Research Article

Comparison of Phytochemical Constituents and Pharmacological Activities of Various Solvent Extracts Obtained from Millettia speciosa Stem Powder

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Millettia speciosa is a plant extensively used as an important component in Chinese herbal medicine and food-based medicines. The present study was carried out to determine the total flavonoid content (TFC), volatile phytoconstituents, and pharmacological activities, i.e., antityrosinase, sunscreen, and anticancer activity, of different fractions of M. speciosa stem. Different organic solvents of increasing polarity, i.e., petroleum ether (PE), ethyl acetate (EtOAc), and methanol (MeOH), were used for extraction. The highest total flavonoid content, i.e., 48.30 ± 0.90%, was reported for PE extract. Various important phytoconstituents were revealed by gas chromatography-mass spectroscopy (GC-MS) analysis. Based on abundance, the major compounds were n-hexadecanoic acid (16.654%), n-hexadecanoic acid (14.808%), and beta-sitosterol (6.298%) for PE, EtOAc, and MeOH extract, respectively. The significant antityrosinase activity, i.e., 70.97 ± 0.66%, with an IC50 value of 4.58 mg/mL was noted for PE extract followed by EtOAc extract, i.e., 59.84 ± 0.67%, with IC50 value of 6.10 mg/mL. The maximum sunscreen activity was reported for PE extract exhibiting the maximum absorbance value (0.633 ± 0.06) in the ultraviolet (UV) region, i.e., UVC, while EtOAc extract showed the second highest level of absorbance in the UVB range, i.e., 0.632 ± 0.07. The strongest anticancer activity (49.73 ± 0.49% cell viability) towards MCF-7 breast cancer cell line was reported for PE extract with IC50 197.51 μg/mL. Our results confirmed the presence of potential therapeutic components for each extract with significant biological functions, showing the importance of the M. speciosa stem as a source of biomedicine. To our knowledge, this is the first report on M. speciosa stem extending comprehensive research about its phytochemical profile and various significant pharmacological activities.

1. Introduction

The use of plants as a source of food and shelter could be traced back to time immemorial, while their use as natural medicine is one of the oldest trends of healthcare known to humanity. The study of plants has made them a better choice to be investigated not only for food but also to find food-based medicines. A medicinal plant can be characterized to have potential therapeutic phytoconstituents that allow it to be used for curing or preventing a certain disease [1]. Numerous studies have explained the therapeutic potential of plants, which may include antitumor, anti-
inflammatory, antidiabetic, antioxidant, and several other disorders [2]. The healing effects of herbal medicines are considered due to the presence of different bioactive compounds like alkaloids, polyphenols, flavonoids, terpenoids, and other important groups [3].

*Milletta speciosa* is a fabaceous plant used as an important ingredient in herbal and food-based medicines for its therapeutic functions. The use of *M. speciosa* as food can trace back to Ming (1368-1644) and Qing (1644-1912) Dynasties. According to famous Chinese traditional medicine monographs during Qing Dynasty, i.e., *Luchuan Bencao* and *Shengcao Yaoxing Beiyao*, it was used both as food and medicine. The development of herbal-based products for food and medicine has grown awareness about *M. speciosa* [4]. In China, *M. speciosa* is used to treat kidney weakness, frequent cough, and bronchitis. It is also consumed as traditional food and mixed with porridge and soup [5]. Cooking in soup may help release more important nutrients for bone and tendon strengthening. Previous chemical studies reported coumarins, alkaloids, flavonoids, terpenes, etc., making the main chemical composition of *M. speciosa* Champ. These components contribute significantly towards its therapeutic properties like hepatoprotection, antibronchitis, and immunity enhancement [6].

To date, no such study has been reported about the phytoconstituents and bioactivities of various extracts of *M. speciosa* stem powder and remained neglected. The aim is to gain new information on bioactive compounds in this part which could be used as a valuable material for new drug and functional food development. Therefore, this study sought to investigate the phytochemical composition and pharmacological activities of different extracts of *Milletta speciosa* stem for the first time. Second, folk healers have used *M. speciosa* to treat various human disorders but lacked scientific validation. Hence, the study was designed to confirm its folklore use and validate its therapeutic potential.

2. Materials and Methods

2.1. Collection of Plant Material. The stem part of *Milletta speciosa* was collected from Wanning city, Hainan province, in May 2018. The sample was thoroughly washed, shade dried, and ground into powder.

2.2. Extraction. Three distinct organic solvents, namely, PE, EtOAc, and MeOH, were used in succession to extract a 300 g powder sample. The sample received about 5 L of PE for 3 days and filtered through a cotton plug and finally through Whatman filter paper. For the following three days, the first step’s leftover material was added back to 5 L of ethyl acetate. The EtOAc fraction was obtained using the same procedure. In order to obtain the crude extract of MeOH, the leftover residues were extracted using 5 L of methanol. The samples were vacuum-evaporated to dryness and kept at 4 °C for further research [7].

