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# PHYTOCHEMICAL ANALYSIS OF *ELAEOCARPUS* SPECIES OF WESTERN GHATS OF KARNATAKA, INDIA

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## ABSTRACT

Western Ghats of India has been known for its rich biological diversity. For this study, nine *Elaeocarpus* taxa were selected; eight taxa from Western Ghats of Karnataka and one from Sri Lanka to evaluate the phytochemicals using solvent extractions. These taxa were grouped into four clusters *viz.*, Munronii, Variabilis, Tuberculatus and Sahyadriensis which showed the presence of 17, 25, 29, 35 molecules respectively through chemotaxonomic analysis using Ultra Performance Liquid Chromatography (UPLC). Among the 29 molecules found in *E. tuberculatus* and *E. madikeriensis*, 13 are common in both the species, 7 are unique to *E. tuberculatus* and 9 are exclusively found in *E. madikeriensis*. In Variabilis, 7 are common in all the three varieties; 2 are common between *E. variabilis* var. *variabilis* and *E. variabilis* var. *saldanhae*; only one molecule is common between *E. variabilis* var. *saldanhae* and *E. variabilis* var. *surlabiensis*. Our findings provided evidence that crude organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases. These can be used as pharmaceutical adjuvants in the formulation of various dosage forms.

Keywords: Elaeocarpus, Phytochemical, UPLC, Western Ghats.

## 1. INTRODUCTION

The dicotyledonous family Elaeocarpaceae includes 12 genera and around 550 species. Elaeocarpus is the largest genus comprising about 350 species distributed mainly in the southern hemisphere and shows local endemism. The family is distributed mainly in tropical and subtropical regions of the world. Their distribution starts in the west from Madagascar and Mauritius up to Japan in the north, Australia and New Zealand in the south [1]. Elaeocarpaceae shows disjunct distribution in India and the species are found distributed in two biodiversity hotspots of India viz., Eastern Himalayan region in the North and Western Ghats in the South. In Karnataka State, the family is represented by a single genus *Elaeocarpus* comprising 11 species which are endemic to the Western Ghats of peninsular India. Herber's enumeration of Elaeocarpus species includes seven species from Western Ghats and Southern Provinces; his collection includes E. munroii from Kodagu district of Karnataka [2]. Species of *Elaeocarpus* are lesser known plants in terms of their uses, except for a few species. E. munronii and E. variabilis are edible. Ripe and unripe fruits of *E. serratus* (Ceylon olive)

are edible; they are used in the preparation of jams, squashes, salads, pickles etc. Leaf is used to get rid of dandruff. The leaf extract shows antioxidant property; it is used to treat rheumatism and is an antidote for poison. Bark extract is useful in the treatment of stomach disorders and as haemostatic agent [3]. A few species are used in traditional medicine. Bark and leaf blend of E. floribundus is used as mouth wash. In Ayurveda, pyrenes of *E. sphaericus* is considered as thermogenic and sedative. Fruit is used to cure asthma, arthritis, epilepsy, hypertension, liver diseases and mental disorders. Extensive studies are conducted about *E. sphaericus* for the scientific validation of its traditional uses and effectiveness against the recent cropping up ailments. The bioactive compounds isolated from E. sphaericus were analyzed pharmacologically against different diseases [4, 5-6]. Various parts of E. sphaericus are used in many ethnomedicines to treat nervous system related problems and in the treatment of arthritis, asthma, fever, headache, skin diseases. Substantial work is done on the scientific validation of curative property of E. ganitrus; Kumar and his colleagues have evaluated antioxidant properties [7].

