



Ethnobotanical Profiles and Phytochemical Constituents of *Barringtonia racemosa* L. for Potential Scrutiny of Bioactive Compounds through Plant Biotechnology

Nurul Izzati Osman¹, Norrizah Jaafar Sidik^{1*} and Asmah Awal²

¹Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.

²Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.

Abstract

This paper reviews the traditional uses and customs of people in the use of *Barringtonia racemosa* L., a type of plant mangrove species in their daily life and medicinal approaches. In addition, the phytochemical constituents and the studies in plant biotechnology carried out on this species were also reviewed and summarized by referring to the available documented literatures. It is aimed to provide a systematic review of such topics to facilitate understanding and providing information regarding that particular species. From the current review of literature, it has been clearly depicted that *B. racemosa* is having promising potentials for natural product discovery through plant biotechnology to be further scrutinized in this species. The information gathered from the ethnobotanical uses of this species as well as its phytochemical constituents are useful to provide a significant background for future works regarding plant secondary metabolites from this species to be further explored by the application of plant biotechnology.

Keywords: *Barringtonia racemosa* L., Ethnobotany, Ethnopharmacology, Plant Bioactive Compounds, Phytochemistry

1. Introduction

Our mother nature has a great diversity of plant species and the existence of plant kingdom provides various benefits towards mankind. Plants may beneficially used for psychological, spiritual, cultural and medicinal elements¹. Traditional uses of plants and its relationships with human are termed ethnobotany and are very well associated with cultural practice and belief. Indeed, the ethnobotanical approach which is based on the indigenous uses of plants has been the basis for modern scientific research in pharmacological studies and phytochemical screening. Furthermore, the usefulness

of plants not only lies in its medicinal properties used in herbal treatment but in fact, plants have been among the most significant element in daily household needs and being incorporated in industrial setting for instance in the production of agrochemicals, flavours, colourants and fragrances due to the presence of bioactive compounds in plant species².

Highly valued as a natural pharmaceutically-important reservoir, plants are also known as an important source of industrially-valuable compounds due to plant bioactive constituents which are inherently exist in plant species. There are numerous studies have

*Author for correspondence

Email: nurulizzati70@gmail.com and norri536@salam.uitm.edu.my

been documented so far reporting the importance of plant bioactive compounds in natural product discovery. However, productions of these compounds are critically affected by environmental factors such as climatic changes, nutrient availability, pathogenic threats and cultivation difficulties³. Therefore, in addition to the studies in the area of natural product chemistry, the application of plant biotechnology to produce bioactive agents by manipulating *in vitro* growth conditions and requirements has emerged as an exciting area of discovery. For instance, the research on cell suspension cultures establishment could be potentially used as a viable alternative to natural plant extraction and functions as a platform provider for high-value secondary metabolite production⁴.

One of the most widely distributed plant species with myriad functional uses is *Barringtonia racemosa* or commonly known as powder puff tree or fish poison tree. It is a type of mangrove species and has been used in various tribes around the world due to its wide geographical distribution from eastern Africa to northern Australia⁵. One of its well known functions is it could be used as fish poison and thus reflects its common name as the 'fish poison tree'. Since *B. racemosa* is widely distributed geographically, therefore it has so many vernacular names⁶ and being called differently in different regions of the world.

Ethnobotanically used as effective remedy for certain ailments, *B. racemosa* had been scientifically proven to have medicinal properties through various pharmacological activity studies as well. Previously, a record on *B. racemosa* pharmacological properties had been documented⁷ and the pharmacological effectiveness of this species therefore could serve as an important clue for further scrutiny of natural drug discovery of lead compounds to be carried out in this species. Even though *B. racemosa* had been used ethnobotanically in various tribes and had been proven to have significant pharmacological properties, however the existing literatures documenting plant biotechnology applications on *B. racemosa* are quite limited. Therefore, more studies could be possibly undertaken in this regard involving the applications of plant tissue culture.

The investigation pertaining to propagation potential through plant tissue culture had been documented in earlier reports⁸ through which the leaves were used

as the explant to regenerate the production of *in vitro* plantlets. In addition to that, the plant biotechnology approach had been incorporated in a very few studies which include the establishment of callus culture and cell suspension culture of the species⁸⁻¹¹. Due to the scarcity of investigations in plant biotechnology-related areas conducted on *B. racemosa*, therefore, there is a great potential for the species to be further studied in such a field.