2.3. GC-MS Analysis. GC-MS analysis of all three extracts was carried out by using an instrument model Agilent 7890A/5975C equipped with a capillary column HP-5 MS (30 m x 250 μm x 0.25 μm). The instrument was run in the Election Impact (EI) mode with an ionization voltage of 70 eV. Helium was employed as the carrier gas, flowing at a constant rate of 1.2 mL/min. Based on their retention times, the compounds were identified by comparing them with authentic standards and their mass spectral records found in the National Institute of Standards and Technology (NIST 08. L) Library [8].

2.4. Determination of Total Flavonoids. Total flavonoid content was determined by the colorimetric method [8]. The absorbance was measured at 510 nm by using a double-beam UV-Vis spectrophotometer (TU-1901, Beijing Puxi General Instrument Co., Ltd.). Different concentrations (0.008, 0.016, 0.024, 0.032, 0.040, and 0.048 mg/mL) of rutin were used to establish a standard calibration \(y = 10.818x − 0.0217, R^2 = 0.997\). The extracts were dissolved with dimethyl sulfoxide, separately. In short, 0.5 mL of each extract was mixed with 2 mL of distilled water. Furthermore, 150 μL of 5% NaNO₂ solution was added. After five minutes, 600 μL of 10% AlCl₃ and 2 mL of 4% NaOH were added. The solution was thoroughly mixed, and distilled water was added to make the volume up to 5 mL. Total flavonoid content was calculated after 15 min as mg of rutin equivalent (RE) per gram dry weight of the extract. The formula is used as follows:

\[
\text{Total flavonoid content} = \text{RE} = \frac{V}{m}. \tag{1}
\]

2.5. Antityrosinase Activity. The assay was performed as reported by Park et al. [9] with slight alterations. Briefly, potato tyrosinase (1 mL) was mixed with 220 μL of phosphate buffer (0.1 M, pH 6.5) and 2 mL of L-tyrosine and 2 mL of different concentrations of each extract. The reading was noted at 490 nm using a UV spectrophotometer after incubation for 30 min at 37°C. Arbutin and vitamin C were used as the positive control. Percent inhibition of tyrosinase was determined according to the following formula:

\[
\text{%Inhibition} = 100 - \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100. \tag{2}
\]

2.6. Sunscreen Activity. The sun protective potential of all three samples was calculated by using the spectrophotometric method as described earlier [10]. Concentrations of 200 μg/mL of the 03 test samples were prepared. The photoprotection activity was recorded in different regions of absorbance, i.e., UVC, UVB, and UVA, using a spectrophotometer (TU-1901, Beijing General Analysis Instruments). The positive controls (rutin and 4-methylbenzylidene camphor) were also run to demonstrate the validity of the results.

2.7. Anticancer Activity

2.7.1. Cell Culture. The human breast cancer cell line (MCF-7) was received from Kunming Cell Bank, Chinese Academy of Sciences, China. The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) having
10% fetal bovine serum (FBS, BOSTER, Wuhan, China) and 1% antibiotic penicillin (5000 units/mL, Biosharp, Hefei, China) which were then kept at 37°C in 5% CO₂ humidified incubator. At 80% confluence, cells were subcultured, and a new medium was added every two to three days.

2.7.2. Cellular Cytotoxicity Measurement. The assay described by Lamyae et al. was used with some modifications [11]. Cancer cells were plated in a 96-well plate at 4 × 10³ cells per well. After 24 hr incubation at 37°C, extracts of different concentrations were added and incubated for 72 hrs. After incubation, the medium was removed, and the crystal violet solution (100 μL of 1%) was added along with fetal bovine serum (1 : 4.5) to each well for least one and a half h. Each well received glacial acetic acid (30%) and gently mixed. The absorbance was measured using an automatic microplate reader (Thermo Scientific Multiskan Go) at 590 nm. The percent viability of cells was calculated with the following formula:

\[
\% \text{Viability} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control cells}} \times 100. \tag{3}
\]

Doxorubicin was used as the positive control (standard drug). The IC₅₀ values were also calculated.

2.8. Statistical Analysis. The data obtained were analyzed using SPSS 22.0 software. A one-way analysis of variance (ANOVA) at α = 0.05 was carried out to establish the significance of the treatments, while IC₅₀ values were calculated by GraphPad Prism™ 8.00.

