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

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ABSTRACT

In this invistigation, we assessed the composition of the essential oil from the leaves of *Artabortrys 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%), βMyrcene (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: *Artabortrys 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 plants[1]. Southern parts of Western Ghats have great diversity of plants with varied ethno medicinal uses and economical importance[2]. The genus Artabotrys are one of the comparatively big genera of the Annonaceae and is composed of 100 species scattered in Africa and Asia[3,4]. *Artabortrys hexapetalus* (L.f.) Bhandari is inherent to India, is commonly dispersed in China, and is used to medicate malaria in Chinese medicine[5]. The fruit and bark are used to cure colic, dysentery, ulcers, tumors, amenorrhea, dysmenorrhea, bruises, cuts, aches, sprains, inflammation, gout, helminthiasis, and diarrhea[6]. 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 effects[7]. The extract

of leaves used as an antimicrobial, anti-fertility, muscle relaxant and cardiac stimulant[8]. 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[9]. 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 Ionozation 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[10-12].

DPPH radical scavenging activity

The DPPH assay was carried out as previously described[13]. 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[14]. 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.

$$\%\text{Inhibition} = [(Ac-As)/Ac]\times 100$$

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. T[15]. 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 CO_2 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%), βMyrcene (5.7%), β3-Carene (5.3%), γ-Muurolene (4.9%) and the minor compounds are βGurjunene (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.

Table 1: Chemical composition of essential oil of *A. hexapetalus* leaves

S. No	Name of the compound	R.T	RI estimated	RI reported	% Composition
1	βMyrcene	4.909	982	988	5.7
2	α-Phellandrene	5.242	998	1002	1.3
3	β3-Carene	5.620	1010	1008	5.3
4	α-Terpinene	5.809	1016	1014	0.7
5	βOcimene	6.031	1030	1032	0.7
6	Isobutyl hexanoate	6.609	1145	1149	0.2
7	α-Cubebene	7.042	1342	1345	0.8
8	Copaene	7.331	1376	1374	12.9
9	α-Santalene	8.431	1413	1416	0.1
10	βGurjunene	9.986	1429	1431	3.5
11	α-Patchoulene	10.375	1450	1454	3.1
12	Caryophyllene	10.542	1463	1466	17.2
13	Geranyl propanoate	10.986	1473	1476	0.2
14	γ-Muurolene	11.364	1481	1478	4.9
15	Bicyclogermacrene	11.508	1502	1500	6.3
16	α-Bisabolene	12.097	1506	1505	8.3
17	Quinoline	12.186	1512	1510	0.7
18	Trans cadina	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	Longipinane	13.064	1646	-	3.5
23	α-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	Phytol	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
			Total identified		92.8
			Monoterpene hydrocarbons		13.7
			Sesquiterpenes hydrocarbons		64.8
			oxygenated compounds		11.4
			Non-terpenes		2.9

According to the literature, the essential oil obtained from Ujjain origin contains different constituents like 2,5-dimethyl tetra decahydro phenenthrene (33.02%), nonanoic acid (19.25%), 2-amino-3-ethyl biphenyl (19.08%)[16]. The essential oil from *A. hexapetulus* in vietnam contains caryophyllene oxide (31.5%), βcaryophyllene (11.4%), humulene epoxide (10.0%), α-copaene (8.1%)[17]. The leaf essential oil from *A. hexapetalus* in southern Karnataka contains major products are 3-Carene (44.91%), βcaryophyllene (19.17%), α-humulene (8.78%), α-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].

In-vitro Antioxidant Activity

The current study examined the scavenging radical efficiency of the essential oil from *A. hexapetalus* leaves using DPPH and ABTS+ assays. The results showed that the essential oil had antiradical action, with IC_{50} values of 104 µL/mL and 122 µL/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 µL/mL. From the results, the essential oil from *A. hexapetalus* leaves has potent antioxidant activity, which may be because it contains a variety of complex terpenes.

Earlier research revealed, the *In vitro* antioxidant activity of ethanolic extract of flowers of *A. hexapetalus* was investigated using ABTS+ radical, nitric oxide radical, reducing ability, and scavenging of Hydrogen peroxide showed IC_{50} values of 280, 200, 130,230 µg/mL respectively[20]. Meanwhile, methanol extract of *A. hexapetalus* leaves were investigated using DPPH assay also produced significant results[21]. We believe that this is the first kind of study to assess the antioxidant activity of *A. hexapetalus* essential oil.

Table 2: *In-vitro* antioxidant activity of essential oil of *A. hexapetalus* leaves-DPPH Assay

Concentration (µL/mL)	DPPHβ%inhibition	Standard %inhibition
25	11.6	35.1
50	21.5	64.2
75	35.0	73.5
100	49.2	89.2
150	73.1	93.2
IC_{50} (µL/mL)	104 (µL/mL)	33.8 (µL/mL)

Table 3: *In-vitro* antioxidant activity of essential oil of *A. hexapetalus* leaves-ABTS Assay

Concentration(µL/mL)	ABTSβ+ %inhibition	Standard %inhibition
25	10.8	34.4
50	16.2	59.9
75	28.5	87.5
100	40.1	90.1
150	62.8	95.2
IC_{50} (µL/mL)	122 (µL/mL)	44.2 (µL/mL)

In-vitro anticancer activity

The MTT assay was used in this work to assess the anticancer efficacies of the essential oil from *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 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 *A. hexapetalus* roots, stems, and leaves[22]. This is the first kind of report for anticancer potential of *A. hexapetalus* essential oil extracted in South India.

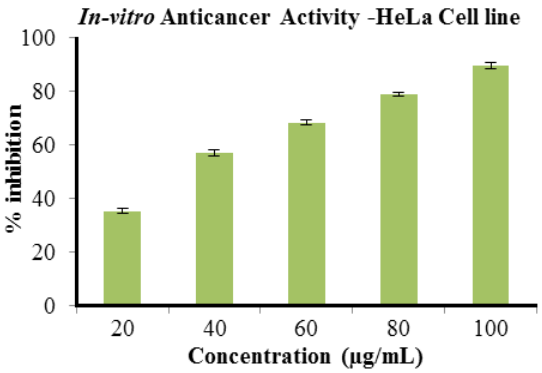

Fig. 1. Percentage of cell inhibition at various concentrations

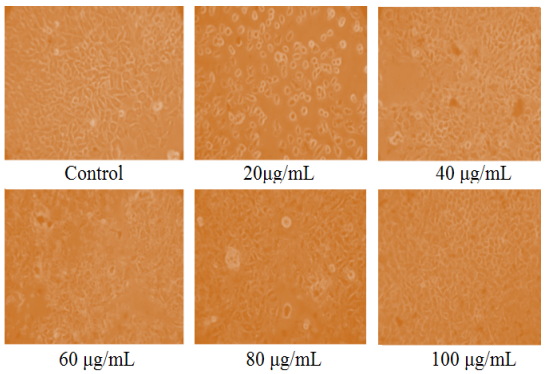

Fig. 2. *In vitro* anticancer activities of essential oil from *A. hexapetalus* leaves at various concentrations against HeLa cell line

CONCLUSION

The following conclusions could be made based on the aforementioned findings. The high effiectiveness of essential oil of *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%), α-Bisabolene (8.3%) as major components. Further studies are under progress.

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