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Biotechnological Approaches for the Improvement of Magnolia Genus Grown in Indonesia

Siti MAESAROH[1*], Çiğdem Alev ÖZEL[2]

[1]Ankara University, Faculty of Agriculture, Department of Field Crops, 06110, Ankara, Turkey
[2]Gazi University, Faculty of Education, Department of Biology Education, 06500, Ankara, Turkey

[1]https://orcid.org/0000-0003-1024-284X
[2]https://orcid.org/0000-0002-5952-1412
*Sorumlu yazar: maesaroh@ankara.edu.tr

Review Article

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ABSTRACT

Magnolia sp., also known as Michelia, are woody fragrant flowering plants that have been used in traditional medicinal purposes. This review covers past, curent and future potential studies of Magnolia species grown in Indonesia. There are 28 species and a hybrid of Magnolia that have been recognized and distributed in Indonesia. Conventional cultivation of Magnolia becomes very hard due to poor seed germination. It is caused by hard seed coat, short-lived seed, a fleshy red outer layer of seed called aeril. There are a few studies about *in vitro* culture and volatile compounds of Magnolia genus at the world literture. *M. champaca*, *M. liliifera*, and *M. alba* (hybrid) and others widely grow in Indonesia, yet the output of studies are anadequate about *in vitro* or *ex vitro* cultivation in Indonesia. This review compiles the works of Magnolia species carried out in the past and approaches for future breeding and production studies. These new approaches will significantly contribute to the economic production of the Magnolia species grown in Indonesia.

Endonezya'da Yetiştirilen Manolya Cinsinin İyileştirilmesine Yönelik Biyoteknolojik Yaklaşımlar

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ÖZET

Michelia olarak da bilinen Manolya türleri, geleneksel tıpta kullanılan kokulu çiçeklere sahip odunsu bitkilerdir. Bu derleme, Endonezya'da *in vitro* koşullarda yetiştirilen Manolya türlerinin geçmişte ve günümüzde yapılan çalışmalar ile gelecekte yapılabilecek potansiyel çalışmaları kapsamaktadır. Endonezya'da tanımlanan ve yayılış gösteren 28 tür ve 1 Manolya melezi (hybrid) vardır. Manolyanın geleneksel ekimi, zayıf tohum çimlenmesi nedeniyle çok zordur. Bunun sebebi sert tohum kabuğu, kısa ömürlü tohum, arillus adı verilen etli kırmızı dış tohum tabakası bulundurmasıdır. Uçucu yağlarının önemi bunun yanında bitkinin geleneksel tarım ile üretiminin zor olması alternatif olabilecek biyoteknolojik çalışmaları teşvik etmektedir. Dünyadaki literatür incelendiğinde Manolya cinsinin *in vitro* kültürü ve elde edilen uçucu yağlar ile ilgili birkaç çalışma bulunmaktadır. *M. champaca, M. liliifera* ve *M. alba* (hibrit) ve diğer türlerin Endonezya'da yetiştirildiğini ancak bu çalışmaların, Endonezya'daki *in vitro* veya *ex vitro* üretimin yetersiz olduğu gözükmektedir. Bu çalışma Manolya türlerinde gelecekte yapılacak ıslah ve üretim çalışmalarına yol gösterici olacaktır. Bu yeni yaklaşımların, Endonezya'da yetiştirilen Manolya türlerinin ekonomik üretimine önemli ölçüde katkı sağlayacağı düşünülmektedir.

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1. Introduction

The genus *Magnolia* known as *Michelia* in Magnoliaceae family is woody flowering plant of about 223 species in the world that are widely distributed in tropical and subtropical regions [1]. Some species of *Michelia* have been used for use in traditional medicine. It has fragrant attractive flowers [2, 3, 4, 5]. The 25 species of Magnoliaceae are discovered in Indonesia [6]. There is limited report on Magnolia research especially species that is commonly grown and distributed in Indonesia known as a region of Indomalaya ecozone. Indomalaya ecozone spreads most of South (Indian subcontinent) and Southeast Asia to the southern part of East Asia (lowland southern China) through Indonesia, Phillipines, lowland Taiwan and Japan Ryuku's islands. These species are native to Indomalaya ecozone especially in Indonesia is *M. champaca* and *M. liliifera* [7, 8, 9].

Previous studies have been conducted to investigate volatile compounds of *M. alba* [3, 5, 10, 11] and chemical composition and bioactive constituents of *M. champaca* [12, 13]. Other, a few studies on the tissue culture report plant regeneration on some species of *Michelia,* [14], especially callus induction, cell culture, somatic embryogenesis and dormancy of *M. champaca* [15, 16, 17, 18] and cell culture of *M. alba* [19]. There are a few tissue culture studies on *M. alba*, *M. liliifera* and Magnolia that grow in Indonesia. Advanced studies are needed to develop genus *Magnolia* that grows in Indonesia; due to its high economic value and advantages to individuals, industries and state.

2. Growth of Magnolia Genus and Their Its Distribution in Indonesia

In contrast to Rozak [6], there are 28 recognized species native to Indonesia (Table 1, Figure 1). There is also a species of hybrid origin, known as *M. alba*. More than half of Magnolia's species are distributed in Borneo with the oldest rainsforest in the world that makes it one of the most biodiverse area in the world. Others species are mostly found in Sumatra, Sulawesi and Jawa. Several species are spread in Maluku, New Guinea and Lesser Sunda Islands.

Table 1. Distribution of Magnonia's species in Indonesia, one of region of Indomalaya ecozone

No	Species	Native
1	*M. ashtonii* Dandy ex. Noot.	Borneo, Sumatra
2	*M. banghamii* (Noot.) Figlar & Noot.	Sumatra
3	*M. bintuluensis (*Agostini) Noot.	Borneo, Sumatra
4	*M. blumei* Prantl **synonim** *M. sumatrana* var. *glauca* (Blume) Figlar & Noot. *M. blumei* var. *blumei* *M. blumei* var. *sumatrana* (Miq.) Figlar & Noot.	Jawa, Lesser Sunda Is., Sulawesi, Sumatra
5	*M. borneensis* Noot.	Borneo
6	*M. calophylloides* Figlar & Noot.	W. Sumatra
7	*M. carsonii* Dandy ex Noot *M. carsonii* var. *carsonii* *M. carsonii* var. *drymifolia* Noot. *M. carsonii* var. *phaulanta* (Dandy ex Noot.) S. Kim & Noot. **synonim** *M. phaulanta*	Borneo, Sulawesi, Sumatra
8	*M. champaca* (L.) Baillon ex Pierre	Borneo, Jawa, Lesser Sunda Is., Sumatra
9	*M. dolichogyna* (Dandy ex Noot.) Figlar & Noot **synonim** *M. utilis* (Dandy) V.S. Kumar	Borneo, Sulawesi
10	*M. elegans* (Blume) Keng	Jawa, Sumatra
11	*M. gigantifolia* (Miq.) Noot.	Borneo, Sumatra
12	*M. koordersiana* (Noot.) Figlar	W. Sumatra
13	*M. lanuginosoides* Figlar & Noot.	Sumatra
14	*M. lasia* Noot.	Borneo
15	*M. liliifera* (L.) Baillon	Borneo, Jawa, Lesser Sunda Is., Maluku, Sulawesi, Sumatra,
16	*M. macklottii* (Korth.) Dandy *M. macklottii* var. *beccariana* (Agostini) Noot. *M. macklottii* var. *macklottii* **synonim** *M. uvariifolia* Dandy ex. Noot.	Borneo, Jawa, Sumatra
17	*M. mariusjacobsia* Noot.	Borneo
18	*M. montana (Blume) Figlar & Noot.*	Borneo, Jawa, Lesser Sunda Islands, Sumatra
19	*M. persuaveolens* Dandy	Borneo
20	*M. praecalva*	W. Sumatra
21	*M. sabahensis* (Dandy ex Noot.) Figlar & Noot.	Borneo
22	*M. sarawakensis* (Agostini) Noot.	Borneo
23	*M. scortechinii* (King) Figlar & Noot.	W. Sumatra
24	*M. sulawesiana* Brambach, Noot. & Culmsee	Sulawesi
25	M. *sumatrae* (Dandy) Figlar & Noot.	Sumatra
26	*M. tsiampacca* (L.) Figlar & Noot.	Borneo, Maluku, New Guinea, Sulawesi, Sumatra
27	*M. villosa (Miq.)* H. Keng	Borneo, Sulawesi, Sumatra
28	M. vrieseana (Miq.) Baill. Ex Pierre	Maluku, Sulawesi

Source: [20]

Rozak [6] reported that there is inadequate information based on IUCN's listing criteria to evaluate the current conservation that lead to the distribution and evaluation of the species distributed in Indonesia yet. Therefore, there is

danger that some of these rare species might become rare and threatened as a consequence of restricted distribution, threats of deforestation, illegal logging or natural disasters. The rare species are *M. ashtonii* and *M. bintulensis* (grow in swamp and Sundaland heath forest-Kerangas forest), *M. borneensis* (grows in the old-growth forest), *M. macklotti* var. *macklotti* (grows in the old-growth forest) and *M. macklotti* var. *beccariana* (grows on the mountains).