3. Results

3.1. GC-MS Analysis. Investigation for bioactive compounds of M. speciosa stem was conducted according to experimental conditions as described in Section 2.3. GC-MS chromatograms for extracts of different solvents are given in Figures 1–3. The data for chemical compounds recorded at different retention times along with similarity index, molecular weight, and relative content are given in their respective Tables 1–3. The mass spectra of phytochemical constituents were compared with the National Institute of Standards and Technology (NIST) library to characterize and identify the number and nature of compounds.

The GC-MS chromatogram of petroleum ether extract depicted different peaks resulting from the presence of 57 compounds. Based on abundance, the top compound identified was n-hexadecanoic acid (16.654%), followed by hexadecanoic acid, ethyl ester (9.710%), octadecanoic acid (4.526%), cyclononasiloxane, octadecamethyl (3.209%), and octadecane (2.840%). The analysis of ethyl acetate extract resulted in 35 compounds based on their retention time. Out of these compounds, n-hexadecanoic acid (14.808%) was found a major chemical constituent followed by octadecanoic acid (2.288%), 2-pentadecanone, 6,10,14-trimethyl (1.541%), and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.398%).

The GC-MS analysis of the methanolic extract of M. speciosa stem led to the identification of 13 chemical compounds. Among these compounds, beta-sitosterol (6.298%) was the most significant phytochemical. The other prevalent compounds were n-hexadecanoic acid (5.369%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (4.435%), and 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (3.566%). The compound n-hexadecanoic acid was recorded for all three extracts but was found as the most abundant for both PE and EtOAc extracts at different retention times. The overall composition of bioactive compounds of all three extracts was found significantly different. Those which were found similar have a significant variation in their amount of existence.

3.2. Total Flavonoid Content (TFC). The total flavonoid content of various extracts of M. speciosa stem was measured by
a spectrophotometric method which has been summarized in Table 4. The amount of flavonoid content in the tested extracts ranged from 6.30% to 48.30%. The results indicated the highest content (48.30 ± 0.90) for PE extract followed by EtOAc extract (28.30 ± 1.00).

3.3. Antityrosinase Activity. The tyrosinase inhibition properties of various extracts of M. speciosa stem were carried out, and IC$_{50}$ values were calculated (Table 5). The results of all three extracts revealed an increase in tyrosinase inhibition values upon dose increment (Figure S1). The PE extract was the most effective and exhibited a maximum value of enzyme inhibition, i.e., 70.97 ± 0.66% with an IC$_{50}$ value of 4.58 mg/mL. The second-highest antityrosinase activity was recorded for EtOAc extract which displayed an IC$_{50}$ value of 6.10 mg/mL. The MeOH extract also showed inhibitory activity but the least.

3.4. Sunscreen Activity. The absorbance values of different extracts of M. speciosa stem recorded in three different ultraviolet (UV) regions, i.e., UVA, UVB, and UVC, are presented in Table 6 (Figure S2). The UV values indicated...
Table 1: GC-MS analysis of petroleum ether extract of *M. speciosa* stem.

