Research Article

Phytochemical Profiling, Antioxidant Activity, and In Silico Analyses of Sterculia villosa and Vernonia patula

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Our study aims to evaluate the chemical profiles and antioxidant activities of a methanolic extract of Sterculia villosa bark (MESV) and a methanolic extract of the Vernonia patula whole plant (MEVP). The chemical profiling of MESV and MEVP was performed via gas chromatography-mass spectrometry (GC-MS), which identified 52 and 33 chemical compounds, respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay indicated that both MESV and MEVP displayed concentration-dependent scavenging activities, and half-maximal inhibitory concentration (IC50) values for MEVP, MESV, and ascorbic acid were 305.30, 555.44, and 36.32 μM/mL, respectively. The total flavonoid content (TFC) and total phenolic content (TPC) of MESV were 81.44 ± 2.70 mg quercetin equivalents (QE)/g dry extract and 62.58 ± 1.93 mg gallic acid equivalent (GAE)/g dry extract, whereas these values for MEVP were 291.31 ± 6.61 mg QE/g dry extract and 58.99 ± 3.16 mg GAE/g dry extract, respectively. Molecular docking studies were also evaluated, and absorption, distribution, metabolism, and excretion (ADME) and toxicological properties were assessed. Therefore, these two plants, S. villosa and V. patula, showed potential options for further advanced studies into oxidative stress.

1. Introduction

Plants containing natural bioactive compounds have been used in traditional medicinal practices worldwide since ancient times, and plants represent a source of potential medicines [1]. The scope of plants as a source of new drugs remains generally unexplored, as only a small fraction of approximately 250,000–500,000 plant species have been biologically or pharmacologically screened [2]. Phytochemicals with antioxidant properties are of particular interest because chronic disorders [3] exacerbated by oxidative stress (OS) have become the leading cause of death [4]. Plant-derived compounds possess potent antioxidant properties that may inhibit OS by countering reactive oxygen species (ROS) and maintaining redox homeostasis [5, 6]. Several attempts have been undertaken to identify phytochemical compounds [4, 7] and assess their potential as antioxidants [8, 9], antimicrobials [10], antidiabetics, and anti-inflammatory compounds [11, 12]. The therapeutic potential of plants is commonly associated with their antioxidant and anticancer properties [2, 13–16].

Oxidation refers to the removal of electrons during a reaction by an atom, molecule, or ion and can occur...
following the formation of elevated amounts of ROS. ROS are formed during natural cellular metabolic processes by living organisms, including byproducts of aerobic metabolism. ROS include hydrogen peroxide (H$_2$O$_2$), superoxide anion (O$_2^{-}$), and hydroxyl radicals (OH•), which all have inherent chemical properties and provide reactivity to various biological objectives [17]. ROS are also correlated with the concept of OS, and ROS can damage lipids, proteins, and DNA [18]. OS is a complicated process involving the generation of ROS and reactive nitrogen species (RNS) [19, 20]. ROS are produced by persistent metabolic processes and can regulate various biological and pathological processes, such as lipid peroxidation, immune response, and phagocyte activation [21]. Furthermore, excessive ROS generation can trigger oxidative damage by targeting the unsaturated fatty acids in membranes and thiol groups in proteins [22, 23]. Many chronic health problems have been associated with excessive lipid peroxidation, and free radicals have been implicated in the induction of several neuropsychiatric conditions and might mediate neuronal malfunctions associated with depression [24, 25]. OS has been identified as a significant contributor to the progression of degenerative and chronic diseases, such as malignant growths [26], diabetes, immune problems, joint inflammation, and cardiovascular and neurodegenerative diseases [27].

*Sterculia villosa* (Family: Sterculiaceae, Bengali name: Udal) is a deciduous tree with large, long-stalked, deeply lobed leaves and yellow flowers. *S. villosa* can be found in subtropical and tropical regions, including Bangladesh [7]. The plant is traditionally used as a diuretic and aphrodisiac agent [28] and is often used by Indian people to cure inflammation through traditional medicinal practices [29]. *Vernonia patula* (Family: Asteraceae, Bengali name: Kukshim) is an annual weed that is geographically disseminated throughout Bangladesh and is often used by Indian people to cure inflammation through traditional medicinal practices [29]. *V. patula* is used for fever reduction, headaches, malaria, common cold, and intestinal and stomach problems [30]. The bioactive compounds derived from these two plants have been reported to have antioxidant activities in prior studies [31, 32]. However, prior studies did not attempt to identify specific bioactive compounds.

Therefore, the present research attempted to identify the bioactive constituents of these two species through gas chromatography-mass spectrometry (GC-MS) analysis and explored the antioxidant efficacy of the compounds found in *S. villosa* and *V. patula*. GC-MS has been widely highlighted as an important analytical tool for secondary metabolite profiling, such as steroids, phenolics, and alkaloids, and can also identify sugars, fatty acids, amino acids, and other macromolecules found in plants and nonplant sources [33–36]. Identifying the bioactive profile may improve the identification of the key components responsible for various biological activities and contribute to the discovery of underlying principles of these effects.

To explore the possible mechanisms of action associated with the compounds identified from *S. villosa* and *V. patula*, we also performed molecular docking and absorption, distribution, metabolism, and excretion (ADME)/toxicity (T) studies to reveal the potential target(s) of the identified antioxidant components.

