Chemical Profile and Biological Activities of Essential oil from *Artabotrys hexapetalus* (L.f.) Bhandari Grown in Southern Parts of Western Ghats

VIMALADEVI KRISHNASAMY1*, SELLADURAI MADHIYAN2, POONKODI KATHIRVEL1*, PRABHU VELLIANGIRI1, MINI RAMAN1 and ABINAYA ANANDHAN1

1,2Department of Chemistry, Nallamuthu Gounder Mahalingam College Pollachi-642001, Tamil Nadu, India.
*Corresponding author E-mail: k.vimala83@gmail.com, ngmvac2020@gmail.com

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ABSTRACT

In this investigation, we assessed the composition of the essential oil from the leaves of *Artabotrys hexapetalus* (L.f.) Bhandari, *in vitro* antioxidant and anticancer activities. The hydrodistilled essential oil from *A. hexapetalus* leaves cultivated in the southern Western Ghats was evaluated by GC/MS for its chemical composition. 34 compounds were present, according to the results of GC/MS analysis. The predominant constituents include Caryophyllene (17.2%), Copaene (12.9%), α-Bisabolene (8.3%), Biocyclogermacrene (6.3%), α-Cadinol (6.2%), β-Myrcene (5.7%), β-3-Carene (5.3%), and γ-Muurolene (4.9 %). The minor constituents are Gurjunene (3.5%), Longipinane (3.5%), Patchoulene (3.1%), Trans cadinal (2.8%), Ledol (1.4%), α-Phellandrene (1.3%), and Patchouli alcohol (1.3%). The DPPH and ABTS assays were used to measure the antioxidant activity of the *A. hexapetalus* essential oil, with ascorbic acid as a reference. The essential oil demonstrated antioxidant activity by having IC$_{50}$ values of 104 and 122 µL/mL, respectively. Further the essential oil has tested its *in vitro* anticancer potential using the MTT assay on the HeLa cancer cell line and showed significant anticancer activity with an IC$_{50}$ value of 36.7 µg/mL.

Keywords: *Artabotrys hexapetalus*, GC/MS, DPPH, ABTS, MTT, HeLa cell line.

INTRODUCTION

The Western Ghats hilly region is home to a large number of highly valuable medicinal plants1. Southern parts of Western Ghats have great diversity of plants with varied ethno medicinal uses and economical importance2. The genus *Artabotrys* are one of the comparatively big genera of the Annonaceae and is composed of 100 species scattered in Africa and Asia3,4. *Artabotrys hexapetalus* (L.f.) Bhandari is inherent to India, is commonly dispersed in China, and is used to medicate malaria in Chinese medicine5. The fruit and bark are used to cure colic, dysentery, ulcers, tumors, amenorrhea, dysmenorrhea, bruises, cuts, aches, sprains, inflammation, gout, helminthiasis, and diarrhea6. Alkaloids, sesquiterpenes, flavonoids, fixed oils and volatile oils are only a few of the chemical compounds that have been discovered to exhibit a variety of pharmacological effects7. The extract
of leaves used as an antimicrobial, anti-fertility, muscle relaxant and cardiac stimulant. Antioxidants are created by the human body through a number of mechanisms that either occur naturally in the body or are supplied externally through diet and/or supplements to the body. A cellular redox imbalance brought on by oxidative anxiety has been reported to be present in a different types of cancer cells as opposed to normal cells. Despite extensive research and survey efforts, there are a few findings about the antioxidant and anticancer properties of the essential oil from the leaves of *A. hexapetalus*. Therefore, the objective of the current study is to evaluate the chemical composition, antioxidant and anticancer properties of the essential oil from *A. hexapetalus* plant that is native to India.

MATERIALS AND METHODS

Plant material

Fresh leaves of *A. hexapetalus* were collected near Pollachi, Tamil Nadu, South India. The plant sample was identified and authenticated by Botanical Survey of India, Coimbatore.

Isolation of essential oil from *A. hexapetalus* leaves

*A. hexapetalus* leaves were subjected to hydrodistillation for 4 hours. The water content was removed using anhydrous sodium sulphate before being subjected to GC/MS analysis. In order to obtain the necessary amount of oil for further examination, the essential oil extraction procedure was repeated.

