Preliminary Study on Medicinal Uses and Phyto-Chemicals of the *Anoectochilus albolineatus* Parish & Rchb.f. in Tarmoe-Nye Township, Northern State

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Preliminary study on medicinal values and phyto-chemicals of the
Anoectochilus albolineatus Parish & Rchb.f. in Kutkai Township,
Northern Shan State

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Abstract

Anoectochilus albolineatus, commonly known as Hywet-Hla thit-khwa, belongs to the family Orchidaceae are the lesser known medicinal plants in Myanmar. It is interested greatly at the present for their high commercial value, which is one of the top marketing items of medicinal plants on sale. In this paper, their morphology and key characteristics to the species are studied. Preliminary studies on the medicinal value and phyto-chemical constituents are also made and discussed and market information is also introduced.

Key words; Anoectochilus albolineatus, medicinal uses, phytochemicals, market information, Orchidaceae
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1. Introduction

The genus *Anoectochilus* consists of about 50 species belongs to the family Orchidaceae in the world. *Anoectochilus* spp. is one of the lesser known medicinal herbs and endemic species in Myanmar and it is include Listed in CITES Appendix II. This genus is a native perennial and small terrestrial plant grows wild in the forests of Myanmar. They are sometime called 'Jewel orchids' because of their attractive foliar venation (Tauscher, 1978). It is distinguished by green to brownish or blackish purple velvety leaves with glistening metallic veins of copper, silver or bright gold coloured. The name *Anoectochilus* is derived from the Greek 'aniktos'= open and 'cheilos'= lip or labellum a reference to the open aspect of the flowers. The labellum or lip is much longer than the flower, which is clawed and has nodules or projections off both lateral sides so that it resembles a filleted fish. The characteristics of *Anoectochilus* are mostly terrestrials but a few are lithophytes. The plants are anchored by wiry roots that appear woolly succulent above ground creeping rhizomes. The inflorescences are terminal, short stalked and on a hairy shoot. Among them, three species could be identified so far in Myanmar such as *A. albolineatus*, *A.burmannicus* and *A. roxburghii*. All of them are top of the demanding items.

The distribution of the genus traced from the Himalayan foothills in India to mountains of Southeast Asia, Japan, South China, Sri-lanka, Indonesia, Malaysia, Australia and some of the pacific Islands including Taiwan. *A.albolineatus* is found in Myanmar, Thailand and Vietnam. In Myanmar *Anoectochilus* species are growing wild in Shan State, Chin State, Kachin State and Tannithayi region. They are typically found growing in shady broad-leafed, evergreen and deep shade of humid forests or on mossy rocks. The diversity ranges is high elevation, cold, sandy soil to clay and the ecological nature is that plants prefer moist and shady situation.

To collect the information and the specimens, field trips were made in which discussion was held with the local people and representative of the trade.

Objectives

1. To proved the information on the medicinal uses and phyto-chemical constituents of *A. albolineatus*
2. To reveal the economic market for Non Wood Forest Products in Myanmar
3. To create a new trend in potential extra income generation of the local people
2. Literature Review

Chemical constituents

Pharmacological studies indicated _Anoectochilus formosus_ that kinsenoside possesses anti-hyperglycaemic, anti-hyperlipidemic and anti-hyperliposis effects (Takeshita et al., 1995; Du et al., 2001). Hsieh et al. (1997) reported that gastrodin and p-hydroxybenzyl alcohol (gastrodigenin) facilitates memory consolidation and retrieval on the passive avoidance tasks in rats. Recently, (Wang et al. 2002) reported that a new compound kinsenone and a number of known flavonoid glycosides such as quercetin and isorhamnetin were isolated from _Anoectochilus formosus_ and with strong antioxidant properties. (Huang, 1991) _A. formosanus_ and _A. koshunensis_ have been identified to contain some aliphatic, butanoic acid glucosides, sterol, aromatic glucosides, flavanoids and flavanoid glucosides (Yanasaki K, Sugimoto H, 1993).

Mei- Hua and Yan- ping (2008) also analysed the extract of the whole plants of _A.roxburghii_ led to the isolation of known sorghumol, two novel sorghumol acyl esters, 3-O-Z-p-coumarate and sorghumol 3-O-E-p-coumarate, and a novel alkaloid, anoectochine.