2. Materials and Methods

2.1. Chemicals. Phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Folin-Ciocalteu Reagent were all purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Collection and Preparation of *S. villosa* and *V. patula* Extracts. Whole *S. villosa* and *V. patula* plants were collected from the Chittagong Hill-Tracts region of Bangladesh, and plants were authenticated and identified by a renowned taxonomist from the Bangladesh Council of Scientific and Industrial Research (BCSIR). The bark of *S. villosa* and the whole *V. patula* plant were washed with distilled water. The plant parts were cut into small pieces and dried. The dried materials were crushed into a fine pure powder using an electric blender. The powder was then stored in separate airtight containers. To obtain the extracts, the powders were placed in airtight containers, hexane was added at a sample to solvent ratio of 1:3, and the sample was subjected to uninterrupted stirring at 150 rpm for 90 minutes. The stirring was discontinued, and the sample was allowed to sit for 30 minutes, after which the hexane was decanted from the sample by filtration. Fresh hexane was added to the sample, and the process was repeated three times. After the final repetition, the hexane was decanted, and the sample was filtered under vacuum to completely remove all hexane, resulting in a defatted sample. The defatted crude powder was placed in an airtight container, and aqueous (95%) methanol was added at a sample to solvent ratio of 1:10. The sample was then subjected to 7 days of repeated 40/20-minute shaking/sonication cycles of uninterrupted agitation on a shaker machine at 150 rpm and ultrasonic vibrations in a sonicator machine at 55°C. The mixture was then filtered through Whatman #1 filter paper, and the filtrate was collected. This procedure was repeated thrice to extract all phytochemicals from the sample. All obtained filtrates were combined, and the methanol was evaporated in a rotary evaporator machine (Buchi, Postfach, Switzerland). The filtrates were lyophilized to complete dryness at −70°C in a freeze drier (SP Scientific, Stone Ridge, NY, USA), collected into a Petri dish, covered and wrapped properly, and stored at 4°C until further experiments.

2.3. GC-MS Analysis. The methanolic extract of *S. villosa* (MESV) and the methanolic extract of *V. patula* (MEVP) were evaluated in a mass spectrometer (TQ 8040, Shimadzu Corporation, Kyoto, Japan) using the electron impact ionization (EI) technique and a gas chromatograph (GC-17A, Shimadzu Corporation) with a merged silica capillary column (Rxi-5 ms; 0.25 m film, 30 m long and internal diameter 0.32 mm) coated with DB-1 (J&W). The oven temperature was set at 70°C (0 min); 10°C, 150°C (5 min); 12°C, 200°C (15 min); and 12°C, 220°C (5 min), with a clamp time of
10 min. The inlet temperature was 260°C. The flow rate of the column was 0.6 mL/min helium gas at constant pressure (90 kPa). The GC to MS interface temperature was 280°C. The MS was used in scanning mode, with a scanning range of 40–350 amu. The ionization mode was EI, and the mass range was 50–550 m/z. One microliter of the sample was injected in the splitless injection mode. The total GC-MS course time was 50 min. The compounds in the peak areas

| Sl. no. | Name | Molecular formula | Nature | RT (min) | m/z | Area (count) |
|--------|------|-------------------|--------|----------|-----|-------------|
| 1      | Heptanal | C7H14O | Aldehyde | 4.146 | 44.00 | 879969     |
| 2      | Benzaldehyde, 2-methyl | C6H8O | Aldehyde | 4.872 | 44.00 | 19425      |
| 3      | Glucitol, 6-O-nonyl | C13H26O6 | Sugar alcohol | 6.119 | 44.00 | 357524     |
| 4      | L-Arabinol | C5H12O5 | Ketone | 6.134 | 150.00 | 531076     |
| 5      | α-Isomethyl ionone | C14H22O | Monoterpenoid | 6.566 | 154.00 | 273748     |
| 6      | Eucalyptol | C10H18O | Phenolic aldehyde | 7.246 | 151.00 | 375524     |
| 7      | Vanillin | C9H8O3 | Glucocorticoid | 7.922 | 44.00 | 18826      |
| 8      | Prednisone | C21H26O5 | Ester | 7.974 | 137.00 | 267302     |
| 9      | Bioallethin | C10H18O | Carbohydrate | 8.134 | 117.00 | 406631     |
| 10     | Sorbitol | C6H14O6 | Sugar alcohol | 8.872 | 137.00 | 507045     |
| 11     | β-D-glucopyranose, 4-O-β-D-galactopyranosyl | C12H22O11 | Carbohydrate | 9.665 | 43.00 | 71620      |
| 12     | Santolinatriene | C14H24O3 | Monoterpenoid | 9.949 | 151.00 | 228315     |
| 13     | Vanillin, acetate | C10H12O2 | Phenyl acetate | 10.092 | 151.00 | 375524     |
| 14     | Guanosine | C10H12O5 | Purine nucleoside | 10.349 | 44.00 | 19425      |
| 15     | Trans-11-Tetradecenyl acetate | C20H38O2 | Fatty acid methyl ester | 11.494 | 44.00 | 26500      |
| 16     | Isopulegol | C10H18O | Terpenoid alcohol | 11.520 | 71.00 | 332135     |
| 17     | Vanillin, acetate | C10H10O4 | Phenyl acetate | 11.697 | 151.00 | 205675     |
| 18     | Prednisone | C21H26O5 | Steroid | 11.974 | 272.00 | 407509     |
| 19     | 3-hexadecanoylglycerol | C39H72O3 | Monodecanoylglycerol | 12.780 | 67.00 | 321453     |
| 20     | Spiro[3.4]octan-5-one | C11H22O | Carotenoids | 13.014 | 124.00 | 283819     |
| 21     | 2-dodecen-1-yl(-)succinic anhydride | C16H30O3 | Fatty acid methyl ester | 13.306 | 43.00 | 135871     |
| 22     | Phytol | C20H40O | Diterpene alcohol | 13.520 | 44.00 | 22833      |
| 23     | Undec-10-ynoic acid | C11H20O2 | Fatty acid | 13.949 | 43.00 | 139829     |
| 24     | Undecanal | C10H20O | Fatty acid | 13.949 | 44.00 | 139829     |
| 25     | 6-octadecenoic acid, methyl ester, (Z) | C19H36O2 | Fatty acid methyl ester | 16.280 | 43.00 | 81791      |
| 26     | Dodecanal | C12H24O | Aldehyde | 16.566 | 43.00 | 139829     |
| 27     | Nerolidol | C15H26O | Sesquiterpene | 19.001 | 69.00 | 148917     |
| 28     | Glycerol 1-palmitate | C19H38O4 | Monoacylglycerols | 20.134 | 44.00 | 114505     |
| 29     | Hexadecanal | C16H32O | Glucocorticoid | 20.515 | 149.00 | 114505     |
| 30     | Meprobamate | C15H24N2O4 | Carbamate | 22.199 | 83.00 | 96437      |
| 31     | Mebutamate | C10H20N2O4 | Carbamate | 22.870 | 207.00 | 124804     |
| 32     | Androsta-3,5-dien-3-ol, 17-acetyl-3-O-(t-butyldimethylsilyl) | C27H44O2Si | Steroids | 25.401 | 207.00 | 39398      |
were classified by comparison with the national institute of standards and technology (NIST) GC-MS library version 08-S [37].