Some studies about Magnolia have been conducted including *M. liliifera*, *M. champaca*, and *M. alba*. Information about the Magnolia species growing in the Indomalaya ecozone is described below:

2.1. Magnolia liliifera

Magnolia liliifera, commonly known as egg magnolia has various varieties with white, yellow, purple and red flower colours. It is valuable ornamental fragrant tree and has fragrant flowers that release stronger smell like a wafting pineapple in the morning [21, 22].

Some *ex situ* studies of saprobic fungi on *M. liliifera* leaves in Thailand has been reported by Promputtha et al. [23, 24]. It had been concluced that its large and thick leaves are a good source for saprobic fungi. Another study has mentioned that saprobic fungi could be a potential resource of bioactive compounds for medicinal utilization [25].

Figure 1. Republic of Indonesia

2.2 Magnolia champaca

M. champaca known as champak in English and is locally known as Cempaka with fragrant yellow flower is one of Magnoliaceae family that distributed in Indomalaya ecozone especially widely grows in Java, Lesser Sunda and Sumatra and Sulawesi of Indonesia. The two varieties of *M. champaca*: *M. champaca var. champaca* (S India and Lesser Sunda Island) and *M. champaca var. pubinervia* (Blume) Figlar & Noot. (Java and Malay Penn.). Their utilization is commonly known as alternative of forest wood in Indonesia to make household utensils [26, 27].

Propagation

Various authors have classified different storage behaviour of *M. champaca* as closer to ortodhox [28] and intermediate or semi-recalsitrant [18]. Their storage behaviour has been classified as recalcitrant seeds with its characteristic fast loss of viability by Bahuguna et al. [29], Robbins [30], Bisht and Ahlawat [31], Yuniarti and Nurhasybi [32] and Pudjiono [27]. Seed of *M. champaca* has aeril lead to reduction of seed viability and small structure called elaiosomes that can enchant animal dispersors due to its colour and lipids and protein compounds [33]. It is difficult to cultivate this plant by seeds due to these mentioned factors

like recalcitrant seed which means short-lived seeds, aeril layer, elaiosomes and hard seed coat. The physical, physiological and morphological characteristics as type of dormancy also have been noted by many authors. Yuniarti and Nurhasybi [32] mentioned that appropriate drying time and storage methods could be applicated to maintain seed viability. The room (ambient) temperature is better than DCS (4-8°C, RH 40-60%) and refrigator (0-5°C, RH 40-50%) storage for *M. champaca* storage to keep their viability.

In order to solve these problems of conventional propagation some studies have been established by many authors. Candiani et al. [34] noted that aeril on dropped seeds can be removed by ants and rodents with higher removal in the old seeds compared to the new eucalypt and related seeds parts to improve seed germination of *M. champaca*. Fernando et al. [18] notified scarcity of natural regeneration and trouble of seed propagation due to its physiological dormancy; which has been solved by using GA_3. The study also showed morphological dormancy of *M. champaca* identified by elongation of embryos inside seeds at warm temperatures following radicle emergence.

The cutting propagation using softwood and semi-hard wood of *M. champaca* IBA treatment has been carried by Tan et al. [35]. Their result showed that *M. champaca* could be propagated by cutting and IBA application to support rooting with the best rooting percentage using 12000 mg/L IBA and softwood cutting. There is also a conventional propagation study of *M. champaca* in Indonesia. The effecetiveness of combination of media with or without plant growth regulator on *M. champaca* cutting had been reported by Danu and Putri [26]. They noted three of best combination for *M. champaca* cutting: sand media only, combination of coconut husk, rice husk and 100 ppm IBA and combination of coconut husk, rice husk, charcoal of rice husk and 50 ppm IBA. Pudjiono [27] studied effectiveness of coir dust and roasted rice hull as growth medium on survival percentage and root growth of *M. champaca* shoot cuttings.

Constituent of M. champaca

The volatile compounds of *M. Champaca*'s concrete, absolute and essential oilisolated and extracted from various part of plant contain esters, alcohols, fatty acid, terpenes (monoterpenes and sesquiterpenes). Phenol and oxydes group are also found from the extracts. Dwicandra et al. [36] noted that stem barks of *M. champaca* extracted by 80% ethanol contain essential oils, triterpenoids, polyphenols, and flavonoids. The obtained total component with different concentration (percentage) is influenced by source, type of oil and isolation or extraction methods (Table 2). Rout et al. [37] reported that the lower sesquiterpene hydrocarbons which compose more than 60% of headspace were found at absolute and concrete compared to essential oil components of *M. champaca* flower. The absolute and concrete contents contained higher amounts of esters while they were no or had decreasing in concentrations in the essential oils. The essential oil contained several monoterpenes such as linalool which were not isolated at the absolute, concrete and headspace. Linalool is also identified at the concrete and absolute level through distilation method [38, 39, 40]. Indoles which were found at the absolute, concrete and headspace dissapeared at essential oil due to loss through distillation. The more components are identified by simultaneous distilation extraction method than other methods [39, 40].

Ananthi and Anuradha [41, 42] noted the availability of phenol compounds (gallic acid, caffeic acid, rutin, quercetin, ferulic acid from flower of *M. champaca*. Previous study conducted by Ahmad et al. [43] also reported gallic acid present in leaves and stem-bark of *M. champaca.*

Table 2. The various component of *M. champaca* from different source extracts and extraction methods

No	Source	Extraction method	Volatile components	Reference
1	Root bark	- Hexane extract - Silica gel chormatography	Sesquiterpene: parthenolide	Sethi et al. [44]
2	The absolute and three concretes of flower	- Flash distilation - GC-MS	- > 240 components Main compound - Commercial absolute and 2 concretes: henylacetonitrile (1,2-4,5%), phenylethyl alcohol (25-34%), *co* + P- ionone (1,0-5%), methyl anthranilate (2,1-9%), indole (2,9-12%) and methyl linoleate (10-18%) - Lab-prepared concrete: linalool (11%), cis-linalool	Kaiser [38]

