Sarawak
Biodiversity Centre

Bunga Ta'ang

(Adenosma nelsonioides)

An essential oil producing herb from Sarawak

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Aloysius K.P. Sim, S.L. Sim, and the late Alexander L. Toyad

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Foreword

by

Dr Yeo Tiong Chia

Chief Executive Officer

Sarawak Biodiversity Centre

Traditional Knowledge is an important component of SBC's focus on research and development. It is one of the Centre's major undertaking where database on information of the plant uses by the indigenous communities, species identification, botanical description and cultivation are documented in systematic manner to preserve the knowledge and to discover new applications.

The book, "Bunga Ta'ang (*Adenosma nelsonioides*): An Essential Oil Producing Herb from Sarawak" was a result of the efforts of many people. *Adenosma nelsonioides* or commonly known as Bunga Ta'ang among the Bidayuh community in Sarawak has been used by practitioners for many generations as traditional medicine. Laboratory analysis indicated that its phytochemical properties has potential to be developed for several products such as for poultry, pet care products, larvicade, food preservative, healthcare, perfumery, aromatherapy and anti-cancer compound.

I would like to convey my deepest gratitude to the late William Sauu, who made this plant known to the world.

DR YEO TIONG CHIA

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Preface

Preface

For thousands of years man has traditionally relied on plants not only for food and materials but also to treat illnesses and relieve aches and pains. Man's relationships with plants have resulted in the development of crop varieties, unique materials for crafts, botanical medicines, perfumes, dyes and even bio-pesticides. Despite the development of modern medicines there is growing interest worldwide in natural products and products that contain active ingredients especially from plants. It is for this reason that traditional knowledge on the use of plants is constantly being mined for compounds that may become the lead in the discovery of a new product.

Sarawak, located in the island of Borneo, is a haven for making such a discovery. There are no less than 30 different ethnic communities living in close proximity to one of the oldest and most biodiversity rich tropical rainforests. Communities located in the interior and less accessible areas still rely heavily on the forest especially for food and medicine. However, communities located nearer to urban areas have become less dependent on traditional practises. As more and more communities become urbanised, traditional knowledge and practises are seldom used and sometimes forgotten especially by the younger generations.

The Sarawak Biodiversity Centre (SBC) is actively involved in documenting the traditional knowledge on the use of plant resources by various ethnic communities. In doing so, the SBC now maintains a database of information not only on plant uses but also its identification, botanical description, cultivation and potential uses.

The first in this series, *Adenosma nelsonioides*, is an herbaceous annual first introduced to SBC by the late Mr. William Sauu of Kampung Semadang. William, as he is fondly remembered, is an elderly but very well informed gentleman dearly missed especially for his knowledge of plants traditionally used in his village. Realising the dearth of information on the herb, SBC has selected it for documentation and further research.

This document is the result of cooperation between the Bidayuh community, SBC and some prominent scientists in Malaysia. It is intended as the first of a series of monographs on plants traditionally used by ethnic communities in Sarawak. The plant featured may not necessarily be endemic to the region but its use may be unique to the community it is associated with. Through this publication it is hoped that interests can be generated to do further research on its potential uses but at the same time create awareness of the relationship between the plant resource and the ethnic communities should any product be developed and commercialised using any part of the plant or its essential oil.

The late Mr. William Sauu (right) with the late Mr. Rimong recording traditional uses of plants.

Acknowledgement

Acknowledgement

The successful completion of this publication would not be possible without support from the State Government of Sarawak through the Sarawak Biodiversity Council. The dedication and hard work of officers and staff of the SBC must be mentioned. Others that provided valuable information and assistance are:

- The late Mr. William Sauu ak Nange, the late Mr. Rimong ak Sutur, Puan Roddiem ak Sabod and the Community of Kampung Semadang
- Agriculture Research Centre, Semengoh
- Institute of Medical Research Malaysia
- Rita Manurung, Paul P.K. Chai, Sim Soon Liang and Goh Swee Hock

A scenic view of the suspension bridge leading towards Kampung Semadang by the bank of the Sarawak River

Acronyms and abbreviations

CAS	Chemical Abstract Service
DDA	Disc Diffusion Assay
DMSO	Dimethyl sulfoxide
GC-MS	Gas chromatography-mass spectrometry
GI_{50}	Growth Inhibition 50
IUPAC	International Union of Pure and Applied Chemistry
Kpg	Kampung, village
LC_{50}	Lethal Concentration 50
MIC	Minimum Inhibitory Concentration
MTP	Microtiter plate
PPM	Parts per million
SK	Sekolah Kebangsaan, National type school
SRB	Sulforhodamine B

Chapter 1

Introduction

Introduction

by
Margarita Naming

Adenosma nelsonioides (Miq.) Hallier *f. ex* Bremek locally known as Bunga Ta'ang to the Bidayuh community of Kampung Semadang in the Padawan area, is a strongly aromatic herbaceous plant. Officers and staff of the SBC first came across Bunga Ta'ang during a documentary filming at the village in April 2007. The film was made to illustrate the rich traditional knowledge on the use of biological resources by the Bidayuh community in that village.

During the filming, the late Mr. William Sauu was happy to share his rich knowledge and used Bunga Ta'ang as an example of a plant known to the community. In one scene he took some leaves, crushed them between his fingers and a strongly aromatic odour immediately filled the air. The odour is not unlike that of 'diesel' which made the villagers refer to the herb as a 'diesel plant'. The scent lingered on for quite a while and is present in various parts of the plant throughout its life cycle. The scent can also be detected in dry leaves and inflorescences even after one year.

According to William, the plant has been used by villagers for generations to treat skin ailments such as rashes and itch and to get rid of fleas and ticks on domestic animals especially chickens. For skin ailments, the whole plant is boiled for half an hour and left to cool. The water is then used to treat the affected skin. It can also be used to improve unpleasant body odour. Dried plants are also used as a repellent to get rid of bed bugs when placed under pillows or mattresses. In the case of chickens infested with fleas the dried plants are placed in the four-corners of the chicken coop.

Bunga Ta'ang can be found growing in the open field, paddy field, around cocoa plants and pepper gardens. It is often regarded as a weed especially in paddy fields where its growth is most vigorous. It is likely that for the first half of its life cycle, the herb may depend on the burning for nutrients while in the second half it competes with the paddy plant for fertilizers

applied by the farmers. From these observations it is assumed that the herb should do well if planted as an intercrop with rice, cocoa or pepper (Fig. 1).

Information on *A. nelsonioides* is scanty. In Sarawak, the first record of collection was made by Hanne Christensen and mentioned in her book "Ethnobotany of the Iban & the Kelabit" (2002). The Iban people of Nanga Sumpa in Sri Aman Division call the plant *Bangkit Perawan*. It is used in religious events. The herb is boiled with the shoots of wild ginger *Etlingera punicera* (Tepus) and agarwood or Gaharu *(Aquilaria* spp*.)*. The concoction is used to decorate the hornbill image "to add to the pleasant and potent aroma of the hornbill image". The plant is also used as a freshener and the leaves are used in perfume mixture. Hanne's specimen (No. 1559) is deposited in the Sarawak Forest Department herbarium and the plant was subsequently identified as *Adenosma nelsonioides*. Later collections are all made by SBC from several locations in Kampung Semadang, Temurang, SK Kambug and Annah Rais in the Padawan area near Kuching (Fig. 2).

Reports of other *Adenosma* species are known from China, India, Vietnam and Laos. Traditional uses by local communities have led SBC to study the properties and potential uses of essential oil produced by the plant. This publication contains the results of various studies on cultivation, propagation and steam distillation of essential oil and its components, and its potential for the development of new products.

Fig. 1. Habitats of Bunga Ta'ang (a) open field, (b) rice or paddy field, (c) cocoa garden, and (d) pepper garden

Fig. 2. Known distribution of *Adenosma nelsonioides* in Sarawak

Chapter 2

Botanical Description

Botanical Description

by
Jovita E. Ripen & Paul P.K. Chai

Adenosma nelsonioides (Miq.) Hall. *f. ex* Bremek

Family: Scrophulariaceae

Local name: Bunga Ta'ang (Bidayuh from Padawan)

Distribution: Lowland from near sea level up to 1,000 m altitude in the Kelabit highlands.

Strongly aromatic herb up to 60 cm tall, branching near ground. **Stem** 4-angled or square in cross section, young stem soft and light green, turning purple with age and becoming semi-woody, 2 to 3.5 mm in diameter, often trailing. **Stipules** absent. **Leaves** simple opposite and sessile (stalkless), bearing numerous tiny translucent oil glands which can be seen when the leaf is held against the light or with a hand lens. Leaf shape highly variable from linear to linear-ovate and lanceolate and resembling a spear, base heart-shaped; leaf blade size variable from 1.5 x 0.4 cm to 2.2 x 0.6 cm when young, 3 x 0.8 cm to 4.4 x 0.9 cm when fully mature; leaf margin smooth to slightly wavy towards the apex. Dry leaves dark brown and remain attached to the lower stem.

