| Apigenin | 604509.5 | 23.9276009 | $C_{15}H_{10}O_5$ | 270.24 | Anti-inflammatory, antioxidant, antibacterial, and wound-healing activities [16-18] |
| Biochanin A | 1482551 | 20.2746162 | $C_{36}H_{46}N_2O_{10}$ | 284.26 | Anti-inflammatory, anticancer, and antidiabetic activities [20, 21] |
| Cassaine | 13366663 | 32.8654671 | $C_{24}H_{39}NO_4$ | 405.6 | Anti-inflammatory and antiangiogenic activities [25, 26] |
| Epicatechin-(4beta->8)-ent-epicatechin | 365248.1 | 23.9955158 | $C_{30}H_{26}O_{12}$ | 578.5 | antioxidant, free radical scavenging, anti-inflammatory, and wound healing activities [44] |
| Estrone 3-sulfate | 1143817 | 22.600317 | $C_{18}H_{22}O_5S$ | 350.4 | Unknown |
| Gambiriin C | 125185.5 | 27.2848167 | $C_{30}H_{26}O_{11}$ | 562.5 | Anticancer, antidiabetic, anti-inflammatory, wound healing activities and for cardiovascular diseases treatment, neurodegeneration, geriatric disorders, and allergies [45, 46] |
| Isobrucein A | 3211901 | 21.9123001 | $C_{26}H_{34}O_{11}$ | 522.5 | Anticancer activity [30] |
| Ketoprofen | 3859513 | 19.9645672 | $C_{16}H_{14}O_3$ | 254.28 | Anti-inflammatory activity [31] |
| Pheophorbide a | 318584 | 37.2061653 | $C_{35}H_{36}N_4O_5$ | 592.7 | Anti-inflammatory activity [32] |
| Salidroside | 245591.3 | 26.9745503 | $C_{14}H_{20}O_7$ | 300.3 | Wound healing activity [37] |
| Secologanate | 228088.2 | 17.4841995 | $C_{16}H_{22}O_{10}$ | 374.34 | Unknown |
| Tingenone | 107825.1 | 30.9713841 | $C_{28}H_{36}O_3$ | 420.6 | Analgesic and anti-inflammatory activities [42] |

**Table 4.** Compounds identified in PSAE by LC-MS.

| Compound | Peak area | Retention time | Molecular formula | Molecular weight (Da) | Biological activity |
|---|---|---|---|---|---|
| Biliverdin | 65326.89 | 26.9065495 | $C_{33}H_{34}N_4O_6$ | 582.6 | Antioxidant activity and for the treatment of polymicrobial sepsis [47, 48] |
| Cassaine | 22635809 | 32.8654671 | $C_{24}H_{39}NO_4$ | 405.6 | Anti-inflammatory and antiangiogenic activities [25, 26] |
| Cetylpyridinium chloride | 297768.1 | 32.4001656 | $C_{21}H_{38}CIN$ | 340 | Antimicrobial and wound healing activities [49] |
| Evocarpine | 124371.9 | 20.4293995 | $C_{23}H_{33}NO$ | 339.5 | Antibacterial activity [50] |
| Glycerol | 439316.7 | 27.0277672 | $C_3H_8O_3$ | 92.09 | Antibacterial and wound healing activities [51] |
| Isobrucein A | 3375756 | 21.9123001 | $C_{26}H_{34}O_{11}$ | 522.5 | Anticancer activity [30] |
| N-Arachidonyldopamine | 94521.7 | 24.6150169 | $C_{28}H_{41}NO_3$ | 439.6 | Anti-inflammatory activity [52] |
| Polhovolide | 1023803 | 22.5321007 | $C_{23}H_{32}O_8$ | 436.5 | Anti-inflammatory, anticancer, antimicrobial, and antioxidant activities and for cardiovascular disease treatment [53] |
| Reserpine | 127492.5 | 31.5238495 | $C_{33}H_{40}N_2O_9$ | 608.7 | Hypertension, cardiovascular disease and nervous disorder [35] |
| Salidroside | 4110341 | 26.9745503 | $C_{14}H_{20}O_7$ | 300.3 | Wound healing activity [37] |
| Tingenone | 216750.9 | 30.9713841 | $C_{28}H_{36}O_3$ | 420.6 | Analgesic and anti-inflammatory activities [42] |