This review was presented by fragmenting the information gathered on *B. racemosa* into ethnobotanical uses which encompassing its non-medicinal and medicinally-related uses respectively as well as its phytochemical constituents. The reviews compiled in this paper are meant to facilitate better understanding and to provide systematic documentation on ethnobotanical uses of this plant species and its phytochemical constituents.

2. Botanical Description

2.1 Taxonomy and Nomenclature

Barringtonia racemosa is one of the species in Plantae kingdom from genus *Barringtonia* which are classified under the family of Lecythidaceae. The *Barringtonia* genus got its name after Hon. Daines Barrington, 1727-1800, an English nobleman, lawyer, antiquary and naturalist who wrote a book on English trees^{5,12}. Botanically, the 'racemosa' term at the later part of the scientific name are reflecting the racemes structure of the species that it possesses which depicts a string-like arrangement of stalked flowers⁵. The taxonomic hierarchy of *B. racemosa* could be arranged in the following order¹³:

- Kingdom: Plantae.
- Subkingdom: Viridiplantae.
- Infrakingdom: Streptophyta.
- Superdivision: Spermatophytina.
- Class: Magnoliopsida.
- Superorder: Asteranae.
- Order: Ericales.
- Family: Lecythidaceae.
- Genus: *Barringtonia*.
- Species: *Barringtonia racemosa* (L.).

2.2 Vernacular Names

Owing to its widespread geographic distribution, the vernacular names of *B. racemosa* exist in various tribes and regions of the world. Different vernacular names are tabulated in Table 1.

2.3 Botanical Features

The trees of *Barringtonia racemosa* is a small to medium-sized evergreen shrub of around 4 to 8 m⁵ or occasionally large up to 20 to 27 m tall¹⁴ or even up to 30 to 47 m in height¹⁷. The bole may reach up to 50 cm¹⁴ to 100 cm in diameter and branch-less for up to 18 m¹⁷. Sometimes the bole having buttresses, smooth bark or fissured with grey or yellow hue. According to Orwa et al.⁵, no aboveground roots are present but they may have spreading surface roots. However, other record claimed that knee roots (pneumatophores) are sometimes present¹⁴.

Leaves of *B. racemosa* are alternate, crowded towards the ends of twigs, simple; with the stipules which are very small and caducous. The petioles are approximately up to 1.5 cm long, slightly winged with elliptical to obovate-oblong or oblanceolate blade of 5-42 cm × 2-16 cm in size. The characteristic of leaf base is cuneate and narrowly tapering running into very short petiole, while the apex is deep green, acuminate, broadly tapering. The leaf appears to be entire to shallowly toothed or scalloped margin^{5,14}.

Flowers of *B. racemosa* are attractive and fragrant with the colours of white to pale pink arranged in many-flowered pendulous sprays of up to 60 cm in length or even more, hanging on axillary inflorescence^{5,17} as presented in Figure 1. The pendulous raceme could reach up to 70 to 100 cm long. Flowers are bisexual and sepals joined at the base, separating in 3-4 lobes. The flower petals are elliptic, up to 3 x 1 cm, attached to the staminal tube with numerous, long, white or pinkish stamens forming a large central mass of 3.5 cm in diameter. The ovary is 2 to 4 chambered and the style is red⁵.



Fig. 1. Pinkish white flowers of *B. racemosa* are hanging on axillary inflorescence.

Table 1: The vernacular names of *B. racemosa* according to geographical regions

Language/ Country / Region	Vernacular Name	Reference
English	Barringtonia, brack-water mangrove, common putat, hippo apple, powderpuff tree, wild guava.	6, 14
Malaysia	Putat kampung, putat padi, putat darat, putat kedul.	15, 16
Brunei	Angas gimpalang, angas gimplang, putat aying.	6, 17
Indonesia	Butun, alakan, butun darat, kungkungan, putat.	6, 17
Myanmar	Kye-bin, kyi	6
Cambodia	Dawm trojiekbres, pchek tekbray	6
Philippines	Botong, ulam, kasouai, putat, tuba-tuba, kutkut-timbalon, nuling, paling	6, 17
Thailand	Chik	17
Laos	Som pawng	6
Papua New Guinea	Paniak, paopao	6
China	Yu rui	6
India	Samudrapandu	15
East Africa	Mtomondo	6
Southern Africa	Poeierkwasboom, iBhoqo, iBoqo (Zulu)	6
Tanzania	Mkuvumkuvu, mtomondo	6
Madagascar	Fotatre, magnondro, manondro, manontro	6