			oxide (pyranoid, 7%), dihydro-P-ionone (10%) and ***a***+Ij-ionone (26,8%)	
3	Root bark	- Methylene chloride - Silica gel chormatography	Sesquiterpene: parthenolide, costunolide, 8 α-acetocyparthinolide, magnograndiolide, michampanolide	Jacobsson et al. [45]
4	Flower	- Petroleum ether - Silica gel chormatography	- N-alkane (hydrocarbon) - Unsaturated aliphatic keton - Natural β- sitosterol	Kapoor and Jaggi [46]
5	Fresh flower and headspace fragrance (emitted from flower)	- Pentane extraction - Hydrodistilation - Solid phase micro-extraction - GC, GC-MS	- Concrete: 42 compounds - Absolute: 46 compounds - Essential oil: 73 compounds - Esters (methyl benzoate, ethyl benzoate, phenyl ethyl formate, phenyl ethyl benzoate, methyl anthranylate, *Z*-methyl jasmonate, *Z*-methyl *epi*-jasmonate and phenyl acetonitrile) - Headspace fragrance: *E*-β-Ocimene, Methyl benzoate, Phenyl ethyl alcohol, Phenyl acetonitrile, Indole, Methyl anthranylate, δ-Elemene, α-Copaene, β-Copaene, β-Elemene, *E*-Caryophyllene, γ - Elemene, α-*E*-Bergamotene, *epi*-α-Muurolene, *E*-β-Farnesene, 9-*epi*-*E*-Caryophyllene, Germacrene D, *E*-β-Ionone, Zingiberene, (*E*, *E*)-α-Farneseneδ-Cadinene	Rout et al. [37]
6	Leaves	- Hydrodistillation - Dichloromethane - GC, GC-MS	- 13 compounds - monoterpene (α-terpinolene) - six sesquiterpene hydrocarbons (β-elemene, β-caryophyllene, α-humulene, β-selinene, α-selinene, and γ-cadinene) - four oxygenated sesquiterpenes [(*E*)-nerolidol, α-cadinol, β-bisabolol, and (*Z,E*)-farnesol] - two aliphatic alcohols (pentadecanol and hexadecanol).	Lago et al. [47]
7	Fresh flower	- Pentane - Liquid CO_2 extract, concrete, absolute, Liquid CO_2 1st fraction, Liquid CO_2 2nd fraction - GC, GC-MS	- Main compound at all: methyl linoleate, methyl benzoate, phenyl acetonitrile, Methyl linolenate, phenyl ethyl alcohol, Methyl palmitate, Palmitic acid, indole, methyl anthranilate - Fatty acid/esters/alcohols and benzenoid most amount at concrete, absolute, liquid CO_2 extract, liquid CO_2,1st fraction, liquid CO_2 2nd fraction - Hydrocarbon most at concrete and absolute - Monoterpenes most at absolute, liquid CO_2extract, concrete	Rout et al. [48]
8	Flower	- Simultaneous distillation-extraction (SDE) - Headspace-solid phase microextraction (SPME)	- SDE: 67 compounds, main compound 1,8-cineole (22,8%), n- tricosane (8,3%), linalool (5,9%) - SPME: 34 compounds, main compound methyl benzoate (30,3%), indole (16,6%) and β-elemene (10,4%)	Baez et al. [39]
9	Different stage of flower (Figure 2)	- Headspace solid-phase microextraction (HS-SPME) - GC-MS	- 51 compounds, - I-V: 1, 6-cyclodecadiene, 1- methyl-5-methylene-8-(1-methylethyl)-, caryophyllene - I-VI: 1, 3, 6-Octatriene, 3, 7-dimethyl- - II-V: Benzoic acid, methyl ester - II-VI: 1, 6-octadien-3-ol, 3, 7- dimethyl- - III- VI2H-pyran-3-ol, 6- ethenyltetrahydro-2, 2, 6-trimethyl - VI: beta pinene, eucalyptol	Jiang et al. [49]
10	Flower, absolute	- Solvent extraction (SE)-dichloromethane/methanol - Simultaneous distillation extraction (SDE) - Supercritical fluid extraction (SFE) - GC-MS	Main compound - SDE: Linalool, phenylethyl alcohol, phenyl acetonitrile, epoxylinalool, cis-linalool pyran oxide, 4, tetpineol, azulene, carvone oxide, varamol, methyl anthranilate, *β*-elemene, cis-jasmone, *α*, bergamotene, *β*-santalene, *β*-caryophyllene, *α*-selinene - SE: similar to SDE, no: tetpineol, cis-jasmone, *α*, bergamotene, *β*-santalene, *α*-selinene - SFE: similar to SDE, no: azulene, carvone oxide	Samakradhamrongthai et al. [40]

			Minor compound: data not avilable	
11	Flower	- Ethtanol - Microencapsulation of flavor powder - 5%, 10%,15%, 20% extract - GC-MS	- All extracts: β-thujene, camphene, 3-carene, limonene, γ-terpinene, α- terpinolene, copaene, β-elemene, β-caryophyllene - α-pinene (no in 20% extract) - para-cymene (no in 15% extract) - α-cubebene (only in 20% extract) - α-humulene (no in 5% extract) - aromadendrene (only in 20% extract) - δ-cadinene -(no in 5% extract)	Utama-Ang et al. [50]
12	Flower	- Headspace method - GC-MS	- 43 compounds - 46,9% were terpenoids, 38,9% were volatile esters and 5,2% belonged to phenylpropanoids/benzenoids - Main compound: Methyl 2-methylbutanoate, Ethyl 2-methylbutanoate, indole, (E)-Furanoid linalool oxide, β-Linalool, phenyethy alcohol, (E)-β-Ocimene, δ-Elemene, (Z)-β-Ocimene	Dhandapani et al. [51]

Figure 2. Different stage of *M. champaca* flower [49]

Tissue culture of M. champaca

The Table 3. showed tissue culture reaserches of *M. champaca* conducted by many authors. Successful on some *in vitro* studies of *M. champaca* had been reported. The various explants and plant growth regulators were used to get appropriate protocol for callus and shoot initiation of *M. champaca*. The auxillary bud and petiole explants responsed to both single auxin and cytokinin on callus induction of *M. champaca* [17, 52]. Although all of hormones inducted callus, the best callus formation is noted on 2,4-D, single exogenous auxin with different medium. The B5 medium showed high callus formation of petiole compared to MS medium. The high callus formation might be caused by combined medium and high concentration of 2,4-D. The previous study conducted by Abdelmageed et al. [17] used MS medium with lower concentration of 2,4-D showed low percentage of callus formation compared to Shukla [52]. Armiyanti et al. [15] also reported effectivity of 2,4-D for callus induction of *M. champaca* seed. The percentage of callus formation (90%) of *M. champaca* on MS medium containing 2,4-D was higher than on MS medium containing NAA. The study also emphasized that high concentration of 2,4-D is capable to get high callus formation. Previous study conducted by Lai and Lee [53] had reported succesfull of 2,4-D to initiate calli of *M. champaca* rachises with fast rate callus initiation and quickly browning at low concentration and

slow rate and hardly browning at high concentration.

Although 2,4-D hormone encouraged callus formation of *M. champaca* more than other hormones, the high embryonic callus had been reported in the MS medium containing NAA compared to MS medium containing 2,4-D Armiyanti et al. [15]. The medium containing NAA had also obtained somatic embryo formation on the studies conducted by Armiyanti et al. [15, 16]. The cell suspension technique is effective to derive somatic embryo that germinate more planlets compared to somatic embryo derived from solid culture. The adding GA_3 on the medium had no impressive effect on germination of *M. champaca*'s somatic embryo showed by the increasing concentration of GA_3 following the decreasing of germinated planlets.

The BAP is effective for shoot initiation of *M. champaca* compared to IAA and 2,4-D [17]. Sinha and Varma [54] also emphazised the effectiveness of cytokinin for shoot initiation of *M. champaca*. The high concentration of combination BAP and Kinetin optimized to initate shoot of *M. champaca*. They reported the effectiveness of IAA is better than 2, 4-D and BAP showing by percentage of survival rate. It had been underlined the importance of auxin for the survival of explants showed by survival rate [17]. The results showed that auxin and cytokinin roles on tissue culture studies. The auxin plays role on callus initiation and somatic embryogenesis while cytokinin has effect on shoot initiation. However, the difference response of callus induction and somatic embryogenesis are also influenced by explant sources and species

