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Phytochemical Survey of Orchids in the Tirunelveli Hills of South India

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Introduction

The conservation of plant biodiversity is one of the priorities for many nations to practice sustainable development. The knowledge of natural resources is necessary to carry out sustainable development. In this sense, the searching and finding medicinal properties in plants is a reason to conserve them and to favor of the *ex situ* culture. This result was greater control of plants whose improper use can have serious repercussions in natural and the well being of the population.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as well as valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, plant products still remain as one of the best reservoirs of new structural types. About 25% of all prescriptions sold in the United States are from natural products (Farnsworth, 1990).

Orchidaceae is a highly evolved and widely distributed monocotyledonous family with a large number of terrestrial, saprophytic and epiphytic species. It comprises more than 30, 000 species in approximately 750 genera (Kong *et al.*, 2003), and new ones are being discovered by almost every botanical expedition in tropical areas. Many orchids are used in traditional system of medicine as a remedy for a number of ailments. The tubers and pseudobulbs of several orchids like *Orchis latifolia*, *Orchis mascula*, *Cymbidium aloifolium*, *Zeuxine strateumatica*, and some species of *Dendrobium*, *Eulophia* and *Habenaria* are used as a restorative and in the treatment of various diseases (Puri, 1970). The seeds of *Cymbidium aloifolium* used for healing of wounds. The powdered roots of *Vanda tessellate* is considered as antidote for poisoning. It is also used in rheumatic pains and abdominal complaints. *Dendrobium fimbriatum* has been used for liver upsets and nervous debility, while *Dendrobium teretifolium* for headache and pain reliever in other parts of body. Other orchid genera like *Oberonia*, *Eria*, *Bulbophyllum*, *Eulophia*, *Geodorum*, *Grammatophyllum*, and *Hetaeria* are also reported to be used as medicine in different parts of the world to cure various diseases (Hawkes, 1944; Withner *et al.*, 1974).

Phytochemical investigations of the orchid family were performed for alkaloid constituents (Luning, 1974),

identification and inheritance of flower pigments in the species of ornamental value (Arditti and Fischer, 1977). Apart from the presence of chlorophyll in green flowered forms and carotenoids in some yellow flowers, anthocyanidins are predominated. The cyaniding, pelargonidin and petunidin, and complex mixture of their glycosides and acylated derivatives are often present in a single flower. William *et al.*, (1978) has made an extensive survey of the leaf flavonoids of Orchidaceae family. In the present investigation of the preliminary phytochemical study of leaf flavanoids contents of Orchidaceae family members in the Tirunelveli hills of South India were analyzed and surveyed

Materials and Methods

All plant materials were collected from their natural habitats in the period of 1999-2006 in the Tirunelveli hills area.

Plant materials

Table 1. List of orchid and its collection locality in Tirunelveli hills.