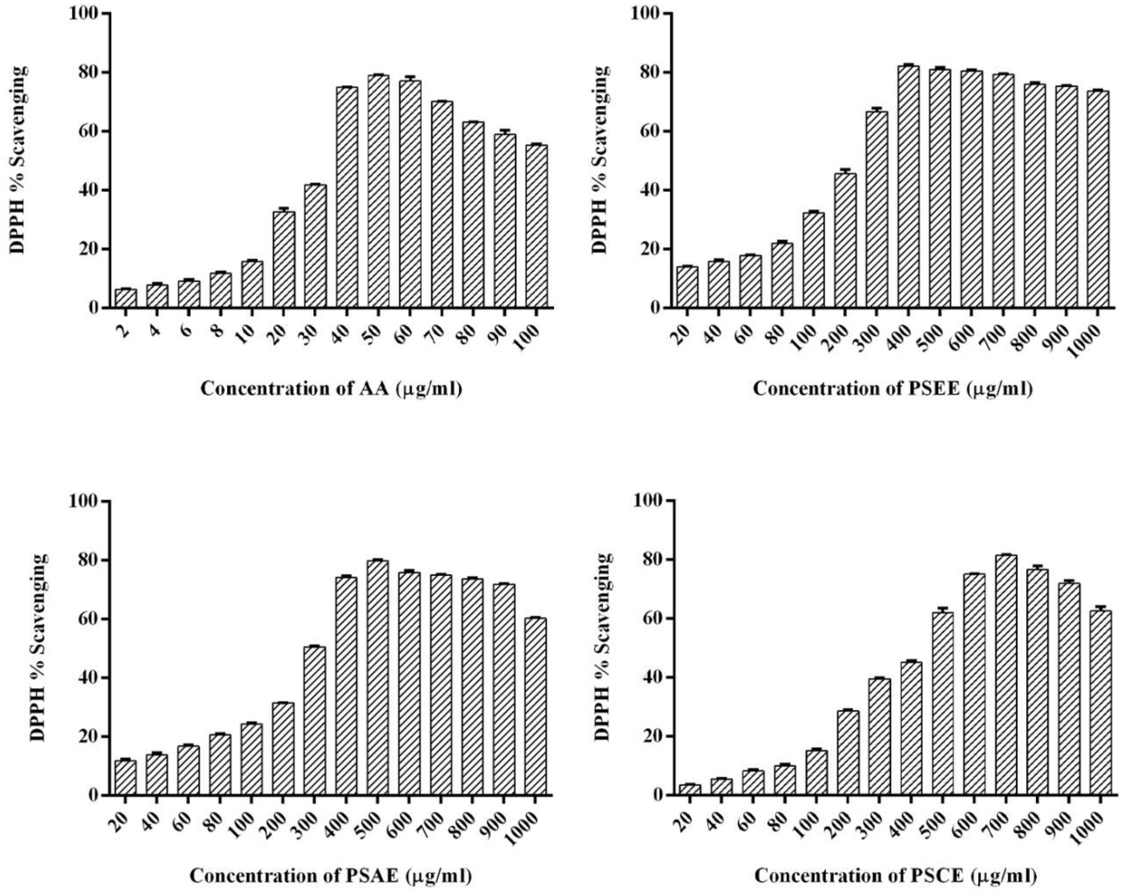


**Figure 2.** DPPH scavenging activity of *P. symmeria* extracts and standard ascorbic acid. Values were expressed as mean ± SEM, n=3, p<0.001. AA=Ascorbic Acid, PSEA= *P. symmeria* ethanol extract, PSCE= *P. symmeria* chloroform extract, PSAE= *P. symmeria* aqueous extract.

### *3.6. ABTS Radical Scavenging Activity*

ABTS radical scavenging activity also elevates with increasing dosage. The maximum scavenging activity was recorded at 400µg/ml for the ethanol and aqueous extracts and 700µg/ml for the chloroform extract. The scavenging activities gradually declined after that. The ethanol extract has the lowest $IC_{50}$ value (105.50 ± 4.59 µg/ml), followed by the aqueous extract (179.60 ± 5.90 µg/ml) and chloroform extract (398.73 ± 5.17 µg/ml). $IC_{50}$ for the positive control, ascorbic acid, is 15.71±2.64µg/ml (**Fig.3**).

### *3.7. Superoxide Anion $O_2^{\bullet(-)}$ Scavenging Activity*

Superoxide anion radical (O2•(-)) scavenging activity also elevates with the increase in concentrations. The maximum scavenging activity was observed at 900µg/ml for chloroform extract and 400µg/ml for aqueous and ethanol extract. The $IC_{50}$ of the positive control, ascorbic acid, was recorded at 19.65 ± 0.22 µg/ml. Among the three extracts, ethanol extract (182.83 ± 6.08 µg/ml) proved to be the most potent as it shows the lowest $IC_{50}$ value, followed by aqueous extract (274.70 ± 5.40 µg/ml). Chloroform extract (389.73 ± 2.12 µg/ml) is the least effective, with an $IC_{50}$ value at a higher dose (**Fig. 4**).