Yang (2009) described that the chemical constituents of _A. roxburghii_ were isolated by various column chromatographic methods and structurally elucidated by IR, NMR and MS evidences. Fourteen compounds were obtained and among then ten were identified as beta-sitosterol, ferulic acid, oleanolic acid, lanosterol, p-hydroxy benzal dehyde, 3-methoxyl-p-hydroxybenzaldehlde, daucosterol ,3’,4’,7-trimeth oxy-3, 5-dihydro xyflavone , isorhamnetin-3-O-beta-D-rutinoside and rutin.

Market information

In Taiwan, 2006, the greenhouse- cultivated products of _A. formosus_ were still around US$120/kg on a fresh weight basis. _A. formosus_, fresh or dried, was boiled in water and taken orally for the treatment of chest and abdominal pains. In 2007, these plants are collected and sold at more than 300 US$/kg in fresh weight. (Lin, 2007) But in 2010, the current market price of the wild fresh herb collected from _A. formosus's_ natural habitat was around US$320/kg, while the dried herb sells for US$3200/kg. (Hu, 1971).
Medicinal uses

In China, several species of this genus *Anoectochilus* were used in Chinese folk medicines, such as *A. formosanus, A.koshunensis, A. roxburghii.* and *Anoectochilus lanceolatus* which grew in forests at below 1,000 meters above sea level, in Guangdong, Guangxi and south Yunnan. The whole plant could be used to make medicine. Although not included in the current Chinese pharmacopoeia, Jin- Xin- Lian was derived from the genus *Anoectochilus.* Three species were well recognized. *A. formosanus*, *A. roxburghii* and *A. koshunensis* are called same name Jin- Xin- Lian in Chinese. They were widely used in Southern China to treat nephritis and pneumonia. Moreover, (*A.roxburghii*) was considered to treat some infections or diseases related to "fire". According to experimental studies Jin- Xin- Lian decreases blood glucose levels and was hence used to treat diabetes. *A. formosanus* (AFE) Hayata was one of the original plants of crude drugs used as a folk remedy in Taiwan for liver cirrhosis, diabetes and for treatment of cardiovascular diseases and in underdeveloped children. (Kan,1979).

The school of Medical technology, Medical School Yang-Ming in Taiwan, had made studies using *A. formosanus* like a anti-inflammatory agent, anti- febrifuge agent, anti- depressing agent and even against the virus influenza A. Especially, the leaves of some species of *Anoectochilus* spp. had been used in China to make a tea believed to be helpful in curing problems associated with the diseases of liver and lung. Some *Anoectochilus* species had been used to cure diseases, such as cancer prevention, high blood pressure, diabetes, snake bite (Yen et al., 1996).

Moreover, In Taiwan, fresh and dried jewel orchids are boiled in water to treat for respiratory distress, diabetes, nephritis, fever, hypertension, impotence, liver and spleen disorders, and chest pains. It also has anti-oxidant effects.

In India, stem and leaves of the *A. regalis* are one of the ingredients in certain medicinal oils (Dr. A. Nageswara, 2004).

*A.roxburghii* (Wall) Lindl. (common name: jewel orchid) is a well- recognized medicinal plant in China, where it is called ‘King Medicine’, and is known to possess various medicinal properties, especially diverse pharmacological effects, such as liver
protection, cancer prevention, and for treatment of cardiovascular diseases and diabetes. (He et al., 2005a)

*Anoectochilus* (Herba Anoectochili) is a rare perennial herb. Chinese are used for folk medicine broadly, there are cool detoxification, anti-inflammatory analgesic efficacy of the unknown swelling, pain, fever, diarrhea, snakebite have a significant effect, and non-toxic side effects, the use of safety (J., Shoyana, Y 2001). More people with its treatment of diabetes, hyperlipidemia, hepatitis B and other diseases (Jiang Haiyan, 1997). In recent years, gold wire lotus in Taiwan, favored, folk call it 'King of Medicine', dual-use food and medicine for the treatment of hypertension, cancer and other difficult cases. With the in-depth study to highlight *A. roxburghii* medicinal, edible, ornamental, and many other fine quality, the world increasingly popular, and has broad prospects for development and utilization. In Taiwan, China and Japan, decoction of the whole plant of the *A. roxburghii* was used for treatment of fever, pleurodynia, snake bite, lung and liver disease, hypertension (Fan et al. 1997)

### Used for Food

In China & Taiwan, the local people were used *Anoectochilus* spp., such as *Anoectochilus* Energy Soup, *Anoectochilus* jelly and Taiwan Jewel Orchid Tea (He C-N 2005a). *Anoectochilus* leaves are used as vegetables in Indonesia and Malaysia (Hkkjrh, 2009).