Table 3. Tissue culture studies of *M. champaca*

No	Purpose	Method	Result	Reference
		M. champaca		
1	To initiate callus	**Explants source** - petals, leaves and remainders **Sterilization:** - 70% ethanol (10 sec) → 1,2% Sodium hyphochlorite (0,5% Tween 20) (10 min) **Culture medium** - MS medium + 3% sucrose + 0,9% gelrite agar + combination of 2,4-D and BAP	- Calli grown on media containing 2,4-D and BAP	Lai and Lee [53]
2	To induce somatic embryo	**a. Somatic embryo induction** **Explant source:** - immature seed **Sterilization:** - 0,2% (w/v) benlate solution (15 min) → 70% (v/v) of ethanol (2 min) → 20% (v/v) Sodium hyphochlorite (Clorox) (15 min) → distilled water (2-3 times) **Culture medium:** - Basal MS medium + 30 g/L sucrose + 3,9 g/L gelrite agar + 0, 2, 4, 6, 8 and 10 mg/L of 2,4-D and NAA **b. Germination** **Explant source:** - somatic embryo calli **Culture medium:** - MS medium hormone free (two weeks) - Tranferred to medium with 0, 0,5, 1,0 and 1,5 mg/L GA_3	- The high callus formation noted on medium + 2,4-D with 90% intense in 6 mg/L and 8 mg/L 2,4-D compared to NAA - The embryonic callus in medium with NAA were noted higher than 2,4-D after 6 month culture - The somatic embryo were noted on medium + NAA with the highest percentage with 2 mg/L NAA, no somatic embryo was noted on medium + 2,4-D - The germinated somatic embryo (solid culture medium): ○ 56% planlets (45% normal planlets, 11% abnormal planlets) on MS medium hormone free ○ 12% planlets (8% normal planlets, 4% abnormal planlets) on 0,5 mg/L GA_3 ○ 8% planlets (4% normal planlets, 4% abnormal planlets) on 1 mg/L GA_3 ○ 6% planlets (1% normal planlets, 5% abnormal planlets) on 0,5 mg/L GA_3	Armiyanti et al. [15]
3	To regenerate embryogenic callus with cell	**a. Embryogenic callus induction** **Explant source:** - immature seed **Sterilization:** - 0,2% (w/v) benlate solution (15 min) →	- Liquid MS medium containing 2 mg/L NAA induced high frequency of somatic embryos formation (47,67 ± 4,53 per ml) at 5th subculture	Armiyanti et al. [16]

	suspension culture	70% (v/v) of ethanol (2 min) → 20% (v/v) Sodium hyphochlorite (Clorox) (15 min) → distilled water (2-3 times) **Culture medium:** - Basal MS medium + 2 mgL/L NAA **b. Enhancing high frequency somatic embryo formation** **Explant source:** - Embryonic callus **Culture medium:** - Liquid MS medium + 2 mg/LNAA **c. Cell suspension** **Explant source:** - 1 gr of four months of embryonic calli **Culture medium:** - 10 ml MS liquid medium supplementing 30 gr/L sucrose, 2 mg/L (w/v) NAA, pH 5,8, 100 rpm - Filtering with polypropylene meshes (250 µm pore size), stored at 25 ± 2°C, a daily fluorescence light of 16 h. **d. Germination of somatic embryo** **Explant source:** - Three months of cotyledonary somatic embryo (6th subculture) **Culture medium:** - Solid MS hormone free + 30 gr/L sucrose + 3,9 g/L gelrite agar (maturation and revoming residual effect) - Tranferred to medium containing 0, 0,5, 1,0 and 1,5 mg/L GA_3 (after two weeks)	- The succesfull of plant regeneration using cell suspension culture technique which used to produce mass planting materials - The germinated somatic embryo (cell suspension): ○ 34% planlets (29% normal planlets, 5% abnormal planlets) on MS medium hormone free ○ 16% planlets (8% normal planlets, 8% abnormal planlets) on 0,5 mg/L GA_3 ○ 11% planlets (6% normal planlets, 5% abnormal planlets) on 1,0 mg/L GA_3 ○ 10% planlets (6% normal planlets, 4% abnormal planlets) on 0,5 mg/L GA_3	
4	To induce calli, and shoots proliferation	**Explant source:** - Axillary bud **Sterilization** - Clorox (3 min) → running tap water (1 h) → 6% NaOCl (35 min) → 6% NaOCl + 0,01% Tween 20 (15 min) → distilled water (7 times) → explants cutting (2 cm) **a. Callus and shoot induction** **Culture medium:** - Basal MS + 30 g/L sucrose + 5 g/L gelrite agar - BAP, NAA and 2,4-D (0, 0,1, 0,25, 0,5, 1,0 and 2,0 mg/L) **b. Callus proliferation** - Sub-cultured with various combination of BAP, IAA and 2,4-D	- All PGRs had positive effect on callus induction - The callus formation were noted with yield 67% (2,4-D), 56% (IAA) and 44% (BAP) - The 22%, 19% and 11% of callus and shoot induction rate were noted on BAP, IAA and 2.4-D respectively - The 81%, 78% and 69% of survival rate were noted on IAA, 2,4-D and BAP respectively. Highest callogenesis: 2 mg/L BAP + 0,5 NAA	Abdelmageed et al. [17]
5	To induce callus	**Explant source:** - Apical shoot tip, nodal segment and petiole **Sterilization** - Labolene (10 min) → distilled water (2-3 times) → 1% sodium hypochlorite (5 min) → distilled water (2-3 times) → LAF with 0,1% $HgCl_2$ (2-3 min) **Culture medium:** - MS, SH and B_5 medium - Best explant + best medium + 2, 4, 6 and 8 mg/L 2,4-D	- The callusing rate: 100% (B5), 66,6% (MS) - Increasing callus formation percentage correlated to increasing concetration of 2,4-D - The best callus formation with 80% callus: petiole + B_5 medium + 8,0 mg/L of 2,4-D.	Shukla [52]
6	To induce shoot initiation	**Explant source**: - Axillary meristem and apical meristem **Sterilization**: - Clorox (3 min) → kept under running tap water for 1 h → rinsed in 6% NaOCl (35 min) → 2% NaOCl (15 min) + 0,01% Tween 20 as surfactants → rinsed with tap water 3-4 times. Put inside laminar air flow (LAF) with 0,1% HgCl2 (2-3 min) **Shoot initiation**:	- The best result: MS media + 2,5 mg/l BAP, 1,0 mg/l KN and 0,5 mg/l IAA + activated charcoal	Sinha and Varma [54]

	- agar-solidified MS media + different concentrations of cytokinin (0,1, 0,5, 1,0, 2,0 and 2,5 mg/L BAP and (0,1, 0,5 and 1,0 mg/L KN) and auxin (0,5 mg/L IAA) + chorcoal.		

2.3 Magnolia alba

M. alba syn. *M. longifolia* Blume is known as white champaca or white jade orchid tree or "Cempaka Putih" in Indonesia and its flowers are used as flower garland with Jasmine at traditional wedding ceremonies. Although it is a typical plant from the province of Central Java, *M. alba* is spread throughoutIndonesia. Similar to *M. champaca*, its essential oil can be used as key topnote of perfumes due to various compounds from flower or leaf.

Propagation

Propagation by seed is rarely used for *M. alba* due to its hybrid origin, infrequent seed availability, erratic germination and long germination period [55]. Vegetative methods such as cutting, grafting and air-layering is an alternative to propagate *M. alba*. The ethepon and IBA application on softwood cutting of *M. alba* had been also conducted by Tan et al. [35]. It differs from *M. champaca,* the ethepon and IBA application showed no significant effect on rooting of softwood cutting due to short period application of ethepon and lower concentration of IBA.

Constiuent of M. alba

There are various compound obtained from different part of *M. alba* that are directly taken from tree with fresh, withered or frozen material (Table 4). The most abundant major chemical compound of *M. alba*'s flowers, leaves and stem is linalool, a monoterpene alcohol, which is widely found as odorous component in several aromatic plants. The linalool percentage of total volatile compounds vary depending on part source extract, stage of flower and extrction method. The high concentration of linalool is commonly obtained by using distilation extraction. While the concentration of indole, a heterocyclic organic compounds extracted during enfleurage is higher compared to linalool [56, 57]. Linalool and indole are known as fragrance and flavor subtances due to their strong and pleasant odor which can be used for medicinal or aromatherapy purposes [58, 59]. Dihydrocarveol is reported as a major compound of three choosen flower bud of *M. alba* [60] and S5-S8 stage [5, 19]. Rusdi [60] also reported that linalool without dihydrocarveol is a major compound of volatile component on callus extract obtained from petals. He also noted that the adding bioelicitor (jasmonic acid, yeast extract and pectinase) in the *in vitro* culture medium lead to decreasing callus production followed by increasing new volatile compound production. Shang et al. [3] noted that fresh flowers contain low concentration of terpenes and esters compared to frozen and withered.