### *3.8. Antibacterial Susceptibility Tests*

Different concentrations (5, 10, and 20 mg/ml) of *P. symmeria* extracts were tested against the bacteria, namely, *E. coli* (ATCC- 25922), *B. subtilis* (ATCC- 11447), and *K. pneumoniae* (ATCC- 10031). The present study revealed that *P. symmeria* extract significantly inhibits the growth of the test organisms. Aqueous extract was shown to have lower activity with a smaller zone of inhibition than ethanol and chloroform extracts, which have more or less the same zone of inhibition (**Table 5**).

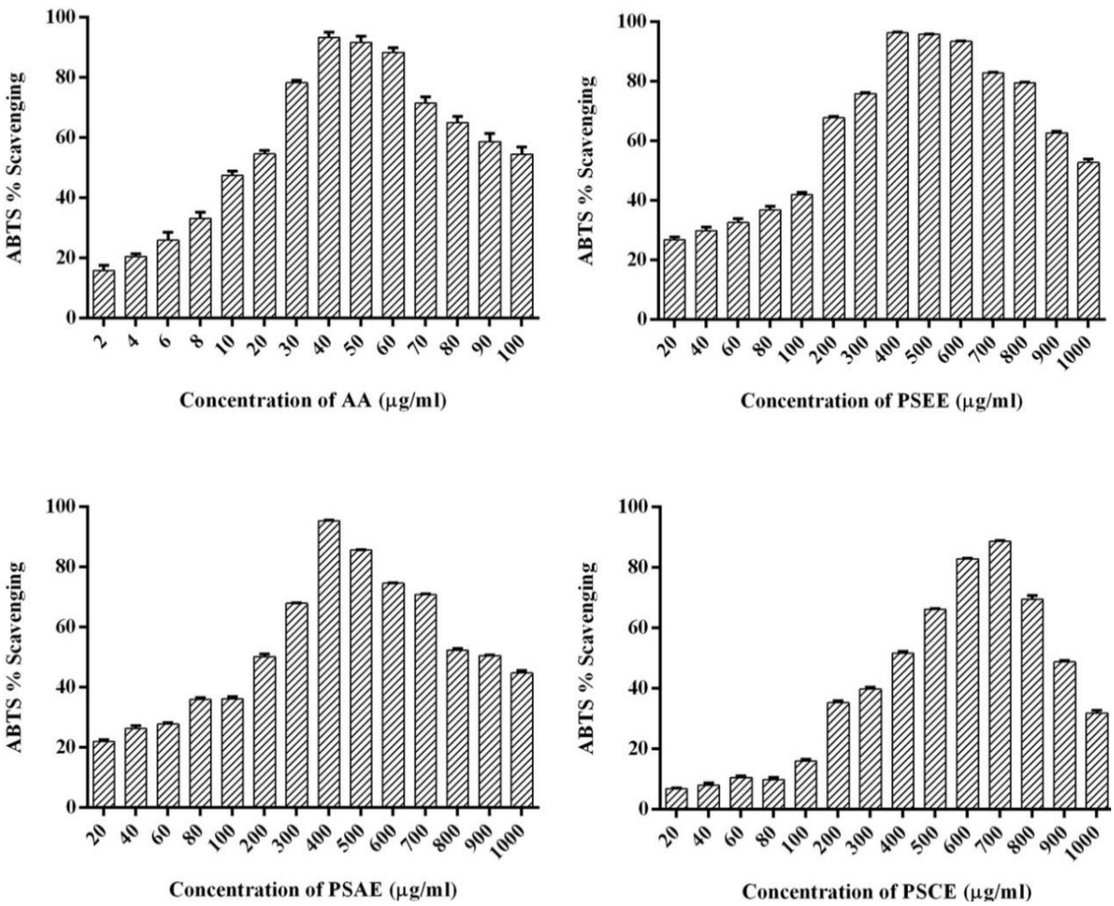


**Figure 3.** ABTS scavenging activity of *P. symmeria* extracts and standard ascorbic acid. Values are expressed as mean ± SEM, n=3, p<0.001. AA=Ascorbic Acid, PSEE= *P. symmeria* ethanol extract, PSCE= *P. symmeria* chloroform extract, PSAE= *P. symmeria* aqueous extract.