2.4. Antioxidant Activity. The experiments were performed in triplicate.

2.4.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity. The extracts were evaluated for antioxidant activity using DPPH, as described in the literature [38]. In this experiment, 3 mg of each extract was added to 1 mL 50% methanol (v/v), and ascorbic acid (0.3 g) stock solution was added to 1 mL 50% methanol (v/v) as a positive control. The serial dilution technique was applied to obtain MEVP, MESV, and ascorbic acid at concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 μg/mL. A 0.1 mL aliquot of each concentration of extract solution in methanol was combined with 1.0 mL freshly formulated DPPH-methanol solution (0.1 mM) and 0.45 mL 50 mM Tris (hydroxymethyl) aminomethane (THAM) hydrochloride buffer (pH 7.40). The reaction was allowed to develop for 30 minutes, and absorbances were estimated at 517 nm. The corresponding inhibition rates were measured using the following equation:

\[
\text{DPPH scavenged (\%)} = \left( \frac{A - B}{A} \right) \times 100, \tag{1}
\]

where A is the absorbance in the presence of extract or standard and B is the absorbance of the control.

2.4.2. Total Phenolic Content (TPC). The total phenolic content (TPC) of MESV and MEVP was determined using an oxidizing agent, Folin-Ciocalteu Reagent (FCR), according to the method described by Ali Reza et al. [39]. A 1 mL volume of FCR was diluted in 9 mL purified water, and then, 2.5 mL of Na₂CO₃ and 500 μg/mL extract was added to obtain a final volume of 10 mL. The solution was
incubated for 20 min (250°C), and the absorbance was observed at 765 nm in triplicate. Gallic acid was used as the standard for the calculation of TPC, and the values were obtained according to the standard gallic acid curve ($y = 0.0039x + 0.0406; R^2 = 0.9981$). TPC was determined according to the following equation, in gallic acid equivalents (GAE; mg/g):

$$\text{TPC} = \text{equivalent reagent (Conc.)} \times \frac{\text{volume of total content}}{\text{conc. of sample taken}}$$

2.4.3. Total Flavonoid Content (TFC). The total flavonoid content (TFC) of MESV and MEVP was evaluated as previously described by Ali Reza et al. [39]. The TFC was calculated by mixing 0.5 mL extract with 1.5 mL methanol and adding 0.1 mL AlCl$_3$ (10%), 0.1 mL CH$_3$CO$_2$K (1 M), and 2.8 mL distilled water. The mixture was incubated at 25°C for 30 min, and later, the absorbance was taken at 415 nm. The blank solution contained all of the reagents except for the extract. The TFC calculation was measured in quercetin equivalents (QE; mg/g), using quercetin as the standard.

2.5. Statistical Analysis. Values are reported as the mean ± standard error of the mean (SEM; $n = 3$). *P < 0.05, *P < 0.01, and *P < 0.001 are used to identify significant differences for extract values compared with those for ascorbic acid, two-way analysis of variance (ANOVA), followed by Dunnett’s test.

2.6. In Silico Molecular Docking

2.6.1. Protein Preparation. The 3D structure of urate oxidase (PDB: 1R4U) [40] and glutathione reductase (PDB: 3GRS) [41] was retrieved from the Protein Data Bank (pdb format) [42] to evaluate the antioxidant effect. The 3D protein structure was assembled and refined using the method described by Uddin et al. [43].

2.6.2. Ligand Preparation. Identified compounds from *S. villosa* and *V. patula* were obtained from the PubChem databases in SDF format. Ligprep (Schrödinger v11.1) was used to prepare the ligand by maintaining OPLS3 force field [44]. The possible ionization state was generated at specific pH values (7.0 ± 2.0).

2.6.3. Receptor Grid Generation. Receptor grid generation was performed in Schrödinger v11.1, using the default parameters, with the van der Waals scaling factor and charge cutoff set to 1.00 and 0.25, respectively. A cubic box was placed on the geometrical center of the selected active site of the selected receptor, and a size setting of 14 Å × 14 Å × 14 Å was used for molecular docking.

2.6.4. Glide Standard Precision (SP) Ligand Docking and MM-GBSA Calculation. Standard Precision (SP) flexible docking was performed using Glide (Schrödinger v11.1) [43, 45, 46]. The default parameters for the van der Waals scaling factor (0.80) and partial charge cutoff (0.15) were retained, and the docking score was recorded. The Schrödinger Prime MM-GBSA (OPLS3) was used for determining the binding energy of each ligand and the targeted receptor (kcal/mol) [47–49].

2.6.5. In Silico Study: Determination of Pharmacokinetic Parameters by SwissADME. The pharmacokinetic parameters used to assess the drug-likeness properties of the identified compounds were determined using SwissADME (http://www.swissadme.ch/). An orally active drug requires that each of the compounds adheres to the drug-like properties established by Lipinski and Veber’s rule [50].

2.6.6. In Silico Study: Toxicological Properties Prediction by ProTox Webserver. The toxicological properties of the compounds were predicted with the assistance of the ProTox online server. The current study evaluated the toxicity profiles of selected compounds based on mutagenicity, carcinogenicity, hepatotoxicity, and toxicity class [51].