Inflorescences terminal, 1.5 to 4 cm long. Floral bracts closely spiral and leaf-like, generally ovate and narrowing towards apex. **Flowers** bisexual, solitary in leaf axils, up to 0.9 cm long, 0.4 cm wide; corolla tubular, light purple to light blue, 2-lipped, lower lip spreading, tinged light yellow and slightly longer than the upper lip. Stamens 4 and arranged in two pairs of unequal lengths, anthers yellow; stigma club-shaped becoming bi-fid and very light purple in colour. **Fruit** capsules in pairs and enclosed in dry floral bracts, ovate and flattened on one side, apex pointed, 2 x 1.2 mm in size; **seeds** tiny and numerous, dark brown and visible through the thin translucent fruit wall.

Ecology: Occurs in abundance in paddy fields after harvest on damp clayey soils, also found in waste land and open areas with similar soil type. When the plant is cleared together with other weeds before the next planting season, the tiny seeds with tough seed coats remain dormant in the soil for several months and germinate after the paddy is harvested.

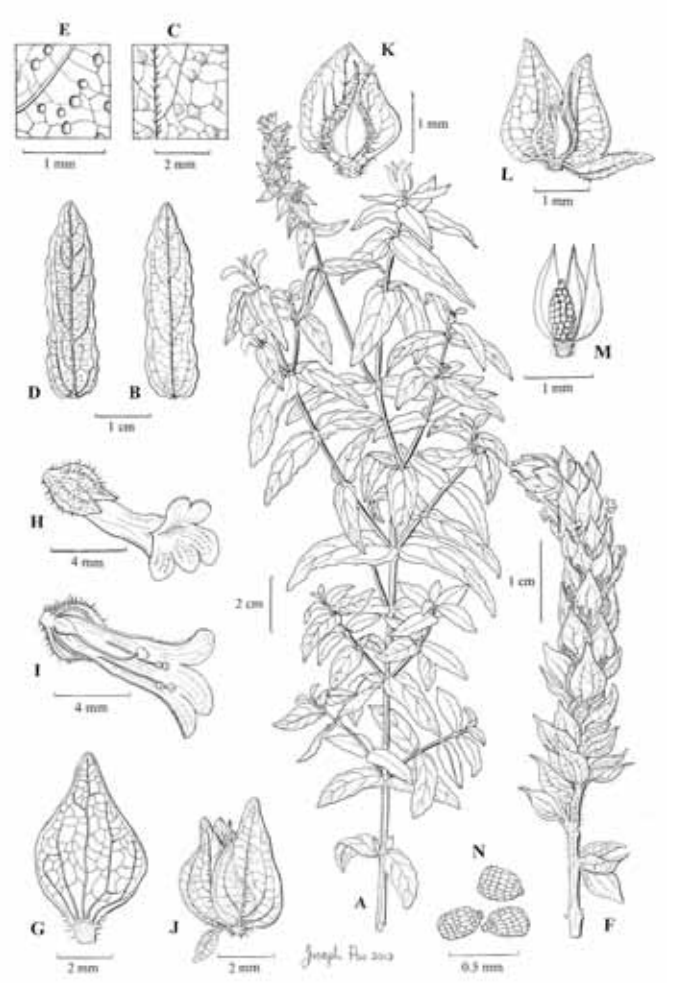

Fig. 3 ***Adenosma nelsonioides.*** A – Habit (X1), B – Upper surface of leaf (X2), C - Upper surface of leaf (X10), D - Lower surface of leaf (X2), E - Lower surface of leaf (X30), F – Inflorescence and infructescence (X3), G – Flower bract (X10), H – Flower (X7), I - L.S. of flower (X7), J – Fruit surrounded by 6 bracts (X10), K – 3 Bracts taken off showing part of the fruit (X10), L – Bract taken off showing the slightly open capsule (X10), M - Fruit with one capsule taken off showing the seeds (X12) and N – Seeds (X50).

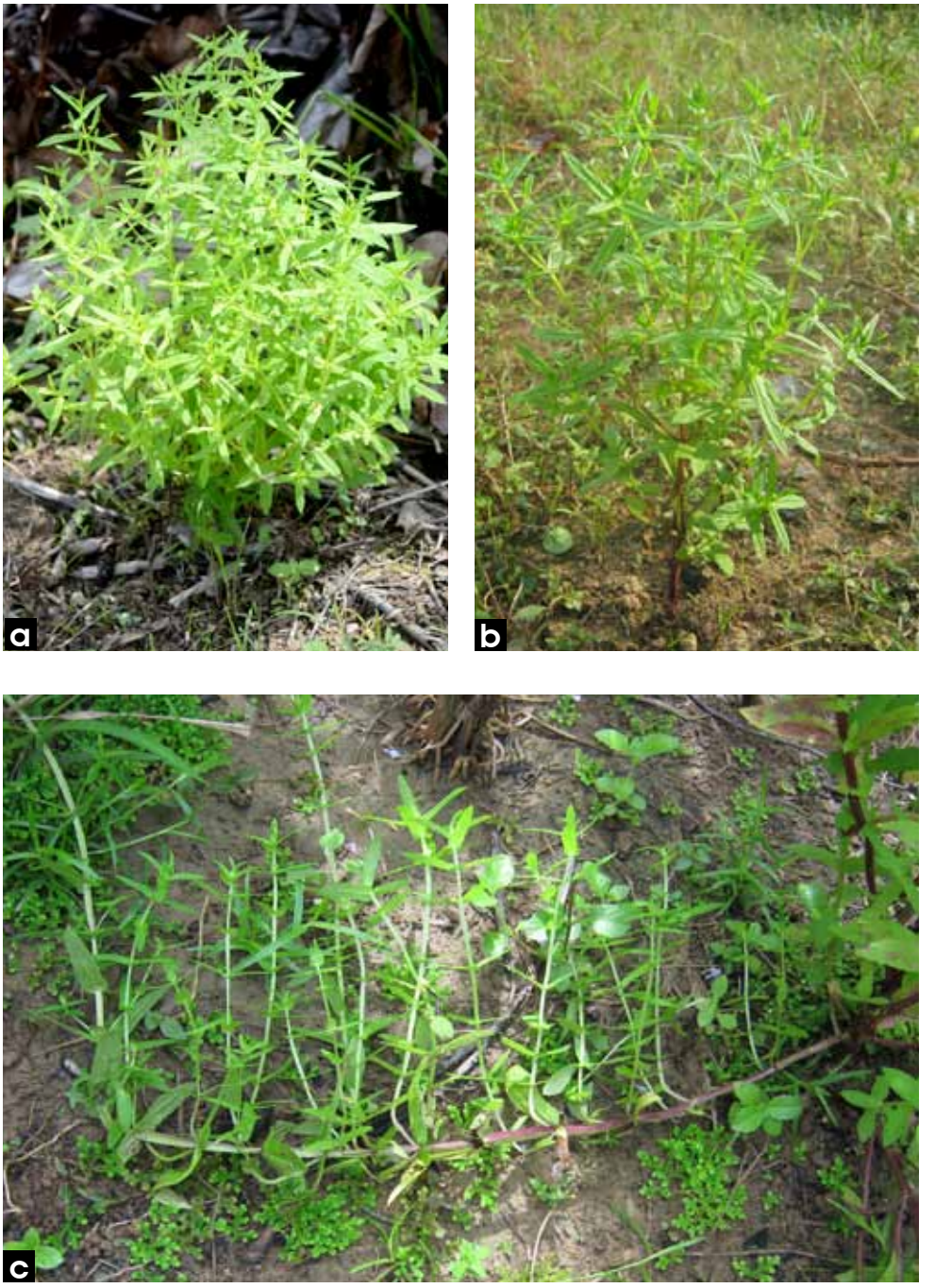

Fig.4 Bunga Ta'ang growing in paddy field. (a) Bushy plant with profuse branching, (b) a single upright plant and (c) plant with trailing stem producing roots from the nodes.