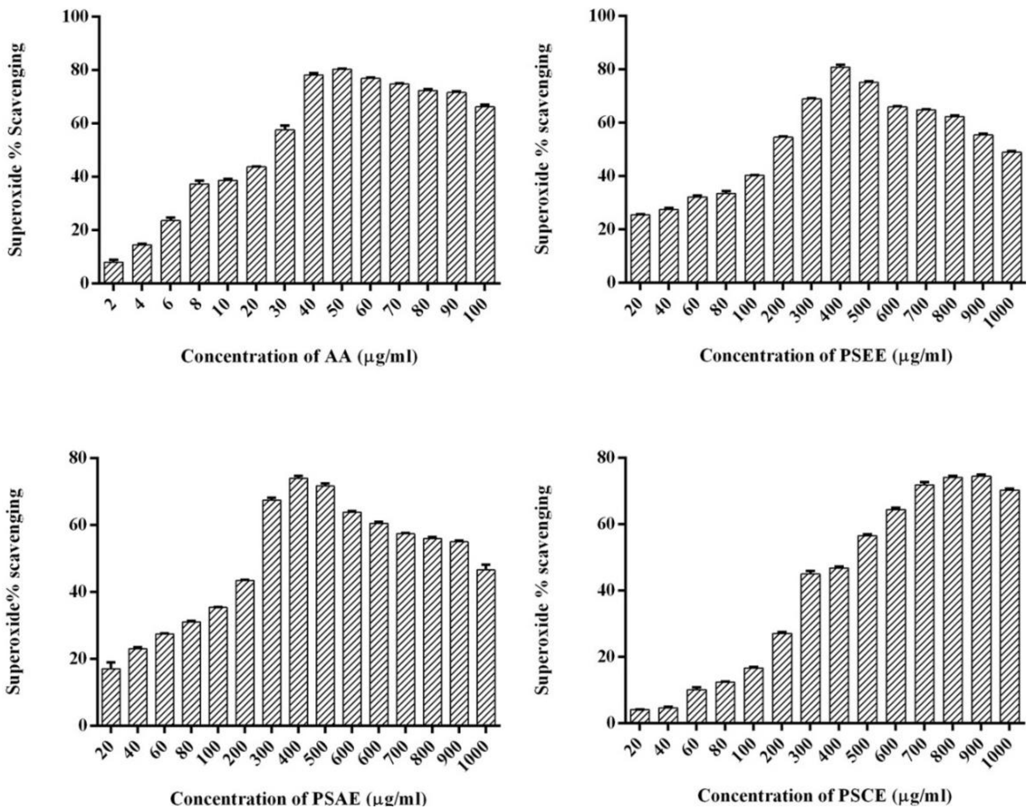


**Figure 4**. $O_2^{\bullet(-)}$ scavenging activity of *P. symmeria* extracts and the standard ascorbic acid. Values are expressed as mean ± SEM, n=3, p< 0.001. AA= Ascorbic Acid, PSEE= *P. symmeria* ethanol extract, PSCE= *P. symmeria* chloroform extract, PSAE= *P. symmeria* aqueous extract.

**Table 5.** Diameter of zone of inhibition of the leaf extract of *P. symmeria* against the tested organisms.

| Extracts (10µg/disc) | Conc. (mg/ml) | Zone of Inhibition (mm) | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***B. subtilis*** | ***K. pneumoniae*** |
| **Ethanol** | 5 | 8.20 ± 0.34 | 9.33 ± 0.57 | 10.86 ± 0.50 |
| | 10 | 12.16 ± 0.28 | 11.20± 0.34 | 11.53 ± 0.92 |
| | 20 | 14.86 ± 1.50 | 14.20± 0.34 | 13.96± 0.05 |
| **Standard** | 25µg | 31.23 ± 0.40 | 31.06 ± 0.11 | 21.26 ± 0.46 |
| **Chloroform** | 5 | 9.06 ± 0.11 | 9.76 ± 0.32 | 10.86 ± 0.90 |
| | 10 | 11.30 ± 0.51 | 11.43 ±0.75 | 11.43 ± 0.75 |
| | 20 | 14.40 ± 0.69 | 14.53 ± 0.92 | 14.13 ± 0.23 |
| **Standard** | 25µg | 29.10 ± 0.17 | 30.13 ± 0.23 | 22.20 ± 0.34 |
| **Aqueous** | 5 | 7.43 ± 0.75 | 7.23 ± 0.40 | 7.83 ± 0.44 |
| | 10 | 9.16± 0.28 | 8.53 ± 0.92 | 9.13 ± 0.23 |
| | 20 | 12.53 ± 0.92 | 10.20 ± 0.34 | 10.13 ± 0.23 |
| **Streptomycin** | 25µg | 30.13 ± 0.23 | 31.26 ± 0.46 | 21.26 ± 0.46 |

### *3.9. Minimum Inhibitory Concentration (MIC)*

MIC results illustrate that *P. symmeria* chloroform and ethanol extracts have a 1.25mg/ml MIC value against *E. coli*, *B. subtilis*, and *K. pneumoniae*. In comparison, the aqueous extract has a 2.5mg/ml MIC value against *E. coli* and 5 mg/ml against *K. pneumoniae* and *B. subtilis* (**Table 6**).

### *3.10. Minimum Bactericidal Concentration (MBC)*

The growth of the bacteria sub-cultured from wells with MIC concentrations and above MIC on fresh nutrient agar was observed. MBC was reported to be 2.5 mg/ml for chloroform and ethanol extracts, while aqueous extract shows an MBC value of 10mg/ml against *E. coli* and 20mg/ml against *B. subtilis* and *K. pneumoniae* (**Table 6**).