Sl. No	Species	Locality
1.	<i>Acampe praemorsa</i> (Roxb.)	Muthukuzhiviyal
2.	<i>Acampe rigida</i> (Buch. – Ham. ex J.E. Smith)	Muthukuzhiviyal
3.	<i>Aenhenrya rotundifolia</i> (Blatter) Sathish. & Rasmussen	Muthukuzhiviyal
4.	<i>Anoectochilus elatus</i> Lindl.	Muthukuzhiviyal
5.	<i>Aphyllorchis montana</i> Reichb. f.	Muthukuzhiviyal
6.	<i>Brachycorythis iantha</i> (Wight) Summerh.	Muthukuzhiviyal
7.	<i>Brachycorythis splendida</i> Summerh.	Muthukuzhiviyal
8.	<i>Bulbophyllum aureum</i> (Hook. f.)	Muthukuzhiviyal
9.	<i>Bulbophyllum fischeri</i>	Muthukuzhiviyal
10.	<i>Bulbophyllum macraei</i> (Lindl.)	Muthukuzhiviyal
11.	<i>Bulbophyllum neilgherrense</i> Wight	Muthukuzhiviyal
12.	<i>Bulbophyllum tremulum</i> Wight	Muthukuzhiviyal
13.	<i>Bulbophyllum xylophyllum</i> Par & Reichb. f.,	Muthukuzhiviyal
14.	<i>Calanthe masuca</i> (D. Don) Lindl.,	Muthukuzhiviyal
15.	<i>Cheirostylis flabellata</i> Wight	Muthukuzhiviyal
16.	<i>Chrysoglossum maculatum</i> (Thwaites) Hook	Upper Kodaiyar
17.	<i>Coelogyne nervosa</i> A.	Muthukuzhiviyal
18.	<i>Crepidium purpureum</i> (D. Don)	Muthukuzhiviyal
19.	<i>Cymbidium aloifolium</i> (L.)	Upper Kodaiyar
20.	<i>Cymbidium ensifolium</i> (L.)	Muthukuzhiviyal
21.	<i>Dendrobium aqueum</i> Lindl.	Muthukuzhiviyal
22.	<i>Dendrobium barbatulum</i> Lindl.,	Muthukuzhiviyal
23.	<i>Dendrobium didon</i> Reichb.	Muthukuzhiviyal
24.	<i>Dendrobium herbaceum</i>	Muthukuzhiviyal
25.	<i>Dendrobium heterocarpum</i> Wall.	Muthukuzhiviyal
26.	<i>Dendrobium heyneanum</i> Lindl.,	Muthukuzhiviyal
27.	<i>Dendrobium macrostachyum</i> Lindl.,	Muthukuzhiviyal
28.	<i>Dendrobium microbulbon</i> A. Rich.	Muthukuzhiviyal
29.	<i>Dendrobium nanum</i> Hook. f.	Muthukuzhiviyal
30.	<i>Dendrobium nutans</i> Lindl.,	Muthukuzhiviyal
31.	<i>Dendrobium panduratum</i> Lindl. subsp. <i>villosum</i>	Upper Kodaiyar
32.	<i>Dendrobium wightii</i> A. Hawkes	Muthukuzhiviyal
33.	<i>Disperis neilgherrensis</i> Wight,	Muthukuzhiviyal
34.	<i>Epipogium roseum</i> (D. Don)	Muthukuzhiviyal

35.	<i>Eria muscicola</i> (Lindl.)	Muthukuzhiviyal
36.	<i>Eria nana</i> A. Rich.	Muthukuzhiviyal
37.	<i>Eria pauciflora</i> Wight,	Vaniyankalpodavu
38.	<i>Eria reticosa</i> Wight,	Muthukuzhiviyal
39.	<i>Eulophia epidendraea</i> (Koen.)	Muthukuzhiviyal
40.	<i>Gastrochilus acaulis</i> (Lindl.)	Muthukuzhiviyal
41.	<i>Goodyera procera</i> (Ker-Gawl.)	Vaniyankalpodavu
42.	<i>Habenaria crinifera</i> Lindl.,	Muthukuzhiviyal
43.	<i>Habenaria dichopetala</i> Thwaites,	Muthukuzhiviyal
44.	<i>Habenaria longicornu.</i> Lindl.,	Muthukuzhiviyal
45.	<i>Habenaria plantaginea</i> Lindl.,	Muthukuzhiviyal
46.	<i>Habenaria virens</i> (Lindl.)	Vaniyankalpodavu
47.	<i>Liparis atropurpurea</i> Lindl.,	Muthukuzhiviyal
48.	<i>Liparis elliptica</i> Wight,	Muthukuzhiviyal
49.	<i>Liparis viridiflora</i> (Blume)	Muthukuzhiviyal
50.	<i>Liparis wightiana</i> Thwaites,	Vaniyankalpodavu
51.	<i>Luisia zeylanica</i> Lindl.,	Muthukuzhiviyal
52.	<i>Malleola gracilis</i> (Lindl.)	Muthukuzhiviyal
53.	<i>Nervilia aragoana</i> Gaud.,	Muthukuzhiviyal
54.	<i>Oberonia brunoniana</i> Wight,	Muthukuzhiviyal
55.	<i>Oberonia santapau</i> Kapadia	Muthukuzhiviyal
56.	<i>Oberonia tenuis</i> Lindl.,	Muthukuzhiviyal
57.	<i>Oberonia verticillata</i> Wight,	Vaniyankalpodavu
58.	<i>Paphiopedilum druryi</i> (Bedd.)	Muthukuzhiviyal
59.	<i>Papilionanthe subulata</i> (Koen.)	Muthukuzhiviyal
60.	<i>Vanda tessellata</i> (Roxb.) Hook.	Nondimongadu
61.	<i>Vanda testacea</i> (Lindl.)	Nondimongadu

