

## CHEMICAL PROFILE AND *In-vitro* BIOLOGICAL ACTIVITIES OF *Eupatorium adenophorum* SPRENG

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### ABSTRACT

*Eupatorium adenophorum* Spreng is an invaded species found in the Western Ghats region, which is used for many diseases in our traditional medicinal system. The essential oil of the plant leaves was examined by Gas chromatography-Mass spectroscopy and evaluated for its biological performance. The antioxidant capacity was found by DPPH radical and ABTs assays. The anticancer activity was determined by MTT assay by using Jurkat E 6.1 cancer cell line along with commercial cancer drug Etoposide. Camphene (11.34%), Bornyl acetate (8.09%), Sabinene (6.75%), Iso borneol (7.96%), were identified as major constituents. It showed remarkable antioxidant performance against DPPH radical and ABTs with IC<sub>50</sub> values of 66.7 µg/mL and 70.1 µg/mL respectively. Essential oil exhibited the strongest cytotoxic effect on Jurkat E6.1 cancer cells with an IC<sub>50</sub> value of 29 µg/mL compared to commercial cancer drug Etoposide with an IC<sub>50</sub> value of 30 µg/mL. This one is the first type of report on the chemical constituents of *E. adenophorum* leaves from the Western Ghats region and its pharmacological activities. *E. adenophorum* plant is existing throughout the year and is easily available for essential oil isolation.

**Keywords:** GC/MS, *Eupatorium adenophorum*, DPPH, ABTs, and Anticancer Activity.

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### INTRODUCTION

*Eupatorium adenophorum* Spreng (syn. *Ageratina adenophora*) is a member of the Asteraceae family which is native to Mexico and distributed all over the world<sup>1,2</sup>, which is an invasive exotic weed species along the terrains of hilly areas of northern and north-eastern India and other lower hilly regions like Valparai and Kodaikanal Hills in Tamil Nadu, South India.<sup>3,4</sup> *E. adenophorum* plant is typically known as “cat weed, sticky snakeroot, and Crofton weed”. The plant has a high reproductive capacity, it propagates quickly and inhibits the growth of other usual plants which always causes serious problems to cropland and forestry.<sup>5,6</sup> Since the leaves and inflorescence of *E. adenophorum* have a characteristic odor, which can be used in perfume, pharmaceutical, and food industries. Many species of *Eupatorium* have been used in indigenous medicine worldwide due to their therapeutic properties like insecticidal, antimicrobial, antipyretic, anti-rheumatic, blood coagulant and analgesic, antioxidant, antitumor activity, cytotoxic activity, hepatotoxicity and antifungal.<sup>7-13</sup>

There are few studies on the chemical composition of aerial parts of *E. adenophorum* of the plant have been investigated in various parts of the world.<sup>2,3,14-20</sup> Except for a few spasmodic efforts on pharmacological studies of *E. adenophorum* essential oil (EAO), no comprehensive research was carried out for its Chemical profile, anticancer and antioxidant properties of the plant grown in Western Ghats, South India. Hence, our investigation was intended to explore the essential oil or volatile oil composition of *E. adenophorum* and its potential as a source of natural anticancer and antioxidant agent.

## EXPERIMENTAL

### Plant Collection

The fresh parts of *E. adenophorum* were brought from Valparai Hills (10.3270° N, 76.9554° E), Tamil Nadu, South India between the periods of February 2021. The plant specimen was identified and authenticated by the Department of Botany, and the voucher specimen (21PCY16) was preserved in the Postgraduate Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi.

### Abstraction of Essential Oil

About 2 kg of freshly collected plant parts were taken to carry out hydro distillation in order to extract essential oil for 3h. The oil was removed from the water using a separating funnel by petroleum ether and the excess solvents are evaporated. Traces of water was removed by anhydrous sodium sulphate and then kept in a container at 4°C. The essential oil yield was also calculated. The process was repeated thrice for enough quantity of oil for further analysis. 1mL of essential oil was dissolved with 1mL of petroleum ether. GC/MS analysis was assessed using an Agilent GC System 7890A gas chromatograph with an Agilent 5975C (MSD) mass-selective detector under the following conditions, DB-5MS capillary column 30 m x 0.25 mm (film thickness 0.25 µm). Helium was taken as the carrier gas, and the run a speed of 1ml/min. The ionization energy used to take the mass spectra was 70 eV. The components were identified by NIST-Wiley 2.0 version.<sup>21</sup>

### In-vitro Antioxidant Activity of EAEO

#### DPPH Method

The ability of EAEO to snatch the free radicals by using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) technique.<sup>22</sup> With a freshly prepared 2.9696mL DPPH (0.1mM) solution in methanol, different concentrations of EAEO (10, 25, 50, 75, and 100µg/mL) were added. The combinations were kept at 25°C in a dark area for 30 minutes and were studied at 517 nm by a UV-Visible spectrophotometer. In the absence of the EAEO, the control experiment was also carried out with the same approach. The ascorbic acid solution was taken as a standard.