3. Results and Discussion

3.1. GC-MS Analysis. In a previous study, four triterpenoids were isolated and identified as bauerenyl acetate (I), friedelin (II), epifriedelanol (III), 20 (30)-taraxastene-3 betas, and 21 α-diol (IV) [30]. Several phytoconstituents from *S. villosa*
Table 3: Total phenolic content (TPC) and total flavonoid content (TFC) of methanolic extract of Sterculia villosa (MESV) and methanolic extract of Vernonia patula (MEVP).

| Subject       | TPC (mg GAE/g extract) | TFC (mg QE/g extract) |
|---------------|-------------------------|-----------------------|
| MESV          | 62.58 ± 1.93            | 81.44 ± 2.70          |
| MEVP          | 58.99 ± 3.16            | 29.31 ± 6.61          |

Regression equation

\[ y = 0.0039x + 0.0406; R^2 = 0.9981 \]

\[ y = 0.0102x - 0.0637; R^2 = 0.9693 \]

Table 4: Molecular docking scores for identified compounds in Sterculia villosa.

| Sl. no. | Compounds                                      | 1R4U (kcal/mol) | 1R4U (MM-GBSA) | 3GRS (kcal/mol) | 3GRS (MM-GBSA) |
|---------|-----------------------------------------------|-----------------|-----------------|-----------------|----------------|
| 1       | Heptanal                                       | −                | −               | −1.966          | −23.399        |
| 2       | Benzaldehyde, 2-methyl                         | −2.475           | −26.2735        | −5.789          | −52.545        |
| 3       | Glucitol, 6-O-ethyl                             | −3.25            | −42.1814        | −5.897          | −62.624        |
| 4       | L-Arabinitol                                    | −3.335           | −34.4304        | −4.144          | −28.63         |
| 5       | α-Isomethyl ionone                             | −3.844           | −33.9885        | −5.359          | −33.515        |
| 6       | Eucalyptol                                      | −4.195           | −19.6369        | −4.072          | −12.525        |
| 7       | Vanillin                                        | −4.843           | −29.0303        | −5.561          | −27.446        |
| 8       | Prednisone                                      | −                | −               | −               | −              |
| 9       | Bioallethrin                                    | −2.753           | −31.6861        | −4.487          | −35.818        |
| 10      | Sorbitol                                        | −2.968           | −31.4369        | −4.206          | −36.397        |
| 11      | β-D-glucopyranose, 4-O-β-D-galactopyranosyl    | −                | −               | −               | −              |
| 12      | Santolactriene                                  | −                | −               | −3.349          | −22.086        |
| 13      | Vanillin, acetate                               | −4.874           | −29.5427        | −5.452          | −35.791        |
| 14      | Guanosine                                       | −5.706           | −42.3974        | −7.029          | −54.903        |
| 15      | Trans-11-tetradecenyl acetate                  | +1.755           | −35.467         | −0.89           | −54.627        |
| 16      | D-galactonic acid, y-lactone                    | −5.106           | −37.522         | −5.364          | −42.959        |
| 17      | Isopuleleg                                     | −3.824           | −32.1878        | −6.033          | −37.453        |
| 18      | Benzaldehyde, 4-hydroxy-3,5-dimethoxy           | −5.606           | −37.7501        | −5.56           | −29.723        |
| 19      | Naphthalene, 2-butyldecahydro                   | −                | −               | −5.423          | −28.689        |
| 20      | 3-buten-2-one, 3-methyl-4-(3,5,6-trimethy1-3-cyclohexen-1-yl)- | −3.845           | −32.3724        | −4.502          | −32.991        |
| 21      | Cis-p-metha-2,8-dien-1-ol                       | −3.743           | −21.7445        | −4.478          | −16.731        |
| 22      | Trans-sesquisabinene hydrate                   | −3.878           | −24.5582        | −4.885          | −31.788        |
| 23      | 2-methoxy-6-methyline                           | −4.43            | −26.2716        | −5.738          | −24.997        |
| 24      | β-carotene                                      | −2.503           | −48.5021        | −6.133          | −52.201        |
| 25      | Aprobarital                                     | −6.266           | −42.6018        | −4.711          | −23.22         |
| 26      | Spiro[3,4]octan-5-one                           | −4.707           | −24.2297        | −4.684          | −24.887        |
| 27      | 2-dodecen-1-yll-(succinic anhydride             | −1.704           | −41.2212        | −3.745          | −53.132        |
| 28      | Phytoil                                        | −1.004           | −48.5855        | −4.22           | −36.0226       |
| 29      | Digitoxin                                       | −6.249           | −61.3721        | −7.396          | −66.1619       |
| 30      | Chrysanthemic acid                              | −3.833           | −33.8038        | −4.539          | −32.3551       |
| 31      | n-hexadecanoic acid                             | +0.47            | −42.1025        | −0.504          | −44.2263       |
| 32      | β-asarone                                       | −4.783           | −40.5895        | −5.033          | −34.2406       |
| 33      | Benzenepropanoic acid, 2,5-dimethoxy            | −3.937           | −42.1905        | −5.359          | −39.4505       |
| 34      | Decanoic acid, 2,3-dihydroxypropyl ester        | +0.681           | −44.3904        | −0.803          | −52.772        |
| 35      | 9,12-octadecadienoic acid, methyl ester, (E)    | +0.566           | −45.4668        | −1.483          | −55.2765       |
| 36      | 7-hexadecanoic acid, methyl ester, (Z)          | +0.652           | −44.276         | −1.171          | −55.5193       |
| 37      | Citronellol                                     | −1.556           | −26.2224        | −3.03           | −24.686        |
| 38      | Undec-10-ynoic acid                             | +2.833           | −38.9058        | +2.186          | −42.1098       |
| 39      | 6-octadecenoic acid, methyl ester, (Z)          | −0.038           | −44.8963        | −0.423          | −53.3609       |
| 40      | Dodecanal                                       | +2.452           | −35.2648        | +1.413          | −40.0126       |
| 41      | Nerolidol                                       | −0.608           | −34.4431        | −2.001          | −42.6634       |
| 42      | Cyclohexane, eicosyl                            | −1.655           | −46.9117        | −2.967          | −44.6644       |
| 43      | Glycerol 1-palmitate                            | −2.579           | −43.8518        | −5.126          | −47.6573       |
| 44      | Hexadecanal                                     | +1.286           | −43.6651        | −0.438          | −46.5658       |
| 45      | Meprobamate                                     | −5.174           | −45.9961        | −5.719          | −42.9626       |
| 46      | Daucol                                          | −                | −               | −               | −              |
| 47      | Methotrexate                                    | −5.849           | −61.4026        | −8.457          | −58.4485       |
| 48      | Estradiol                                       | −5.068           | −33.4644        | −5.8            | −33.9768       |
| 49      | Octadecanoic acid, 2-hydroxy-1,3-propanediyl    | −                | −              | −1.109          | −51.6491       |
| 50      | Mebutamate                                      | −5.359           | −40.317         | −6.05           | −29.296        |
| 51      | Androsta-3,5-dien-3-ol, 17-acetyl-3-O-(t-butyl)| −                | −              | −               | −              |
| 52      | Ascorbic acid (control)                        | −4.655           | −37.8208        | −5.965          | −33.9373       |
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and V. patula were identified in the current study. The MESV and MEVP may have potential therapeutic properties due to the presence of these bioactive phytoconstituents. The study was performed using GC-MS, one of the most widely used methods for phytoconstituent separation. The investigation of MESV and MEVP via GC-MS identified 52 and 51 compounds, respectively. The major phytoconstituents in MESV included terpenoids, phytosterols, esters, acids, and other organic compounds. The major phytoconstituents in MEVP, respectively. Figures S1 and S2 depict typical chromatograms for MESV and MEVP, respectively. Figures S3 and S4 depict the typical fragmentation pattern of compounds identified from MESV and MEVP, respectively.