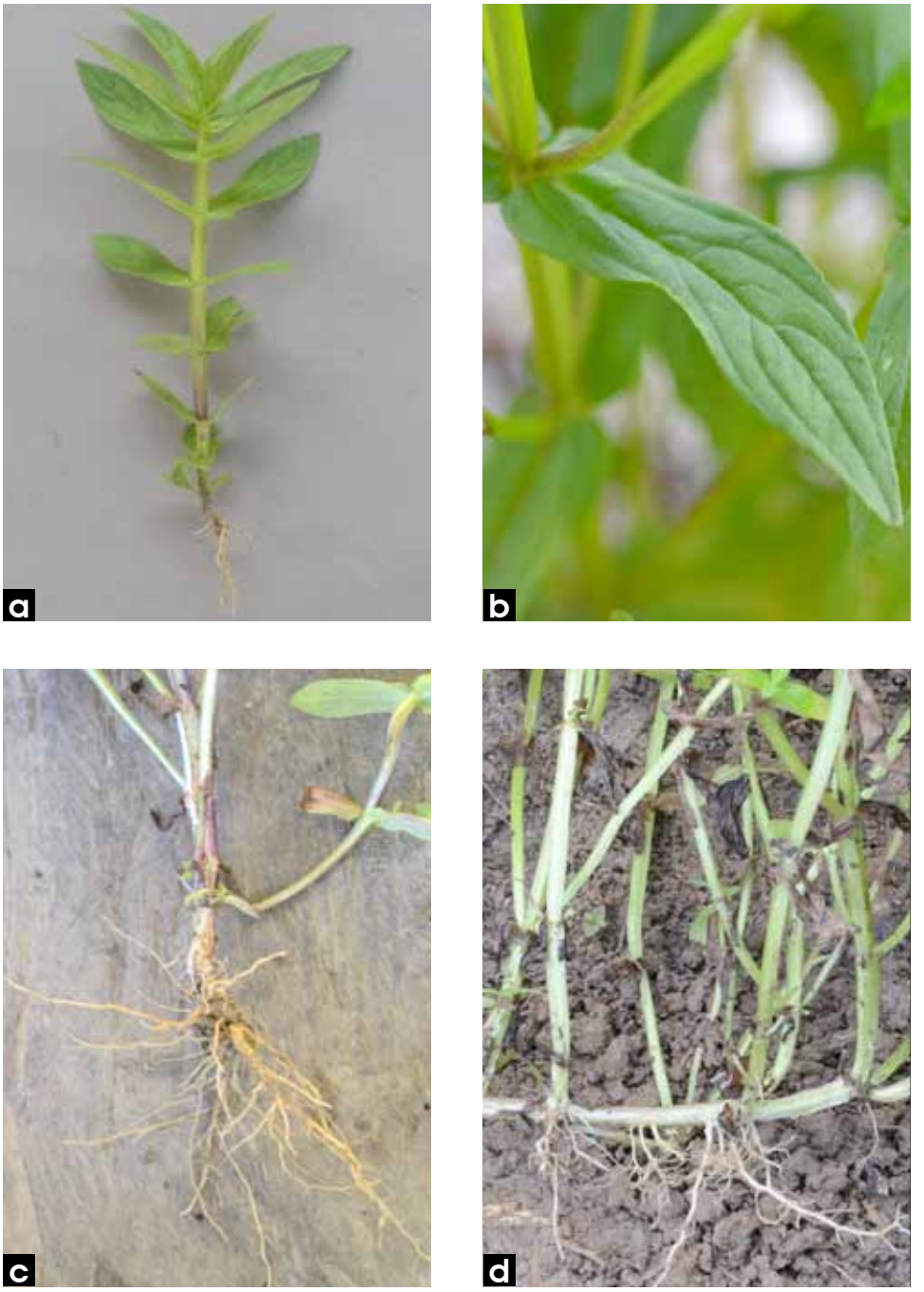

Fig. 5. Vegetative characters (a) Young plant with its root system, (b) leaf shape, (c) roots of a mature plant, and (d) adventitious roots arising from a trailing stem.

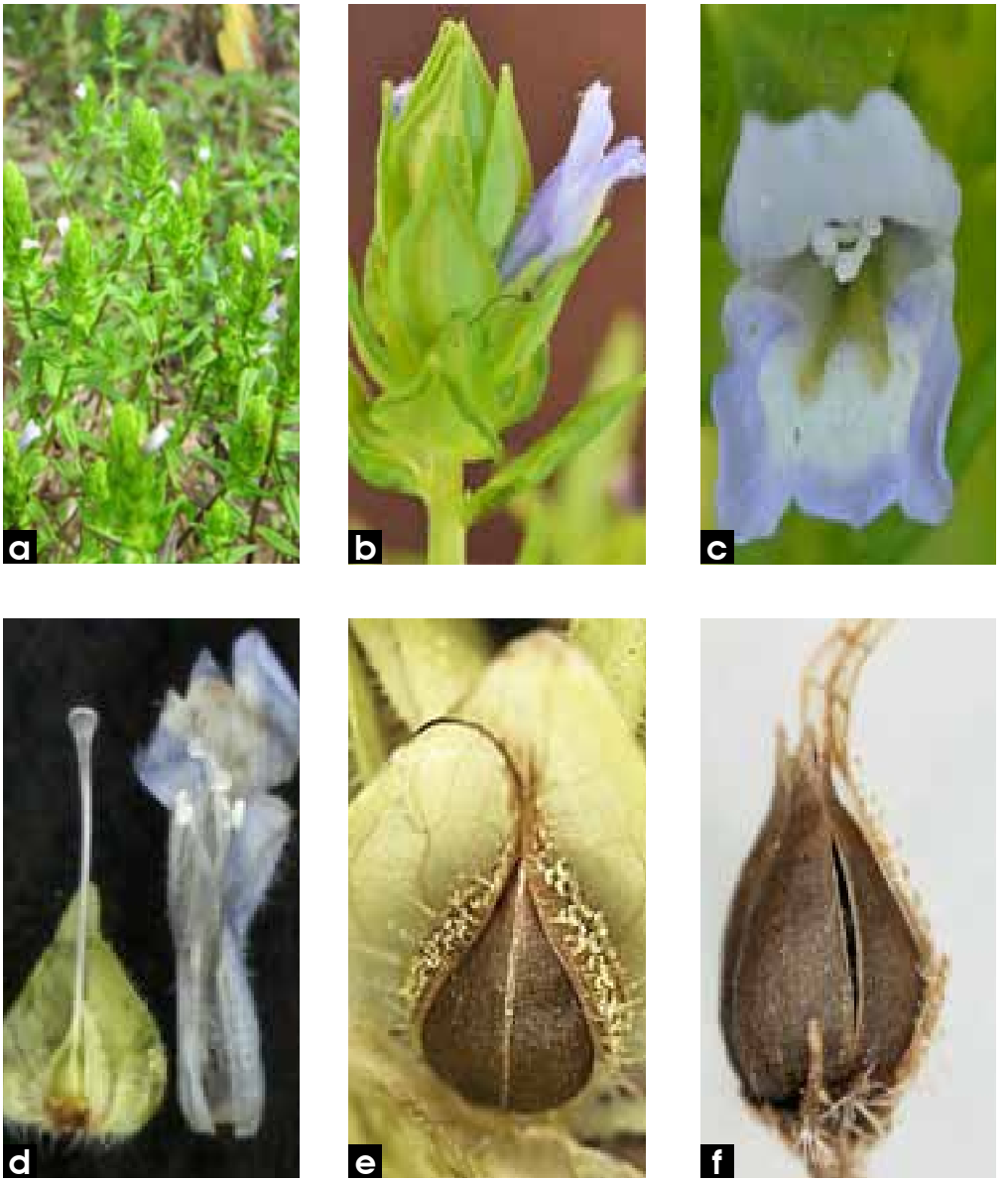

Fig. 6. Inflorescences and flowers (a) Flowering plant, (b) side view of a flower (c) top view of an open flower to show arrangement of the petals, (d) gynoecium (stigma, style, ovary and stamen), (e) capsule, and (f) dehisced capsule.

Chapter 3

Growth and Development

Growth and Development

by

Aloysius K.P. Sim, Alexander L. Toyad & S.L. Sim

3.1 Natural life cycle

In the Penrissen and Padawan areas, growth of Bunga Ta'ang is more often closely associated with the paddy season than with other crops like vegetables, maize, cocoa and pepper. In Kampung Semadang, the paddy season normally begins with clearing and burning the fields from August until September. This is followed by direct sowing of paddy seeds in October. From October to February the following year, the paddy crop will be tended, weeded and fertilised by the villagers until it is ready for harvest from mid-February to early March. After harvest, the field is left to fallow until August when the season begins again with clearing and burning.

The natural life cycle of Bunga Ta'ang is observed by randomly placing several quadrats in the paddy field where the herb is seen to grow in the previous year. The seedlings begin to appear about two months after burning around November. More seedlings continue to germinate and by December certain parts of the paddy field are covered by vigorously growing young plants (Fig. 7).

From early January to March the following year many small light blue or light purple flowers appear on the terminal inflorescences. One species of wasp has been seen visiting the flowers (Fig. 12f). Fruits are produced by April as the plants begin to dry and turn brown. Many tiny seeds are enclosed in the dry bracts.

Because growth of Bunga Ta'ang between September and March coincides with the paddy growing season, it is often considered by the villagers as a weed. Distribution of the plant

is uneven; while some areas of the field may be thickly covered, other areas may be patchy or with only a few plants. Most of the seedlings are found near mother plants suggesting that dispersal may be limited.