Gas chromatography-Mass spectrometry analysis

An Agilent GC 7890A gas chromatograph, coupled with MS5975C mass spectrometer running in Electron Ionization mode at 70 eV, coupled with injector and a flame ionisation detector, was used for the analysis of the essential oils. The capillary column was an Agilent DB5MS (30m 0.25mm; film width, 0.25m) and the carrier gas was helium (1 mL/minute). The temperature settings had a split ratio of 1:10 and extended from 60 to 280°C at a rate of 3°C/min and 60 to 260°C at a rate of 3°C/min, respectively. Identification of constituents was performed on the basis of Retention indices and mass spectra compared with those of authentic samples and NIST library version 2.0 g. The DPPH radical scavenging activity

The DPPH assay was carried out as previously described. The essential oil were blended with 1 mL of DPPH and then mixed with MeOH. The samples ranged in concentrations from (25, 50, 75, 100, 150 μL/mL). The absorbance of the mixture was measured at 517nm by UV-Vis Spectrophotometer, 3 mL of DPPH was taken as control.

ABTS+ Decolorization Assay

It was performed using an enhanced ABTS decolorization technique that has been utilised for both lipophilic and hydrophilic substances. The sample concentrations varied from 25, 50, 75, 100, 150 μL/mL respectively. The antioxidant activity of the essential oil was determined using the following formula.

\[
\%\text{Inhibition} = \left(\frac{\text{Ac}-\text{As}}{\text{Ac}}\right) \times 100
\]

In-vitro Anticancer Activity

The effect of essential oil of *A. hexapetalus* on HeLa cells was assessed by MTT assay to determine its *in-vitro* anticancer activity. The anticancer activity was evaluated according to Mosmann. The cells (2×105 cells) were exposed with various concentrations of essential oil (20, 40, 60, 80, and 100 μg/mL) separately and incubated at 37°C for 48 h means of a CO2 incubator. The test was performed in triplicates for accuracy.

RESULTS AND DISCUSSION

The presence of phytochemical constituents of essential oil of *A. hexapetalus* leaves were analyzed by GC/MS method. The GC/MS analysis indicated the presence of 34 compounds. The major compounds are Caryophyllene (17.2%), Copaene (12.9%), α-Bisabolene (8.3%), Biocyclogermacrene (6.3%), α-Cadinol (6.2%), β-Mycrene (5.7%), β-3-Carene (5.3%), γ-Muurolene (4.9%) and the minor compounds are β-Gurjunene (3.5%), Longipinane (3.5%), α-Patchoulene (3.1%), Trans cadina (2.8%), Ledol (1.4%), α-Phellandrene (1.3%), Patchouli alcohol (1.3%), were present in *A. hexapetalus* leaves essential oil. Results are given in Table 1.
According to the literature, the essential oil obtained from Ujjain origin contains different constituents like 2,5-dimethyl tetra decahydro phenethrene (33.02%), nonanoic acid (19.25%), 2-amino-3-ethyl biphenyl (19.08%)\(^{16}\). The essential oil from \textit{A. hexapetulus} in Vietnam contains caryophyllene oxide (31.5%), \(\beta\)-caryophyllene (11.4%), humulene epoxide (10.0%), \(\alpha\)-copaene (8.1%)\(^{17}\). The essential oil from \textit{A. hexapetulus} in southern Karnataka contains major products are 3-Carene (44.91%), \(\beta\)-caryophyllene (19.17%), \(\alpha\)-humulene (8.78%), \(\alpha\)-copaene (6.59%) and caryophyllene oxide (5.55)%\(^{18}\). The obtained results are almost similar in Thailand, Vietnam and southern Karnataka origin. Since there were some differences in their composition, due to climatic and geographical changes\(^{19}\).

