Volatile Compounds and Bioactivities of Non Polar Extracts of Horsfieldia macrothyrsa leaves and twigs

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Abstracts. The purpose of this study is to ascertain the chemical composition of Horsfieldia macrothyrsa leaves and twigs by using Gas Chromatography/Mass Spectrometry (GC/MS) to extract the hexane (non-polar) fraction, which has antioxidant, antidiabetic, and anticancer properties against the MCF-7 breast cancer cell line. As per the findings, the primary constituents of this fraction were fatty acids and sesquiterpenes, with the other constituents being sesamin (13.32% twigs), palmitic acid (20.58% leaves), squalene (2.7% twigs), and linolenic acid (25.56% leaves). According to the antidiabetic activity test, the hexane fraction of H. macrothyrsa twigs exhibited an IC50 of 23.230 ± 0.22 µg/mL. In contrast, the antioxidant values of the leaves and twigs ranged from 50 µg/mL. The breast cancer cell line MCF-7 exhibited cytotoxic capability in an in vitro anticancer assay.

1 INTRODUCTION

Horsfieldia is a major genus from the Myristicaceae family[1], with 11% of its species thriving in South Asia [2]. The species Horsfieldia has been utilized in traditional medicine to treat a variety of illnesses, including cytotoxic [3–7], inflammatory [1, 8], antidiabetic [9–11], and antioxidant [9, 10-12].

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According to earlier research on the genus’s chemical constituents, Horsfieldia is known to have a large amount of lignans and flavonoids [1, 3, 4, 5, 7, 9, 10, 13-15, 17]. An unusual class of flavonoids with a linear C6-C3-C6 structure is diarylpropanes (DAPs) [1, 3, 5, 6, 7, 13, 14, 15], also known as reduced chalcones. This encouraging genus’s chemical composition has consequently seldom been investigated in twigs or leaves [18].

The body's overabundance of free radicals can lead to degenerative diseases, including cancer, diabetes, and heart disease. However, consuming more antioxidants can lower the chance of developing these conditions [19]. In particular, the anti-cytotoxic, anti-inflammatory action of H. macrothyrsa species is one of the little-studied bioactivity profiles of compounds isolated from the genus Horsfieldia.

2 MATERIALS AND METHODS

2.1 Plant Materials

Leaves and twigs of H. macrothyrsa were collected from Cibinong, West Java, Indonesia in March 2023. A voucher specimen 04-SHU-12-2023 was determined at determined at the Traditional Medicine Raw Material Standarization Laboratory- BRIN.

2.2 Instrumentation and Chemicals

Instrumentation used in this study were glassware, extractor, rotavapor (Buchi R214-Switzerland), micropipette (humapette, Germany), ovens, UV-Vis spectrometer (Hitachi U-2000, Series No. 0372-026). Chemicals used were n-hexane, ethyl acetate, n-butanol, methanol pa. (E. Merck), DPPH (1,1-diphenyl-2-picrylhidrazil) (Aldrich), quercetin (positive control), Dragendorff reagent, Chloroform, Anhydrous acetic acid, sulfuric acid,10%, FeCl3 solution, HCl, 1 N NaOH, Sodium carbonate. Mayer reagents, iron (III) chloride Mg metal Lieberman-Bouchard reagents, TPTZ, trolox, ABTS (TCI), potassium sulphate.

2.3 Methods

2.3.1 Extraction and isolation

The dried powdered H. macrothyrsa leaves (650 g) and twigs (374.8 g) were macerated at room temperature with a methanol solvent (3 x 5 L). The filtrate was evaporated under a vacuum to obtain methanol leaves extracts (163.25 g) and twigs (33.387 g). The extracts of methanol leaves (100g) and twigs (100g) partitioned with n-hexana solvent. The n-hexane fraction was evaporated to give hexane oily residue leaves (LH) 13.129 g and twigs (TH) 1.6338g. After obtaining the hexane fraction, a GC/MS spectrometer was used to determine its chemical composition. Antioxidant, anti-diabetic, and MCF-7 bioactivity tests were subsequently conducted. Solvents used for extraction and preparative chromatography were of technical grade and distilled before use.