Fig. 7. Bunga Ta'ang after paddy harvest

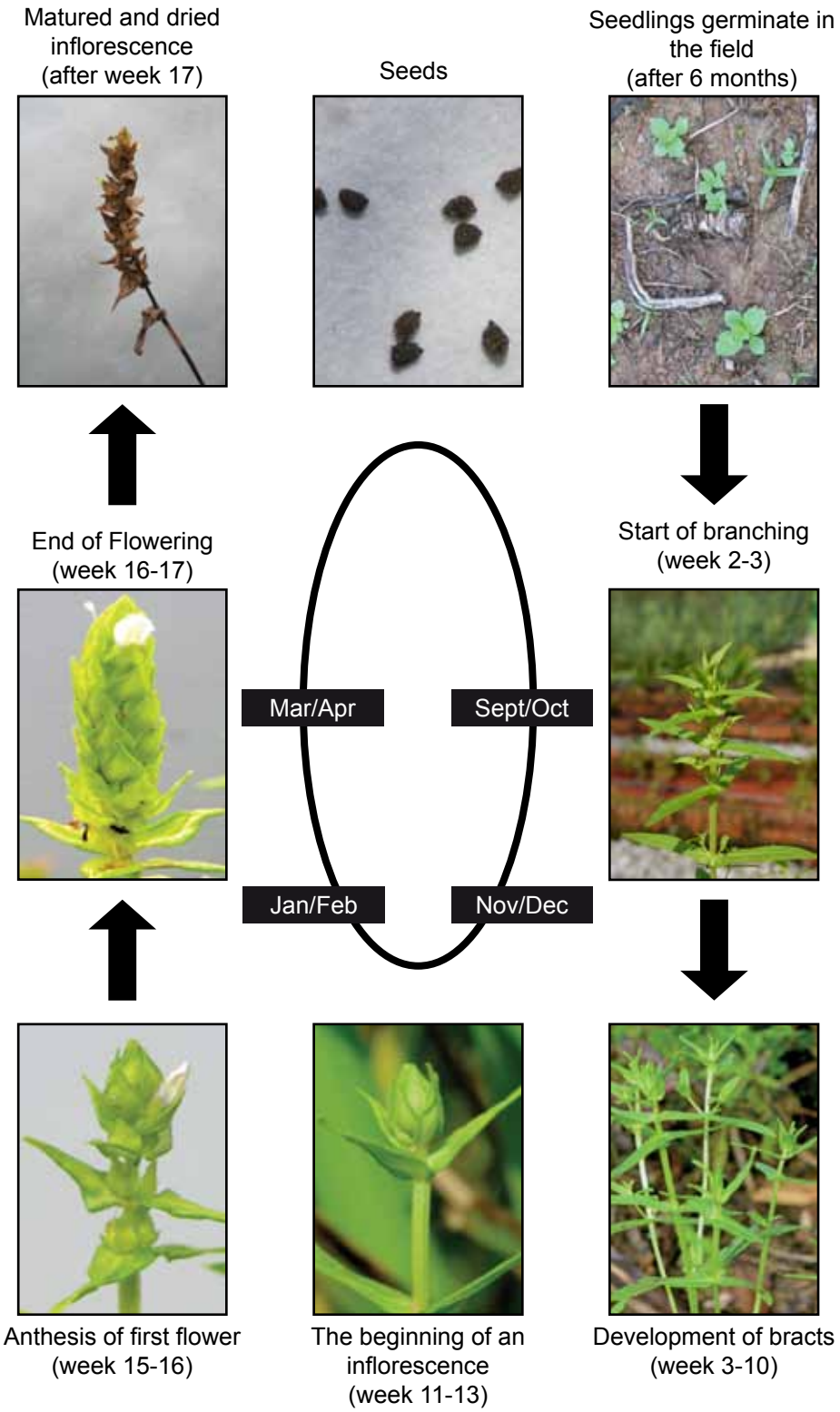

Figure 8. The natural life cycle of Bunga Ta'ang.

3.2 Vegetative growth

Under nursery conditions without any treatment Bunga Ta'ang seeds begin to germinate after about six months. This seems to indicate a period of dormancy or that the seeds may require some stimulus to trigger rapid germination. In the paddy field, germination begins about 2 months after burning. This suggests that heat could play a role in hastening germination.

During the first three weeks the seedlings grow upright without branching (Fig. 9a). Branches begin to appear in the axils of the leaves after four weeks. After two months, a healthy plant will grow to about 42 cm tall with 13 nodes (Figs. 10a and 10b), and up to 20 primary and 50 secondary branches. After five weeks, the lower part of the main stem becomes woody and dark red in colour (Fig. 4c and Fig. 10c), similar to the colour caused by the presence of anthocyanin. This coloration also appears on older branches.

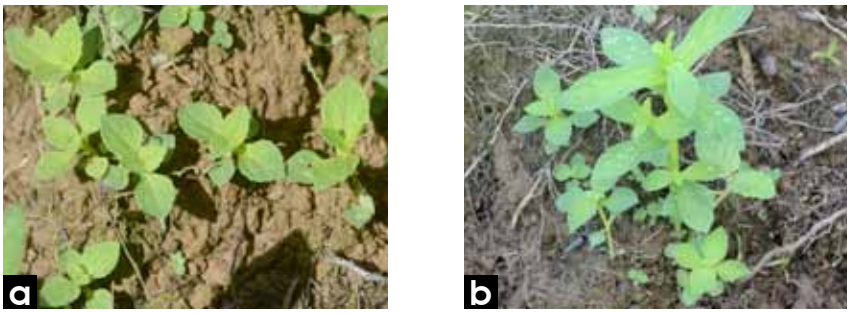

Fig. 9. Young seedlings growing in the paddy field (a) one week old, and (b) after 3 weeks.

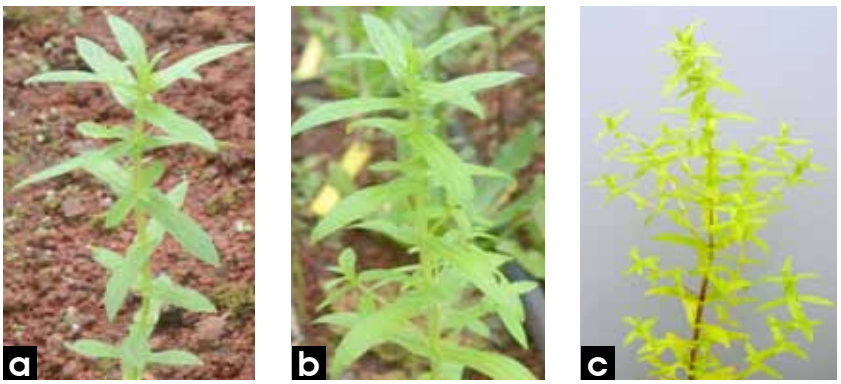

Fig. 10. Branching of Bunga Ta'ang (a) Plants at 4 weeks old, (b) primary branches at 6 weeks old, and (c) profuse branching after 8 weeks.

Observations on height increment of ten plants indicate vigorous growth during the first eight weeks after which growth begins to slow down (Fig. 11). The plant enters the reproductive phase during the 7th and 8th weeks with the emergence of floral bracts.

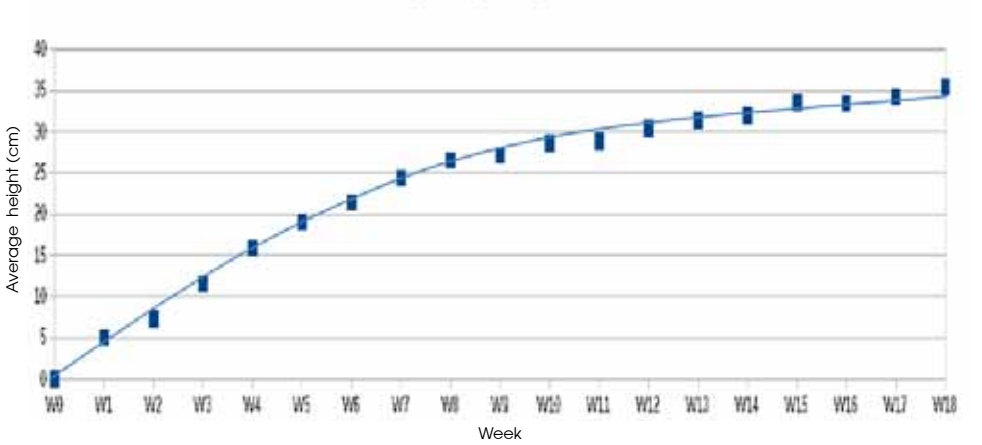

Fig. 11. Average heights of Bunga Ta'ang over a period of 18 weeks.

Plants that grow among the paddy crop grow more vigorously and attain an average height of 35.5 cm, branching profusely and producing many flowers. In contrast, plants that appear after the paddy season are weak and attain a mean height of only 23.3 cm, probably due to the lack of nutrients (fertilizers) and the dryer weather towards the middle of the year.

3.3 Reproductive Growth

The first sign of flowering is the emergence of floral bracts when the plant is 7 to 8 weeks old (Figs. 12a and 12b). The bracts can be distinguished from the leaves by their ovate shape and spiral arrangement whereas the leaves are linear and opposite. The change from vegetative to reproductive phase is indicated by the appearance of narrow and pointed bracts at the end of the terminal shoot.

Flowering occurs after ten weeks. The terminal inflorescence (Fig. 12c) slowly elongates and up to three flowers will bloom each time (Figs. 12d and 12e). The mature inflorescences range in length from 3 to 5 cm. Likely pollinators include a species of wasp (Fig. 12f). As the flowers are produced, the leaves will begin to dry up starting from the base of the stem. After 16 weeks the inflorescence will slowly turn brown and become dry. Tiny seeds contained in small capsules are enclosed in the bracts (Figs. 13a and 13b). Seed dispersal appears to be by mechanical means as the dry seed pods dehisce and push the seeds out. Animal dispersal is also a possibility.