**Table 6.** MIC & MBC of leaf extract of *P. symmeria* against the tested organisms.

| Extract | *E. coli* | | *B. subtilis* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|
| | **MIC (mg/ml)** | **MBC (mg/ml)** | **MIC (mg/ml)** | **MBC (mg/ml)** | **MIC (mg/ml)** | **MBC (mg/ml)** |
| **Ethanol** | 1.25 | 2.5 | 1.25 | 2.5 | 1.25 | 2.5 |
| **Chloroform** | 1.25 | 2.5 | 1.25 | 2.5 | 1.25 | 2.5 |
| **Aqueous** | 2.5 | 10 | 5 | 20 | 5 | 20 |

### *3.11. Discussion*

Herbal medicines are gaining popularity for treating many disorders due to their low cost, effectiveness, and lesser side effects than modern drugs [54]. Bioactive compounds, including polyphenols obtained from plants, are known to be the main contributors to their healing properties [55]. *P. symmeria* extracts were found to contain various phytochemicals. Phenols are crucial plant components that can scavenge free radicals and are therefore utilized to screen antioxidant activity present in plants [56]. Flavonoids are a category of plant secondary metabolites recognized for their potent chelating and antioxidant properties [57]. The extracts of *P. symmeria* were found to contain an ample amount of phenols and flavonoids, which may have contributed to their antioxidant and anti-bacterial properties. LC-MS analysis results also showed the presence of many compounds with anti-inflammatory, antibacterial, and wound-healing activity, supporting the previous findings. DPPH is a stable free radical that, when receiving hydrogen from antioxidants, its nitrogen atom odd electron is reduced to hydrazine [58]. The presence of antioxidants turns violet or purple DPPH methanolic solutions to yellow [59]. The present studies and reports from other studies using the roots of P. symmeria ethanol extract confirm the DPPH radical scavenging activity, thereby demonstrating the plant's usefulness as a possible radical scavenger [60]. ABTS is a stable free radical that arises from ABTS salt reaction with potent oxidizing agents such as potassium permanganate/potassium persulfate. In this analysis, with a blue-green color, the ABTS solution transformed into colorless ABTS by an antioxidant, allowing for spectrophotometric measurement [61]. Antioxidant compounds in *P. symmeria* extract prevent ABTS free radical formation, indicated by color-change from blue-green to colorless solution, thereby rapidly decreasing optical density(734nm) [62]. Leaks of the electron transport chain produced superoxide anion radicals ($O2^{\bullet(-)}$) [63]. This free radical can signal apoptosis, aging, and senescence. Superoxide radicals can further produce more reactive free radicals, including hydroxyl radicals ($OH^{\bullet}$), by the Haber-Weiss reaction [64]. Thus, inhibiting or neutralizing superoxide anion radicals ($O2^{\bullet(-)}$) may indirectly prevent the generation of more free radicals. The current study revealed the superoxide anion radical ($O2^{\bullet(-)}$) scavenging ability of all three extracts, suggesting its role as a potential antioxidant. Among the three extracts, ethanol extract had the lowest IC50 value and was the most potent in scavenging the various radicals. Infectious diseases are still a major concern regarding global health issues. Antibiotic-resistant pathogens exacerbated the issue. This leads to the need to develop new antimicrobial compounds. This experiment showed that *P. symmeria* extracts have significant antibacterial properties. Another study also reported the antibacterial properties of the roots of *P. symmeria* ethanolic extract [60]. Based on results from current investigations and results from other experiments, *P. symmeria* is proven to be a potent antimicrobial agent. Other species within the same genus have also been found to exhibit anti-inflammatory, anti-bacterial, and antioxidant properties, validating the medicinal potential of this plant [65-68].

## 4. Conclusion

This research examined the scientific characteristics of *P. symmeria* to verify its traditional usage. The experiment's results show proof of the healing ability claimed to be mostly because of its free radical scavenging and antibacterial properties. However, additional investigation is necessary to determine the key ingredients of the plant responsible for healing and how they specifically influence the healing process. An in vivo experiment is also necessary to confirm its potential. Therefore, we can conclude that the plant *P.*

*symmeria* has the potential to be further developed into new phytomedicines for the management of radical-inducing inflammations and bacterial infections.


## Acknowledgments

We thank the University Grant Commission, Ministry of Tribal Affairs, and Government of India for providing fellowship to RK Lalremtluangi (202021-NFST-MIZ-00636) and Lalthansangi (UGC-MZU Fellowship). The authors thank the Department of Biotechnology (DBT), New Delhi, Government of India sponsored Advanced Level State Biotech Hub (BT/NER/143/SP44475/2021) at Mizoram University for the equipment's and infrastructural support.