The distinctive features of the fruit are presented as having conical to ovate chicken egg-sized of about 3 to 9 cm x 2 to 5.5 cm (Figure 2) and crowned by the remains of the persistent calyx. The style is fleshy at first and turns hard, fibrous and yellowish-brown as maturity is attained. The fruits are green with purple and dark red tinge and usually one-seeded. The seed lies in the inner part of the fruit with the size of approximately 2 to 4 cm x 1 to 2.5 cm^{5,14}.



Fig. 2. Fruits of *B. racemosa* which are green with purple and dark red tinge.

2.4 Geographical Distribution and Ecology

The *Barringtonia* genus comprises about 40 to 50 species worldwide. South-East Asia especially the Malesian region is the centre of diversity in which there are approximately 30 to 40 species could be found in the region^{14,17}. The species of *Barringtonia racemosa* is distributed from eastern Africa and Madagascar to Sri Lanka, India, Myanmar, southern China, Taiwan, the Ryukyu Islands (Japan), Thailand, the Andaman and Nicobar Islands (India), throughout the Malesian region towards Micronesia, Polynesia (east to Fiji and Samoa) and northern Australia⁵. Well known as a mangrove species, *B. racemosa* grows well in tropical and subtropical regions in primary and secondary forest, mostly restricted to inundated flood plains on tidal river banks, or in less saline swampy areas^{14,15}. Although being well recognised as mangrove associate, this plant species can also be found in tropical rainforest, open lowlands and thickets⁵. Nevertheless, it cannot tolerate even light frost and thus can only grow in wet and moist tropical and subtropical climates⁵ at the height of 500-900 m altitude¹⁴.

3. Ethnobotanical Profiles

3.1 Construction

The bole and wood from *B. racemosa* had been widely used for construction purposes. It has been documented that the wood derived from this species are used in local house building, general planking, flooring and mouldings¹⁷. In addition, they are also useful in light and temporary construction and used to produce veneer and plywood to form a layer of decorative lining of fine wood to cover the coarse surfaces in construction¹⁴. Due to these properties, its uses in constructive fabrication and furnishing have been extended to produce household utensils and included as an element in interior finishing and in the manufacturing of wooden pallets.

Apart from that, it is also suitable to be used for carving and turnery owing to the properties of the wood which is light and soft yet moderately resistant to pressure impregnation. In Nicobar Island, New Guinea and Pacific islands, the bole of *B. racemosa* had been utilized in the manufacturing of canoe¹⁷. Meanwhile, in South India, the durable characteristic of the wood makes it suitable to be used in cabinet making, boat building, carts and rice pounders¹⁸. The bark of *B. racemosa* had been recorded to be used as a source of fibre and hence suitably used as tying materials. Upon being treated with preservatives, the timber of *B. racemosa* serves as a suitable candidate to make good ties and paving blocks in Philippines⁵.

3.2 Animal Poisons

Being commonly named as fish-poison tree, fish-killer tree and fish-poison wood, it is called as such due to its potential in poisoning fish^{19,20}. It is used to stun fishes and octopus in the area of Pacific islands²¹ and many regions of the world where the species are native to. When the pounded materials of *B. racemosa* passes through the gills of fish or being directly ingested, the fishes are therefore stunned. The grated and pounded parts of *B. racemosa* are introduced into stagnant pool or slow-flowing stream and with that, the poisons are concentrated and not easily being washed away by the water current. The uses of this species as animal poisons are attributed to its tannin and saponin content²⁰. In stunning fishes, the saponins from *B. racemosa* are taken

in directly into bloodstream through gills. Even though the saponins effects are powerful, the results on fishes are not fatal. In addition, the toxins which act on fishes' respiratory organs are not affecting their edibility. *B. racemosa* and other species of *Barringtonia* genus are widely used for this purpose and most frequently, the seeds²¹ and leaves parts are being used²² in addition to bark and fruits²⁰.