Table 4. The various components of *M. alba* from different sources, extracts and extraction method

No	Source	Extraction method	Volatile components	Reference
1	Fresh flower, fresh leaves (July-summer)	- Extraction: Petroleum ether (30-60ºC) - Isolation: Simultaneous Steam Distillation Extraction (SDE) - GC-MS	Main compound - Flower: linanool (72,8%), α-terpineol (6,04%), P-phenylethyl alcohol (2,58%), P-pinene (2,39%), methyl 2 methylbutyrate (1.46%), limonene (1,42%), geraniol (1,23%), 1,8-cineole (1,03%) - Leaves: linanool (80,1%), P-caryophyllene (3,0%), p-elemene (1,7%), caryophyllene oxide (1,68%), and nerolidol (1,19%), a-humulene (1%) Other compound - Flower: α-pinene, myrcene, methyl hexanoate, methyl angelate, ρ-cimene, terpinolene, (Z)-3-hexenol, α-ρ dimethylstyrene, trans-linalool oxide (furanoid), beta elimene, p-mentha-8,9-dien-1-ol, methyl chavicol, cis-linalool oxide (pyranoid), trans-linalool oxide (pyranoid), nerol, p-phenylethyl propionate, methyl eugenol - Leaves: a-pinene, camphene, P-pinene, sabinene, myrcene, limonene, (Z)-p-ocimene, (E)-p-ocimene, p-cymene, cis-linalool oxide (furanoid),	Ueyama et al. [11]

			rans-linalool oxide (furanoid), a-cubebene, a-copaene, methyl chavicol, borneol, y-muurolene, germacrene D, (E, E)-a-farnesene, &cadi nene, nerol, geraniol, methyl eugenol, T-muurololb,	
2	Fresh, frozen, withered flower	- Headspace solid phase microextraction technique - The simulated natural environment, combined with SPME–GC–MS	- 61 volatile compound of fresh flower Main compound - Fresh: a-myrcene, (S)-limonene, (R)-fenchone, linalool, camphor, caryophyllene, germacrene D and their isomer - Withered: similar to fresh with lower concentration of mono- or sesquiterpene hydrocarbons, oxidates (present in traces or not at all), n-alkanes (low) - Frozen: several main volatiles of fresh flowers are not show at frozen flowers, most oxidates, the mono- or sesquiterpene hydro- carbons and *n*-alkanes present in trace or absent) Minor compound - Camphene, b-Pinene, Eucalyptole, b-Phellandrene, g -Terpinene, 2-Nonanone, 2-Undecanone, a-Muurolene	Shang et al. [3]
	Flower, Oct-Feb	- Extraction a. Steam distilation b. Hexane c. Enfleurage (buffalo fat) - GC-MS	Main compound - Steam distilation: linalool (66,92%), methyl-2-methylbutyrate (7,77%), ethyl-2-methyl butyrate (6,76%), eugenol (4,52%). trans-ß-ocimene (2,26%), cis-ocimene (1,81), ß -elemene (1,34%) - Hexane: 2-methylbutanoic acid (33,01%), linalool (28,92%), methyl benzoate (5,06%), phenyl ethylalcohol (4,52%), phenetyl-2- and methyl eugenol (3,07%), cis-linalool oxide (1,43%), trans-lina- lool oxide (1,14%) - Enfleurage: indole (1H-indole) (35,49%), hexadecanoic acid (13,18%), phenylethyl alcohol (8,28%), phenylethyl-2- methyl butyrate (6,80%), germacrene D (6,02%), methyl-2-methylbutyrate (5,24%), ethyl-2-methyl butyrate (3,18%), linalool (3,09%) Minor compound - Steam distilation: trans-linalool oxide (<0,5%), cis-linalool oxide (0,28%), trans- caryophyllene (0,72%) - Hexane: cis ocimene (<0,5%), trans- ß -ocimene (<0,5%), methyl benzoate (0,40%), epoxylinalool (0,53%) - Enfleurage: 2-methylbutyl-2-methylbutyrate (0,79 %), trans linalool oxide (< 0,5%), cis linalool oxide (0,90%), 2-methyl butanoic acid (<0,5%)	Pensuk et al. [56]
3	Different stage of flower S5-S11 (Figure 3)	- Isolation: Simultaneous Steam Distillation Extraction (SDE) - GC-MS	Main compound - linalool (S9-S11), dihydrocarveol (S5-S8), butanoic acid-2-methyl and methyl ester (S9), cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methyl- ethenyl) (S6-S7) and eugenol methyl ether - 77-78 compound: 93–98% of the overall volatiles - 33 of these compounds: isoprenoid group, comprising 30–50% of the total volatile compounds Other compound - Fatty acid derivatives and benzenoid, phenylpropanoid, and other hydrocarbon compounds. - α-terpineol (p-menth-1-en-8-ol) (S8), α-cadinol (S9), α-santalene(S6–S7), α-cubebene (S5–S6), α-pinene (S11), β-farnesene (S5), caryophyllene (S7–S9), camphene (S9, S11), caryophyllene oxide (S6–S7, S9, S11), cineol (S6, S9–S11), D-limonene (S9), germacrene D-(S5–S9), *cis*-geraniol (S6–S7), ocimene (S5–S8), linalool (S9–S11), nerolidol (S8), sabinene (S9), τ-muroolol	Sanimah et al. [5], Ibrahim et al. [19]

			(S7–S9), bicyclo[3.1.1] hept (S5–S6, S8–S9), isobutanol (S9), phenylethyl alcohol (S7–S9), eugenol methyl ether (S5–S11)	
4	Leaves, stems (autumn)	- Steam distillation method - GC-MS	- Leaves: 63 compounds, 95,7% essential oil - Stem: 78 compounds, 97,3% essential oil Main compounds - Leaves: Linalool (63,31%), nerolidol (7,4%), caryophyllene (4,41%), isoaromadendrene epoxide (3,53%), ß-cubebene (2,6%), trans-citral (2,02%), (+)-2-bornanone (1,86%), α-humulene (1,78%), α-cadinol (1,63%) - Stem: Linalool (69,62%), germacrene D (4,49%), caryophyllene (3,35%), α-asarone (2,65%), E)-ocimene (2,07%ß -cubebene (1,81%), isoledene (1,66%), nerolidol (1,59%), α-cubebene, (1,47%), caryophyllene oxide (1,23%), α-humulene (1%) Minor compounds - b-pinene, camphene, borneol, copaene, isoeugenyl methyl ether, eudesma-4(14),11-diene, b--chamigrene, α- selinene, isoledene etc	Huang et al. [61]
5	Fresh flower	- Extraction a. Distilation (water, steam and water-steam) b. Enfleurage (Cold and hot) c. Solvent (Hexane and petroleum ether) - GC-MS	Main compounds - Water: linalool (85,78%) and 6 volatile compound - Steam: linalool (91,74%), and 8 volatile compound - Water-steam: linalool (83,38%) and 8 volatile compound - Cold: linalool (20,43%), phenyl ethyl alcohol (11,67%), indole (67,89%) - Hot: linalool (91%), ethyl2-methylbutyrate (9%) - Hexane: linalool (13,3%), phenylethyl alcohol (39,10%), indole (25,98%) and 6 volatile compound - Petroleum: linalool (34,86%), phenyl ethyl alcohol (34,93%), indole (17,4%) and 3 volatile compound	Punjee et al. [57]
	Stem	- Extraction: Methanol-MeOH (room temperature - Silica gel chormatography	- 20 compounds - Six aporphines:(–)-anonaine, (–)-norushinsunine,(–)-ushinsunine,(–)-N-formylanonaine,(–)-romerine,(–)-asimilobine - two oxoaporphines: liriodenine, oxoxylopine; lignan:(+)-syringaresinol - amide: N-trans-feruloyltyramine - seven benzenoids: 4-hydroxybenzaldehyde, p-anisaldehyde, veratraldehyde, 3,4,5-trimethoxybenzoic acid, 3,4-dimethoxybenzoic acid, eugenol, methyl isoeugenol; triterpenoid, ficaprenol - two steroids: i-sitosterol stigmasterol	Lo et al. [62]
6	Leaves, March, Spring	- Extraction: Methanol-MeOH (room temperature - Thin layer chromatography (TLC)	(-) -N-Formylanonaine, (-)-oliveroline, (þ)-nornuciferine, lysicamine, (þ)-cyperone (þ)-epi-yangambin ficaprenol-10, pheophytin a, aristophyll C, michephyll A (a new compound, antioxidant activity)	Wang et al. [63]
7	Flower	- Extraction: Maseartion method, n-hexana - GC-MS	1,3-Benzioxole,5-(2-propenyl), cyclohexane; 1-ethenyl-1-methyl-2,4-bis (1- methylethenyl); Butanoic acid; 3-methyl-,2-phenylethyl ester; 9,12-Octadecadienoic acid, methyl ester; Tricosane; Pentacosane.	Bawa [64]
8	Flower (juvenile, middle, whitening phase) leaves (tender, grow-up, fallen phase), tender twigs	- Ultrasound extract - HPLC	- Flower: Linalool (1,63-4,89%), juvenile is highest - Leaves: Linalool (0,21-0,65%), fallen is highest - Tender twigs: Linalool (0,43%)	Xia et al. [9]
9	Fresh, fallen, and	- Solid-phase	Main compound	Qin et al. [65]

	dried leaves	microextraction - GC-MS	- Fresh: linalol (26,10%), isocaryophyllene, aromadendrene, α-caryophyllene and (-)-γ-cadinene, total volatile (40%) - Fallen: linalool (40,52%), β-elemene (11,94%), β-caryophyllene (10,78%) - Dried: linanol (36,52%), β-elemene, β-caryophyllene, α-selinene, α-cubebene, total volatile (70%)	