Extraction

The collected plant's leaf materials (Table1) were air-dried and powdered. Ten grams of each dried plant material were separately extracted with 75% methanol (Plant materials and Solvent in the ratio of 1:4). The collected extracts were evaporated under reduced pressure to a volume of 25ml. The qualitative identification of the flavonoids were detected by the method of Shinoda test (1ml of the methanol extracts were evaporated, diluted with 1 ml of water + 1 ml methanol + 3 - 4 mg magnesium + 3 - 4 drops of concentrated hydrochloric acid). The result of colouring of the solutions from pink to red was an indication for presence of flavonoids. Cyanogenic glycosides were identified by subjecting 1ml of extract in 10 ml sterile water and filtered. Sodiumpicrate paper was added to the filtrate and heated to boil. The extract was also tested for carbohydrates using resorcinol solution. Fehling's solution was added to the extract and heated to detect reducing sugar. Liebermann-Burchardt test is conducted for steroids and terpenoids - to 1 ml of methanolic extract of drug, 1 ml of chloroform, 2-3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green colouration of the solution indicated the presence of Steroids and dark pink or red colouration of the solution indicated the presence of terpenoids). For alkaloids - a drop of methanolic extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicated the presence of alkaloids). Braemer's test is used for identification of tannins: 10% alcoholic ferric chloride solution was added to 2-3 ml of methanolic extract (Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the extract) (Maridas, 2006).

Results and Discussion

The results of qualitative phytochemical analysis of the leaf extracts of Orchidiaceae family members were shown in Tables 2. A total of 27 genus and sixty one species were qualitatively identified for the presence of flavonoids. 37 orchid species are given positive results to reducing sugar and other 21 species were shown negative results to it. In 48 orchid species, cyanogenic glycosides are found and other 13 species such as *Anoectochilus elatus* Lindl., *Bulbophyllum neilgherrense* Wight., *Bulbophyllum tremulum* Wight, *Bulbophyllum xylophyllum* Par & Reichb. f., *Calanthe masuca* (D. Don) Lindl., *Cheirostylis flabellata* Wight, *Cymbidium ensifolium* (L.), *Dendrobium macrostachyum* Lindl., *Epipogium roseum* (D. Don), *Eria reticosa* Wight, *Liparis atropurpurea* Lindl., *Malleola gracilis* (Lindl.), *Papilionanthe subulata* (Koen.), *Vanda testacea* (Lindl.) did not cyanogenic glycosides in their leaf extract. Therefore, further studies should be done on isolation of the individual components of Orchidiaceae family members and also analysis the phytochemicals properties for its pharmacological studies. 45 orchid species of Tirunelveli hills area contained tannin in its flowers and 14 species of orchids showed Terepenoids in its flowers. Only eight orchid species were contained all types of flavonoid contents.

Table-2: List of Leaf flavonoids found in the orchid species of Tirunelveli Hills

Sl.No	Species	Phytochemicals				
		Flavonoids	Reducing Sugar	Cyanogenic glycosides	Terpenoids	Tannin
1.	<i>Acampe praemorsa</i> (Roxb.)	+	-	+	-	-
2.	<i>Acampe rigida</i> (Buch. – Ham. ex J.E. Smith)	+	-	+	-	-
3.	<i>Aenhenrya rotundifolia</i> (Blatter) Sathish. & Rasmussen	+	-	+	-	-
4.	<i>Anoectochilus elatus</i> Lindl.	+	+	-	-	-
5.	<i>Aphyllorchis montana</i> Reichb. f.	+	-	+	-	-
6.	<i>Brachycorythis iantha</i> (Wight) Summerh.	+	+	+	-	+
7.	<i>Brachycorythis splendida</i> Summerh.	+	-	+	-	-
8.	<i>Bulbophyllum aureum</i> (Hook. f.)	+	+	+	-	+
9.	<i>Bulbophyllum</i>	+	-	+	-	+