Table 5: Molecular docking scores for identified compounds in Vernonia patula.

| Sl. No. | Compounds                  | 1R4U (kcal/mol) | 1R4U (MM-GBSA) | 3GRS (kcal/mol) | 3GRS (MM-GBSA) |
|---------|----------------------------|-----------------|----------------|-----------------|----------------|
| 1       | Cystine                    | −4.03           | −41.7644       | −3.399          | −36.8961       |
| 2       | D-alanine                  | −5.953          | −15.5837       | −5.036          | −15.1612       |
| 3       | Propanamide                | −2.634          | −18.48         | −3.847          | −26.3392       |
| 4       | (−)-norephedrine           | −5.79           | −25.9004       | −6.047          | −29.0084       |
| 5       | Norpseudoephedrine         | −5.79           | −25.9004       | −6.047          | −29.0084       |
| 6       | DL-phenylephrine 3TMS derivative | −4.823     | −31.1556       | −5.544          | −34.4102       |
| 7       | Octodrine                  | −2.424          | −26.1646       | −3.653          | −25.4794       |
| 8       | 1,2-ethanediamine, N-(2-aminomethyl) | −2.227   | −19.1913       | −3.182          | −22.3522       |
| 9       | Chlorodifluoroacetamide    | −4.071          | −15.6083       | −5.072          | −23.672        |
| 10      | Cathine                    | −5.79           | −25.9004       | −6.047          | −29.0084       |
| 11      | Phloroglucitol             | −5.858          | −25.276        | −5.854          | −24.6614       |
| 12      | 2-octynoic acid            | −2.58           | −32.7105       | −3.556          | −29.6861       |
| 13      | Glutaraldehyde             | −3.026          | −19.8291       | −2.555          | −25.0008       |
| 14      | Methyl stearate            | +1.332          | −40.0523       | −0.447          | −51.2734       |
| 15      | Dibutyl phthalate          | −1.334          | −37.2661       | −2.763          | −45.1367       |
| 16      | Epinephrine, β−, 3TMS derivative |          |                |                |                |
| 17      | 1-dodecane                 | +5.184          | −24.7357       | +3.225          | −39.3134       |
| 18      | 10-undecenol               | +2.981          | −32.3244       | +2.797          | −34.283        |
| 19      | Phytol                     | −1.004          | −48.5855       | −4.22           | −36.0226       |
| 20      | Piperazine                 | −4.933          | −18.7799       | −4.204          | −21.361       |
| 21      | D-galactonic acid, γ-lactone | −5.106      | −37.522        | −5.364          | −42.9587       |
| 22      | 3,3′-iminoisobispolyamine  |                |                |                |                |
| 23      | Glutaraldehyde             | −3.026          | −19.8291       | −2.555          | −25.0008       |
| 24      | Hexanal                    | −1.698          | −24.1357       | −2.712          | −19.7671       |
| 25      | Folic acid                 | −6.038          | −49.7423       | −8.243          | −55.9741       |
| 26      | Undecanola                 | +1.756          | −32.3068       | +1.482          | −39.983        |
| 27      | 3,3′-dimethylpiperidin derivative |          |                |                |                |
| 28      | Nonanol                    | −1.868          | −28.3973       | −1.738          | −32.1394       |
| 29      | 1-Eicosanol                | +1.381          | −54.2076       | −2.03           | −52.5786       |
| 30      | Ascorbic acid (control)    | −4.655          | −37.8208       | −5.965          | −33.9373       |

3.2. Antioxidant Activity. Compared with the standard antioxidant, ascorbic acid, the inhibitory rate of MEVP gradually increased with increasing concentrations, ranging from 15.625 to 500 µg/ml. A similar increase in inhibition was observed for increasing concentrations of MESV in the same concentration range. The maximum scavenging activity was observed at 500 µg/ml for all the three tested compounds, with values of 52.42%, 53.89%, and 98.33% for MESV, MEVP, and ascorbic acid, respectively (Figure 1). The IC50 values for MEVP, MESV, and ascorbic acid were determined to be 305.30, 555.44, and 36.32 µg/ml, respectively.