Figure 3. Eleven stages of *M. alba* flower development [5, 19]

Tissue culture of M. alba

Table 5 showed tissue culture studies of *M. alba* that had been conducted by some authors in Indomalaya ecozone. It had also been reported the succesfull of using single auxin and single cytokinin on callus induction a of *M. alba* rachises [19]. Similar to *M. champaca*, somatic embryonic callus with globular structure of *M. alba* petals had been noted on medium containing 2,4-D. While petals on medium containing BA showed caulogenesis after 20 days culture. Previous studies conducted by Evachristy [55] had also reported that the concentration of 2 mg/l (ppm) NAA and 3 ppm BAP is optimum to induce calli of *M. alba* young leaves compare to other combinations of NAA and BAP with 1-5 mg/l (ppm) concentration range. Similarly, the appropiate combination of NAA and BAP stimulated calli growth [60]. The auxin and cytokinin roles on callus initiation and somatic embryogenesis and their responses are also influenced by explant sources.

Table 5. Tissue culture studies of *M. alba*

No	Purpose	Method	Result	Reference
		M. alba		
1	To induce callus and investigate monoterpens production	**Explants source:** **-** Flower bud (petal), **Culture medium:** a. Solid MS media + different combination of NAA and BAP b. Callus + casein hydrolisate **c.** Medium + bioelicitor (jasmonic acid	- Different concentration of combination of NAA + BAP obtained callus which produce different main compound of volatile component - Pectinase encouraged highest callus growth - Jasmonic acid decreased callus growth, increased new volatile components	Rusdi [60]
2	To evolve cell culture system (callus, suspension cell cultures, and somatic embryos production	**a. Callus production** **Explant source:** - Freshly flowers (light green to yellowish white colour of petals + rachis) **Sterilization:** - 20% clorox solution + a few drops of Tween 20 (10 min) **Culture medium:**	- Both 2,4-D and BA induced callus and globular stuctures from rachises explant - Direct somatic embryogenesis with globular structure observed from petal explants on solid MS media + 2,4-D - Caulogenesis of petals explant	Ibrahim et al. [19]

	and essential oil production)	- Solid MS media + 1,0 mg/L benzyladenine (BA) and 4,0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) (15 days) **Culture condition:** - 16 h photoperiod at 23 ±2°C and dark at 25 ±2°C. **b. Suspension cell culture** **Explant source:** - 18 gr somatic embryo of R and CH lines **Culture medium:** - 3 L liquid MS medium + 1,0 mg/L 2,4-D + 0,5 mg/L BA + 30 g/L sucrose in a 5 L balloon type air-lift bioreactor **Culture condition:** - dark at 25 ±2°C (30 days) **c. Production of secondary metabolites** -liquid culture system + L-phenylalanine (0,05-0,10 mg/L) to bioreactor (2-3 weeks)	occurred on MS media + BA after 20 days

3. Improvement Study of Magnolia in Indonesia

Many studies under *in vitro* conditions mentioned above were conducted with the same source explant grown outside Indonesia. There are large chances and opportunities to establish studies under *in vitro* conditions about both conservation and development of natural resourses like Magnolia that grow in Indonesia.

3.1. Propagation

Germination study to solve problem on seeds can be carried out by different pre-treatments. Scarification and stratification are an alternative to solve seed dormancy in seeds of several types of legume plants to improve their germination. The cheap and easy application of methods to optimize germination of seeds can be conducted on Magnolia genus in Indonesia and will be recommended to germinate them both under in vitro and and *ex situ* conditions to establish their nurseries and orchards.

3.2. Tissue culture

Tissue culture which cover plant breeding and plant propagation could be applied to solve problems of Magnolia seed dormancy. Micropopagation *in vitro* condition is also an alternative when the plant is difficult to propagate by conventional methods. Seeds or others parts of a plant could be used as explant source. Different and combination of plant growth regulator and medium could be used to develop a protocol for callus induction, shoot initiation or root regeneration. The successful acclimatization of appropriate explants and methods can be selected to provide nursery transplant stocks for supporting cultivation of Magnolia in Indonesia. It can also be used for production of synthetic seeds from somatic embryos obtained by tissue culture techniques.

Tissue culture or cell culture methods can be used for production of seconder metabolites from callus or under stress conditions providing large opportunities to study and screen them against antimicrobial, antifungal, antibacterial and antioxidant and antiviral activities in other Magnolia's species of Indonesia which has not been conducted yet. The antimicrobial, antibacterial and antioxidant activities of of *M. champaca* grown in Bangladesh and India have been carried out by using direct extracts from flowers [66] and leaves and stems [67]. It also contains essential oil from flower [68], fresh leaves [69] and bark [70]. Iyer and Panda [71] reported the potential of callus extract of *M. champaca* as renewable bio-resource that could be used to control biocompatible gold and silver nanoparticles synthesis due to great size and shape diversity and stability compared to flower extract-generated particles. However, there is limited information about biological activities of Magnolia' species in Indonesia especially from calli obtained from various explants source under normal or stress conditions that are stimulated for production of more phytochemical components.

Protoplast culture and hybridization of plants by developing cybrids could serve as an alternative to improve colour and aroma of flowers from different varieties and *M. liliifera* or species in genus Magnolia for use in ornamental plants industry or making garlands. Genetic transformation of species in genus Magnolia could

open new chances and avenues to make these tolerant against prevalent biotic or abiotic stresses.

3.3. Volatile component

The phytochemical screening and volatile component analysis of *M. champaca* and *M. alba* have been reported by many authors in many countries, especially in Indomalaya ecozone. Volatile compounds at the concrete, absolute and essential oil which have different percentage depending on extraction methods and extract sources. These can be developed for medicinal and industrial purposes including their use in perfume, splash cologne, air freshener and aromatherapy industries. *M. liliifera* and others Magnolia species native to Indonesia could be reviewed on their volatile compound of concrete, absolute and essential oil etc. can be used to extract these directly or from callus under *in vitro* tissue culture conditions.

4. Conclusions

This study describes tissue cultures, micropropagation and comparison of compounds obtained from these trees. The results describe variations among different species of genus magnolia. The results indicated more work on the plant in reference to Indonesia and suggest a substantial improvement in vegetative growth and multiplication through these techniques. Differences in species among genus may reflect possible physical or hormone related factors that could prevent growth and release of metablites under in vitro tissue culture conditions of thee plants.

Statement of Conflict of Interest
Authors have declared no conflict of interest.

Author's Contributions
The contribution of the authors is equal.

References

[1] Frodin DG., Govaerts R. World checklist and bibliography of Magnoliaceae. Kew, United Kingdom: Royal Botanic Gardens; 1996.

[2] Kirtikar KR., Basu BD. Indian medicinal lant, New Delhi, India: Periodical Expert Book Agency; 1991.

[3] Shang C., Hu Y., Deng C., Hu K. Rapid determination of volatile consituents of *Michelia alba* flowers by gas chromatography-mass spectrometry with solid-phase microextraction, Journal of Chromatography A 2002; 942: 283-288.