2.3.2 Phytochemical Screening

Phytochemical screening is a qualitative technique to reliably assess the concentration of secondary metabolite groups in the methanol extract and hexane fraction. Six categories of secondary metabolites were targeted for confirmation: alkaloids, which were confirmed with...
Mayer reagents; phenolic, which were confirmed with iron (III) chloride solutions; flavonoids, which were confirmed with magnesium metal and a few drops of HCl (p); saponin, which were confirmed by vigorously shaking the sample solution by hand; terpenoids and steroid Reagents for Lieberman-Bouchard [20].

2.3.3 Preparation of GC-MS

Examination of the extraction of the n-hexane fraction using Gas Chromatography-Mass Spectrometry (GC-MS). Utilizing an Agilent Technologies 7890 B mass detector and a DB-5 capillary column (5% diphenyl and 95% dimethylpolysiloxane) with a length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 μm, GC-MS analysis of the n-hexane fraction was performed. One milli Liter per minute of ultrapure helium was utilized as the carrier gas. At 250 °C, the injector and interface were. The column temperature was designed to increase at 10 °C per minute between 40 and 280 °C. Initial and ultimate temperatures were kept for one and five minutes, respectively. The prominent peaks were identified by comparing the mass fragment patterns with standard spectra found in the NIST collection.

2.3.4 Determination of antioxidant activities

Three antioxidant tests DPPH, ABTS, and FRAP were used in these investigations. Since those methods yield rapid, repeatable findings and only call for relatively standard equipment, they have been frequently employed to assess the antioxidant capabilities of plant extracts. The antioxidant activity (DPPH) [21] by [22] with little modification. The variation of concentration (100, 50, 25, 10 ppm) repeated three times, added with 0.5 mL of 1 mM DPPH in MeOH. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 517 nm. Radical ABTS was prepared by mixing ABTS (7 mM) and potassium persulfate (140 mM) in the dark for 18 hours. The ABTS reagent was diluted in water (1:3, v/v) until it reached an absorbance value of 0.7 at 734 nm [23]. The FRAP radical scavenging assay was done based on a previously described protocol (2012) [24]. The absorption of mixtures was recorded from measured 593 nm.

2.3.5 Method α-Glucosidase Inhibitory Activity Assay

The α-glucosidase inhibitory activity test was assessed chiefly per the prior methodology [25]. The concentration variations used 100, 50, 25, 10, 5 ppm. Using linear regression analysis, the IC50 value is determined from the primary resistance value. The usual reference was a Quercetin.

2.3.6 Method Preparation of cancer cell line

Breast carcinoma (MCF-7) was the cancer cell line employed in this investigation. Seven concentrations (4-300 ppm) and established protocols [26, 27] were employed in the trials. Using a linear regression analysis between sample concentration and % survival, the IC50 of the samples was determined. Positive control was provided with antimycin

\[
\% \text{Viability} = \frac{\text{Optical Density (cells + sample)} - \text{Optical Density (negative control)}}{\text{Optical Density (cells) - Optical Density (negative control)}} \times 100
\]
3 RESULTS AND DISCUSSION

The extracts of *H. macrothyrsa* leaves and twigs that were non-polar (n-hexane) yielded yields of 2.07% and 0.45% following the evaporation procedure. The secondary metabolite content of the *H. macrothyrsa* leaves and twig extracts was determined by phytochemical screening, a qualitative technique. Alkaloids, flavonoids, saponins, and terpenoids chemical groups were found in *H. macrothyrsa* leaves hexane (LH) extracts, whereas twig (TH) extracts had lower levels of flavonoids, terpenoids, and saponins than leaf extracts. This information is shown in Table 1. This demonstrates that, in comparison to twig extracts, leaves extracts contain far more chemicals. The saponins test showed more foam in the leaves extracts than in the twigs, suggesting that the twig extracts from *H. macrothyrsa* exhibited lower antibacterial activity.[28]

Table 1. Phytochemical screening of *H. macrothyrsa* leaves and twigs hexane fraction extracts