### Used for perfumes

*A. sandvicensis* was the most common orchid species found in Hawaii. It was popularly referred to as "the jewel orchid" because of its splendid foliage. It also grew up to 20 inches and its flowers were yellow and mostly bloom from August to December, though sometimes they bloom of all year round. The scent of these exotic orchids was used in creating world famous Hawaiian perfumes. The Orchid perfume truly captures the mesmerizing scent of Hawaiian orchids. The floral aroma of this delightful perfume
was something that was certain to awaken feelings of romance and fascination (Yang XW, 2009).

3. Materials and Methods

Materials were collected from Htar-mo-nye village, Kutkai Township, Northern Shan State, in the year of 2010. The identification of *Anoectochilus albolineatus* was approved by Dr. Hubert Kurzwail (Senior Orchid Researcher) from Botanical Garden, Singapore. Measurements for morphological characters were taken directly from living or dried herbarium specimens. Ecological data were recorded and photographs of living specimens were taken in the field. Preliminary phyto-chemical tests were conducted at the Chemistry laboratory, Department of Chemistry, University of Yangon.

4. Results

4.1 Taxonomy

4.1.1. Generic Description

*Anoectochilus*

Terrestrial herbs. Rhizome creeping, terete, several noded, flashy, roots narrowly filiform to fibrous, arising singly from rhizome nodes. Stem erect or ascending, with 1 to a few loose tubular sheaths at base, a few clustered leaves, glabrous. Leaves green to purplish black with white, pinkish, and golden reticulate venation on the surface, ovate or elliptic, velvety and often slightly fleshy, with a long petiole-like base dilating into tubular amplexicaual sheath. Inflorescence erect, terminal, racemose, pubescent, peduncle with a few scattered sheathing bracts, rachis laxly 2-10 flowered. Flower resupinate or not; ovary slender, twisted or not. Sepals free, similar, spreading, surface usually pubescent; dorsal sepal forming hood with petals. Petals obliquely ligulate- oblong, membranous; lip basally adnate to base; hypochile subtubular, lateral margin erect; mesochile short to elongate, canaliculated, both external lateral margins with an entire pectinate or filamentous flange; epichile entire to deeply bilobed, lobes divergent, rarely divided; spur containing a pair of irregular calli or septa. Column short, dorsally dilated, ventrally with 2 intra marginal lamellate or fleshy appendages wings; anther erect, ovoid, bilocular,
pollinia 2, clavate, sectile, granular- farinaceous, each attenuate into a narrow stalk attached to a solitary ovate viscidium; rostellum usually erect, remnant shortly bifid, stigma lobes 2, separate, convex, placed laterally toward apex of column. Capsule narrowly ellipsoid.

4.1.2 *Anoectochilus albolineatus* Parish& Rchb.f.

Terrestrial herb with 2 to 3 shortly petiolate leaves in basal rosette. Leaves broadly ovate, acute, 1.5 - 4cm long, velvet nearly black. Often with broad irregular median longitudinal reticulation of pink veins. Scape pubescent, with 2 or 3 acuminate yellowish bracts, 6 - 15 cm tall, with 1 - 8 lax white flowers. Floral bracts ovate, acuminate, pubescent. Ovary pubescent, 5 - 10 mm long. Sepals sub-similar, ovate-oblong, 7 - 10mm long, acute, reddish-hairy. Petals narrowly-ovate, slightly oblique, as long as sepal, shortly acumate. Lip 5 - 7 mm long, spurred. Spur directed parallel with ovary and claw, 1.5 - 3mm long, broadly – conical, notched at the apex, with 2 large peltate, warty glands. Mesochile with 4 - 7 pairs of long fringes. Epichile lobes narrowly-ovate to oblique – flabellate 5 - 7 mm long. Column at front with 2 large narrowly – triangular, parallel wings not entering to the spur and 2 short broad rosetellar arms. Anther narrowly – ovate, 3 - 4 mm long. Flowering period is from October to December.