Phytochemical analysis of leaf, bark, fruit and seed of different species of *Elaeocarpus* were studied by various investigators. Collins and his colleagues opined that Elaeocarpaceae is one of the major alkaloid containing families [8]. Various alkaloids reported from species of *Elaeocarpus* include alloelaeocarpiline, elaeocarpidine, epiialloelaeocarpiline, indolizidine, isoelaeocarpiline, etc [9, 10-11]. Chand and his colleagues have shown the presence of either myricetin or gallic acid in 7 Elaeocarpus species [12]. In addition to alkaloids, other compounds found are anthraquinone, fixed oils, flavonoids, glycosides, ninhydrin, reducing sugar, saponins, steroids, terpenoids, quinines [13, 14]; flavonoids such as methylmyricetin, myricetin [15]; gallic acid and tannins like ellagic acid derivatives, geraniin etc are reported from various species of Elaeocarpus [16, 17]. Extensive work has been done using various plant parts of E. ganitrus, bioactive compounds from this species [18-20]. Phytochemical profiling of *E. serratus* was carried out by various workers [21-23]. They have published review on phytochemicals and their therapeutic activities of Elaeocarpus species and noted that many chemical constituents have potential medicinal value. Indolizidine alkaloids and cucurbitacins are obtained from *Elaeocarpus* species are believed to have the potential to cure diseases like cancer, diabetes and HIV [24]. Methods used for chemical profiling in various species of Elaeocarpus include HPTLC, GC-MS TLC etc. [14, 20, 25]. In the present investigation, an attempt was made to resolve the problem of species delimitation based on phytochemical analysis of aqueous and solvent extractions.

## 2. MATERIAL AND METHODS

### 2.1. Solvent extraction

Fresh and healthy leaves of nine Elaeocarpus taxa viz., E. madikeriensis (CAL0000027231), E. munronii, E. sahyadriensis (CAL0000027226), E. serratus, E. tuberculatus, E. variabilis var. saldanhae (CAL0000027223), E. variabilis var. surlabiensis (CAL0000027219), E. variabilis var. variabilis and E. viridisepalus (CAL0000027221), collected from the natural habitat were used for phytochemical analysis (eight taxa from Western Ghats of Karnataka and one from Sri Lanka). Solvent extraction was done using methanol solvent. Standard protocol was followed for solvent extraction [26]. Shade dried leaves were ground using electric blender to obtain fine homogenized particles and the homogenized powder was used for Soxhlet extraction. Finely ground sample was kept in the thimble using Whatman filter paper 1 and placed in thimble holder. Solvent taken in a round bottom flask was fixed to the Soxhlet and heated using burner. Plant extract collected in the flask was condensed to obtain dry extract. The solvent extract was stored for further studies.

## 2.2. Ultra Performance Liquid Chromatography (UPLC) analysis

Methanol (AR grade) leaf extracts of the above mentioned taxa of *Elaeocarpus* were analysed for different compounds by Ultra Performance Liquid Chromatography-Electrospray Ionization-Quadropole-Time of Flight-Mass Spectrometer (Synapt G2, Waters, USA). Analysis of metabolites of above mentioned nine taxa of genus Elaeocarpus was done by the UPLC system. Water used was ACQUITY UPLC (Waters, USA) quaternary pump equipped with column thermostat and the auto sampler. Aquity BEH C18 column (100 mm  $\times$  4.6 mm, 2.7 μm, 110 Å USA) at 40°C, auto sampler was at 10°C and flow rate was 0.3 ml/min. The mobile phase consisted with (A) 0.1% formic acid in water and (B) 0.1% formic acid in Acetonitrile. The eluting conditions used were as follows: 0 min, 5% B; 3.5 min, 95% B; 6 min, 95% B; 6.5 min, 5% B; 8 min, 5% B. The UPLC system was connected to Qudrupole-Time-of-Flight (Synpat G2, Waters corp., USA) which is an orthogonally accelerated Q-TOF mass spectrometer, furnished with electrospray ionization source (ESI). Parameters for analyses were set using positive mode and the spectral range was set to 100-1500 m/z. The parameters of the MS optimized as Polarity, ES+; Analyser, High Resolution Mode; Capillary (kV),1.8; Source Temperature 150°C; Sampling Cone voltage, 40V; extraction Cone voltage, 4.0V; Desolvation Temperature, 200°C; Desolvation Gas Flow, 500.0 L/Hr; trap Collision Energy, 4.0 and Nitrogen was used as carrier gas and Nitrogen-Argon were used as collision gas. The MS data were processed using retention time and high-resolution mass of the compounds present in these plant specimens were noted. Using these data, the probable empirical formulas were calculated using Mass Lynx SCN781 software (Water's corp, USA).