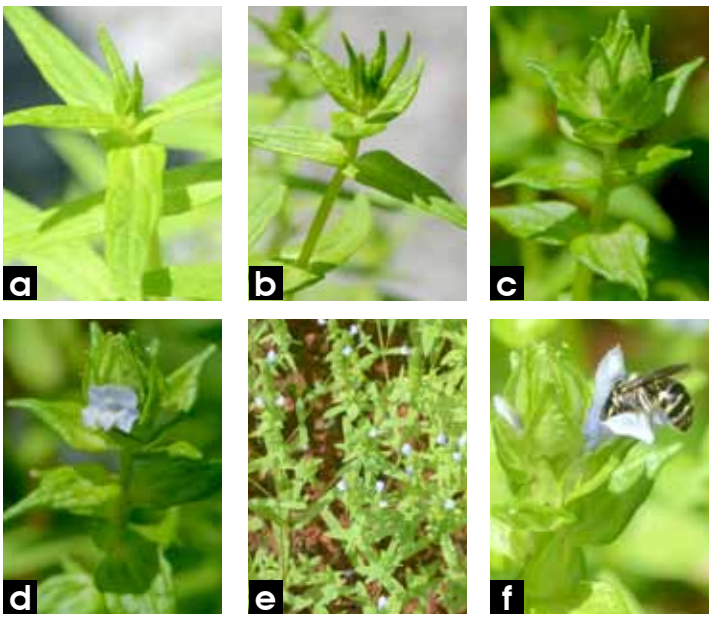

Fig. 12. Development of floral bracts, inflorescences and flowers (a&b) Development of floral bracts, (c) emergence of inflorescence, (d) opened flower, (e) elongation of inflorescence, and (f) a wasp visiting one of the flowers.

Fig. 13 (a&b). End of the reproductive phase with fruit development and drying of floral bracts.

3.4 Natural Mode of Propagation

The tiny seeds probably remain dormant in the soil until the next burning season to prepare the field for paddy planting. Many seedlings can be seen growing together with young paddy plants one to two months after planting. Bunga Ta'ang also propagates naturally by vegetative means, notably from stems trailing on the ground (Fig. 15) and also fallen branches (Fig. 14). From these stems, shoots are produced that grow upright to form new plants. The stems anchor themselves by producing roots from the nodes and internodes (Fig. 15).

Fig. 14. New shoots arising from a fallen branch

Fig. 15. Adventitious shoots produced on a fallen stem

3.5 Nursery propagation

3.5.1 By layering

In layering, the whole plant is placed horizontally with the nodes on the main stems and branches covered in the soil. Rooting and production of new shoots from the nodes occur after one week (Figs. 16a and 16b). After four weeks, the new shoots are ready to be removed from the stems for potting (Fig. 16c). Some of the shoots can be re-layered to produce more new plants.

By using this method, a single mother plant can potentially produce 6 to 10 rooted plantlets or axillary shoots from each branch depending on the number of nodes present. Some of the new axillary shoots can further proliferate producing up to 20 plantlets after three weeks.

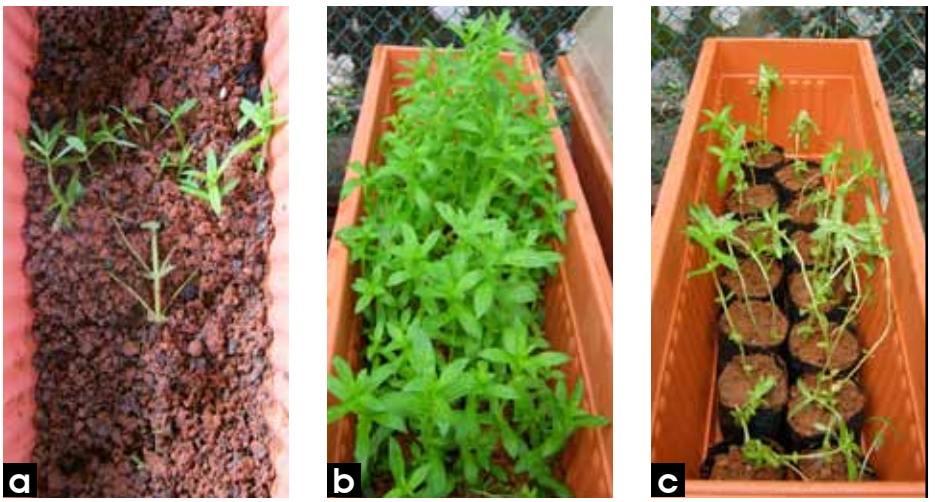

Fig. 16. Stages in propagation by layering (a) A mature plant placed horizontal and partly covered with potting medium, (b) new growth after one month, and (c) rooted shoots potted in polybags.

3.5.2 By rooting of cuttings

Cuttings can be made from the main stem and main branches. Sections containing three-nodes are potted in sand, top soil mix or perlite mix media (Fig. 17a). Perlite is a naturally occurring white siliceous rock that is lightweight and can expand by absorbing water when wet. Rooting percentage is high with mortality recorded at only 5% for all the media.

Roots are observed in the second week when rooted cuttings are ready to be potted into polybags. Cuttings grown in the top soil produce more widespread and extensive root system than those grown in the other two media (Fig. 17c). The newly rooted plants are acclimatized and fertilized (Fig. 17b). They are ready for transplanting in the field after six weeks (Fig. 18).

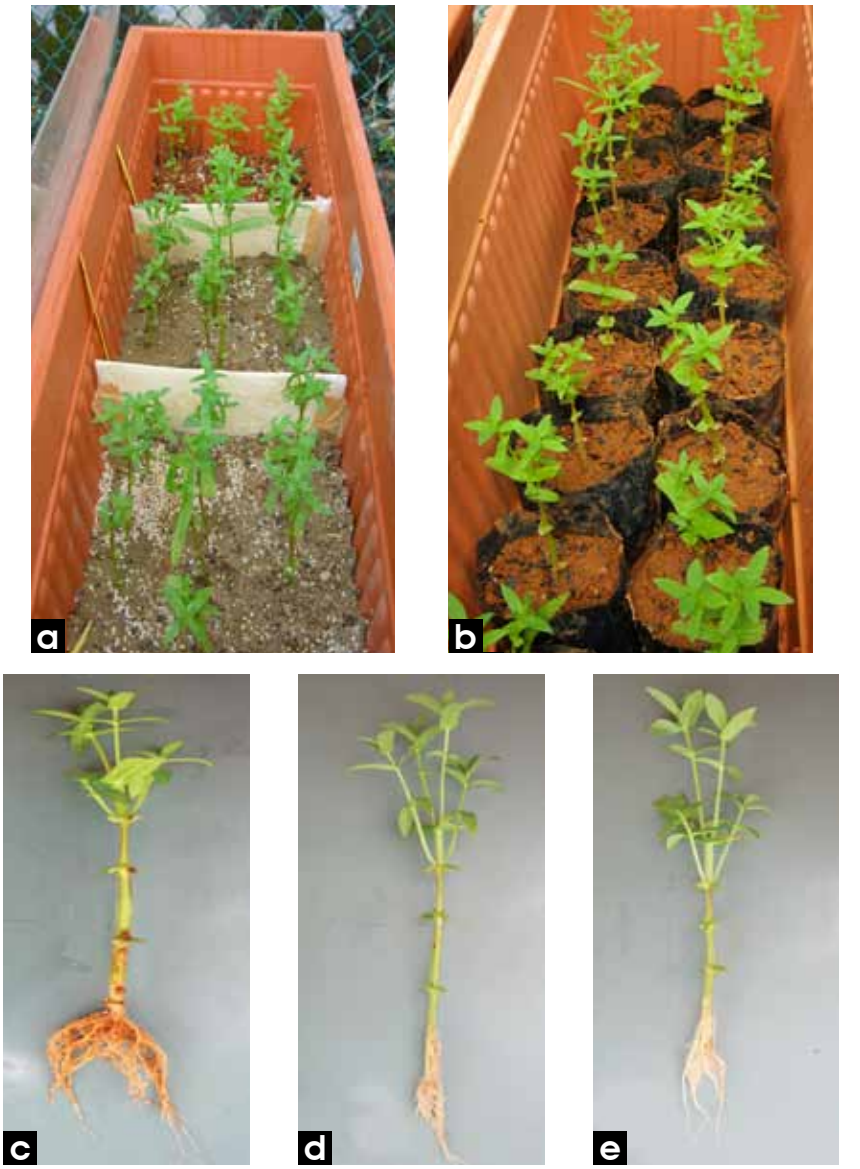

Fig. 17. An experiment to test suitability of medium for rooting in the nursery
(a) Growth in 3 types of medium, (b) healthy rooted cuttings being weaned, (c) healthy root system after 20 days in top soil mix, (d) root system after 20 days in sand and perlite mix, and (e) root system after 20 days in perlite.

Fig. 18. A healthy plant with many branches after 2 months

3.5.3 Rooting of cuttings in water

Alternatively, cuttings can be rooted in water. Cuttings with 4 nodes are dipped in water with two of the nodes immersed (Fig. 19a). Roots begin to appear after three days; and after one week, 70% of the cuttings have successfully produced roots (Fig. 19b). However, prolonging submersion in water for more than a week will cause the stem to rot. Rooted cuttings must be removed from the water after one week, otherwise, 10 to 30% of the cuttings will rot and die.

Rooting of cuttings in water is quick and easy. This method of propagation requires minimal preparation and space, and can be done in a pail of water.