Apart from effectively used as fish poison, the species is also used to poison wild pigs in Philippines¹⁷. The effectiveness of this species to poison animals had also been verified scientifically. The toxicity of *B. racemosa* extract had been proven to be useful in combating agricultural pest threats. It had been proven that this species exerted toxic effects against golden apple snail by using the seed kernel²³. In addition to that, the ethnobotanical records of this plant species in Palawan Island, Philippines had shown that it is used as insecticide²⁴. *B. racemosa* was reported to have insecticide potential against citrus aphids¹⁴. For instance in certain district of Bangladesh, it is used as insect repellent²⁵ and snake repellent^{26,27}. Besides, the potency of this species to produce toxic effects towards mosquitoes, snails and brine-shrimp had been documented as well²⁸. Interestingly, scientific studies had proven that fruit and seed extracts of this mangrove species are having molluscicidal, cercariacidal, larvicidal, antiplasmodial^{29,30} and piscicidal properties²⁹. The possible role of *B. racemosa* to control snail intermediate hosts of schistosomiasis and dengue fever had been proven by Adewunmi et al.,²⁹ in a research conducted on *Biomphalaria glabrata* snails and *Aedes aegypti*. In such assay, the pericarp extracts were more potent in molluscicidal activities than those of seed extracts while the seed extracts had shown promising larvicidal activities.

3.3 Culinary

The shoots and young fruits are usually eaten raw and mostly favoured by the elderly. The young fruits are consumed and favoured due to its crunchy texture³¹. Its leaves are usually served as salad³² especially in Malay custom and eaten together with shrimp paste³¹ in main meals of lunch and dinner. Additionally, it is also being added in Malay's cooking as sauté paste³¹. In certain region of the world, its use as an element in culinary is

also manifested in which the seeds are milled to produce edible flour. They are pounded to extract the starchy content whereby the pounded seeds are used to make foods such as cakes^{5,14}.

3.4 Household and Other Daily Uses

According to Kitalong et al.,³³ *B. racemosa* is used for cleaning purpose of personal hygiene such as soap by the people of Republic of Palau. The suitability of *B. racemosa* to be used as soap might be owing to its saponin content whereby the presence of saponin in this plant species had been identified and validated by a number of previous studies^{23,34}. The presence of saponin is useful in soap making since saponin has been recognized to be able to exhibit distinct foaming properties and feasible to be added to shampoos, liquid detergents, toothpastes as emulsifier and long-lasting foaming agent^{35,36}.

Other than being used as cleaning agent, the pulp of *B. racemosa* is used in paper industry¹⁴. This species is also used as a good source of dye which produces reddish brown dye due to its tannin content which has been associated to its bark of stems and roots. The tannins found in the species belong to the group of condensed proanthocyanidins which produce reddish leather and used to dye vegetable fibres.

In addition, the wood of this plant species is used as a source of fuel and firewood¹⁴. It had been reported that oil from the seeds is used for lighting³⁷. Apart from being plucked and extracted for multiple ethnobotanical purposes, this species also being appreciated as ornamental plant species and planted for decorative purposes^{5,14,17} which might be due to its attractive characteristics of delicate white flowers with masses of pinkish white stamens and its unique hanging fruits found on the hanging racemes.

3.5 Ethnopharmacological Uses

Ethnopharmacological term is used to refer to the traditional uses of plant species specifically for medicinal purposes. *Barringtonia racemosa* L. has been traditionally used in various tribes around the world as a type of medicinal approaches. The ethnomedicinal properties of the species according to the related areas and ethnicity were presented in Table 2.