#### ABTS Method

The ability of *E. adenophorum* essential oil to act as an antioxidant against ABTS<sup>•+</sup> radical cation was determined by the procedure.<sup>23</sup> EAEO (10, 25, 50, 75, and 100 µg/mL) was added with the required quantity of ABTS substance. After half-hour incubation, the absorbance was examined at 734 nm.

### In-vitro Anticancer Activity

The Anticancer effect of EAEO was tested on the Jurkat E6.1 cancer cell line by means of an MTT assay.<sup>24</sup> Different concentrations of EAEO (range from 10 to 50µg/ml) were added with MTT to each well, the mixture was stored for 72 hr at 37°C. The medium with MTT was then turned off and the generated formazan crystals were solubilized into 100µl of Dimethyl sulfoxide before being quantified using a microplate reader at 570 nm.

## RESULTS AND DISCUSSION

### GC/MS Results

The hydro distilled EAEO was green in colour with a strong fragrance and yielded 0.92% (w/w). Table-1 shows a total of 51 compounds identified by GC/MS analysis based on retention duration, retention index, and concentration (peak area) and account for 99.42%. The essential oil is dominated by monoterpenes (60.5%), sesquiterpenes (20.21%), oxygenated monoterpenes (5.01%), and esters. The main components in the oil were Camphene (11.34%), Bornyl acetate (8.09%), Iso borneol (7.96%), Sabinene (6.75%), and minor compounds were Cis-limonene oxide (4.46%), α-Pinene oxide (3.96%), 3-Hexenyl butanoate (3.64%), Norbornen-2-ol acetate (3.75%), Thujene (3.3%), Carveol (2.76%), Fenchyl acetate (2.79%), trans-pinenehydrate (2.51%) and cis-α-terpineol (2.47%). From the literature, variations are noticed in the major and minor composition of EAEO extracted from the Western Ghats of south India, when compared to the EAEO collected from North India and other parts of the world. The essential oil comprised primarily of Torreyol (30.10%), aristolone (11.54%), α-bisabolol (9.2%), and α-curcumin (7.88%) was reported for

the whole plant in China.<sup>25</sup> Similar results were found in Nepal, with the major constituents like torreyol (16.8%), and  $\alpha$ -bisabolol (5.1%) with smaller variations in the percentage.<sup>18</sup> Acoradiene, p-cymene, and camphene were found in the essential oil of the whole plant from North India.<sup>19</sup> Further, the essential oil dominated by amorph-4-en-7-ol was collected from different regions of the Himalayas.<sup>17</sup> Similar composition was identified in Spain.<sup>4</sup> The composition of aerial part of essential oil of EAEO showed varied major compositions.<sup>15,26</sup> Another study revealed that the essential oil extracted from the flower of *E.adenophorum* contains  $\gamma$ -cadinene and roots exhibited  $\gamma$ -Murolene as major compounds collected from the same region.<sup>20</sup> The composition of *E.adenophorum* essential oils taken from various regions of India as well as from various nations varies greatly. In general, the EAEO obtained were mono terpenoid in nature. Outcomes of our current research have shown that the major components of the EAEO isolated in South India are dominated by monoterpenes than sesquiterpenes. The predominant component of the EAEO identified in South India is dominated by monoterpenes rather than sesquiterpenes, according to the results of our current research. This dissimilarity may be due to different chemotypes of *E.adenophorum* available in Western Ghats, South India.