4.1.3 Synoptic key to the species of *Anoectochilus*

1. Terrestrial herb with 2-3 shortly petiolate leaves, Leaves broadly ovate, acute, nearly black with broad irregular reticulation of pink veins. Flowers white, Ovary pubescent. Sepals sub-similar, ovate-oblong, reddish-hairy. Petals narrowly-ovate, Lip 5-7 mm long, spurred, Spur directed parallel with ovary, Mesochile with 4-7 pairs of long fringes, Column at front with 2 large narrowly triangular -------------------------- *Anoectochilus albolineatus*
4.2 Phytochemistry

4.2.1 Preliminary phyto-chemical Investigation of the whole plant of *Anoectochilus albolineatus*

*Anoectochilus* spp. (Ywet Hla Thit Khwa or Hnyte Phat Hla Thit Khwa.) is one of the lesser known medicinal herbs and endemic species not only in Myanmar but in the world widely also. When referring to the publications on Myanmar traditional medicine so far there were no previous study on it. Expect a few information from the local people in KutKai area and lack of uses and chemical analysis. But China, Japan, Taiwan and Austral have already been tested *Anoectochilus* spp by many other modern machines at laboratory and they were published so many research papers. Therefore, in order to fulfill the requirement on chemical constituents a phyto-chemical investigation was undertaken and physicochemical characterization was also carried out in Table 1& 2.

Table 1 Preliminary phyto-chemical Investigation of the whole plant of *A. albolineatus*

<table>
<thead>
<tr>
<th>No.</th>
<th>Constitution</th>
<th>Extract</th>
<th>Reagents</th>
<th>observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Glycoside</td>
<td>D/W</td>
<td>10% lead acetate</td>
<td>White ppt.</td>
</tr>
<tr>
<td>3</td>
<td>Reducing sugar</td>
<td>D/W</td>
<td>1. Benedict’s solution</td>
<td>Reddish brown ppt</td>
</tr>
<tr>
<td>4</td>
<td>Saponin glycoside</td>
<td>D/W</td>
<td>Distilled water</td>
<td>Sponge</td>
</tr>
<tr>
<td>5</td>
<td>Cyano genetic glycoside</td>
<td>D/W</td>
<td>Sulphuric acid</td>
<td>Colour does not discharged</td>
</tr>
<tr>
<td>6</td>
<td>Terpene</td>
<td>Ethanol</td>
<td>Acetic anhydrate</td>
<td>Colour does not discharged</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compound</td>
<td>D/W</td>
<td>10% ferric chloride</td>
<td>green</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrate</td>
<td>10% alcoholic napthol&amp; sulphuric acid</td>
<td>Molisch’s solution</td>
<td>brown</td>
</tr>
<tr>
<td>9</td>
<td>α- amino acid</td>
<td>D/W</td>
<td>Ninhydrin solution</td>
<td>Deep pink</td>
</tr>
<tr>
<td>10</td>
<td>Tannin</td>
<td>D/W</td>
<td>1 %Ferric chloride</td>
<td>Colour does not discharged</td>
</tr>
<tr>
<td>11</td>
<td>Flavonoids</td>
<td>Ethanol</td>
<td>Ferric chloride</td>
<td>Yellow</td>
</tr>
<tr>
<td>12</td>
<td>Polyphenol</td>
<td>Ethanol</td>
<td>1% Ferric chloride solution</td>
<td>Blue</td>
</tr>
</tbody>
</table>
4.2.2 Physicochemical characterization of *Anoectochilus albolineatus*

The quantitative analyses, namely determination of moisture content, determination of total ash, acid insoluble ash and extractive values for various solvents were made according to the methods described in the British pharmacopoeia (1980) and Myanmar Traditional formulary (1989). The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away. The acid insoluble ash is the part of the total ash which is insoluble in dilute hydrochloric acid. The results are shown in table 2.

Table 2. Quantity determined percentage of *A. albolineatus*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Physiochemical characters</th>
<th><em>A. albolineatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture content</td>
<td>71.09</td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>15.10</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>25.40</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble matter</td>
<td>17.05</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol soluble matter</td>
<td>6.30</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl acetate soluble matter</td>
<td>2.80</td>
</tr>
<tr>
<td>7</td>
<td>Petroleum soluble matter</td>
<td>2.25</td>
</tr>
</tbody>
</table>

4.2.3 Medicinal uses

According to local people of Man Ban, Nam Kut, Kho Lone, Kho Tin villages, Kutkai Township, *A. albolineatus* is called Ywet Hla Thit Khwa or Hnyte Phat Hla Thit Khwa. In China, the local people called it jin xian lan. In these villages, the juice of the whole plant is used in jaundice for children.
Moreover, the fresh or dried leaves are used for drink as tea regularly to decrease hypertension. Some people the fresh leaves are eaten as salads or boiled with rice. The local people used them for fever, snake bite, diabetes, and tuberculosis and liver diseases.