[4] Sobhagini N., Soumit KB., Malaya KM. Ethno-medico botanical survey of Kalahandi district of Orissa, Indian Journal of Traditional Knowledge 2004; 3(1): 72-79.

[5] Sanimah S., Suri R., Nor Azizun R., Hazniza A., Radzali M., Rusli I., Hassan MD. Volatile compounds of essential oil from different stages of *Michelia alba* (cempaka putih) flower development, Journal of Tropical Agriculture and Food Science 2008; 36(1): 109-119.

[6] Rozak AH. Status taksonomi, distribusi dan kategori status konservasi *Magnoliaceae* di Indonesia (Taxonomy, distribution and conservation status of Magnoliaceae in Indonesia), Buletin Kebun Raya 2012: 15(2): 81-91.

[7] Germplasm Resources Informatıon Network (GRIN). *Magnolia champaca.* United States Department of Agriculture (USDA): Agricultural Research Service (ARS); 1880.

[8] Chua LSL. *Magnolia liliifera*. IUCN Red List of Threatened Species, 1998. https://www.iucnredlist.org/details/39004/0. Retrieved: Feb 15, 2020.

[9] Xia EQ., Song Y., Ai XX., Guo YJ., Xu XR., Li HB. A new high-performance liquid chromatographic method for the determination and distribution of linalool in *Michelia alba*, Molecules 2010; 15(7): 4890-4897.

[10] Likhitwitayawuid K., Ruangrungsi N., Boriboon M., Lange GL., Decicco CP. Constituents of the stem bark of *Michelia longifolia*, Journal of The Science Society of Thailand 1988; 14: 73-76.

[11] Ueyama Y., Hashimoto S., Nii H., Furukawa K. The chemical composition of the flower oil and the leaf oil of *Michelia alba* D.C., Journal of Essential Oil Research 1992; 4: 15-23.

[12] Makhija IK., Vignes H., Chandrashekar KS., Ricard L., Prasanna KS. Isolation of 3β-16α-dihydroxy-5-cholesten-21-al, n-Docosanoic acid and Stigmasterol from petroleum ether

extract of stem bark of *Michelia champaca*, Archives of Applied Science Research 2010; 2(6): 344-348.

[13] Yeh YT., Huang JC., Kuo PL., Chen CY. Bioactive constituents from *Michelia champaca*, Natural Product Communications, 2011; 6(9): 1251-1252.

[14] Merkle SA. Somatic embryogenesis in Magnoliaceae (Liriodendron and Magnolia). In: Bajaj YPS. (ed.) Somatic embryogenesis and synthetic seed I, biotechnology in agriculture and forestry 30, Y.P.S. Bajaj, Berlin: Springer-Verlag; 1995. p. 388-403.

[15] Armiyanti Kadir MA., Kadzimin S., Panjaitan SB. Plant regeneration of *Michelia champaca* L., through somatic embryogenesis, African Journal of Biotechnology 2010; 9(18): 2640-2647.

[16] Armiyanti Kadir MA., Kadzimin S., Panjaitan SB., Aziz MA. Establishment of plant regeneration of *Michelia champaca* L. through cell suspension culture technique, Journal of Medicinal Plants Research 2012; 6(8): 1394-1402.

[17] Abdelmageed AHA., Faridah QZ, Nor Shuhada K., Julia AA. Callus induction and plant regeneration of *Michelia champaca* (Magnoliaceae): A multipurpose tree, Journal of Medicinal Plants Research 2012; 6(17): 3338-3344.

[18] Fernando MTR., Gehan Jayasurya KMG., Walck JL., Wijetunga ASTB. Identifying dormancy class and storage behaviour of champak (*Magnolia champaca*) seeds, an important tropical timber tree, Journal of the National Science Foundation of Sri Lanka 2013; 41(2): 141-146**.**

[19] Ibrahim R., Hussein S., Noordin N., Azlan E., Manan MA., Adnan H., Vellupillai M. Advanced cell culture technology for essential oil production and microarray studies leading to discovery of genes for fragrance compounds in *Michelia alba* (cempaka putih), Acta Horticultura 2008; 765: 95-100.

[20] Plants of the world online. 2020. http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:933059-1. (Retrieved 03.01.2020)

[21] Nooteboom HP., Chalermglin P. The Magnoliaceae of Thailand, Thai Forest Bulletin (Botany) 2009; 37: 128-129.

[22] Germplasm Resources Information Network (GRIN), *Magnolia liliifera* (L.) Baill. United States Department of Agriculture (USDA): Agricultural Research Service (ARS); 2019.

[23] Promputtha I., Promputtha I., Lumyong S., Lumyong P., Mckenzie EHC., Hyde KD. Fungal succession on senescent leaves of Manglietia garrettii in Doi Suthep-Pui National Park, Northern Thailand, Fungal Diversity 2002; 10: 89-100.

[24] Promputtha I., Lumyong S., Lumyong P., Mckenzie EHC., Hyde KD. Fungal saprobes on dead leaves of Magnolia lillifera (Magnoliaceae) in Thailand, Cryptogamie Mycologie 2004; 25: 43-47.

[25] Monkai J., Chukeatirote E., Chamyuang S., Synytsya A., Rumi T., Hyde KD. Antimicrobial activity of some saprobic fungi isolated from *Magnolia liliifera* and *Cinnamomum iners* leaves, Mycology 2013; 4(2): 82-84.

[26] Danu Putri KP. Penggunaan media dan hormon tumbuh dalam perbanyakan stek bambag lanang (*Michelia champaca* L.), Jurnal Perbenihan Tanaman Hutan 2015; 3(2): 61-70.

[27] Pudjiono S. Pengaruh perbedaan media tanam terhadap perkembangan perakaran dan keberhasilan stek pucuk manglid (*Magnolia champaca* var *pubinervia* (Blume) Figlar & Noot.) (The effect of different growing media on root development and the success of manglid shoot cuttings (*Magnolia champaca* var *pubinervia* (Blume) Figlar & Noot.)). Seminar Nasional Pendidikan Biologi dan Saintek II, 20 Mei 2017, pp: 22-27, Universitas Muhammadiyah, Surakarta, Indonesia.

[28] Hong TD., Ellis RH. A protocol to determine seed storage behaviour. IPGRI Technical Bulletin No. 1. Rome, Italy: International Plant Genetic Resources Institute (IPGRI) Publication; 1996.

[29] Bahuguna VK., Rawat MMS., Naithani KC. Investigation on the seed of *Michelia champaca* Linn. for perfection of optimum conditions of storage, Indian Forester 1987; 113: 243-248.

[30] Robbins AMJ. Storage of champ (*Michelia champaca*) seed, Banko Janakari 1988; 2: 55-57.

[31] Bisht NS., Ahlawat SP. Seed technology. Itanagar, India: SFRI Information Bulletin No 7; 1999.

[32] Yuniarti NN. Perubahan viabilitas dan biokimia benih bambang lanang (*Michelia champaca* Linn.) pada berbagai tingkat pengeringan dan metode penyimpanan (The change of viability and seed biochemistry on bambang lanang (*Michelia champaca* Linn.) in different of drying time and storage method), Jurnal Perbenihan Tanaman Hutan 2015; 3(1): 36-48. (In Indonesian)

[33] Guimaraes JrP., Cogni R. Seed cleaning of *Cupania vernalis* (Sapindaceae) by ants: Edge effect in a highland forest in south-east Brazil, Journal of Tropical Ecology 2002; 18(2): 302-307.

[34] Candiani G., Galetti M., Cardoso VJM. Seed germination and removal of *Michelia champaca* L. (magnoliaceae) in eucalypt stands: the influence of the aril, Revista Arvore 2004; 28(3): 327-332.

[35] Tan SH., Abdullah TL., Go R. Effect of plant growth regulators on cutting propagation of cempaka putih (*Magnolia alba*) and cempaka kuning (*Magnolia champaca*), Transactions of The Malaysian Society of Plant Physiology 2018; 25: 124-127.

[36] Dwicandra NMO, Astuti MAP., Ariantari NP., Yowani SC. Skrining kandungan kimia ekstrak etanol 80% kulit batang *Michelia champaca* L. (Screening chemical content of stem bark of *Michelia champaca* L. extracted by 80% ethanol), Jurnal Farmasi Udayana 2013; 2(3): 43-47.