The extracts were found to be potently active in the DPPH scavenging activity assay. The antioxidant activity of MESV and MEVP against DPPH is thought to be attributable to their hydrogen-donating capacity, suggesting that the extracts have the capacity to donate protons and can be used as primary antioxidants. The TPC and TFC were assessed using the regression equations for gallic acid \( y = 0.0039x + 0.0406; \ R^2 = 0.99981 \) and quercetin \( y = 0.0102x - 0.0637; \ R^2 = 0.99693 \), respectively. The TPC for MESV was higher (81.44 ± 2.70 mg GAE/g dry extract) than the TPC (62.58 ± 1.93 mg GAE/g dry extract; Table 3). In MEVP, the TFC was higher (291.31 ± 6.61 mg QE/g dry extract) than the TPC (58.99 ± 3.16 mg GAE/g dry extract; Table 3).

Based on the TFC and TPC assays, the S. villosa and V. patula extracts contained significant amounts of flavonoid and phenolic contents, indicating that these plants'
Table 6: Interactions and bond distances between selected compounds identified in *Sterculia villosa* and the receptors following: urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS) binding sites.

| Proteins | Ligands | Hydrogen bond interactions | Hydrophobic interactions |
|----------|---------|-----------------------------|--------------------------|
|          |         | Amino acid residue | Distance (Å) | Amino acid residue | Distance (Å) |
| Aprobarbital | ARG-176 | 2.27, 2.35 | HIS-256 | 4.39 |
|           | VAL-227 | 1.89 | LEU-170 | 5.20 |
|           | GLN-228 | 2.02, 2.02 | PHE-159 | 4.13 |
|           | — | — | SER-226 | 3.66 |
|           | LEU-163 | 2.09 | ASP-165 | 2.69 |
|           | ASP-165 | 1.79 | TYR-167 | 2.70 |
|           | TYR-167 | 2.17 | PHE-258 | 5.49, 4.36, 4.21 |
|           | ILE-177 | 2.38 | LEU-170 | 5.20, 5.39 |
| Digitoxin | — | — | GLU-259 | 2.38 |
|           | — | — | ARG-176 | 2.83, 2.34 |
|           | — | — | TYR-167 | 2.70 |
|           | — | — | LEU-170 | 5.20, 5.39 |
|           | GLU-259 | 3.06 | HIS-256 | 4.72 |
|           | HIS-256 | 1.98 | ARG-176 | 2.71 |
|           | ILE-177 | 1.88 | LEU-170 | 4.81, 4.48, 4.44 |
| 1R4U | Methotrexate | LEU-170 | 2.72 | THR-168 | 2.72 |
| | THR-169 | 2.54 | — | — |
| | THR-168 | 2.14 | — | — |
| | ASN-254 | 1.98, 2.01 | PHE-159 | 2.69 |
| | ARG-176 | 1.90 | GLN-228 | 2.42 |
| | GLN-228 | 1.96 | — | — |
| | LEU-287 | 2.50 | — | — |
| Guanosine | HIS-265 | 2.58 | ARG-176 | 2.73, 5.03 |
| | ILE-177 | 1.99 | TYR-257 | 2.34 |
| Benzaldehyde, 4-hydroxy-3, 5-dimethoxy | GLU-259 | 2.14 | — | — |
| | TYR-257 | 5.46 | TYR-257 | 6.50 |
| Ascobic acid | ILE-177 | 3.63, 4.43 | — | — |
| | HIS-256 | 4.03 | — | — |
| | GLU-259 | 4.18 | — | — |
Table 6: Continued.

| Proteins | Ligands  | Hydrogen bond interactions | Hydrophobic interactions |
|----------|----------|-----------------------------|--------------------------|
|          |          | Amino acid residue | Distance (Å) | Amino acid residue | Distance (Å) |
|          |          | PHE-181 | 5.46 | VAL-61 | 5.99, 4.88 |
| Methotrexate |            | ASP-104 | 3.81 | GLY-50 | 5.20 |
|          |            | ASN-60 | 3.18 | THR-156 | 4.62, 3.40 |
|          |            | THR-57 | 4.17 | — | — |
|          |            | GLU-50 | 4.76 | — | — |
|          |            | SER-51 | 3.01 | — | — |
|          |            | LYS-296 | 5.25, 4.24 | MET-159 | 4.99 |
| Digoxin  |            | THR-162 | 3.08 | PRO-160 | 6.97 |
|          |            | GLY-158 | 3.93 | HIS-158 | 4.87 |
|          |            | THR-156 | 4.50 | VAL-61 | 5.19 |
|          |            | GLU-50 | 3.87 | GLY-56 | 3.78 |
|          |            | — | — | GLU-50 | 4.43 |
|          |            | — | — | HIS-52 | 4.87 |
|          |            | GLU-50 | 3.76 | GLU-50 | 5.28, 5.38 |
| Guanosine |            | HOH-482 | 3.24 | GLY-56 | 3.61 |
|          |            | ASP-331 | 4.05 | THR-156 | 4.85 |
|          |            | GLY-158 | 3.87 | ALA-155 | 4.28 |
|          |            | THR-57 | 4.22, 3.74 | GLY-157 | 3.75 |
| 3GRS     |            | HOH-490 | 3.63 | GLY-330 | 3.78 |
|          |            | — | — | ALA-342 | 7.47 |
|          |            | — | — | CYS-63 | 4.63, 4.21 |
|          |            | — | — | PHE-372 | 6.26 |
| β-Carotene |          | — | — | VAL-370 | 5.21, 4.21 |
|          |            | — | — | LEU-338 | 5.55, 4.45 |
|          |            | — | — | PRO-340 | 4.49 |
|          |            | — | — | TYR-197 | 6.03 |
|          |            | — | — | HIS-52 | 6.18 |
|          |            | — | — | HIS-129 | 5.57 |
| Mebutamate |          | THR-156 | 4.74 | VAL-61 | 4.73 |
|          |            | ARG-291 | 6.02 | LYS-53 | 4.06 |
|          |            | HOH-490 | 3.68 | HIS-52 | 4.17 |
|          |            | THR-57 | 3.85 | — | — |
|          |            | ASP-178 | 4.21 | — | — |
|          |            | GLU-50 | 4.60, 4.15, 4.93 | GLY-157 | 3.71 |
| Ascorbic acid |        | HOH-490 | 3.11 | GLY-27 | 3.38 |
|          |            | THR-57 | 3.67 | — | — |
|          |            | ALA-155 | 4.10 | — | — |
phenolic components may predominantly consist of flavonoids in glycosidic forms; glycosidic flavonoids tend to concentrate in polar solvents, which are more effective than less polar solvents for the removal of phenolic compounds from plant materials [52, 53]. Previous research showed that the presence of phenolic compounds, such as flavonoids, correlates with high levels of antioxidant activity and health benefits [54].