<table>
<thead>
<tr>
<th>No</th>
<th><em>H. macrothyrsa</em></th>
<th>Alkaloid Mayer</th>
<th>Dragendorff</th>
<th>Bouchardat</th>
<th>Flavonoids</th>
<th>Terpenoids</th>
<th>Tanins</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TW</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>LH</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Noted: TH = (Twigs Hexane), LH = (Leaves Hexane), + = (weak colour), ++ = (strong colour), - = (no colour)

The chemical composition of *H. macrothyrsa* leaves and twigs of hexane fraction was analyzed using the GC-MS technique. The chromatogram of the GC-MS experimental results is shown in Fig.1 and Fig.2. The GC-MS analysis of the *n*-hexane extract has led to the assistance and to 20 different compounds (with similarity > 98) presented in Table 2.

![Fig. 1. Chromatogram of GC-MS analysis from Twigs Hexane (TH) of *H. macrothyrsa* extracts](image-url)
RESULTS AND DISCUSSION

The extracts of *H. macrothyrsa* leaves and twigs that were non-polar (n-hexane) yielded yields of 2.07% and 0.45% following the evaporation procedure. The secondary metabolite content of the *H. macrothyrsa* leaves and twig extracts was determined by phytochemical screening, a qualitative technique. Alkaloids, flavonoids, saponins, and terpenoids chemical groups were found in *H. macrothyrsa* leaves hexane (LH) extracts, whereas twig (TH) extracts had lower levels of flavonoids, terpenoids, and saponins than leaf extracts. This information is shown in Table 1. This demonstrates that, in comparison to twig extracts, leaves extracts contain far more chemicals. The saponins test showed more foam in the leaves extracts than in the twigs, suggesting that the twig extracts from *H. macrothyrsa* exhibited lower antibacterial activity.

![Fig. 2. Chromatogram of GC-MS analysis from LeavesTwigs Hexane (LH) of *H. macrothyrsa* extracts](image)

These studies indicate that the primary constituents of the *n*-hexane fraction of *H. macrothyrsa* leaves and twigs are the following compounds: sesamin, palmitic acid, squalene, linolenic acid, methyl stearate, and sarcosine. These chemicals demonstrate the primary constituents of the hexane fraction from the twigs and leaves of *H. macrothyrsa* as the fatty acid and sesquiterpene groups [29]. Fig. 1 and 2 display the standard spectra from the NIST collection, which were compared to the fragment mass patterns of the critical peak chemicals. However, the main ingredients from *Horsfieldia* twigs include copaene, linolenic acid, and octadecenoic acid [30].

**Table 2. Chemical composition hexane fraction of *H. macrothyrsa* leaves and twigs**

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>% Content</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>twigs</td>
<td>leaves</td>
</tr>
<tr>
<td>1</td>
<td>Sesamin</td>
<td>13.32</td>
<td>2.20</td>
</tr>
<tr>
<td>2</td>
<td>Hydrazinecarboxamide</td>
<td>0.82</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>palmitic acid</td>
<td>12.25</td>
<td>20.58</td>
</tr>
<tr>
<td>4</td>
<td>7 Octadecenoic acid</td>
<td>9.42</td>
<td>9.42</td>
</tr>
<tr>
<td>5</td>
<td>9,12 Octadecadienoic acid</td>
<td>7.05</td>
<td>8.34</td>
</tr>
<tr>
<td>6</td>
<td>linolenic acid</td>
<td>-</td>
<td>25.56</td>
</tr>
<tr>
<td>7</td>
<td>Squalene</td>
<td>2.70</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Methyl stearate</td>
<td>1.91</td>
<td>5.38</td>
</tr>
<tr>
<td>9</td>
<td>Ortetamine</td>
<td>1.78</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Tetracosanoic acid</td>
<td>1.59</td>
<td>1.42</td>
</tr>
<tr>
<td>11</td>
<td>Fluoxetine</td>
<td>1.53</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Eicosane</td>
<td>-</td>
<td>1.20</td>
</tr>
<tr>
<td>13</td>
<td>Phenylephrine</td>
<td>-</td>
<td>1.23</td>
</tr>
</tbody>
</table>
The n-hexane fraction's cytotoxic activity (Fig. 3) was significantly active at IC₅₀ 170.82 µg/mL (LH) and 130.76 µg/mL (TH), with antimycin serving as a positive control (1.24 µg/mL). The presence of several lignans, including sesamin, in this fraction might cause its poor activity [4, 31, 32]. In contrast to its modest anti-cancer action, the lignan group exhibits several biological activities, including anti-inflammatory and antibacterial properties [32].