\textbf{In-vitro Antioxidant Activity}

The current study examined the scavenging radical efficiency of the essential oil from \textit{A. hexapetulus} leaves using DPPH and ABTS\(^+\) assays. The results showed that the essential oil had antiradical action, with IC\(_{50}\) values of 104 \(\mu\)L/mL and 122 \(\mu\)L/mL, respectively, as shown in Tables 2 and 3. Ascorbic acid was used as standard with IC\(_{50}\) values of 33.8 and 44.2 \(\mu\)L/mL. From the results, the essential oil from \textit{A. hexapetulus} leaves has potent antioxidant activity, which may be because it contains a variety of complex terpenes.

\begin{table}[h]
\centering
\caption{Chemical composition of essential oil of \textit{A. hexapetulus} leaves}
\label{tab:chemical_composition}
\begin{tabular}{llllll}
\hline
S. No & Name of the compound & R.T & RI estimated & RI reported & % Composition \\
\hline
1 & -Myrcene & 4.909 & 982 & 988 & 5.7 \\
2 & \(\alpha\)-Phellandrene & 5.242 & 998 & 1002 & 1.3 \\
3 & -3-Carene & 5.620 & 1010 & 1008 & 5.3 \\
4 & \(\alpha\)-Terpinene & 5.809 & 1016 & 1014 & 0.7 \\
5 & -Ocimene & 6.031 & 1030 & 1032 & 0.7 \\
6 & Isobutyl hexanoate & 6.609 & 1145 & 1149 & 0.2 \\
7 & \(\alpha\)-Cubebene & 7.042 & 1342 & 1345 & 0.8 \\
8 & Copae & 7.331 & 1376 & 1374 & 12.9 \\
9 & \(\alpha\)-Santalene & 8.431 & 1413 & 1416 & 0.1 \\
10 & -Gurjene & 9.986 & 1429 & 1431 & 3.5 \\
11 & \(\alpha\)-Patchoulen & 10.375 & 1450 & 1454 & 3.1 \\
12 & Caryophyllene & 10.542 & 1463 & 1466 & 17.2 \\
13 & Geranyl propanoate & 10.986 & 1473 & 1476 & 0.2 \\
14 & \(\gamma\)-Murolene & 11.364 & 1481 & 1478 & 4.9 \\
15 & Bicyclogermacrene & 11.508 & 1502 & 1500 & 6.3 \\
16 & \(\alpha\)-Bisabolene & 12.097 & 1506 & 1505 & 8.3 \\
17 & Quinoline & 12.166 & 1512 & 1510 & 0.7 \\
18 & Trans codina & 12.530 & 1536 & 1533 & 2.8 \\
19 & Hexenyl benzoate & 12.675 & 1564 & 1565 & 0.6 \\
20 & Ledol & 12.864 & 1608 & 1602 & 1.4 \\
21 & Aromadendrene epoxide Bicyclogermacrene & 12.930 & 1641 & 1639 & 0.8 \\
22 & Longipiane & 13.064 & 1646 & - & 3.5 \\
23 & \(\alpha\)-Cadinol & 13.219 & 1650 & 1652 & 6.2 \\
24 & Patchouli alcohol & 13.375 & 1658 & 1656 & 1.3 \\
25 & Cedren-13-ol<8-> & 13.475 & 1684 & 1688 & 0.9 \\
26 & Heptadecane & 13.586 & 1697 & 1700 & 0.5 \\
27 & Farnesol & 13.741 & 1712 & 1714 & 0.3 \\
28 & Methyl tetradecanoate & 14.119 & 1724 & 1722 & 0.2 \\
29 & Nonadecane & 14.486 & 1892 & 1900 & 0.5 \\
30 & Phyto & 15.341 & 1947 & 1942 & 0.7 \\
31 & Eicosane & 16.174 & 2012 & 2000 & 0.2 \\
32 & Heneicosane & 17.041 & 2108 & 2100 & 0.4 \\
33 & Tetracosane & 22.262 & 2412 & 2400 & 0.2 \\
34 & Heptacosane & 23.685 & 2691 & 2700 & 0.4 \\
\hline
Total identified & & & 92.8 & \\
Monoterpene hydrocarbons & & & 13.7 & \\
Sesquiterpenes hydrocarbons & & & 64.8 & \\
oxxygenated compounds & & & 11.4 & \\
Non-terpenes & & & 2.9 & \\
\hline
\end{tabular}
\end{table}
Earlier research revealed, the \textit{In vitro} antioxidant activity of ethanolic extract of flowers of \textit{A. hexapetalus} was investigated using ABTS+ radical, nitric oxide radical, reducing ability, and scavenging of Hydrogen peroxide showed IC\textsubscript{50} values of 280, 200, 130, 230 μg/mL respectively\textsuperscript{20}. Meanwhile, methanol extract of \textit{A. hexapetalus} leaves were investigated using DPPH assay also produced significant results\textsuperscript{21}. We believe that this is the first kind of study to assess the antioxidant activity of \textit{A. hexapetalus} essential oil.