### 3. RESULTS AND DISCUSSION

Methanol extracts of mature shade dried leaf materials belonging to 8 taxa belonging to 4 clusters *viz.*, Serratus, Tuberculatus, Variabilis and Munronii of genus *Elaeocarpus* subjected to LC analyses in positive and negative ionization modes showed the following results.

## 3.1. Ultra Performance Liquid Chromatography (UPLC) analysis

Chemotaxonomic analysis using Ultra Performance Liquid Chromatography revealed the presence of 17, 29, 35 and 25 molecules in Munronii, Tuberculatus, Sahyadriensis and Variabilis clusters, respectively. The tentative empirical formulas for the above molecules derived with the help of MassLynx software are listed in tables 1-4. The tentative empirical formulas of 17 molecules found in *E. Munronii* derived using LC chromatogram of Methanol extract is given in fig. 1 and table 1.

### 3.1.1. UPLC analysis of Tuberculatus cluster

The tentative empirical formulae of 29 molecules found in *E. tuberculatus* and *E. madikeriensis* are derived using LC chromatogram of Methanol extract (Fig. 2 & 3) are given in table 2. Among the 29 molecules found, 13 are common in both the species, 7 are unique to *E. tuberculatus* and 9 are exclusively found in *E. madikeriensis*.

Table 1: Retention time (Rt), Mass and tentative Empirical formulas of *E. munronii* 

S. No.	Rt	Mass	Empirical formula
1	0.46	218.99	C <sub>9</sub> H <sub>3</sub> N <sub>2</sub> O <sub>3</sub> S
2	0.50	332.08	$C_{15}H_{15}N_{3}O_{4}P$
3	1.30	1,051.02	$C_{27}H_{52}N_{10}O_6PS_4Cl_8$
4	1.31	782.99	$C_3H_{28}N_{16}O_{17}PS_6$
5	1.72	449.04	$C_{16}H_{17}O_{13}S$
6	2.08	356.27	$C_{22}H_{34}N_3O$
7	3.11	601.33	$C_{20}H_{46}N_{12}O_7Cl$
8	3.35	518.26	$C_{30}H_{37}N_{3}O_{3}P$
9	3.59	520.27	$C_{22}H_{43}N_5O_5PS$
10	3.77	496.27	C <sub>21</sub> H <sub>39</sub> N <sub>9</sub> OPS
11	4.23	485.22	$C_{22}H_{35}N_6O_2Cl_2$
12	4.51	609.19	$C_{15}H_{30}N_{16}O_7PS$
13	4.58	593.20	$C_{12}H_{34}N_{16}O_6PS_2$
14	4.80	623.21	$C_9H_{24}N_{26}O_6P$
15	4.92	607.22	$C_{22}H_{46}N_6O_3PS_2Cl_2$
16	5.02	621.23	$C_{30}H_{38}N_8OPS_2$
17	6.97	601.19	$C_{24}H_{46}N_2O_4Cl_5$