| No. | Rt (min) | Compound name                                      | Similarity | MW       | Rc (%) |
|-----|----------|---------------------------------------------------|------------|----------|--------|
| 1   | 3.594    | Heptanal                                           | 97         | 114.104  | 0.023  |
| 2   | 4.727    | Cyclotetrasiloxane, octamethyl-                   | 91         | 296.075  | 0.146  |
| 3   | 5.477    | 3-Octen-2-one                                     | 95         | 126.104  | 0.068  |
| 4   | 5.755    | 2-Octenal, (E)-                                   | 93         | 126.104  | 0.043  |
| 5   | 6.388    | Nonanal                                           | 94         | 142.136  | 0.224  |
| 6   | 6.957    | Cyclopentasiloxane, decamethyl-                  | 94         | 370.094  | 0.165  |
| 7   | 7.847    | Decanal                                           | 90         | 156.151  | 0.065  |
| 8   | 9.049    | Nonanoic acid                                     | 95         | 158.131  | 0.112  |
| 9   | 9.355    | Cyclohexasiloxane, dodecamethyl-                 | 93         | 444.113  | 0.172  |
| 10  | 10.327   | 2-Tetradecene, (E)-                              | 97         | 196.219  | 0.268  |
| 11  | 10.432   | Tetradecane                                       | 98         | 198.235  | 0.070  |
| 12  | 10.626   | 1,1-Dodecanediol, diacetate                      | 91         | 286.214  | 0.067  |
| 13  | 11.495   | Dimethyl phthalate                                | 95         | 194.058  | 0.048  |
| 14  | 11.641   | 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)- | 98         | 220.146  | 0.244  |
| 15  | 11.842   | Cycloheptasiloxane, tetradecamethyl-             | 91         | 518.132  | 0.420  |
| 16  | 11.995   | Pentadecane                                       | 98         | 212.25   | 0.107  |
| 17  | 12.558   | Phenol, 2,4-bis(1,1-dimethylethyl)-               | 96         | 206.167  | 0.215  |
| 18  | 13.308   | 1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl- | 98         | 178.063  | 0.162  |
| 19  | 13.67    | Dodecanoic acid                                   | 98         | 200.178  | 0.163  |
| 20  | 13.843   | 1-Hexadecene                                      | 98         | 224.25   | 0.562  |
| 21  | 13.989   | Hexadecane                                        | 98         | 226.266  | 0.234  |
| 22  | 14.365   | Hexadecanal                                       | 95         | 240.245  | 0.065  |
| 23  | 18.68    | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde          | 92         | 234.162  | 0.034  |
| 24  | 18.972   | Tetradecanoic acid                                | 96         | 228.209  | 0.474  |
| 25  | 19.256   | E-15-Heptadecenal                                 | 99         | 252.245  | 0.769  |
| 26  | 21.063   | 2-Pentadecanone, 6,10,14-trimethyl-              | 99         | 268.277  | 1.078  |
| 27  | 21.681   | Oxacyclotetradecane-2,11-dione, 13-methyl-       | 91         | 240.173  | 0.063  |
| 28  | 22.349   | Pentadecanoic acid                                | 98         | 242.225  | 0.433  |
| 29  | 22.807   | Benzenamine, N-[4-(1-methylethyl) benzylidene]-4-(1-pyrrolidylsulfonil)- | 90         | 356.156  | 0.455  |
| 30  | 23.981   | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 99         | 276.173  | 2.136  |
| 31  | 24.572   | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 91         | 292.204  | 0.028  |
| 32  | 25.441   | 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester | 95         | 278.152  | 2.494  |
| 33  | 26.566   | Hexadecanoic acid, ethyl ester                   | 95         | 284.272  | 9.710  |
| 34  | 27.254   | n-Hexadecanoic acid                              | 99         | 256.24   | 16.654 |
| 35  | 30.381   | Ethyl 14-methyl-hexadecanoate                    | 91         | 298.287  | 0.384  |
| 36  | 34.307   | Octadecanoic acid                                | 95         | 284.272  | 4.526  |
| 37  | 38.31    | Heptadecane                                       | 96         | 240.282  | 0.599  |
| 38  | 40.401   | 4,8,12,16-Tetramethylheptadecan-4-olide          | 98         | 324.303  | 0.862  |
| 39  | 41.27    | 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide | 90         | 264.093  | 0.099  |
| 40  | 41.971   | 1-Docosene                                        | 99         | 308.344  | 0.456  |
| 41  | 42.208   | Tetracosane                                       | 99         | 338.391  | 1.368  |
| 42  | 44.487   | Decane, 3,6-dimethyl-                            | 86         | 170.203  | 0.083  |
| 43  | 45.967   | Pentacosane                                       | 99         | 352.407  | 1.563  |
| 44  | 46.606   | 1-Tricosene                                       | 95         | 322.36   | 0.163  |
| 45  | 47.1     | Tetrapentacontane, 1,54-dibromo-                 | 90         | 914.682  | 0.092  |
| 46  | 47.683   | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | 91         | 278.152  | 0.555  |
| 47  | 49.636   | Hexacosane                                        | 99         | 366.423  | 1.909  |
| 48  | 56.626   | Eicosane                                          | 95         | 282.329  | 1.821  |
### Table 1: Continued.

| No. | Rt (min) | Compound name                                      | Similarity | MW     | Rc (%) |
|-----|----------|-----------------------------------------------------|------------|--------|--------|
| 49  | 57.877   | (Z)-14-Tricosenyl formate                            | 94         | 366.35 | 1.203  |
| 50  | 58.655   | Octacosane                                           | 98         | 394.454| 0.154  |
| 51  | 59.385   | Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-| 92         | 281.078| 0.151  |
| 52  | 60.017   | Octadecane                                           | 96         | 254.297| 2.840  |
| 53  | 61.268   | 2-Dodecen-1-yl(-)succinic anhydride                  | 92         | 266.188| 0.123  |
| 54  | 62.067   | Heptacosane                                          | 93         | 380.438| 0.290  |
| 55  | 63.617   | Triacontane                                          | 98         | 422.485| 1.388  |
| 56  | 65.423   | Oxirane, hexadecyl-                                  | 93         | 268.277| 1.832  |
| 57  | 68.335   | Octadecane, 1-iodo-                                  | 97         | 380.194| 3.166  |