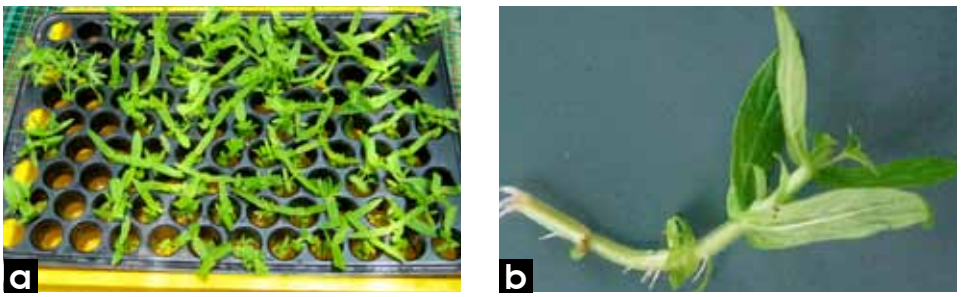

Fig. 19(a&b). Cuttings successfully rooted in water

3.6 Diseases, pests and their control

In the nursery, mite infestation is common causing the plants to weaken. As a result, secondary infection by the fungus *Colletotrichum* sp. occurs (Fig. 20). The fungal infection appears as necrotic spots on the leaves which eventually wither. If left unattended, the infection can spread to the whole plant and also to surrounding plants within a week.

Early prevention can minimize damage caused by the fungus. If infected plants are treated with fungicides, they may produce healthy new shoots. However, a small percentage of the infected plants failed to recover even after treatment, and any new shoots produced were stunted and weak.

To prevent fungal infection, the control of mite infestation is crucial. Miticide with the active ingredient Propagite or Amitraz can be sprayed once a month. At the onset of fungal infection, all infected leaves are to be removed and the fungicides Mancozeb and Benomyl can be applied alternately every two weeks (Table 1).

Fig. 20. Leaves infected by *Colletotrichum* sp.

Table 1. Pest and disease control

Type of infection	Causal organism	Method of control
Pest	Mite	Spray with 0.05-0.1% Propagite or Amitraz
Fungal	*Colletotrichum* sp.	Remove infected leaves and spray with 0.1% Mancozeb and 0.3% Benomyl alternately every two weeks.

Chapter 4

Chemistry

Chemistry

by
Noor Pahtiwi Bt Bohari & S.H. Goh

4.1 Essential oil from *Adenosma nelsonioides*

The essential oil from Bunga Ta'ang is a complex mixture of compounds obtained by steam and hydro-distillations from the aerial parts of the plant. The distillation process yields between 0.4 and 0.6% (v/w) of essential oil based on fresh weight. The oil is pale yellow to yellow in colour and has a pungent/spicy scent (Figs. 21a and 21b).

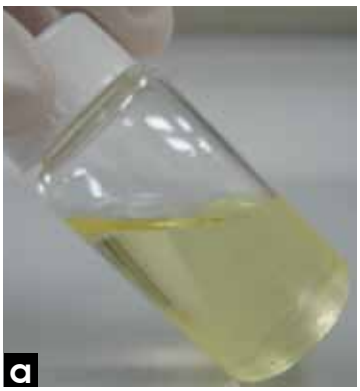

Fig. 21. (A) A sample of the essential oil and (B) its physical properties

Physical Properties	
Colour	**: Pale yellow to yellow**
Appearance	**: Liquid**
Solubility in water	**: Not soluble**
Boiling point	**: 55°C**
Specific gravity	**: 0.9192 (Min) and 0.9194 (Max)**
Refractive index	**: 1.4979 (Min) and 1.4980 (Max)**
pH	**: 3.03**
Relative density	**: 0.92011 g/cm³**

b

The GC-MS analysis has identified 7 compounds which represents the major chemical constituents of the oil. Fig. 22 shows the total-ion peak abundance and retention times. The two most abundant components, carvacrol (51.52%) and γ-terpinene (18.35%), are indicated based on peak areas. Other smaller components of the oil with more than 2% relative peak areas are given in Table 2.

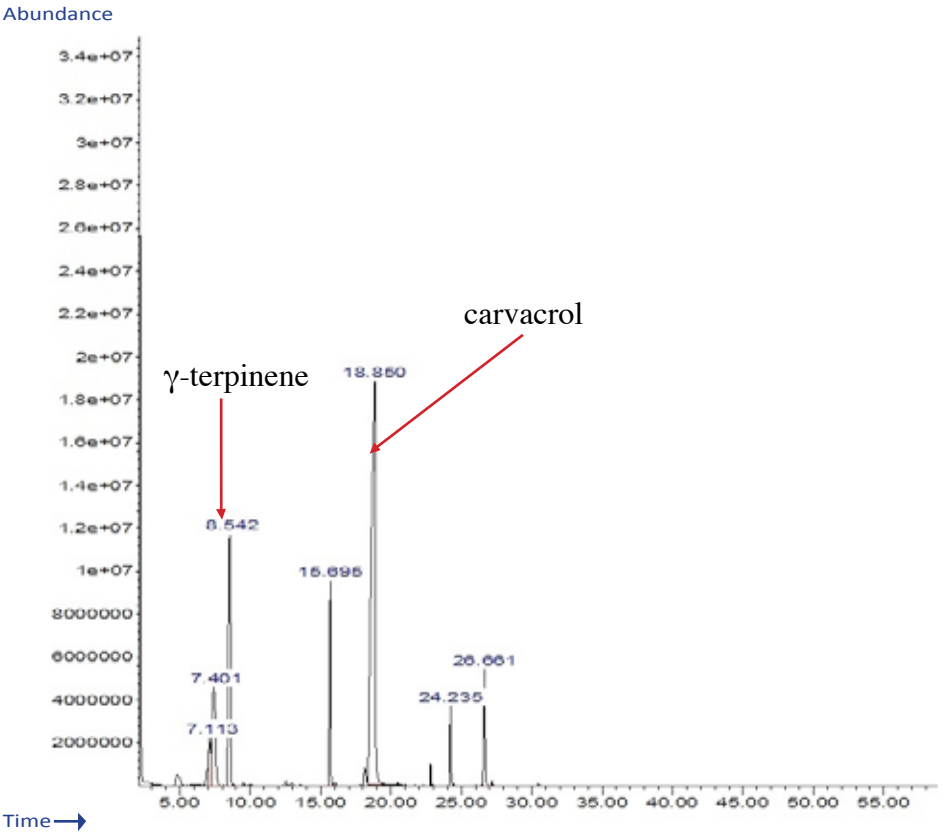

Fig. 22. Chromatogram of Bunga Ta'ang essential oil

Table 2. Chemical constituents of Bunga Ta'ang essential oil

Compounds	Relative Retention Time (min)	Molecular Formula	Relative Peak Areas (%)
2-Carene	7.11	$C_{10}H_{16}$	3.53
o-Cymene	7.40	$C_{10}H_{14}$	10.62
γ-Terpinene	8.54	$C_{10}H_{16}$	18.35
Carvacrol methyl ether	15.70	$C_{11}H_{16}O$	8.40
Carvacrol	18.85	$C_{10}H_{14}O$	51.52
α-Caryophyllene	24.24	$C_{15}H_{24}$	2.58
β-Bisabolene	26.66	$C_{15}H_{24}$	5.01

(Column type HP-5MS 5% Phenyl Methyl Siloxane capillary column 30 m x 0.25 mm x 0.25 µm Agilent)

Carvacrol, $C_{10}H_{14}O$

IUPAC Name : 5-isopropyl-2-methylphenol

Synonyms : 2-Methyl-5-isopropylphenol;Phenol, 3-isopropyl-6-methyl; Isothymol; *o*-Thymol; 2-Hydroxy-*p*-cymene, 2-*p*-Cymenol, 5-Isopropyl-*o*-cresol

CAS Name : 2-methyl-5-(1-methylethyl)phenol
(No. 499-75-2)

The structure of carvacrol is evident from GC and GC-MS as shown in Fig. 25. A summary of properties and known uses of carvacrol is given in Table 3.

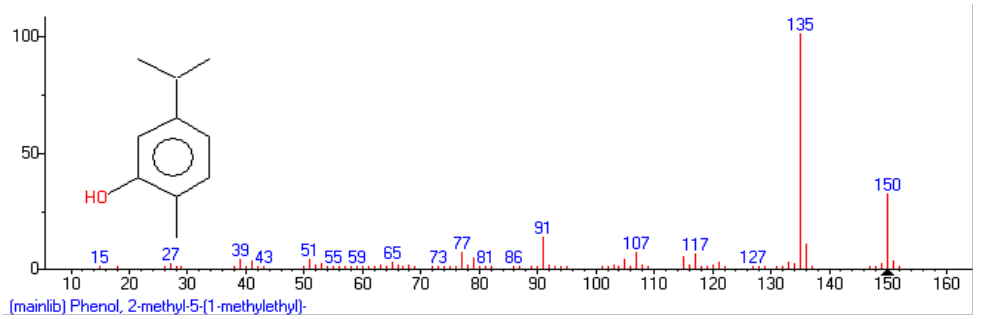

Fig. 23. Structure and Mass Spectrum of carvacrol

Table 3. Known uses and properties of carvacrol

Known uses	Properties
Cosmetics	Fragrance used in perfumes, personal care and household products such as soaps and creams
Food Industry	Flavouring agents in candies, pickles, meat sauces, seasonings and baked goods Antimicrobial properties to eliminate the growth of microorganisms that cause food spoilage
Green Pesticide	Insecticidal and acaricidal activities against pest arthropods Biocidal activities against ticks, fleas and mosquitoes

ɣ-terpinene, $C_{10}H_{16}$

IUPAC Name : 1-methyl-4-(propan-2-yl)cyclohexa-1,4-diene

Synonyms : 1-Methyl-4-isopropyl-1,4-cyclohexadiene; 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-; *p*-Menthadiene;1-Isopropyl-4-methyl-1,4 cyclohexadiene

CAS Name : 1,4-Cyclohexadiene, 1-methyl-1-(1-methylethyl)- (No. 99-85-4)

The structure of the second largest component was determined by GC and GC-MS (Figure 26) as ɣ-terpinene. A summary of properties and known uses of ɣ-terpinene is given in Table 4.