Table-1: Chemical constituents of *E.adenophorum* essential oil

S. No.	RT	RI (Evaluated)	RI (Literature)	Compound	% Relative Content
1	8.07	1008	1006	1,8-cineole	1.1
2	8.39	1015	1015	p-cymene	1.47
3	9.56	1046	1044	bornyl acetate	<b>8.09</b>
4	10.10	1063	1062	$\gamma$ - elemene	0.94
5	10.45	1065	1065	Octenol	<b>1.26</b>
6	10.53	1070	1070	Cis- citral	0.83
7	10.76	1080	1080	$\alpha$ -Phellandrene-8-ol	1.36
8	11.21	1092	1092	Norbornen-2-ol acetate	<b>3.75</b>
9	11.27	1094	1094	$\alpha$ -farnesene	2.01
10	11.34	1098	1096	Linalool	0.96
11	11.45	1099	1099	$\alpha$ -pinene oxide	<b>3.96</b>
12	11.59	1100	1100	1,3,8-p-Menthatriene	0.74
13	11.77	1103	1105	Trans-Pinene hydrate	2.51
14	11.86	1108	1108	methyl octanoate	1.57
15	12.07	1113	1114	2E, 4E-Octadienol	2.19
16	12.13	1114	1115	Cis - p-Menth-2-en-1-ol	2.42
17	12.29	1118	1118	limonene oxide	<b>4.46</b>
18	12.41	1121	1120	cis-p-menth-1-en-2-ol	0.88
19	12.59	1125	1124	Sabinene	<b>6.75</b>
20	12.73	1128	1128	$\beta$ -Terpineol	0.51
21	12.88	1132	1134	Isopulegol	1.51
22	12.98	1134	1139	Nerol oxide	0.98
23	13.48	1145	1144	Camphene	<b>11.34</b>
24	14.06	1160	1156	Iso Borneol	<b>7.96</b>
25	14.29	1165	1165	Linalool oxide	0.7
26	14.39	1167	1167	3-Hexenyl butanoate	<b>3.64</b>
27	14.49	1169	1169	Cis- $\alpha$ - nerol	0.52
28	14.55	1172	1172	Cis- camphone	1.02
29	14.64	1173	1173	Trans -linalool oxide	0.47
30	14.72	1175	1175	Iso amyl aceto acetate	0.67
31	14.82	1177	1176	$\alpha$ -terpinene	1.51
32	14.98	1181	1181	Thujene	<b>3.33</b>
33	15.13	1184	1186	Cis- $\alpha$ -terpineol	2.47
34	15.59	1195	1195	melhylchavicol	2.05
35	15.64	1196	1196	$\beta$ -cyclocitral	0.94
36	15.83	1201	1201	Trans- carveol	0.57
37	16.10	1207	1205	Trans-piperitol	<b>2.76</b>

38	16.34	1212	1210	fenchyl acetate	0.35
39	16.44	1215	1214	Thymol	0.44
40	16.57	1218	1218	Carveol	2.79
41	17.19	1232	1230	Chavicol	1.72
42	17.35	1235	1234	$\beta$ -ocimene	0.78
43	18.76	1268	1267	3-iso-thujanol acetate	0.32
44	19.46	1285	1283	p-cymen-7-ol	1.32
45	20.22	1300	1302	Terpinyl acetate	0.04
46	20.39	1306	1308	Methyl decanoate	0.06
47	21.00	1326	1330	Limonene	0.01
48	21.40	1340	1344	$\delta$ -elemene	0.13
49	22.00	1348	1346	$\alpha$ -cubebene	0.24
50	22.51	1515	1510	$\gamma$ -cadinene	0.30
51	23.32	1581	1580	Caryophyllene oxide	0.99
Total identified					99.69%
Monoterpene hydrocarbons (S.Nos-1,2,7,10-12,16-23,27-29,31-33,35-40,42,44,45,47)					57.76%
Sesquiterpenes hydrocarbons (S.Nos-4,9,48-51)					4.61%
Oxygenated Compounds (S.Nos-3,5,6,8,13-15,24-26,30,34,41,43,46)					37.32%

### In-vitro Antioxidant Assays

Figure-1 depicts the results of the DPPH and ABTs assays carried out to assess EAEO's antioxidant abilities. The antioxidant ability of EAEO was compared with commercial standard ascorbic acid. With IC<sub>50</sub> values of 66.7g/mL for EAEO and 40.4g/mL for commercial standard ascorbic acid, EAEO demonstrated the strongest radical scavenging activity against DPPH radical and ABTs assays which showed the IC<sub>50</sub> value of 70.1 $\mu$ g/mL for EAEO and 42.0 $\mu$ g/mL for standard Ascorbic acid.