### Table 2: GC-MS analysis of ethyl acetate extract of *M. speciosa* stem.

| No. | Rt (min) | Compound name                                      | Similarity | MW     | Rc (%) |
|-----|----------|-----------------------------------------------------|------------|--------|--------|
| 1   | 5.186    | Phenol                                              | 91         | 94.042 | 0.397  |
| 2   | 6.388    | Phenol, 2-methoxy-                                  | 92         | 124.052| 0.669  |
| 3   | 8.181    | Decanal                                             | 91         | 156.151| 0.155  |
| 4   | 10.745   | Cyclohexasiloxane, dodecamethyl-                    | 90         | 444.113| 0.310  |
| 5   | 13.566   | Vanillin                                            | 97         | 152.047| 0.601  |
| 6   | 15.81    | Cycloheptasiloxane, tetradecamethyl-                | 93         | 518.132| 0.318  |
| 7   | 17.846   | 1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl- | 97         | 178.063| 0.253  |
| 8   | 18.805   | Dodecanoic acid                                     | 94         | 200.178| 0.230  |
| 9   | 18.993   | 1-Hexadecene                                        | 98         | 224.25 | 0.284  |
| 10  | 22.029   | Benzaldehyde, 4-hydroxy-3,5-dimethoxy-              | 97         | 182.058| 0.365  |
| 11  | 23.155   | Octadecanal                                         | 92         | 268.277| 0.297  |
| 12  | 24.6     | 2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-          | 90         | 178.063| 0.173  |
| 13  | 24.781   | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol       | 99         | 180.079| 0.666  |
| 14  | 25.379   | Tetradecanoic acid                                  | 93         | 228.209| 0.483  |
| 15  | 25.677   | 1-Octadecene                                        | 98         | 252.282| 0.663  |
| 16  | 27.449   | 2-Pentadecanone, 6,10,14-trimethyl-                 | 99         | 268.277| 1.541  |
| 17  | 28.596   | Pentadecanoic acid                                  | 99         | 242.225| 0.549  |
| 18  | 29.979   | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | 99         | 276.173| 1.398  |
| 19  | 31.264   | Dibutyl phthalate                                   | 97         | 278.152| 0.609  |
| 20  | 32.112   | n-Hexadecanoic acid                                 | 99         | 256.24 | 14.808 |
| 21  | 34.773   | Heptadecanoic acid                                  | 96         | 270.256| 0.512  |
| 22  | 35.809   | Phytol                                             | 90         | 296.308| 0.234  |
| 23  | 36.983   | Oleic acid                                          | 93         | 282.256| 0.254  |
| 24  | 37.775   | Octadecanoic acid                                   | 99         | 284.272| 2.288  |
| 25  | 38.96    | 1-Docosene                                          | 96         | 308.344| 0.598  |
| 26  | 41.798   | Oxirane, heptadecyl-                                | 93         | 282.292| 0.236  |
| 27  | 42.521   | 4,8,12,16-Tetramethylheptadecan-4-oxide             | 97         | 324.303| 0.457  |
| 28  | 43.688   | Octadecane                                          | 95         | 254.297| 0.570  |
| 29  | 46.28    | Heptadecane                                         | 96         | 240.282| 0.457  |
| 30  | 47.128   | Oxirane, hexadecyl-                                 | 94         | 268.277| 0.680  |
| 31  | 47.599   | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | 91         | 278.152| 0.601  |
| 32  | 52.061   | 13-Tetradecen-1-ol acetate                          | 91         | 254.225| 0.276  |
| 33  | 53.264   | 13-Docosenamide, (Z)-                              | 97         | 337.334| 1.370  |
| 34  | 53.541   | Eicosane                                           | 96         | 282.329| 0.588  |
| 35  | 56.404   | 1-Hexacosene                                       | 97         | 364.407| 0.957  |
cell viability) was exhibited by all three extracts at the present in Table 7 (Figure S3). Doxorubicin was used as a reference. The cytotoxic activity of PE, EtOAc, and MeOH extracts was determined by the cell viability assay. The results revealed that the EtOAc extract showed the highest cytotoxic activity, followed by PE and MeOH extracts. The overall data ranged between 49.73 ± 0.49 % and 90.74 ± 0.00 % for PE extract, 80.94 ± 0.21 % for EtOAc extract, and 70.61 ± 0.34 % for MeOH extract.