4.2.4. Market Information

According to the local people of Northern Shan State in 2009, the black market offered Kyats 25,000 - 30,000 for a viss of fresh plant and Kyats 60,000 - 100,000 for a viss of dried orchids. But in 2011, the market price increased up to 40,000 - 50,000 Kyats/viss of fresh orchids and 200,000- 300,000 Kyats/viss of dried ones respectively. The market price is higher in from September to November and most of the collectors are races of Palaung, Kachin and Shan. The fresh and dried orchid plants are usually smuggled out to China from the Kutkai via Muse route.

5. Discussion

5.1 Chemical analysis

The results of Preliminary phyto-chemical investigation showed that the whole plant of this species has consist mainly of the alkaloids and glycosides, saponin glycoside, phenolic compound, carbohydrate, acid, base, α-- amino acid, tannins and flavonoids are also present.

The results of Physiochemical characterization of A. albolineatus is moisture 71.09%, ash 15.10%, acid insoluble ash 25.40%, water soluble matter 17.05%, ethanol soluble matter 6.30%, ethyl acetate soluble matter 2.80%, petroleum soluble matter 2.25%.

5.2 Medicinal uses

In many centuries, orchids are valued locally for their medicinal, nutritional and ornamental qualities. Averyanove et al (2000) commented on the quantities of Anoectochilus spp., Cymbidium aloifolium, Dendrobium chrysanthum, Dendrobium fimbriatum, Goodyera spp. and Nervilia punctata being collected in northern Vietnam for the Chinese Medicinal Plant market. Some of the species used in Chinese herbal medicine, such as Dendronbium officinals are now very rare in the wild.
In Kutkai Township, the local people, especially Chinese, the juice of the whole plant are used for eye drop and some are used for tea.

5.3 Market Information

China, northern neighbor, has bought as much as possible the orchids from local people. Local brokers buy up orchids by offering enticing prices to villagers. In that area, the local people have been collected large amount of *Anoectochilus spp.* for selling. In the past, traders began buying in that area, local people have earned their extra income by selling orchids collected from the forest. Now, this species is becoming very rare and demand is high for medicinal orchid trade. Commercial collecting and selling benefit local people to a limited period, where the orchids are abundant. The villagers who are the main collectors actually earn very little compared their work. However, the immediate profit is going to the middlemen brokers and dealers based in the large cities or abroad as had been described by (Cribb et.al 2003).

6. Conclusion

Economically some members of the Orchidaceae family are important as they are prized for their spectacular beautiful flower. Among these some are in danger of extinction through the destruction of natural habitats. In Shan state, although orchid plants grow abundantly, now they became endangered due to over- collection and deforestation.

Cribb et al, (2003) stated that the main damage or extinction was caused by repeated collections in the same location. For example; a local collector collected the mature plants of *Anoectochilus* spp. But other local collectors led to removal of the remaining youngest juvenile specimens, and even mature seedlings. Due to this fact, the Northern Shan State forests and hills have been nearly depleted. Such destruction should be controlled and prevented by forest law and measures taken by local activities concerned.

An education programme should be implemented to higher public awareness of the need for conservation. Reproduction through modern techniques could result in cultured plants which could then be distributed rurally to enhance the villager's income as well as legalized the trade.
The natural resources of Myanmar could be prevented and prohibiting collection and selling of orchids without due consideration. Hence there should be selection of areas rich in orchids as "orchid preserves" at suitable levels in the hot spot areas to prevent deforestation, habitat destruction and collection by orchid lovers and exploitation by tradesman (Rao Anand T., 1996.). So, Protecting and conserving are forests in a National duty for the state and future generation.

Moreover, the Government of the Union of Myanmar has been raised the standard of Myanmar Traditional Medicine. As a rule, the aim of the government strategy is to upgrade the benefit of research to be useful and applicable purposes. On this altitude various medicinal research programs were carried out to improve and to standardize the preparations as were as to fulfill the country's needs and local needs.

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