3.3. Molecular Docking Study. The results for the molecular docking simulation study for the five compounds and control with the highest docking scores identified from *S. villosa* and *V. patula* extracts are shown in Tables 4 and 5, respectively. To evaluate the antioxidant attributes, the selected compounds from each plant extract were subjected to docking against urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS) binding sites. For the compounds identified in *S. villosa*, the five selected compounds, ordered according to docking score for urate oxidase (PDB: 1R4U), were as follows: probarbital > digitoxin > methotrexate > guanosine > benzaldehyde, 4-hydroxy-3,5-dimethoxy-. Then, the order for docking scores when docked with glutathione reductase (PDB: 3GRS) in case of compounds identified in *S. villosa* is as follows: methotrexate > digitoxin > guanosine > β-carotene > mebutamate. For *V. patula* extract, the five selected compounds according to docking scores for urate oxidase (PDB: 1R4U) were as follows: folic acid > d-alanine > phloroglucitol > (−)-norephedrine ≥

Table 7: Interactions and bond distances between selected compounds identified in *Vernonia patula* and the receptors following: urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS) binding sites.

| Proteins | Ligands | Hydrogen bond interactions | Hydrophobic interactions |
|----------|---------|----------------------------|--------------------------|
|          |         | Amino acid residue | Distance (Å) | Amino acid residue | Distance (Å) |
| Folic acid | ARG-176 | 6.43 | — | — |
| D-alanine | GLN-228 | 4.01 | — | — |
| (−)-norephedrine | TRP-160 | 1.79, 2.04 | — | — |
| Norpseudoephedrine | ALA-225 | 1.97 | — | — |
| Ascorbic acid | GLU-50 | 4.75, 3.36 | — | — |
| Folic acid | THR-57 | 3.59 | — | — |
| (−)-norephedrine | ALA-130 | 6.54 | — | — |
| Norpseudoephedrine | THP-156 | 3.73 | — | — |
| Cathine | GLU-155 | 4.10 | — | — |
| Phloroglucitol | GLU-50 | 4.93, 4.15 | — | — |
| Ascorbic acid | GLU-50 | 4.93, 4.15 | — | — |

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When the docking simulation was carried out on the compounds from *V. patula* with glutathione reductase (PDB: 3GRS), the following order of docking score was found: folic acid > (−)-norpseudoephedrine ≥ norpseudoephedrine > cathine > phloroglucitol. The molecular docking simulations between the selected compounds and the protein are further demonstrated in Tables 6 and 7. Furthermore, the 2D representations of the ligand–protein interactions are presented in Figures 2 and 3 for compounds in *S. villosa* and in Figures 4 and 5 for compounds in *V. patula*.

### 3.4. ADEME and Toxicological Study

The ADME properties are markers of pharmacokinetic characteristics and were used to assess oral bioavailability based on the Lipinski and Veber rules. The data for each compound were retrieved from the SwissADME online server, as shown in Tables 8 and 9 for *S. villosa* and *V. patula*, respectively. Digitoxin was found to violate three of the parameters, and β-carotene violated two parameters, while one parameter of the Lipinski rule was violated by methotrexate and guanosine, among compounds identified in *S. villosa*. Only folic acid, which
violated one parameter, was found to violate these rules among the compounds identified in *V. patula*.

The toxicity profiles for each of the selected compounds in the two extracts are evaluated through the ProToxon online server and are presented in Tables 10 and 11. These results showed that none of the selected compounds from *S. villosa* are associated with the hepatotoxic property. β-carotene was found to have mutagenicity while two of the compounds, namely, aprobarbital and mebutamate, were associated with carcinogenic properties among the compounds identified in *V. patula*.

In this study, molecular docking simulations were performed to associate and reciprocate the in vitro experimental findings. The extracts from *S. villosa* and *V. patula* were subjected to GC-MS to identify chemical compounds, and those compounds with potential pharmacological activity were subjected to a molecular docking simulation against urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS) to evaluate the antioxidant properties in silico. The five compounds from each plant extract with the highest docking scores against two distinctive receptors were
selected for further analysis. Aprobarbital had the highest docking score (−6.266) among the compounds identified in *S. villosa* when the docking simulation was preceded against urate oxidase (PDB: 1R4U). It exhibits a better score than the control, ascorbic acid (−4.655). Aprobarbital formed conventional hydrogen bonds with the following amino acid residues in urate oxidase: ARG-176, VAL-227, and GLN-228. Aprobarbital also forms alkyl and pi-alkyl bonds with HIS-256, LEU-170, and PHE-159 and a carbon–hydrogen bond with SER-226 when binding with the active site of urate oxidase to exert an antioxidant effect. When the compounds were docked against glutathione reductase and compared to the control, methotrexate was found to have a significant binding affinity (−8.457). When bonded with methotrexate, it formed a conventional hydrogen bond with SER-51, ASN-60, PHE-181, ASP-104, THR-57, and GLU-50. One carbon–hydrogen bond with THR-156, one amide-pi stacked bond with GLY-50, and one pi-alkyl bond with VAL-61 were also reported. Folic acid possessed the best result (−6.038) for the compounds identified in *V. patula* in case of urate oxidase (PDB: 1R4U). Folic acid formed conventional hydrogen bond (ARG-176, HIS-256, ASN-254, ILE-177, and TYR-257); (c) phloroglucitol: conventional hydrogen bond (ARG-176, GLN-228); (d) (−)-norephedrine: conventional hydrogen bond (TRP-160, ALA-225); (e) norpseudoephedrine: conventional hydrogen bond (TRP-160, ALA-225); (f) ascorbic acid (control): conventional hydrogen bond (TYR-257, ILE-177, HIS-256, GLU-259).