3.5. Anticancer Activity. The cytotoxic activity of PE, EtOAc, and MeOH extracts against MCF-7 cells was evaluated using the MTT assay. The results showed that all extracts have sunscreen capacity in all regions. The maximum value of absorbance (0.633 ± 0.06 %) was noted for PE extract in the UVB region. While in the UVC region, the highest absorbance value, i.e., 0.663 ± 0.07 %, was noted for EtOAc extract. The overall order of the UV absorption of the tested extracts in all given zones was PE > EtOAc > MeOH. The results were not in comparison to that of pure compounds (rutin and 4-methylbenzylidene camphor); however, the EtOAc extract showed almost the same value (0.632 ± 0.07) in the UVB region compared to that of rutin, i.e., 0.663 ± 0.32.

Table 4: Total flavonoid content of various M. speciosa stem extracts.

| Extract            | Total flavonoid content (%) |
|--------------------|-----------------------------|
| Petroleum ether    | 48.30 ± 0.90                |
| Ethyl acetate      | 28.30 ± 1.00                |
| Methanol           | 06.30 ± 0.90                |

that all extracts have sunscreen capacity in all regions. The maximum value of absorbance (0.633 ± 0.06 %) was noted for PE extract in the UVB region. While in the UVC zone, the highest absorbance value, i.e., 0.663 ± 0.07 %, was noted for EtOAc extract. The overall order of the UV absorption of the tested extracts in all given zones was PE > EtOAc > MeOH. The results were not in comparison to that of pure compounds (rutin and 4-methylbenzylidene camphor); however, the EtOAc extract showed almost the same value (0.632 ± 0.07) in the UVB region compared to that of rutin, i.e., 0.663 ± 0.32.

4. Discussion

4.1. GC-MS Analysis. Studies about medicinal plants have shown their importance as a store for natural medicine. They are used both as a source of purified drugs and as such in folk medicine [12]. GC-MS is a proven and well-recognized technique to identify phytoconstituents along with other biologically important components like hydrocarbons, esters, and alcohols that exist in medicinal plants [13].

In the present study, GC-MS analysis of various extracts of M. speciosa stem was carried out. The most abundant compound in PE extract was n-hexadecanoic acid, which has anti-inflammatory, anticancer [14], antioxidant, and hypocholesterolemic properties [15]. Hexadecanoic acid ethyl ester has been reported to have antioxidant activities [16]. The compound octadecanoic acid was previously identified as an antibacterial, anticancer, and antiasthmatic agent [17]. Cyclononasiloxane, octadecamethyl- has also exhibited its bioactivity as an antifungal [18], while octadecane was detected as sesquiterpene hydrocarbon in the stem-bark extract of Adansonia digitata which has anti-inflammatory and antiallergic properties [19].

The EtOAc extract also demonstrated important biologically active compounds. The phytoconstituent, 2-pentadecanone, 6,10,14-trimethyl- is one of the major compounds listed for EtOAc extract, which has demonstrated hypocholesterolemic, antioxidant, and lubrication properties [10]. The compound, 7,9-ditert-buty1-oxaspiro(4, 5)deca-6,9-diene-2,8-dione was identified from EtOAc extract of Penicillum citrinum Strain ND7c and reported to have strong antimicrobial activities [20].

The GC-MS analysis also revealed various important bioactive compounds, but 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol and 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester were found as major components. The former is a phenolic compound and is used for anti-inflammatory, antiasthmatic, and antiallergic activities [19].

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Table 5: Antityrosinase activity and IC_{50} (mg/mL) of various *M. speciosa* stem extracts.

| Compound Extract | Concentration (mg/mL) | Petroleum ether IC_{50} (mg/mL) | Ethyl acetate IC_{50} (mg/mL) | Methanol IC_{50} (mg/mL) |
|------------------|-----------------------|---------------------------------|-------------------------------|--------------------------|
|                  | 0.5                   | 31.22 ± 0.70                    | 29.54 ± 0.73                  | 13.72 ± 0.70             |
|                  | 2.0                   | 36.29 ± 0.63                    | 33.52 ± 0.65                  | 19.49 ± 0.68             |
|                  | 3.5                   | 44.53 ± 0.62                    | 38.79 ± 0.66                  | 26.27 ± 0.63             |
|                  | 5.0                   | 51.67 ± 0.64                    | 45.39 ± 0.55                  | 32.57 ± 0.90             |
|                  | 6.5                   | 59.99 ± 0.72                    | 52.61 ± 0.83                  | 39.92 ± 0.72             |
|                  | 8.0                   | 70.97 ± 0.66                    | 59.84 ± 0.67                  | 46.67 ± 0.67             |

Positive Control:

| Compound | Concentration (mg/mL) | Petroleum ether IC_{50} (mg/mL) | Ethyl acetate IC_{50} (mg/mL) | Methanol IC_{50} (mg/mL) |
|----------|-----------------------|---------------------------------|-------------------------------|--------------------------|
| Arbutin  | 0.5                   | 27.23 ± 0.29                    | 50.89 ± 0.56                  | 71.65 ± 0.66             |
|          | 2.0                   | 38.19 ± 0.72                    | 62.16 ± 0.81                  | 82.65 ± 0.66             |