Table 2: Ethnopharmacological uses of *B. racemosa* in various regions of the world

Ethnomedicinal properties	Area of use /Ethnic involved	Parts used / Method of Application	References
Used as deobstruent, in the treatment of cough, asthma and diarrhoea. Also applied for skin diseases, colic and parturition	Sri Lanka	The fruits are used for the treatment of cough, asthma and diarrhoea. It is powdered to be used in skin diseases. The aromatic seeds are used in colic and parturition.	38
Treatment of otitis media	Palau, Micronesia	Young leaves are crushed to produce herbal mixtures and injected into the ears of patients.	39
Treatment of hallucination	Eastern part of former Zaire by Nyindu people	The pounded bark is soaked in water and the solution is used to wash patient's head.	40
Treatment of hypertension, itchiness and chicken pox	Malaysia	Its leaves are used to reduce high blood pressure and used as a depurative, in which the pounded leaves, roots and barks are used to reduce itchiness and chicken pox.	5, 41
Treatment of cancer-like diseases, jaundice, dog-bite wounds	Kerala, India	Not specified	42
Treatment of malaria, cough, asthma, diarrhoea, eye inflammation, sores, rheumatism, fever and used for women in parturition	Africa (South Africa, Eastern Africa)	The Zulus use the fruit to treat malaria while the pulverized fruit is used as snuff. Seeds are used for the treatment of eye inflammation. Meanwhile, midwives use them for parturition. Bark decoction is applied externally for rheumatism treatment. Root decoction is used as febrifuge.	5, 14
Treatment of snake bite	Bagerhat district and Balidha village, Jessore district, Bangladesh by <i>kavirajes</i>	Leaves are used as a snake repellent and in Jessore and Bagerhat districts of Bangladesh, the leaves are smashed and the decoction is orally consumed for snake bite treatment.	26-27, 43
Treatment of heartburn, bleeding and abortion	Palauan archipelago, Western Micronesia	Not specified	33
Treatment of cough	Buton, Indonesia	Not specified	44
Used as anti-diabetic, anti-malaria, antidote	Tamil Nadu, India	Seed is used for the therapeutic purposes	45
Treatment of cough, asthma, diarrhoea, stomachache, ophthalmia and jaundice	Uttara Kannada, India	Aromatic seeds used in stomach ache and ophthalmia; seed kernel with milk for treating jaundice. Also used in the preparation of skin ointment.	46
Treatment of scabies, tetanus and for placental apposition in women	Southeastern Madagascar	Leaves are used for scabies, tetanus and placental apposition.	47
Treatment of pain, joint pain and bone fracture	Kagrachari, Bangladesh	Murong tribes use the leaves to be applied externally.	48
Treatment of body ache, fever, joint pain, snake bite	Nancowry group of islands (Andaman and Nicobar Island) by Nicobarese	Applied topically to the affected area	49
Treatment of snake poisoning	Songkhla, Thailand	The root is prepared to make decoction	50

4. Phytochemical Constituents

4.1 Terpenoids

Terpenoid metabolites are one of the largest and most diverse classes of chemical compounds derived from plants. Plants use terpenoids for both basic functions in growth and development as well as for more specialized plants chemical interactions and protection⁵¹. For instance, monoterpenes and diterpenes generally act as allelopathic agents, attractants in plant-plant or plant-pathogen/herbivore interactions or repellants⁵². Through scientific discoveries, a great number of terpenoid metabolites had been isolated as phytochemical constituents in *B. racemosa*.

Saponins for instance are classified as triterpene glycosides which are very well known to be inherently present in *B. racemosa*. The presence of such compounds had been acknowledged to be the reason of its suitability to be used as cleaning agents due to its surface-active properties^{35,53} and may produce long-lasting foam³⁶. In addition, saponins in *B. racemosa* serve as the key factor of its insecticidal and piscicidal activities to stun fish and to stupefy animals and pests. Biologically, the mechanism by which saponins exert its toxicity as animal poison could be understood by the occurrence of hemolysis that they cause. When saponins are in the proximity of animal cell membrane, their interaction with cholesterol may create pores and resulting in hemolysis. The effects of hypersecretion due to such interaction will eventually cause toxicity to the fishes being stunned⁵⁴.

To date, several types of saponins had been identified from *B. racemosa* extract which are baringtonin and sapogenins. Three different metabolites had been further isolated from triterpenoid sapogenins group which are R1-barringenol, barringtogenol^{55,56} and barringtogenic acid⁵⁵. Other two new triterpenoids had been successfully isolated in which the presence of olean-18-en-3beta-O-E-coumaroyl ester and olean-18-en-3beta-O-Z-coumaroyl ester from stem bark of *B. racemosa* had been discovered along with five known compounds which were germanicol, germanicone, betulinic acid, lupeol and taraxerol⁵⁷.