Fig. 1: LC chromatogram of methanol extract of E. munronii

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S No	рт	E. tuberculatus	E. madikeriensis	Empirical formula
<b>5.</b> INO.	NI	Mass		•
1	0.461	219.0027	219.0027	$C_7 H_8 O_6 P$
2	1.092		328.1161	$C_{22}H_{18}NS$
3	1.297		481.034	$C_{25}\overline{H_{10}}N_4O_5Cl$
4	1.314	783.0003	783.0003	$C_8H_{27}N_{14}O_{17}S_6$
5	1.502	797.0137		$C_{27}H_{19}N_{16}O_{2}S_{4}Cl_{2}$
6	1.707	536.3089	536.3092	C <sub>27</sub> H <sub>54</sub> NOS <sub>4</sub>
7	1.844	397.036		CH <sub>5</sub> N <sub>18</sub> O <sub>6</sub> S
8	1.895		501.2983	$C_{14}H_{33}N_{18}O_3$
9	2.048		501.2983	$C_{14}H_{33}N_{18}O_3$
10	2.219	503.2899	503.2893	$C_{23}H_{51}O_5S_3$
11	2.441	501.269		$C_{19}H_{42}N_4O_9P$
12	2.458	501.2690	501.2983	$C_{14}H_{33}N_{18}O_{3}$
13	2.561		532.2819	$C_{24}H_{44}N_5O_4PCl$
14	2.748	471.3008	471.3099	$C_{23}H_{43}N_4O_4S$
15	2.885	1019.5722		$C_{28}H_6NO_{12}S_6Cl_8$
16	3.022		499.3206	$C_{26}H_{47}NO_2O_5S$
17	3.363	518.2772	518.2971	$C_{22}H_{36}N_{11}O_2S$
18	3.585	520.2921	520.322	$C_{16}H_{39}N_{15}OPS$
19	3.79	496.293	496.3125	$C_{18}H_{43}N_9O_5P$
20	4.012		583.3216	$C_{21}H_{42}N_7S$
21	4.217	524.3236	524.3437	$C_{14}H_{46}N_{13}O_4S_2$
22	4.507		609.2482	$C_{27}H_{29}N_{16}S$
23	4.575	593.2239	593.2559	$C_{31}H_{33}N_{10}OS$
24	4.78	623.2329		$C_{24}H_{42}N_{10}OSCl_3$
25	4.763	623.222		$C_{26}H_{48}N_4O_2Cl_5$
26	4.797		535.2451	$C_{23}H_{35}N_8O_5S$
27	4.9	607.2403		$C_{11}H_{44}N_{16}O_{3}PS_{4}$
28	6.607	871.5153	871.554	$C_{29}H_{74}N_{18}O_6PCl_2$
29	6.966	601.2148	601.2363	$C_{21}H_{14}N_6O_8S_3$

Table 2: Retention time (Rt), Mass and tentative Empirical formulas of *E. tuberculatus* and *E. madikeriensis* of Tuberculatus cluster



Fig. 2: LC chromatogram of methanol extract of *E. tuberculatus* (00595-4) and *E. madikeriensis sp. nov*. (00596-5)



Fig. 3: Venn diagram depicting phytochemicals present in the methanol extracts of 2 species of Tuberculatus cluster of genus *Elaeocarpus* 

#### 3.1.2. UPLC analysis of Sahyadriensis cluster

The tentative empirical formulae of 35 molecules found in Sahyadriensis cluster derived using LC chromatogram of Methanol extract (fig. 4 & 5) are given in table 3. Among the 35 molecules, 6 are common in all the three species; 4 are common between *E. sahyadriensis* and *E. viridisepalus*; only one molecule is common between *E. viridisepalus* and *E. serratus*; common molecules are absent between *E. serratus* and *E. sahyadriensis*. 3, 13 and 8 molecules are exclusively found in *E. sahyadriensis, E. viridisepalus* and *E. serratus* respectively.



Fig. 4: LC chromatogram of methanol extract of *E. sahyadriensis* (1600593-2), *E. viridisepalus* (1600594-3) and *E. serratus* (1601555-10)