\textbf{Table 2: \textit{In-vitro} antioxidant activity of essential oil of \textit{A. hexapetalus} leaves-DPPH Assay}

<table>
<thead>
<tr>
<th>Concentration (μL/mL)</th>
<th>DPPH % inhibition</th>
<th>Standard % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>11.6</td>
<td>35.1</td>
</tr>
<tr>
<td>50</td>
<td>21.5</td>
<td>64.2</td>
</tr>
<tr>
<td>75</td>
<td>35.0</td>
<td>73.5</td>
</tr>
<tr>
<td>100</td>
<td>49.2</td>
<td>89.2</td>
</tr>
<tr>
<td>150</td>
<td>73.1</td>
<td>93.2</td>
</tr>
<tr>
<td>\textit{IC}_{50} (μL/mL)</td>
<td>104 (μL/mL)</td>
<td>33.8 (μL/mL)</td>
</tr>
</tbody>
</table>

\textbf{Table 3: \textit{In-vitro} antioxidant activity of essential oil of \textit{A. hexapetalus} leaves-ABTS Assay}

<table>
<thead>
<tr>
<th>Concentration (μL/mL)</th>
<th>ABTS + % inhibition</th>
<th>Standard % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>10.8</td>
<td>34.4</td>
</tr>
<tr>
<td>50</td>
<td>16.2</td>
<td>59.9</td>
</tr>
<tr>
<td>75</td>
<td>28.5</td>
<td>87.5</td>
</tr>
<tr>
<td>100</td>
<td>40.1</td>
<td>90.1</td>
</tr>
<tr>
<td>150</td>
<td>62.8</td>
<td>95.2</td>
</tr>
<tr>
<td>\textit{IC}_{50} (μL/mL)</td>
<td>122 (μL/mL)</td>
<td>44.2 (μL/mL)</td>
</tr>
</tbody>
</table>

\textbf{In-vitro anticancer activity}

The MTT assay was used in this work to assess the anticancer efficacies of the essential oil from \textit{A. hexapetalus} leaves against HeLa. The obtained results showed that the essential oil demonstrated effective anticancer performance. This might be explained by their capacity to penetrate cell membranes, interact with, and alter proteins and other macromolecules. The \textit{IC}_{50} concentration for essential oil was 36.7 μg/mL, indicating good efficacy of essential oil in the therapy of cancer. Fig.1 demonstrate the increased cytotoxic effectiveness of essential oils.

Similarly, based on the literatures, other scientists found stronger anticancer efficacies of crude extracts of \textit{A. hexapetalus} roots, stems, and leaves\textsuperscript{22}. This is the first kind of report for anticancer potential of \textit{A. hexapetalus} essential oil extracted in South India.

\textbf{CONCLUSION}

The following conclusions could be made based on the aforementioned findings. The high effectiveness of essential oil of \textit{A. hexapetalus} leaves may used as anticancer agents. Remarkable antioxidant performance were observed with DPPH and ABTS+ assays at 104 μL/mL and 122 μL/mL respectively. In addition to that, the GC-MS analysis yield Caryophyllene (17.2%), Copaene (12.9%), \textalpha;-Bisabolene (8.3%) as major components. Further studies are under progress.

\textbf{ACKNOWLEDGEMENT}

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