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>IC₅₀ LH (µg/mL)</th>
<th>IC₅₀ TH (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Heptasiloxane</td>
<td>1.20</td>
<td>15.241</td>
</tr>
<tr>
<td>15</td>
<td>Heptadecanoic acid</td>
<td>1.09</td>
<td>1.44</td>
</tr>
<tr>
<td>16</td>
<td>Adenosine</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Sarcosine</td>
<td>0.72</td>
<td>0.41</td>
</tr>
<tr>
<td>18</td>
<td>Cyclotetracosane</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Piperazine</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Phytol</td>
<td>-</td>
<td>3.71</td>
</tr>
</tbody>
</table>

**Fig. 3.** Percent viability of cytotoxic activities of hexane fraction *H. macrothyrsa* leaves (LH) and twigs (TH) extract against MCF-7 cell lines

The tannin concentration of *H. macrothyrsa* will influence antioxidant activity since tannins have biological action as antioxidants. While *H. macrothyrsa* leaves and twigs extracts did not contain tannin in the phytochemical test, they almost had the same inhibition values of 53.960 ± 0.9 and 54.502 ± 1.3 µg/mL in the DPPH method antioxidant activity test. This number nevertheless permits the hexane fraction extracts of *H. macrothyrsa* to suppress the activity of the twigs and leaves [33].

This study reportedly presents the first comparative analysis of several edible portions of the *Myristicaceae* family. The study's findings indicate that *H. macrothyrsa* leaves and twigs extract exhibit more antioxidant activities than DPPH techniques when subjected to FRAP and ABTS techniques. Phenolic compounds from lignans may be the primary source of the more significant main antioxidant activity of the twigs extract *H. macrothyrsa*. The phenylpropene group is responsible for low-density lipoproteins' most potent antioxidant activities [34] and the maximum DPPH radical scavenging activity [35].
Table 3. Inhibition Concentration (IC50) antioxidant and antidiabetic hexane fraction of *H. macrothyrsa* leaves and twigs extracts

<table>
<thead>
<tr>
<th><em>H. macrothyrsa</em></th>
<th>IC50 (μg/mL)</th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>50.303 ± 2.32</td>
<td>54.502 ± 1.3</td>
<td>5.75 ± 0.05</td>
<td>14.585 ± 0.02</td>
</tr>
<tr>
<td>TH</td>
<td>23.230 ± 0.22</td>
<td>53.960 ± 0.9</td>
<td>4.829 ± 0.27</td>
<td>1.85 ± 0.22</td>
</tr>
</tbody>
</table>

One of the flavonoid molecules with the reputation of being antidiabetic is quercetin. Quercetin may function as a glucose transport inhibitor, which lowers blood glucose levels by absorbing glucose in the small intestine [38, 39]. Blood sugar levels are said to drop due to blocking α-glucosidase, which lowers postprandial hyperglycemia, the condition that leads to diabetes [40]. Quercetin (IC50 3.875 ± 0.28 μg/mL), a xanthone group with α-glucosidase inhibitory action, was employed as the positive control in this study [41].

4 CONCLUSION

The use and exploitation of *H. macrothyrsa* have been given scientific support, according to data on the application of various phytochemical substances found in the references. Nevertheless, before such medications are advised, further research is necessary. When the hexane extract of the twig portion of *H. macrothyrsa* is compared to the leaf part, antioxidant, anti-diabetic, and anti-cancer actions are demonstrated; this combination can be employed as a natural medicine candidate.

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