S. No.	RT	E. sahyadriensis	E. viridisepalus	E. serratus	Empirical formulae
1	0.478		342.0899		$C_5H_{14}N_9O_3S$
2	0.648	280.094	280.1013		$C_7H_{18}N_7OS_2$
3	1.024			411.1125	$C_8H_{20}N_{14}S_2Cl$
4	1.348			951.2245	$C_{34}H_{45}N_{16}O_7PS_3Cl$
5	1.468	899.1133			$C_{30}H_{38}N_{16}OPS_5Cl_2$
6	1.605	465.0404	465.0498		$C_{12}H_{22}N_2O_{11}PS_2$
7	1.622			463.1828	$C_{31}H_{28}O_2P$
8	1.724	479.0583	479.0583	479.162	$C_5 H_{23} N_{10} O_8 S_4$
9	1.758			4772044	$C_{26}H_{34}O_6Cl$
10	1.878		463.0696		$C_4H_{25}N_{14}PS_4Cl$
11	2.219		501.2592		$C_9H_{25}N_{24}O_2$
12	2.356		346.2151		$C_5H_{20}N_{19}$
13	2.748	471.2914			$C_{12}H_{37}N_{13}O_5Cl$
14	2.97		316.2448		$C_7 H_{31} N_{11} OP$
15	3.09		318.2563		C <sub>18</sub> H <sub>37</sub> NOCl
16	3.35	518.26	518.2672	518.2781	$C_{30}H_{37}N_{3}O_{3}P$
17	3.38			562.4266	$C_{30}H_{56}N_7OS_2$
18	3.585	520.2722	520.2821	520.2932	$C_{22}H_{43}N_5O_5PS$
19	3.602	478.273			C <sub>18</sub> H <sub>45</sub> N <sub>8</sub> PSCl
20	3.602			584.444	$C_{21}H_{58}N_{15}S_2$
21	3.756	496.2735	496.2833	496.2925	$C_{14}H_{35}N_{15}O_{3}Cl$
22	3.773	435.1693			$C_{22}H_{32}N_2OPS_2$
23	3.79			540.4318	C <sub>37</sub> H <sub>54</sub> N <sub>3</sub>
24	3.875		522.3009	522.3022	$C_{20}H_{44}N_9O_3S_2$
25	4.217	524.3036	524.3136	524.3251	$C_2H_{30}N_{29}O_4$
26	4.507		609.205		$C_{19}H_{33}N_{10}O_{11}S$
27	4.575	593.2026	593.2133	593.209	$C_{12}H_{34}N_{16}O_6PS_2$
28	4.729	535.2045	535.2147		$C_9H_{27}N_{16}O_{11}$
29	4.9	607.2187	607.2295		$C_{12}H_{36}N_{18}O_5PS_2$
30	5.019		621.2457		$C_{19}H_{45}N_{10}O_5S_4$
31	6.027		887.4955		$C_{40}H_{68}N_{14}O_5PS$
32	6.266		490.4681		$C_{22}H_{56}N_{11}O$
33	6.658		871.5023		$C_{20}H_{70}N_{28}O_2SCl_3$
34	6.966		601.204		C <sub>22</sub> H <sub>33</sub> N <sub>8</sub> O <sub>10</sub> S
35	6.983	597.218			$C_{19}H_{35}N_{12}O_6Cl_2$

Table 3: Retention time (Rt), Mass and tentative Empirical formulas of *Elaeocarpus sahyadriensis*, *E. viridisepalus* and *E. serratus* of Serratus cluster



Fig. 5: Venn diagram depicting phytochemicals present in the methanol extracts of 3 species of Sahyadriensis cluster of genus *Elaeocarpus* 

### 3.1.3. UPLC analysis of Variabilis cluster

The tentative empirical formulae of 25 molecules found in Variabilis cluster are derived using LC chromatogram of Methanol extract (fig. 6 & 7) are given in table 4. Among the 25 molecules, 7 are common in all the three varieties; 2 are common between *E. variabilis* var. *variabilis* and *E. variabilis* var. *saldanhae*; only one molecule is common between *E. variabilis* var. *saldanhae* and *E. variabilis* var. *surlabiensis*; common molecules are absent between *E. variabilis* var. *variabilis* and *E. variabilis* var. *surlabiensis*. 7, 2 and 6 molecules are exclusively found in *E. variabilis* var. *surlabilis*, *E. variabilis* var. *saldanhae* and *E. variabilis* var. *surlabiensis* respectively.

In current study, phytochemical data is used as supportive tool in resolving species delimitation problem of *Elaeocarpus* taxa of Karnataka State. The study has revealed the number of phytochemicals which can be extracted from leaves using organic solvent like methanol. This can be further extended to different parts of the plant such as bark, fruit etc. In case of Tuberculatus, Sahyadriensis and Variabilis clusters, the results of phytochemical studies have supported the morphological analysis, where distinct difference between the taxa in each cluster is evident by the presence of unique molecules. It also revealed presence of few common molecules in all the *Elaeocarpus* taxa collected during the current study; unique molecules in each cluster; presence of some common molecules among the taxa in each cluster, relation between and within the clusters.