	<i>fischeri</i>					
10.	<i>Bulbophyllum macraei</i> (Lindl.)	+	+	+	-	-
11.	<i>Bulbophyllum neilgherrense</i> Wight	+	-	+	-	-
12.	<i>Bulbophyllum tremulum</i> Wight	+	+	-	-	-
13.	<i>Bulbophyllum xylophyllum</i> Par & Reichb. f.,	+	+	-	-	+
14.	<i>Calanthe masuca</i> (D. Don) Lindl.,	+	-	-	-	-
15.	<i>Cheirostylis flabellata</i> Wight	+	+	-	-	+
16.	<i>Chrysoglossum maculatum</i> (Thwaites) Hook	+	+	+	-	+
17.	<i>Coelogyne nervosa</i>	+	+	+	-	+
18.	<i>Crepidium purpureum</i> (D. Don)	+	+	+	-	+
19.	<i>Cymbidium aloifolium</i> (L.)	+	+	+	+	+
20.	<i>Cymbidium ensifolium</i> (L.)	+	+	-	+	+
21.	<i>Dendrobium aqueum</i> Lindl.	+	-	+	-	+
22.	<i>Dendrobium barbatulum</i> Lindl.,	+	+	+	-	-
23.	<i>Dendrobium didon</i> Reichb.	+	-	+	-	+
24.	<i>Dendrobium herbaceum</i>	+	+	+	-	+
25.	<i>Dendrobium heterocarpum</i> Wall.	+	+	+	-	-
26.	<i>Dendrobium heyneanum</i> Lindl.,	+	+	+	-	+
27.	<i>Dendrobium macrostachyum</i> Lindl.,	+	-	-	-	-
28.	<i>Dendrobium</i>	+	+	+	-	+

	<i>microbulbon</i> A. Rich.					
29.	<i>Dendrobium nanum</i> Hook. f.	+	+	+	-	+
30.	<i>Dendrobium nutans</i> Lindl.,	+	-	+	-	-
31.	<i>Dendrobium</i> <i>panduratum</i> Lindl. subsp. <i>villosum</i>	+	+	+	-	+
32.	<i>Dendrobium wightii</i> A. Hawkes	+	+	+	-	-
33.	<i>Disperis</i> <i>neilgherrensis</i> Wight,	+	+	+	-	-
34.	<i>Epipogium roseum</i> (D. Don)	+	+	-	-	+
35.	<i>Eria muscicola</i> (Lindl.)	+	+	+	-	+
36.	<i>Eria nana</i> A. Rich.	+	-	+	-	+
37.	<i>Eria pauciflora</i> Wight,	+	-	+	-	+
38.	<i>Eria reticosa</i> Wight	+	-	-	-	+
39.	<i>Eulophia</i> <i>epidendraea</i> (Retz.)Fischer	+	+	+	+	+
40.	<i>Gastrochilus acaulis</i> (Lindl.)	+	+	+	-	+
41.	<i>Goodyera procera</i> (Ker-Gawl.)	+	-	+	-	+
42.	<i>Habenaria crinifera</i> Lindl.,	+	+	+	+	+
43.	<i>Habenaria</i> <i>dichopetala</i> Thwaites,	+	-	+	+	+
44.	<i>Habenaria</i> <i>longicornu.</i> Lindl.,	+	+	+	+	+
45.	<i>Habenaria</i> <i>plantaginea</i> Lindl.,	+	+	+	+	+
46.	<i>Habenaria virens</i> (Lindl.)	+	-	+	+	+

47.	<i>Liparis atropurpurea</i> Lindl.,	+	+	-	-	+
48.	<i>Liparis elliptica</i> Wight,	+	+	+	-	+
49.	<i>Liparis viridiflora</i> (Blume)	+	-	+	-	+
1.	<i>Liparis wightiana</i> Thwaites,	+	-	+	-	+
2.	<i>Luisia zeylanica</i> Lindl.,	+	+	+	-	+
3.	<i>Malleola gracilis</i> (Lindl.)	+	+	-	-	+
4.	<i>Nervilia aragoana</i> Gaud.,	+	-	+	-	+
5.	<i>Oberonia brunoniana</i> Wight,	+	+	+	+	+
6.	<i>Oberonia santapaui</i> Kapadia	+	-	+	+	+
7.	<i>Oberonia tenuis</i> Lindl.,	+	+	+	+	+
8.	<i>Oberonia verticillata</i> Wight,	+	+	+	+	+
9.	<i>Paphiopedilum druryi</i> (Bedd.)	+	-	+	-	+
10.	<i>Papilionanthe subulata</i> (Koen.)	+	+	-	-	+
11.	<i>Vanda tessellata</i> (Roxb.) Hook.	+	-	+	+	+
12.	<i>Vanda testacea</i> (Lindl.)	+	+	-	+	+

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