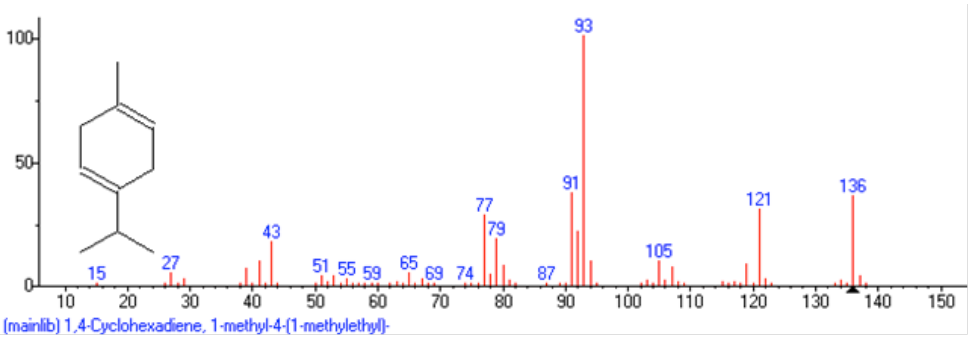

Fig 24. Structure and Mass Spectrum of γ-terpinene

Table 4. Known uses and properties of ɣ-terpinene

Known uses	Properties
Green Pesticide	Insecticidal and acaricidal activities against pest arthropods
Food Industry	Flavouring agents in candies, pickles, meat sauces, seasonings and baked goods

Table 5. Properties and uses of the two largest constituents

Compound	Information
Carvacrol	Approved by the FDA for food and drug (CITE: 21CFR172.515, Revised as of April 1, 2011) Fragrance, preservatives (food and cosmetics), antiseptic and disinfectants Among the most active constituents against insects e.g. mosquitoes, house fly, ticks, fleas and mites (Koul,. *et al*, 2008) Antifungal, antimicrobial, anti-inflammatory
γ-Terpinene	Approved by the FDA for food and drug (CITE: 21CFR172.515, Revised as of April 1, 2011) Inhibited the growth of the fungal root pathogens *Phytophthora cinnamomi* and *Fomes annosus* (Krupa & Nylund, 1972) Limited to the level 0.05-10 ppm in finished foods, GRAS

4.2 Yield and composition of the essential oil from different plant parts

Different parts of the herb were collected (Fig. 25), separated (Fig. 26) and analysed by GC-MS. The results are given in Table 6. As shown, the highest percentage of essential oil yield comes from the inflorescences at 1.89%, followed by the leaves at 1.38%. Stems only yield a low 0.06% and no oil is extracted from the roots. Comparing the essential oils from four different plant parts, the primary difference of the essential oils is the concentration of carvacrol and ß-Bisabolene as shown in Table 7. The highest percentage of carvacrol (60.79%) is found in stems, and the lowest in inflorescences with only 31.13%. ß-bisabolene found in the oil from inflorescences (31.83%) is about three times more than that from the stems (10.60%) and ten times more than that from the leaves (3.40%)

The results of the present study indicate that the chemical composition of the essential oil varies in deferent parts of the plant.

Table 6. Yield of the essential oil from different plant parts

Plant Parts	Fresh weight (g)	Oil weight (g)	Yield (%)
Leaves	151.06	2.08	1.38
Stems	155.57	0.09	0.06
Inflorescences	75.83	1.43	1.89
Roots	128.47	0	0

Table 7. Chemical composition of the essential oil from different plant parts

Compound	Total Peak Area (%)			
	Leaves	Inflorescences	Stems	Roots
2-Carene	3.16	1.41	0	0
o-Cymene	8.08	3.65	5.19	0
γ-Terpinene	15.85	8.37	7.19	0
Carvacrol methyl ether	6.72	17.85	12.82	0
Carvacrol	59.28	31.13	60.79	0
α-Caryophyllene	1.39	3.68	3.41	0
β-Bisabolene	3.23	31.83	10.6	0
β-Sesquiphellandrene	0	1.55	0	0

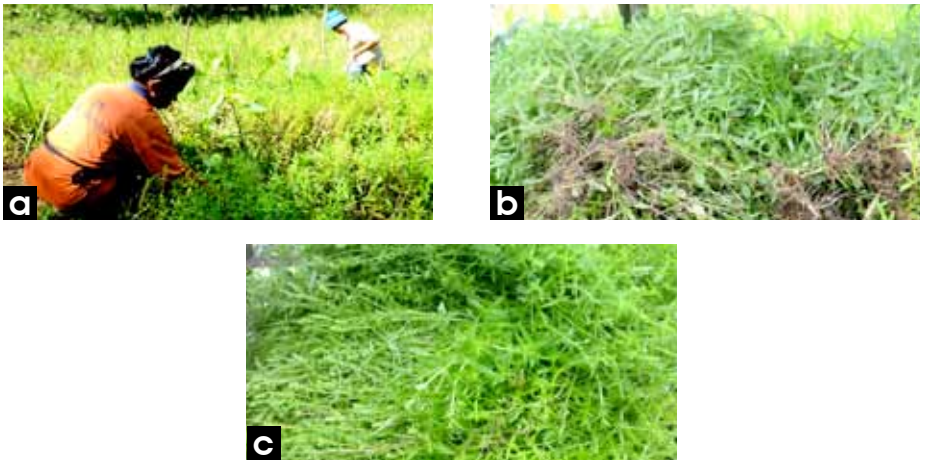

Fig. 25. Collecting plants for essential oils (a) Harvesting Bunga Ta'ang from paddy field, (b) bulk collection of whole plants, and (c) plants collected at the flowering stage.

Fig. 26. Separating plants into different parts for essential oil analysis (a) Leaves, (b) stems, (c) inflorescences, and (d) roots.

Table 8. Chemical composition of essential oils at different stages of plant development

Compound	Total Peak Area (%)		
	Vegetative	Early Flowering	Flowering
2-Carene	2.60	2.57	2.06
o-Cymene	6.04	6.86	5.84
γ-Terpinene	16.50	15.40	12.76
Carvacrol methyl ether	3.23	3.77	7.67
Carvacrol	71.62	71.40	63.88
α- Caryophyllene	0	0	2.46
β-Bisabolene	0	0	7.38
β-Sesquiphellandrene	0	0	0

Table 8 indicates that both the major compounds carvacrol and γ-terpinene are consistently abundant during the vegetative and early flowering stages but decrease during late flowering stage. Thus the result indicates that for essential oil distillation, the plant can be optimally harvested at the vegetative stage.

Figure 27. Separation of samples for oil determination (a) Plant at the vegetative stage, and (b) at the flowering stage.

Chapter 5

Potential Uses of the Essential Oil

Potential Uses

by

Harny Chapy & Yeo Jiun-Tzen

5.1 Anti-microbial and Anti-fungal activities

The essential oil obtained from Bunga Ta'ang has been evaluated for antimicrobial activity using disc diffusion assay (DDA) for primary screening and Microtiterplate (MTP) assay to measure the Minimum Inhibitory Concentration (MIC) against 11 different pathogenic microorganisms, including Gram-positive bacteria, Gram-negative bacteria, yeast and fungus. The values for MIC of the oil are considered as the first concentration where spectrophotometrically 70% or more reduction is measured against tested microorganisms with concentration measured in mg/mL. As for the positive control, Chloramphenicol is used for bacteria and Nystatin for fungus and yeasts, both at concentration of 1.6 mg/mL. Each assay is performed in triplicates and repeated three times separately to ensure the reproducibility of the results. The antimicrobial properties of the essential oil are summarized in Table 9.

Table 9. Antimicrobial activities of the essential oil from Bunga Ta'ang

Microorganisms	Bunga Ta'ang oil		Antibiotic control	
	DDA	MTP	DDA	MTP
Gram positive bacteria				
Staphylococcus aureus	++++	+++	++	++++
Bacillus subtilis	++++	++	++	+++
Micrococcus luteus	+++	++	++	+++
Streptococcus mutans	++	++	+	+++
Gram negative bacteria				
Escherichia coli	++	+	+	++++
Pseudomonas aeruginosa	+	+	+	++++
Porphyromonas gingivalis	+++	++	++	+
Yeast				
Saccharomyces cerevisiae	++++	+++	+	+++
Rhodoturula glutinis	++++	+++	+	++++
Pitirosporum ovale	++++	++	+	+++
Fungus				
Aspergillus niger	+++++	++	+	+++++

(Results are mean of 3 separate experiments)

Strength	MIC (mg/mL)	DDA (mm)	Activity
-	No activity	0	Resistant
+	> 1.60	9 - 20	
++	0.50 - 1.59	21 - 40	Intermediate
+++	0.20 - 0.49	41 - 60	
++++	0.05 - 0.19	61 - 80	Susceptible
+++++	< 0.05	No cell growth	

The results indicate that the essential oil has antimicrobial activity of varying magnitudes against all the tested microorganisms. About 20 µL of oil is used in primary screening using DDA method and the results indicate the strength of antimicrobial activity ranging from 10 to 90 mm. Based on the DDA results as shown in Table 9, the fungus *A. niger* is the most susceptible strain to the essential oil. *A. niger* is found to be completely inhibited by the essential oil as there is no cell growth observed. While the oil is observed to have mild activity on both of the tested Gram negative bacteria, it is found only to partially inhibit *P. aeruginosa*. The values for antibiotic control in DDA are in the range of 15 to 31 mm (antibacterial) and 17 to 20 mm (antifungal).