**Figure 4: 2D representations of the best docking scores between urate oxidase (PDB: 1R4U) and (a) folic acid: conventional hydrogen bond (GLN-228, LEU-287, HIS-256, and TYR-257), pi-alkyl bond (ARG-176), and pi-pi T-shaped bond (PHE-159); (b) D-alanine: conventional hydrogen bond (ARG-176, HIS-256, ASN-254, ILE-177, and TYR-257); (c) phloroglucitol: conventional hydrogen bond (ARG-176, ASN-254, ILE-177, and TYR-257); (d) (−)-norephedrine: conventional hydrogen bond (TRP-160, ALA-225); (e) norpseudoephedrine: conventional hydrogen bond (TRP-160, ALA-225); (f) ascorbic acid (control): conventional hydrogen bond (TYR-257, ILE-177, HIS-256, GLU-259).
Table 8: Physicochemical properties associated with good oral bioavailability for the isolated compounds from Sterculia villosa.

| Compounds                          | MW <500 | HBA <10 | HBD <5 | Log P ≤5 | Lipinski's violations <1 | nRB ≤10 | TPSA ≤140 |
|------------------------------------|---------|---------|--------|----------|--------------------------|---------|-----------|
| Aprobarbital                       | 210.23  | 3       | 2      | 0.82     | 0                        | 3       | 75.27     |
| Digitoxin                          | 764.94  | 13      | 5      | 2.61     | 3                        | 7       | 182.83    |
| Methotrexate                       | 454.44  | 9       | 5      | -0.50    | 1                        | 10      | 210.54    |
| Guanosine                          | 283.24  | 7       | 5      | -2.02    | 1                        | 2       | 139.51    |
| Benzaldehyde, 4-hydroxy-3,5-dimethoxy| 182.17  | 4       | 1      | 0.93     | 0                        | 3       | 55.76     |
| Mebutamate                         | 232.28  | 4       | 2      | 1.05     | 0                        | 8       | 104.64    |
| β-carotene                         | 536.87  | 0       | 11.11  | 0        | 2                        | 10      | 0         |

Here, MW, molecular weight (g/mol); HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; Log P, lipophilicity; nRB: number of rotatable bonds; TPSA: topological polar surface area.
of the compounds having the highest docking score were significantly better than the control in terms of ligand–protein interaction. This suggested that these compounds have good potential as a promising antioxidant agent. All of the compounds were also subjected to the evaluation of ADME and toxicological properties. The Lipinski rule of five states that orally administered agents should have the following properties: molecular weight <500 amu, hydrogen bond acceptor sites <10, hydrogen bond donor sites <5, and lipophilicity value (Log \( P \)) ≤ 5. Veber’s rules recommend a number of rotatable bonds ≤ 10 and topological polar surface area ≤ 140. According to these rules, a compound or potential medicinal agent cannot violate all of the parameters while still presenting good oral bioavailability [55, 56]. Among the two compounds with the highest docking scores identified for each plant species, aprobarbital did not violate any of the parameters, whereas folic acid and methotrexate had violated one parameter of the Lipinski rule of five, which is within the acceptable range. Thus, these compounds are considered safe for in vivo administration to an animal model. In the \( S. \) villosa extract, all other compounds, except digitoxin, methotrexate, guanosine, and \( \beta \)-carotene, also met all of the criteria for the Lipinski rule. In the \( V. \) patula extract, all compounds meet all components of the rules except for folic acid. Additionally, the toxicity prediction showed that none of the identified compounds in \( S. \) villosa is played hepatotoxicity. When it comes to carcinogenicity, all of the compounds were free from carcinogenic properties except aprobarbital and mebutamate. In addition to that, only \( \beta \)-carotene was associated with mutagenic properties. By contrast, in the \( V. \) patula extract, all of the compounds were free from mutagenic, carcinogenic, and hepatotoxic properties. Therefore, the selected compounds from \( S. \) villosa and \( V. \) patula may represent promising antioxidant agents, as further supported by the molecular docking study.

4. Conclusions
This study reported the potential antioxidant effects of methanolic bark extract of \( S. \) villosa and methanolic whole-plant extract of \( V. \) patula; this might be due to their chemical constituents. These chemical compounds may offer antioxidant activities, as assessed by the molecular
docking study. Further advanced studies remain necessary to identify the potential compounds responsible for antioxidant activities displayed by these two plants, *S. villosa* and *V. patula*.

**Data Availability**

Available data are presented in the manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest.

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**Supplementary Materials**

The following are available online at http://www.mdpi.com/xxx/s1. Figure S1: total ionic chromatogram (TIC) of methanol extract of Sterculia villosa (MESV). Figure S2: total ionic chromatogram (TIC) of methanol extract of Sterculia patula (MEVP). Figure S3: fragmentation pattern of compounds identified from the methanol extract of Sterculia villosa (MESV). Figure S4: fragmentation pattern of compounds identified from the methanol extract of Sterculia patula (MEVP). (Supplementary Materials)

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