In year 2000, a research had been documented⁵⁸ which reported the presence of two novel neo-clerodane type diterpenoids which were methyl-15,

16-epoxy-12-oxo-3,13(16), 14-neo-clerodatrien-18, 19-olide-17-carboxylate (nasimalun A) and dimethyl-15,16-epoxy-3, 13(16), 14-neo-clerodatrien-17, 18-dicarboxylate (17-carboxymethylhardwickiic acid methyl ester (nasimalun B). First documentation on the presence of bartogenic acid had been recorded by Sun et al.,⁵⁹ from the extract of stem bark. In 2008, Nurul-Maryam et al.,⁶⁰ reported that great amount of β -carotene and lycopene were found in the ethanolic extract of *B. racemosa* leaf. Later on, further studies had been published which verified the presence of bartogenic acid done by Patil et al⁶¹. In such discovery, they confirmed the isolation of bartogenic acid from the fruits of this plant species. A terpene compound classified under tetraterpenoid had been studied by Behbahani et al.,⁹ and her findings verified the presence lycopene in callus and cell suspension cultures of *B. racemosa*.

4.2 Plant Sterols

Plant sterols are the components occur in plant cells which are generally functions to control membrane fluidity and permeability. The presence of sterol in plants is associated with a number of benefits for instance it has the potential to be used as natural preventive dietary product in lowering plasma cholesterol level⁶². Phytosterols had been proven to play a key role in plant innate immunity against bacterial pathogens as well⁶³. In addition, they also have functions in temperature adaptation of plants. Considered as membrane reinforces which sustain the domain structures of cell membrane, plant sterols also involve in embryonic growth of plants and in fundamental biological process⁶⁴. In *B. racemosa*, the presence of stigmasterol had been isolated and identified from the ethyl acetate extract of stem bark. The isolation was done by using Silica Sephadex LH-20 column chromatography and identified by NMR analysis⁵⁹.

4.3 Phenolics

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly diversified ranging from simple molecules to highly polymerized substances with more than 8000 compounds being categorized into the group. Plant phenolics can be further classified into several sub-groups which are phenolic acids, flavonoids, tannins

(non-flavonoid polyphenols), stilbenes and lignans⁶⁵. Phenolic compounds are frequently associated with antioxidative properties and being recognized as natural antioxidants.

Phenolic acids are the hydroxy derivatives of cinnamic acid or hydroxy derivatives of benzoic acid⁶⁶. Several phenolic acids had been identified in *B. racemosa* extracts. A compound identified as 3',3'-dimethoxy ellagic acid had been discovered from the ethyl acetate extract of *B. racemosa*'s stem bark⁵⁹. From the phytochemical constituent analysis in this species, the findings revealed that the water extract of shoots contained three phenolic acids which were gallic acid, ellagic acid and protocatechuic acid³².

As a large group of polyphenols, flavonoids are important naturally occurring plant products which can be ubiquitously found in all families of plant kingdom. They are chemically characterized by having multiple phenol structural units. Flavonoid compounds can further be classified into five major subgroups which are flavanones, flavones, flavonols, flavanols (catechins) and isoflavones. In addition, proanthocyanidins and anthocyanins are also known as subgroups of flavonoid compounds⁶⁶. The presence of total phenolic content and total flavonoid content in the extracts of aerial parts of the species had been verified as well⁶⁰. This was further seconded by later findings by Amran et al.,⁶⁷ in which they found there were high total phenolic and total flavonoid contents in the fruits of *B. racemosa*. Upon phytochemical analysis done on water extract of *B. racemosa*, the shoots of this species were found to possess three flavonoid compounds which were quercetin, rutin and kaempferol³² whereby it has been acknowledged that quercetin and kaempferol are the two most predominating flavonols found in fruits and vegetables.

Tannin is another group of plant polyphenolic compounds under the phenolic groups which are characterized by having polymerized structure. The term 'tannin' comes from French word which reflects the tanning properties of this species⁶⁸. To date, very little is known about the validated composition of tannin in *B. racemosa* and no data on structural elucidation of the compounds have ever been reported. However, the total tannin content of the bark has been estimated to be 18% which belongs to the group of condensed proanthocyanidins and contributes to its reddish brown dye production¹⁴.