Microtiterplate (MTP) assay is done to determine the lowest concentration of oil that inhibits or reduces the visible growth of each organism. The MIC of the essential oil is detected in the range 0.2 to 6.25 mg/mL against 11 pathogenic microorganisms (Table 9). Yeast species (*S. cerevisiae and R. glutinis*) and Gram positive bacteria (*B. subtilis*) are most susceptible to the oil. Based on the results obtained, Gram-positive bacteria appear to be more susceptible towards the oil compared to Gram-negative bacteria. Antibiotic control for MIC is detected in the range of 0.05 to 6.25 mg/mL (antibacterial) and 0.02 to 0.4 mg/mL (antifungal).

5.2 Cytotoxic activities against cancer derived cell lines

Lung cancer cells (NCI-H460) and hormone-dependent breast carcinoma (MCF-7) cell lines are frequently used as reference cell lines in cytotoxic assays to measure the potential cytotoxic activities of extracts. Preliminary results of *in-vitro* cytotoxic activity of Bunga Ta'ang essential oil and carvacrol (98%, Sigma Aldrich) against NCI-H460 have yielded similar results, but with varying results in the MCF-7 cell lines, both evaluated using Sulforhodamine B (SRB) assay method (Table 10). Extracts with promising activities can be further tested with specific cancer cell lines. Table 10 shows the GI_{50} values of Bunga Ta'ang essential oil and carvacrol on cell lines. After 48 hours exposure, the Bunga Ta'ang essential oil's cytotoxic effects on NCI-H460 cell line (GI_{50}) is 102.0 µg/mL and 71.0 µg/mL for the MCF-7 cell

lines. As shown in Table 10 also, the sensitivity of carvacrol (98%) is found to have GI50 of 100 µg/mL on NCI-H460 cell line and GI_{50} of 21 µg/mL on MCF-7 cell line respectively. These results indicate that the cytotoxic activities of Bunga Ta'ang essential oil may have been contributed by the carvacrol present in the oil.

Table 10. Comparison of cytotoxicities (GI_{50}) of the essential oil and carvacrol (both reconstituted with DMSO to a concentration of 100 mg/mL) against NCI-H460 and MCF-7 cell lines

Extract	GI_{50} (µg/mL)	
	NCI-H460	MCF-7
Bunga Ta'ang Essential Oil	102.0	71.0
Carvacrol 98%	100.0	21.0
Solvent Control (DMSO)	No Activity	No Activity

5.3 Larvicidal activities

The larvicidal potential of the essential oil is evaluated against early-fourth instar larvae of *Aedes aegypti* and *A. albopictus* (studies with researchers from the Institute of Medical Research Malaysia, IMR) using WHO standard of larval susceptibility test method (WHO, 1981). Preliminary investigation has indicated that 24 hours exposure to early fourth-instar larvae of *A. aegypti* and *A. albopictus* with the essential oil results in 50% mortality at 100.0 mg/L and 82.0 mg/L respectively (Table 11). From the LC_{50} value obtained in the study, it clearly indicates that the oil is considered to be effective against both the *Aedes* larvae, and is found to be more toxic against *A. albopictus* than *A. aegypti larvae* based on the lower concentration required to achieve LC_{50}.

Table 11. LC_{50} values of Bunga Ta'ang essential oil on *Aedes aegypti* and *A. albopictus* after 24 hours exposure as reported by IMR

Mosquito	LC_{50}	Regression line
Aedes aegypti	100 ppm (100.0 mg/L)	Y = 10.15x - 14.19
Aedes albopictus	82 ppm (82.0 mg/L)	Y = 8.03x -10.53

This is the first report on the larvicidal activity of the essential oil against *A. albopictus* and *A. aegypti*. Several studies have shown that essential oil containing carvacrol possesses significant larvicidal activity against *A. aegypti* larvae, but limited information is available on the effects of carvacrol-containing oils against *A. albopictus*. Since this study has found that Bunga Ta'ang essential oil is 1.2 times more toxic to *A. albopictus* compared to *A. aegypti*, it demonstrates the potential of the oil as a new source of biocide against the larvae of both *A. albopictus* and *A. aegypti* mosquitoes.

Chapter 6

Research and Development Needs

Research and Development Needs

by
Aloysius K.P. Sim & S. L. Sim

The rich biodiversity of indigenous plants in Sarawak is a valuable resource for research into new crops and new products that can be developed from them. Bunga Ta'ang is a good example of a relatively unknown plant traditionally used by an ethnic community for a specific purpose. The Sarawak Biodiversity Centre, through its documentation of Traditional Knowledge programme came across this plant and have begun systematic research on the properties of its essential oil.

It must be noted that research on biodiversity in Sarawak is controlled under the enactment of The Sarawak Biodiversity Centre Ordinance (1997) and its amendments. The legislation is to ensure that should any product be commercialised from plants traditionally used by the ethnic communities in Sarawak, recognition must be given to the community that provided the plants or the knowledge that leads to the development of the products from the plants.

In the case of Bunga Ta'ang, there is still much to be done before any large scale commercialisation can result. As in other plants or future crops, relevant research on aspects of domestication is necessary in order that a regular supply of quality raw materials can be available for a cottage industry or for prospective entrepreneurs.

Some areas of focus for further research are suggested here.

6.1 Cultivation, Productivity and Cost

6.1.1 Selection and assessment of planting materials. To maximise the essential oil yield, it is important that the Bunga Ta'ang planted is from plus mother trees. Clonal propagation will then be applied to obtain uniform plants with desired traits

such as high production of essential oil, vigorous growth, pest and disease resistance, and other beneficial traits.

6.1.2 Cost-effective propagation techniques. To compare the use of seeds and rooted cuttings as planting material. Preliminary finding indicated germination of Bunga Ta'ang seed could be accelerated by heat treatment and cuttings could simply be rooted in water. Thus, the optimal condition for the heat treatment of seeds and rooting need to be established. A comparison of the cost-effectiveness of these two techniques of propagation is necessary so that the best can be adopted.

6.1.3 Cultivation method and fertiliser requirements. Uneven and patchy growth of the plant indicates the need to initiate a site feasibility study to determine the best environment for growth. Determine the best organic fertilizers for use, the quantity to be applied and frequency of application in order to maximize the vegetative growth and ultimately the yield of essential oil.

6.1.4 Planting density/Commercial planting. Bunga Ta'ang can apparently exist as an intercrop species and grows best with up-land paddy, but less well with cocoa and pepper. Research should focus on growing the plant as a crop by itself, determine suitable sites, planting space, cost of maintenance, productivity per hectare and profits.

6.1.5 Harvesting and post-harvest handling. To determine the stage when the essential oil yield is highest. To harvest only the top without uprooting and leaving about 10 cm above ground for regeneration of new shoots. Post-harvest handling is also required to minimise setbacks from bulk harvest and increase productivity.

6.1.6 Planting duration and cycle. It is important to understand the duration from plant establishment until harvesting to maximize productivity and to minimize material, cost and labour wastages. The study on the number of planting cycles per year will ensure better farm management practices which will increase the raw materials for essential oil extraction and decreases the costs incurred.

6.2 Product Development

6.2.1 Poultry and pet care products. The first idea of making use of carvacrol from Bunga Ta'ang comes from observing and documenting the traditional use of Bunga Ta'ang by the local communities. They placed dried Bunga Ta'ang at four corners of the chicken coop to ward off chicken fleas and ticks. They also place the same under their pillow to get rid of bed bugs. These showed that Bunga Ta'ang or carvacrol (major chemical component at approximately 60%) possesses repellency characteristic against acaricides that can be developed into pet care products.

6.2.2 Larvicide for the control of mosquitoes. From preliminary works conducted on the effects of the essential oil against *Aedes* mosquitoes larvae showed larvicidal activities on 2 common dengue fever and Chikungunya fever vectors, *Aedes aegypti* and *A. albopictus*. With this finding, the plant based essential oil could potentially be used as an alternative for the oil used for fumigation for mosquito control.

6.2.3 Food preservation and healthcare. Anti-microbial studies conducted indicated that the essential oil can suppress the growth of several common Gram positive and negative bacteria. It also arrests the growth of yeasts and *Aspergillus niger* fungus. With these properties, it has the potential to be developed as an anti-microbial, anti-yeast and anti-fungal in healthcare products or as food additives in food preservation.

6.2.4 Perfumery and aromatherapy. The strongly aromatic essential oil could potentially be used in the perfume industry. As the aroma of the essential still persists even after the plants are dried, it can also be used as potpourri.

6.2.5 Anti-cancer compound. Preliminary works to test the cytotoxicity of the essential oil against 2 cancer cell lines namely the lung cancer (MCF-7) and breast cancer (NCI-H460) cell lines has shown that the essential possesses cytotoxic activities. However, further research is still required to probe into the potential use of Bunga Ta'ang essential oil in anti-cancer compounds.

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