## Conflicts of Interest

The authors declare that there is no possible conflict of interest.


## Athors Orcid numbers:

RK Lalremtluangi: 0000-0002-6371-1825
C Lalthansangi: 0009-0003-4239-073X
Esther Lalhmingliani: 0000-0002-2794-2095
Mathipi Vabeiryureilai: 0000-0001-8708-3686


## Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

## References


1. Cheeseman KH, Slater TF. An introduction to free radical biochemistry. Br. Med. Bull. (1993) 49(3): 481-493.
2. Alugoju P, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian. J. Clin. Biochem. (2014) 30(1): 11-26.
3. Farahpour MR, Habibi M. Evaluation of the wound healing activity of an ethanolic extract of Ceylon cinnamon in mice. Vet Med (Praha). 2012 57(1): 53-57.
4. Rozika R. Ramhmul damdawi (Medicinal plants) Mizoram, 1st ed., Medicinal plants board: Mizoram (2005).
5. Evans WC, Evans D, Trease GE. Trease and Evans Pharmacognosy, 15th ed., Saunders: London (2002).
6. Harborne A. Phytochemical Methods A guide to modern techniques of plant analysis, 3rd ed., Chapman and Hall: London (1998).
7. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Wiedemann N (Eds.) In: Methods in Enzymology, Academic Press, New York (1999) 152-178.
8. Barreira JC, Ferreira IC, Oliveira MBP, Pereira JA. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food. Chem. (2008) 107(3): 1106-1113.
9. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food. Chem. (2002) 76(1): 69-75.
10. Re R, Pellegrini N, Proteggente AR, Pannala AS, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free. Radic. Biol. Med. (1999) 26(9-10): 1231-1237.
11. Hyland K, Voisin E, Banoun H, Auclair C. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. Anal. Biochem. (1983) 135(2): 280-287.
12. Balouiri M, Sadiki M, Koraichi SI. Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal. (2016) 6(2): 71–79.
13. Araujo MGS, Silva ALL, Silva-Junior EF, Santos Junior PFS, Santos MS, Bernardo THL, et al. Evaluation of antimicrobial and cytotoxic potential of Argemone mexicana L. J. Chem. Pharm. Res. (2015) 2015(7): 482-489.
14. Datar H, Datar A. Antimicrobial activity of Anthoceplalus cadamba and Scirpus kysoor roxb. against food pathogens. Int. J. Curr. Pharm. Res. (2016) 8(4): 13.
15. Guo L, Dixon RA, Paiva NL. Conversion of vestitone to medicarpin in alfalfa (Medicago sativa L.) is catalyzed by two independent enzymes. Identification, purification, and characterization of vestitone reductase and 7,2'-dihydroxy-4'-methoxyisoflavanol dehydratase. J. Biol. Chem. (1994) 269(35): 22372-22378.
16. Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: anticancer effects and mechanisms of action. Cell. Biosci. (2017) 7(50): 9034071.
17. Suntar I, Akkol EK, Keles H, Yesilada E, Sarker SD. Exploration of the wound healing potential of Helichrysum graveolens (Bieb.) Sweet: isolation of apigenin as an active component. J Ethnopharmacol. (2013) 149(1): 103-110.
18. Yajie X, Li X, Wang H. Protective Roles of Apigenin Against Cardiometabolic Diseases: A Systematic Review. Front. Nutr. (2022) 9: 875826.