Vitamin C:

| Compound | Concentration (mg/mL) | Petroleum ether IC_{50} (mg/mL) | Ethyl acetate IC_{50} (mg/mL) | Methanol IC_{50} (mg/mL) |
|----------|-----------------------|---------------------------------|-------------------------------|--------------------------|
|          | 0.5                   | 32.75 ± 0.59                    | 56.12 ± 0.52                  | 78.73 ± 0.59             |
|          | 2.0                   | 44.35 ± 0.56                    | 67.69 ± 0.53                  | 92.89 ± 1.17             |

Table 6: Sunscreen activity of various extracts of *M. speciosa* stem.

| Compound                  | Concentration (μg/mL) | UVA zone (320~400 nm) | UVB zone (280~320 nm) | UVC zone (200~280 nm) |
|---------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Petroleum ether extract   | 200                   | 0.291 ± 0.05          | 0.384 ± 0.05          | 0.633 ± 0.06          |
| Ethyl acetate extract     | 200                   | 0.369 ± 0.03          | 0.632 ± 0.07          | 0.459 ± 0.06          |
| Methanol extract          | 200                   | 0.212 ± 0.06          | 0.522 ± 0.09          | 0.355 ± 0.10          |
| Rutin                     | 40                    | 1.014 ± 0.28          | 0.663 ± 0.32          | 1.908 ± 0.61          |
| 4-Methylbenzylidene camphor| 40                    | 0.512 ± 0.19          | 3.350 ± 0.52          | 3.485 ± 0.46          |

Table 7: Cell viability (%) and IC_{50} (μg/mL) for various *M. speciosa* stem extracts.

(a)

| Compound | Concentration (μg/mL) | Petroleum ether IC_{50} (μg/mL) | Ethyl acetate IC_{50} (μg/mL) | Methanol IC_{50} (μg/mL) |
|----------|-----------------------|---------------------------------|-------------------------------|--------------------------|
| Extract  | 12.5                  | 100.00 ± 0.00                   | 100.00 ± 0.00                 | 100.00 ± 0.00            |
|          | 25                    | 100.00 ± 0.00                   | 100.00 ± 0.00                 | 100.00 ± 0.00            |
|          | 50                    | 88.20 ± 0.55                    | 90.27 ± 0.58                  | 93.94 ± 0.73             |
|          | 100                   | 76.36 ± 0.51                    | 85.42 ± 0.55                  | 85.66 ± 0.68             |
|          | 200                   | 49.73 ± 0.49                    | 62.10 ± 1.11                  | 77.28 ± 1.08             |

(b)

| Compound | Concentration (μg/mL) | Petroleum ether IC_{50} (μg/mL) | Ethyl acetate IC_{50} (μg/mL) | Methanol IC_{50} (μg/mL) |
|----------|-----------------------|---------------------------------|-------------------------------|--------------------------|
| Doxorubicin | 1.25                  | 81.40 ± 0.54                    | 73.24 ± 0.53                  | 50.34 ± 0.59             |
|          | 2.50                  | 61.40 ± 0.52                    | 50.34 ± 0.59                  | 50.34 ± 0.59             |
|          | 5.00                  | 27.34 ± 0.61                    | 27.34 ± 0.61                  | 27.34 ± 0.61             |
4.2. Total Flavonoid Content. Several studies have shown that flavonoids are responsible for various therapeutic activities like anticancer, hepatoprotective, antibacterial, and antidiabetic [23]. During the current study, the highest flavonoid content was reported for PE extract of *M. speciosa* stem, while the least amount was noted for MeOH extract, which was 8-fold less than that of PE extract. The difference in the quantity of flavonoid content depends on the polarity of the solvent and the flavonoids present in the plant extracts [24]. Several studies have been conducted to determine the flavonoid content of various extracts of whole and different plant parts. Mbinda and Musangi determined the flavonoid content of the methanolic extract of *Calotropis procera* stem and further established its antioxidant and radical scavenging properties [25]. Furthermore, the study is supported by that of Ferdinand et al. who reported the flavonoid content of *Millettia laurentii* seed extract [26]. There are numerous studies about the flavonoid content of medicinal plants, which support our study and confirm the importance of the plant stem as a source of bioactive ingredients.