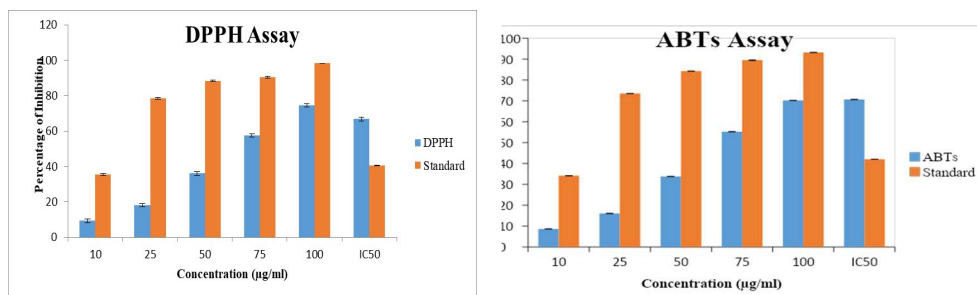


Fig.-1: In vitro Antioxidant Activity Result for DPPH and ABTs Assays

The antioxidant activity of EAEO is concentration-dependent because the scavenging activity of EAEO increases with an increase in concentration in both assays. The high amount of monoterpenes and sesquiterpenes in EAEO contributes to its ability to scavenge free radicals. The major compounds camphene, Bornyl acetate, Linalool, Limonene oxide, and Sabinene interacted with hydroxyl groups and scavenge the DPPH radicals. After scavenging the radical, the DPPH radical turns yellow in colour, and the degree of the antioxidant activity of essential oil is indicated by the intensity of colour produced. The DPPH test is primarily based on electron transfer and hydrogen atom abstraction<sup>27</sup>. Several research has demonstrated the antioxidant activity of *E. adenophorum* essential oil.<sup>10, 18, 20</sup> EAEO exhibits high antioxidant action against both of the tested methods. According to the findings, EAEO is particularly effective when compared to commercial standards, as evidenced by the IC<sub>50</sub> values. Our study is the first of its kind on the antioxidant activity of *E. adenophorum* essential oil from the Western Ghats region of South India.

### Anticancer Results

The results of the anti-cancer potential of EAEO were presented in this study in Table-2. By using the MTT assay, it was determined whether the Jurkat E6.1 cancer cells were still viable after being incubated with various quantities of the essential oil from the leaves of *E. adenophorum* (10–50 g/mL). Our findings revealed that high cytotoxicity was considerably and concentration-dependently induced by the essential

oil. The essential oil had the strongest anticancer properties against the Jurkat E6.1 cell line and Etoposide with IC<sub>50</sub> values comprised of 29 µg/mL and 30 µg/mL respectively. The outcomes demonstrated the essential oil of *E. adenophorum* could be used as a potential source of substitute medicine for treating cancer. There are limited results on the anticancer activity of essential oil of *E. adenophorum* leaves. It has been reported that EAEO was tested for its anti-tumor and anti-cancer activities mainly due to a sesquiterpene that has inhibited the growth of human cancer cell lines HCT8 (colon), Bel7402 (liver), and A2780 (ovary).<sup>28</sup>

Table-2: IC<sub>50</sub> Values of EAEO and Etoposide

Name of the Essential oil	IC <sub>50</sub> µg/ml	Name of the cell line
<i>E. adenophorum</i>	29 µg/mL	Jurkat E 6.1
Etoposide	30 µg/mL	

Additionally, it was determined that *A. adenophora* methanol extract against A549 cells had an anticancer activity with an IC<sub>50</sub> value of 50.08 µg/mL.<sup>29</sup> Anticancer and apoptosis on HCC cells and HepG2-containing bare mice was also studied.<sup>25</sup> EAEO exhibited high cytotoxicity on MCF-7 with IC<sub>50</sub> values of 25.95 µg/mL collected from the Philippines.<sup>30, 32</sup> The major composition and concentrations of essential oils, which are primarily influenced by a number of factors such as plant parts, the period of the vegetative cycle, seasonal deviation, geographical variation, climatic and soil nature, may be endorsed to this variation in chemical composition and pharmacological property<sup>31</sup>. In the present research, we reported the differences in chemical constituents of essential oil *E. adenophorum* leaves collected from Valparai Hills, Western Ghats, South India, when compared to that of essential oil extracted from the Himalayas, India. The above differences were attributed to geographical variation, climatic change, and soil type. The essential oil from the plant varies in content and may belong to distinct chemotypes.

## CONCLUSION

Herein, we have analyzed the chemical constituents of essential oil from *E. adenophorum* and their antioxidant and anticancer activities. According to GC/MS analysis, monoterpenes predominated in the leaf essential oil and the major constituents are Camphene (11.34%), Bornyl acetate (8.09%), iso borneol (7.96%), Sabinene (6.75%), cis-limonene oxide (4.46%) and α-pinene oxide (3.96%). The essential oil exhibited good anticancer activity against the JurkatE6.1 cancer cell line with an IC<sub>50</sub> value of 29 µg/mL. The *in vitro* antioxidant activity by DPPH and ABTs assay displayed concentration-dependent activity compared with commercial standard ascorbic acid. The findings showed that the multifaceted blend of several terpenes present in the essential oil of *E. adenophorum* had powerful anticancer and antioxidant properties. It has the potential to be employed in the food and perfume sectors, but more research is needed to confirm pharmacological actions.

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