[37] Rout PK., Naik SN., Rao YR. Composition of the concrete, absolute, headspace and essential oil of the flowers of *Michelia champaca* Linn., Flavour and Fragrance Journal 2006; 21: 906-911.

[38] Kaiser R. New volatile constituents of the flower concrete of *Michelia charnpaca* L, Journal of Essential Oil Research 1991; 3: 129-146.

[39] Baez D., Morales D., Pino JA. Volatiles from *Michelia champaca* flower: comparative analysis by simultaneous distillation-extraction and solid phase microextraction, Natural Product Communications 2012; 7(5): 659-660.

[40] Samakradhamrongthai R., Utama-ang N., Kopermsub P., Thakeow P. Effect of extraction on volatile compounds and sensory profile of champak flower (*Michelia champaca* L.). Proceedings: 1st ASEAN Plus Three Graduate Research Congress, 1-2 March 2012, pp: 721-727, Chiang Mai, Thailand.

[41] Ananthi T., Anuradha R. Determination of phenolic compounds in flower of *Michelia champaca* (L.) by HPTLC analysis, International Journal of Pharmaceutical Science Review and Research 2015; 33(2): 166-168.

[42] Ananthi T., Anuradha R. Quantitative determination of quercetin in *Michelia champaca* (L.) flowers by HPTLC technique, International Journal of Pharmacognosy and Phytochemical Research 2016; 8(9): 1516-1521.

[43] Ahmad H., Misra A., Gupta R., Saraf SA. Determination of gallic acid in *Michelia champaca* L. (Champa) leaves and stem bark by HPTLC, Der Pharmacia Lettre 2011; 3(5): 307-317.

[44] Sethi VK., Thappa RK., Dhar KL., Atal CK. Constituents of *Michelia champaca* and lewis acid catalysed transformations of parthenolide into guaianolides, Planta Medica 1984; 50(4): 364.

[45] Jacobbson U., Kumar V., Saminathan S. Sesquiterpene lactones from *Michelia champaca*, Phytochemistry 1995; 39(4): 839-843.

[46] Kapoor S., Jaggi RK. Chemical studies on flower of *Michelia champaca*, Indian

Journal of Pharmaceutical Sciences 2004; 66(4): 403-406.

[47] Lago JHG., Favero OA., Romoff P. Chemical composition and seasonal variation of the volatile oils from leaves of *Michelia champaca* L., Magnoliaceae, Brazilian Journal of Pharmacognosy 2009; 19(4): 880-882.

[48] Rout PK., Naik SN., Rao YR. Liquid CO_2 extraction of flowers and fractionation of floral concrete of *Michelia champaca* Linn., Journal of Supercritical Fluids 2011; 56: 249-252.

[49] Jiang DY., Li YH., He F., Lin QP., Pan HT. The components and changes of vocs of *Michelia champaca* L. flower at different developmental stages, Scientia Agricultura Sinica 2012; 45(6): 1215-1225.

[50] Utama-Ang N., Kopermsub P., Thakeow P., Samakradharongthai R. Encapsulation of *Michelia champaca* L. extract and its application in instant tea, International Journal of Food Engineering 2017; 3(1): 48-55.

[51] Dhandapani S., Jin J., Sridhar V., Sarojam R., Chua NH., Jang IC. Integrated metabolome and transcriptome analysis of *Magnolia champaca* identifies biosynthetic pathways for floral volatile organic compounds, BMC Genomics 2017; 18(1): 463.

[52] Shukla S. Callus Induction of *Michelia champaca* L. through petiole-an aromatic tree of high economic value, International Journal of Enhanced Research in Science Technology & Engineering 2014; 3(1): 438-442.

[53] Lai YC., Lee WC. The initiation of callus culture of *Michelia champaca* for essential oil production, Biotechnology Letters 1994; 16(1): 85-88.

[54] Sinha R., Varma R. Effect of growth hormones on shoot initiation of *Michelia champaca* L., International Journal of Life Sciences Research 2019; 7(1): 22-25.

[55] Evachristy E. Penggunaan NAA dan BAP dalam induksi kalus janringan tanaman kantil (*Michelia alba* D.C) (The use of NAA and BAP in the induction of callus of antylum plant (*Michelia alba* D.C), Bachelor Thesis, University of Airlangga, Faculty of Mathematics and Natural Sciences, Surabaya, Indonesia, 1997. (In Indonesian language)

[56] Pensuk W., Padumanonda T., Pichaensoonthon C. Comparison of the chemical constituents in *Michelia alba* flower oil extracted by steam distillation, hexane extraction and enfleurage method, Journal of Thai Traditional & Alternative Medicine 2007; 5(1): 30-39.

[57] Punjee P., Dilokkunanant U., Sukkatta U., Vajrodaya S., Haruethaitanasan V., Pitpiangchan P., Rakthaworn P. Scented extracts and essential oil extraction from *Michelia alba* D.C., Kasetsart Journal Natural Science 2009; 43(5): 197-203.

[58] Peana AT., Moretti MDL. Linalool in essential plants oil: pharmacological effects. In: Watson RR., Preedy VR. (eds.) Botanical medicine in clinical practice. Wallingford, UK: CABI; 2008. p. 716-724.

[59] Hamid HA., Ramli ANM., Yusoff MM. Indole alkaloids from plants as potential leads for antidepressant drugs: a mini review, Frontiers in Pharmacology 2017; 8(96): 1-7.

[60] Rusdi NA. Production and biological activity of monoterpenes from flowers and callus cultures of *Michelia alba* D, Master thesis, University of Putra Malaysia, Faculty of Biotechnology and Biomoleculer Sciences, pp.; 153, Selangor, Malaysia, 2005.

[61] Huang XZ., Yin Y., Huang R., Chen MH., Ge PG., Ma ZJ., Gui H. Study on chemical constituents of essential oils from leaves and stems of *Michelia alba* DC, Food Science 2009; 30(8): 241-244.

[62] Lo WL., Huang LY., Wang HM., Chen CY. Chemical constituents from the stems of *Michelia alba*, Chemistry of Natural Compounds 2010; 46(4): 664-665.

[63] Wang HM., Lo WL., Huang LY., Wang YD., Chen CY. Chemical constituents from the leaves of *Michelia alba*, Natural Product Research 2010; 24(5): 398-406.

[64] Bawa IGAG. Aktivitas antioksidan dan antijamur senyawa atsiri bunga cempaka putih (*Michelia alba*), Jurnal Kimia 2011; 5(1): 43-50.

[65] Qin DQ., Huang RL., Li ZH., Wang SY., Cheng DM., Zhang ZX. Volatile component analysis of *Michelia alba* leaves and their effect on fumigation activity and worker behavior of *Solenopsis invicta*, Sociobiology 2018; 65(2): 170-176.

[66] Parimi U., Kolli D. Antibacterial and free radical scavenging activity of *Michelia champaca* Linn. flower extracts, Free Radicals and Antioxidants 2012; 2(2): 58-61.

[67] Manhas N., Dahiya P. *In vitro* antimicrobial activity and phytochemical screening of leaf and stem extracts of *Michelia champaca* Linn, International Food Research Journal 2017; 24(6): 2672-2676.

[68] Batchu UR., Mandava K., Bhargav PNV., Maddi KM., Syed M., Rasamalla SP., Madhira S. Evaluation of antibacterial and antioxidant activities of essential oil from *Michelia champaka*, Journal of Applied Pharmaceutical Science 2017; 7(3): 113-116.

[69] Ruwali P., Adhikari M., Sharma S. Phytochemical and antioxidant properties of various extracts of *Michelia champaca* leaves, International Journal of Pharmacy and Pharmaceutical Sciences 2019; 11(5): 56-61.

[70] Yesmin R., Huang JC., Kuo PL., Chen CY. *In vitro* antioxidant and antidiabetic assessment of extracts from the bark of *Michelia champaca*, a medicinal plant in Bangladesh, World Journal of Pharmaceutical Research 2019; 8(9): 1505-1526.

[71] Iyer RI., Panda T. Biosynthesis of gold and silver nanoparticles with anti-microbial activity by callus cultures of *Michelia champaca* L, Journal of Nanoscience and Nanotechnology 2016; 16(7): 7345-7457.