Chemotaxonomical study taken up during the current study also helped in documenting the many biomolecules from eight *Elaeocarpus* taxa. These phytochemicals were identified upto tentative empirical formulae. This investigation can be taken up further to characterize and identify the biomolecules which can be exploited for evolving pharmaceutically important drugs. This type of

investigation was done extensively in *E. sphaericus* [22, 27-28]. Similar work was also done on phytochemicals present in different species of *Elaeocarpus*. Such investigations carried out by Chand and his colleagues on *Elaeocarpus grandis*, Ray and his colleagues on *E*. ganitrus, Katavic on E. grandis, Kothale & Rothe on E. tuberculatus, Geetha on E. serratus etc. [11-12, 20, 29-30] helped to elucidate many chemical components present in the respective species. The current investigations have scope of further extension to correlate the chemical components present in the plants to the medicinal properties as done by different researchers [27, 31-32]. In the present investigation phytochemistry is used supplementary tool for solving the problems in taxonomy. This tool was used by many taxonomists as done in the present investigations. Ankanna and his colleagues used phytochemical data in constructing relationship within Monocotyledons; Geetha employed the phytochemical information in resolving problem in Mimosoideae [33-34].



Fig. 6: LC chromatogram of methanol extract of *E. variabilis* var. *variabilis* (1600598-7), *E. variabilis* var. *saldanhae* (1600599-8) and *E. variabilis* var. *surlabiensis* (1600600-9)

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S. No. RT	DT	E. variabilis var.	E. variabilis var.	E. variabilis var.	Empirical
	ΚI	variabilis	saldanhae	surlabiensis	formulae
1	0.461	219.0122			$C_3H_3N_6O_6$
2	0.478		266.1122		$C_6H_{21}N_5PS_2$
3	0.631			357.033	$C_5H_{21}N_6O_2S_5$
4	0.7	224.0941			$C_{12}H_{10}N_5$
5	1.092	926.129			$C_{37}H_{37}N_{15}PS_2Cl_4$
6	1.28	481.0532			$C_{13}H_{30}N_4O_2SCl_5$
7	1.348	783.0615	783.0737	783.086	$C_{16}H_{19}N_{26}O_3S_5$
8	1.468		785.0839	785.0962	$C_{43}H_{26}N_6PS_4$
9	1.724	258.0974			$C_{19}H_{17}N_{14}O_8S$
10	2.1	356.3138			$C_{11}H_{39}N_{11}P$
11	2.97	316.2681			$C_{11}H_{30}N_{11}$
12	3.056	1018.5102			C <sub>61</sub> H <sub>74</sub> N <sub>7</sub> OPSCl
13	3.09			318.2953	$C_{17}H_{40}N_3S$
14	3.363	518.317	518.317	518.327	$C_{12}H_{40}N_{17}O_4S$
15	3.58	520.332	520.332	520.342	$C_{27}H_{46}N_5O_3S$
16	3.773	496.332	496.332	496.3418	$C_{12}H_{40}N_{17}O_4S$
17	3.875			522.361	$C_{27}H_{46}N_5O_3S$
18	4.217	524.3637	524.3637		$C_{12}H_{46}N_{17}O_4S$
19	4.285			758.5739	$C_{23}H_{50}N_{3}O_{6}S$
20	4.575	593.2666	593.2773	593.2773	$C_9H_{27}N_{16}O_{11}$
21	4.729			535.2755	$C_{12}H_{36}N_{18}O_5PS_2$
22	4.746	871.5799	871.5799	871.5928	$C_{19}H_{45}N_{10}O_5S_4$
23	4.9	607.2834	607.2942		$C_{40}H_{68}N_{14}O_5PS$
24	6.966		387.1107		C <sub>22</sub> H <sub>33</sub> N <sub>8</sub> O <sub>10</sub> S
25	6.983	601.2578	601.1182	601.2685	$C_{10}H_{25}N_{12}O_{2}Cl_{2}$





Fig. 7: Venn diagram depicting phytochemicals present in the methanol extracts of 3 varieties of *E. variabilis* of Variabilis cluster of genus *Elaeocarpus* 

### 4. CONCLUSION

In the present investigation, an attempt was made to explore the phytochemical compounds from solvent extractions of few species of *Elaeocarpus*. This study revealed the number of phytochemicals which are extracted from leaves using organic solvent methanol. This study helped in documenting the many biomolecules from eight *Elaeocarpus* taxa. It can be a supportive tool in resolving species delimitation problem of *Elaeocarpus* taxa of Karnataka State. The identified potent biomolecules can be exploited further to evaluate pharmaceutical importance during drug discovery program.

### Conflict of interests

Authors declare that they no conflict of interest exits in this investigation.

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