19. Thawabteh AM, Thawabteh A, Lelario F, Bufo SA, Scrano L. Classification, Toxicity and Bioactivity of Natural Diterpenoid Alkaloids. Molecules. (2021) 26(13): 4103.
20. Yu C, Zhang P, Lou L, Wang Y. Perspectives Regarding the Role of Biochanin A in Humans. Front. Pharmacol. (2019) 10: 793.
21. Sarfraz I, Rasul A, Riaz A, Ucak I, et al. Biochanin A and biochanin B. Mushtaq M, Anwar F (Eds.) In: A Centum of Valuable Plant Bioactives, Academic Press, New York, (2021) 563-588.
22. Al-Akayleh F, Jaber N, Al-Remawi M, Al-Odwan G, Qinna N. Chitosan-biotin topical film: preparation and evaluation of burn wound healing activity. Pharm. Dev. Technol. (2022) 27(4): 479-489.
23. Qing ZX, Hwang JL, Yang XY, et al. Anticancer and Reversing Multidrug Resistance Activities of Natural Isoquinoline Alkaloids and their Structure-activity Relationship. Curr. Med. Chem. (2018) 25(38): 5088-5114.
24. Weber C, Opatz T. Bisbenzylisoquinoline Alkaloids. Alkaloids. Chem. Biol. (2019) 81: 114.
25. Zhong H, Wang L, Xiong S, Wang Z, Chen Z, Huang W, Li M, Tian H. Cassaine diterpenoid glycosides from the seeds of Erythrophleum fordii Oliv. and their antiviral and anti-inflammatory activities. Fitoterapia. (2022) 163: 105348.
26. Chen Z, Mou Y, Zhong H, et al. Cassaine diterpenoids from the seeds of Erythrophleum fordii Oliv. and their antiangiogenic activity. Phytochem. (2022) 203: 113399.
27. Zhang J, Liu YQ, Fang J. The biological activities of quinolizidine alkaloids. Alkaloids. Chem. Biol. (2023) 89: 1-37.
28. Silva JR, Burger B, Kuhl CMC, Candreva T, Dos Anjos MBP, Rodrigues HG. Wound Healing and Omega-6 Fatty Acids: From Inflammation to Repair. Mediat. Inflamm. (2018) 2018: 2503950.
29. McIlwain HH. Fenoprofen calcium versus aspirin in the treatment of acute inflammatory soft-tissue injuries. J. Med. (1985) 16(4): 429-438.
30. Bagheri E, Hajiaghaalipour F, Nyamathulla S, Salehen NA. Ethanolic extract of Brucea javanica inhibit proliferation of HCT-116 colon cancer cells via caspase activation. RSC Adv. (2018) 8(2): 681-689.
31. Yu Y, Yang Q, Wang Z, Ding Q, Li M, Fang Y, He Q, Zhu YZ. The Anti-Inflammation and Anti-Nociception Effect of Ketoprofen in Rats Could Be Strengthened Through Co-Delivery of a H2S Donor, S-Propargyl-Cysteine. J. Inflamm. Res. (2021) 14: 5863-5875.
32. Islam MN, Ishita IJ, Jin SE, et al. Anti-inflammatory activity of edible brown alga Saccharina japonica and its constituents pheophorbide a and pheophytin a in LPS-stimulated RAW 264.7 macrophage cells. Food. Chem. Toxicol. (2013) 55: 541-548.
33. Saha P, Rahman FI, Hussain F, Rahman SMA, Rahman MM. Antimicrobial Diterpenes: Recent Development From Natural Sources. Front. Pharmacol. (2022) 12: 820312.
34. Cronheim G, Brown W, Cawthorne J, Toekes MI, Ungari J. Pharmacological Studies with Rescinnamine, a New Alkaloid Isolated from Rauwolfia serpentina. Exp. Biol. Med. (1954) 86(1): 120-124.
35. Weiss RF, Fintelmann V. Herbal Medicine, 2nd ed., George Thieme Verlag: Germany (2000).
36. Fu C, Dong H, Wang X, Wang H, Zheng Y, Ren D, et al. Antioxidant Effects of Rhodoxanthin from Potamogeton crispus L. on H2 O2 -Induced RAW264.7 Macrophages Cells. Chem. Biodivers. (2023) 20(1): e202200393.
37. Ariyanti AD, Zhang J, Marcelina O, et al. Salidroside-Pretreated Mesenchymal Stem Cells Enhance Diabetic Wound Healing by Promoting Paracrine Function and Survival of Mesenchymal Stem Cells Under Hyperglycemia. Stem. Cells. Transl. Med. (2019) 8(4): 404-414.
38. Guang C, Chen J, Sang S, Cheng S. Biological functionality of soyasaponins and soyasapogenols. J. Agric. Food. Chem. (2014) 62(33): 8247-8255.
39. Sanchez-Quesada C, Lopez-Biedma A, Toledo E, Gaforio JJ. Squalene Stimulates a Key Innate Immune Cell to Foster Wound Healing and Tissue Repair. Evid. Based. Complement. Alternat. Med. (2018) 2018: 9473094.
40. Park JE, Kwon HJ, Lee HJ, Hwang HS. Anti-inflammatory effect of taxifolin in TNF-α/IL-17A/IFN-γ induced HaCaT human keratinocytes. Appl. Biol. Chem. (2023) 66: 8.
41. Sunil C, Xu B. An insight into the health-promoting effects of taxifolin (dihydroquercetin). Phytochem. (2019) 166: 112066.
42. Veloso CC, Soares GL, Perez AC, Rodrigues VG, Silva FC. Pharmacological potential of Maytenus species and isolated constituents, especially tingenone, for treatment of painful inflammatory diseases. Rev. Bras. Farmacogn. (2017) 27(4): 533-540.
43. Murillo AG, Hu S, Fernandez ML. Zeaxanthin: Metabolism, Properties, and Antioxidant Protection of Eyes, Heart, Liver, and Skin. Antioxidants (Basel). (2019) 8(9): 390.