4.3. Antityrosinase Activity. Tyrosinase is a copper-containing enzyme with prime importance for controlling the production of melanin which is responsible for the hyperpigmentation of human skin. Therefore, the suppression of tyrosinase is an eminent approach to the development of melanogenesis inhibitors [27]. The current attempt was to find the tyrosinase inhibition potential of various *M. speciosa* stem extracts. The results indicated the antityrosinase activity of all extracts at different levels. The maximum inhibition was noted for PE extract followed by EtOAc extract. The results were supported by previous studies of *Tamarix nilotica* (Ehrenb.) Bunge stem extract, which demonstrated the L-tyrosine and L-DOPA inhibition values of 79.51% and 53.00%, respectively [28]. The stem extract of *Artocarpus chama* has shown strong antityrosinase activity both in enzymatic and intracellular assays [29]. Similarly, the stem extract of *Astragalus siligusus* exhibited antityrosinase activity as reported by Zarei and his coworkers. However, the difference in inhibition level may be due to several factors like the composition of bioactive compounds, age of the plant, genetic and seasonal variations, and physiological and geographical factors [30].

4.4. Sunscreen Activity. Skin is a natural barrier between the internal parts of the body and the environment that protects against physical and chemical damage to skin tissues. Irreversible skin damage like skin cancer, aging, DNA damage, and oxidative stress can occur due to the presence of UV radiation and especially UVA and UVB. One of the protective measures to counter the effect of UV radiation is the use of medicinal plant extracts, which house natural antioxidants such as flavonoids and polyphenols. These compounds can absorb a wide range of UV light [31–33].

Our results were in good agreement with the available literature as all extracts showed sunscreen properties to some extent in all regions. The maximum photoprotection values were noted for EtOAc extract in the UVB zone while PE extract displayed the highest potential for UV absorption in the UVC region. The study of Preethima et al. confirmed the extract of *Pongamia pinnata* seeds to have high photosorption properties in the UVA and B regions and hence can be used in sunscreen formulations [34]. The study could be further supported by Miguel et al. who reported higher photosorption capacity in the UVB range for the extracts of *Bejaria aestuans* and *Cavendishia pubescence* [35].

4.5. Anticancer Activity. Cancer is the second leading cause of death around the world. Comparing the side effects induced by synthetic drugs, plant-based natural products are a wise option [36]. During the current study, the anticancer activity of different extracts of *M. speciosa* stem powder against human breast cancer cells (MCF-7) was reported for the first time. The highest cytotoxicity value was recorded for PE extract followed by EtOAc at the same concentration of 200 μg/mL. A similar study was carried out by Pham et al. which revealed the strong anticancer activity of crude extract of *Helicteres hirsuta* stem against MCF-7 cell lines [37]. Kumar and coscientists investigated *Millettia pinata* for anticancer activity against lung cancer cells and found a higher cytotoxic effect of EtOAc extract [38]. The results obtained by Zingue et al. also declared notable anticancer activity of *Millettia macrophylla* extract against MCF-7 human breast cancer cells [39]. Several studies exist about the anticancer activity of plant extracts that correlate the anticancer activity with the combination of phytoconstituents present in a specific part of the plant.

The results clearly indicated that *M. speciosa* stem could be a potential source of natural anticancer products, which needs further studies related to isolation and clinical investigation to develop novel herbal-based anticancer medicine.

5. Conclusions

This was the first report about GC-MS analysis, antityrosinase, sunscreen, and anticancer activity of *Millettia speciosa* stem powder. Total flavonoid content was significantly different among all three extracts. The study revealed that various extracts of *Millettia speciosa* stem powder possess a very interesting phytochemical profile. More extractable metabolites were reported for petroleum ether extract. Ethyl acetate and methanolic extracts also showed considerable in vitro biological activities, but petroleum ether extract revealed the highest potential against tyrosinase, ultraviolet radiations, and cancer cell proliferation. The present study suggests that *Millettia speciosa* stem powder may be used as a potential source of natural products for pharmaceutical as well as nutraceutical development. However, future studies are needed to isolate bioactive compounds and in vivo studies to be carried out to establish a true cause-effect of *M. speciosa* stem.

Data Availability

All the data is available in the article. Figures have been submitted as supplementary materials to the journal.
Conflicts of Interest

The authors have no conflict of interest.

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Supplementary Materials

Figure S1: antityrosinase activity of M. speciosa stem extracts, arbutin, and Vit C. Values are expressed as means ± SD (n = 3). Figure S2: sunscreen activity of M. speciosa stem extracts, rutin, and camphor. Values are expressed as means ± SD (n = 3). Figure S3: anticancer activity of M. speciosa stem extracts and doxorubicin. (A) MCF-7 cells were treated with different extracts of various concentrations. (B) MCF-7 cells were treated with standard drug, i.e., doxorubicin, as a positive control. Values are expressed as means ± SD (n = 3). (Supplementary Materials)

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