5. Application of Plant Biotechnology in *B. racemosa*

Plant biotechnology offers advantageous alternative to the conventional breeding methods in the propagation of plant. In addition, plant biotechnology can also be utilized in the *in vitro* production of important plant secondary metabolites. The application of plant tissue culture as one of the pertinent elements in plant biotechnology could confer benefits owing to the totipotency property of plants. Totipotency is a special characteristic possessed by cells in young tissues and meristems⁶⁹. By adopting the practises of plant tissue culture, total genetic potential of the parent plant could be maintained through totipotency. The adoption of micropropagation technology through plant tissue culture method has broadened the potential of greater plant production of selected traits rapidly which can overcome the slow and labour-intensive process with the advantages of season-independent plant breeding⁷⁰.

Nevertheless, the application of plant biotechnology especially in the adoption of plant tissue culture in *B. racemosa* are still very few. The regeneration of this species through plant tissue culture was only recorded in a study published in 2007 whereby they managed to establish a protocol for *in vitro* propagation of *B. racemosa* from leaf explant⁸. They found that the leaf explants which were cultured on Woody Plant Medium (WPM) supplemented with 30 g/l sucrose and treated with 2 mg/l kinetin and 0.2 mg/l IBA had successfully induced optimum shooting response. Meanwhile, the supplementation of 3 g/l activated charcoal and 0.8 mg/l IBA had been used as a good rooting induction medium in this species. Excellently acclimatized, the *in vitro* plantlets of *B. racemosa* were survived after being transferred to mixed soil and were grown well in the glass house.

Meanwhile, the effort in establishing callus culture in *B. racemosa* was previously recorded in a number of studies. The earliest documented protocol for the establishment of callus culture was made in 2011. In such experiment, the researchers found that the fast growing and friable calli formation were initiated in the culture of leaf explant treated with 2.0 mg/l 2,4-D in WPM medium⁹. Later on, in 2013 another research had been documented in describing the protocol of callus culture establishment from leaf and endosperm explants

of *B. racemosa*¹⁰. Upon callus induction percentage assessment, the highest record was identified in the culture of endosperm explant treated with 1.5 mg/l 2,4-D and 0.5 mg/l kinetin in Murashige and Skoog's (MS) medium whereby the calli formed from such treatment were found to be friable and grew intensively. In the most recent study of callus culture establishment which was published in 2016, the endosperm explant of *B. racemosa* was proven to be productive in yielding callus with desirable friable morphology¹¹. However, the optimal combination of Plant Growth Regulators (PGR) was different as compared to the previously documented research whereby the latter found that 1.0 mg/l 2,4-D in the presence of 1.5 mg/l kinetin was the optimal treatment for callus induction.

Research in the area of plant biotechnology which involves the establishment of cell suspension culture and secondary metabolite analysis was extremely limited and only found in a study documented in 2011. In such a study, they established optimum cell suspension culture by using friable calli cultured in liquid B5 medium. Further, they determined the content of lycopene in the cultures by chromatographic analysis. The effect of light towards lycopene level was investigated and it had been determined that the accumulation of lycopene in cell suspension culture was higher in the presence of light than in the darkness.

Due to the rarity of the research in the area of plant biotechnology conducted in this mangrove species, especially in the efforts of producing as well as enhancing its phytochemical constituents, therefore there's tremendous potential for further investigation to be carried out in *B. racemosa*. The involvement of intense studies in such particular area for instance through the application of plant tissue culture, mass production through bioreactor as well as enhancement of phytochemical compounds through the action of elicitor could be made possible by harnessing the resources and competency in plant biotechnology.

6. Conclusion

Considering the usefulness of this plant species and its versatile functions in human lives, *B. racemosa* is indeed having great potentials to be further scrutinized owing to its bioactive compounds. With respect to

its phytoconstituents, the discovery pertaining to the compounds present in the species could be further explored to produce fruitful productions of not only medicinally important compounds, but also those with tanning and colouring properties. Due to the scantiness of research in the area of plant biotechnology in this species, hence the research in the respective area on *B. racemosa* should be intensified and diversified to ensure the benefits of *B. racemosa* could be fully harnessed through further exploratory investigations.

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