44. Kapoor M, Howard R, Hall I, Appleton I. Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats. Am. J. Pathol. (2004) 165(1): 299-307.
45. Nie Y, Sturzenbaum SR. Proanthocyanidins of Natural Origin: Molecular Mechanisms and Implications for Lipid Disorder and Aging-Associated Diseases. Adv. Nutr. (2019) 10(3): 464-478.
46. Vilkickyte G, Zilius M, Petrikaite V, Raudone L. Proanthocyanidins from Vaccinium vitis-idaea L. Leaves: Perspectives in Wound Healing and Designing for Topical Delivery. Plants. (2022) 11(19): 2615.
47. Chowdhury JR, Chowdhury NR, Jansen PLM. Zakim and Boyer's Hepatology: A textbook of Liver Disease, 5th ed., WB Saunders: New York (2006).
48. Overhaus M, Moore BA, Barbato JE, Behrendt FF, Doering JG, Bauer AJ. Biliverdin protects against polymicrobial sepsis by modulating inflammatory mediators. Am. J. Physiol. Gastrointest. Liver. Physiol. (2006) 290(4): 695-703.
49. Rembe JD, Thompson VD, Stuermer EK. Antimicrobials cetylpyridinium-chloride and miramistin demonstrate non-inferiority and no "protein-error" compared to established wound care antiseptics in vitro. AIMS Microbiology. (2022) 8(4): 372-387.
50. Casciaro B, Mangiardi L, Cappiello F, et al. Naturally-Occurring Alkaloids of Plant Origin as Potential Antimicrobials against Antibiotic-Resistant Infections. Molecules. (2020) 25(16): 3619.
51. Stout EI, McKessor A. Glycerin-Based Hydrogel for Infection Control. Adv. Wound. Care. (New Rochelle). (2012) 1(1): 48-51.
52. Lawton SK, Xu F, Tran A, et al. N-Arachidonoyl Dopamine Modulates Acute Systemic Inflammation via Nonhematopoietic TRPV1. J. Immunol. (2017) 199(4): 1465-1475.
53. Chadwick M, Trewin H, Gawthrop F, Wagstaff C. Sesquiterpenoids lactones: benefits to plants and people. Int. J. Mol. Sci. (2013) 14(6): 12780-12805.
54. Aghel N, Iran R, Mombeini A. Hepatoprotective Activity of Capparis spinosa Root Bark Against CCl4 Induced Hepatic Damage in Mice. Iran. J. Pharm. Res. (2007) 6(4): 285-290.
55. Pinto T, Aires A, Cosme F, et al. Bioactive (Poly)phenols, Volatile Compounds from Vegetables, Medicinal and Aromatic Plants. Foods. (2021) 10(1): 106.
56. Tosun M, Ercısli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant Properties and Total Phenolic Content of Eight Salvia Species from Turkey. Biol. Res. (2009) 42(2): 175-181.
57. Shen N, Wang T, Gan Q, Liu S, Wang,L, Jin B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food. Chem. (2022) 30: 383.
58. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J. Food. Sci. Technol. (2011) 48(4): 412–422.
59. Zoremsiami J. Jagetia GC. Phytochemical Analysis and Free Radical Scavenging Activity of Helicia nilagirica. Trends. Green. chem. (2019) 4(1:5): 1-9.
60. Subba B, Basnet P. Antimicrobial and antioxidant activity of some indigenous plants of Nepal. J. Pharmacogn. Phytochem. (2014) 3(1): 62-67.
61. Dasgupta A, Klein K. Methods for Measuring Oxidative Stress in the Laboratory. Dasgupta A, Klein K (Eds.) In: Antioxidants in Food, Vitamins and Supplements, Elsevier, United States (2014) 19-40.
62. Goldschmidt S, Renn K. Amine oxidation IV. Diphenyl-trinitrophenylhydrazyl. Chem. Ber. (1992) 55: 628-643.
63. Wong H, Dighe P, Mezera V, Monternier P, Brand MD. Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. J. Biol. Chem. (2017) 292(41): 16804-16809.
64. Haber F, Weiss J. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. R. Soc. Lond. A. Math. Phys. Sci.(1934) 147(861): 332-351.
65. Chahardehi AM, Ibrahim D, Sulaiman SF. Antioxidant activity and total phenolic content of some medicinal plants in Urticaceae family. J. Appl. Biol. Sci. (2009) 3(2): 27-31.
66. Chahardehi AM, Demirtas I, Sulaiman SF. Antioxidant, Antimicrobial Activity and Toxicity Test of Pilea microphylla. Int. J. Microbiol. (2010) 2010: 826830.
67. Prabhakar K, Veerapur VP, Bansal P, et al. Antioxidant and radioprotective effect of the active fraction of Pilea microphylla (L.) ethanolic extract. Chem. Biol. Interact. (2007) 165(1): 22-32.
68. Bansal P, Paul P, Nayak PG, et al. Phenolic compounds isolated from *Pilea microphylla* prevent radiation-induced cellular DNA damage. Acta. Pharm. Sin. B. (2011) 1(4): 226-235.