

The Fragrance Production from Callus Cultures of Gynoecium and Stamen of *Michelia alba* Flower

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Abstract

Michelia alba Linn., is an aromatic plant from Magnoliaceae family. Pleasant smell of its white flower has been used as fragrance especially in perfumes and cosmetic products. The flower especially gynoeciums and stamens were cultured on woody plant medium supplemented with plant growth regulators. The cultures were conducted both under illumination of 16 hr light and 8 hr dark regimes at 25°C for 7 weeks incubation. The chemical compositions of the essential oils from callus cultures of gynoecium and stamen explants of *Michelia alba* flowers were extracted by hydro-distillation with dichloromethane by using simultaneous distillation extraction (SDE) of modified Liken-Nickerson apparatus and then, analyzed by using gas chromatography-mass spectrometry (GC-MS) technique. There are found 60 peaks present and approximately 15 compounds were chromatographically identified in both oils. The compound with the highest composition found in both essential oils was linalool with 49.978% in gynoecium oil and 28.803% in stamen oil for 0.1g of dry weight callus. The other abundant chemical components of gynoecium oil were found as β -elemen (10.0%), caryophyllene oxide (8.43%), caryophyllene (6.39%), germacrene D (4.20%), δ -cadinene (5.63%) and eugenol methyl ether (2.27%) The other abundant chemical constituents of stamen oil were α -bergamotene (12.52%), β -elemen (4.87%), germacrene-D (3.95%), δ -cadinene (4.76%), caryophyllene (3.43%) and caryophyllene oxide (2.88%). The rest of the compounds were present less than 2%. The composition of essential oil from callus cultures of gynoecium and stamen were not a big difference from the fresh gynoecium and stamen of *M. alba* flower. Therefore it shows that the *in vitro* production have potential in fragrance production from *M. alba* replacing the seasonal bearing fresh flowers.

Keywords: fragrance, callus culture, *Michelia alba*, essential oil, linalool, hydrodistillation

Introduction

Plantation in Malaysia is more focus on food crops and lately diverse in herbal and medicinal plants. Only recently it concentrates on flavour and fragrance produced plants too. The flavour and fragrance industries become enhance due to global market value which getting increase, leading with detergents, cosmetics and toiletries, fine fragrances, household cleaners, air fresheners and other flavour and fragrance products. In Malaysia, production of flavour and fragrance products cannot be denied since the tropical rain forest contribute abundant of sources which comprise of diversified species and most of them are aromatic and highly valued. However most of these species are underexploited and our knowledge and technologies dealing with this industry are still behind.

One of the un-notified plants is *Michelia alba* from the Magnoliaceae family with have very pleasant smell white flower. This plant is different from *Michelia champaca* with stronger odour flower of orange in colour although they are from the same family. *M. champaca* is better well known compared to *M. alba* and it produce lots of capsule bearing seeds rather than *M. alba* which hardly produce seed. Moreover, the flowers being produce mostly after rainy season when new shoots produce together with the flower buds. Nevertheless, *M alba* has been used as an ingredient of costliest perfume of Jadore from Christian Dior.

Natural fragrance production is a new developing business in Malaysia and it is getting rapid when cosmaceuticals, aromatherapy and spa industries getting expand. Local producers beginning to use natural extracts in their products for its therapeutic, rejunaveting, antioxidant, anti-wrinkle, anti-inflammatory and anti-cellulites properties as an attraction to health conscious consumers. Requirement for natural plant materials become demanding but productions become more restricted and highly variable depending on the source plant, location and season of harvest and affected to unpredictable environmental circumstances. The low abundance and uncertain in production have directed into alternate methods of production. In order to overcome the problems, fulfill the demand and maintain the quality of the raw material, there is need to have an alternative system for the production of the desired materials.

In the last century, plant tissue culture has become a promising method for rapid and mass production of plant regeneration. Recently this technique appears as a potential alternative method for rapid and continuous production of secondary metabolites including aromatic compounds. It has been established that under appropriate culture conditions, *in vitro* grown cells / tissue genetically inherit the biosynthetic potential of *in situ* cells and can produce a good amount of secondary metabolites (Shrivastava *et al.*, 2006).

In this study, the potential plant of *M. alba* was used in order to extract the fragrance compounds from flower. The flower part which produce strongest smell come from the base of the flower especially the gynoecia (female reproductive organ) and stamen (male reproductive organ). Since the development of flower involves many stages, the flower chosen is from white flower bud which directly changes from green bud since the tissue is still young, active dividing and ready to bloom with full of aroma to be discharge.

Methodology

Callus culture

Fresh samples of *M. alba* were collected from Serdang, Selangor. Fresh flower bud from stage 7 (white, unopened flower bud) of *M. alba* were sterilized and cut opened. The gynoecia and stamen are cultured separately on woody plant media supplemented with 4mg/l NAA and 2 mg/l kinetin. It was subcultured every 3-4 weeks. The cultures were conducted both under illumination of 16 hr light and 8 hr dark regimes at 25°C for 7 weeks. After 7 weeks incubation under light regime, both cultures were harvest for extraction.

Isolation of the oils

Fresh flowers from stage 7 were collected and the gynoecia and stamen were detached from the flowers separately. Callus cultures prepared earlier from 7 weeks incubation were also harvested. The fresh samples and the callus were then extracted separately by hydro distillation technique

using simultaneous distillation extraction (SDE) method (Sugisawa et al. 1988). The essential oils were collected in dichloromethane and separately dried over 10g of anhydrous sodium sulphate at 4°C for overnight. The dried essential oils obtained were kept cold in tight glass vial with rubber septum and ready for further experiments.

Gas Chromatography-Mass Spectrometry

All analysis of each oil were carried out using a Shimadzu QP5050A GC-MS system, operating with 30m x 0.25mm, 0.25 µm film thickness, fused silica capillary column from Australia. He (2 ml/min) was used as carrier gas. The initial temperature of the column was 40°C and it was then heated to 240°C at a rate of 5°C /min and followed with 270°C at a rate of 10°C /min. Injector and transfer line temperatures were set up at 250°C and 280°C respectively. The identification of the individual compounds was based on comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS, Wiley library spectra and the literature. The percentage compositions of the essential oils are usually based on peak areas obtained. Data are as mean of duplicate (n=2) determinations.

Result

Extractions of essential oils from fresh flowers (white, unopened bud) were characterized by their main compound of linalool. The percentage of linalool was a bit higher in gynoecium (74.56%) compared to stamen (74.41%). The second abundant compound in flower was β-elemen in stamen (4.78%) and gynoecium (5.17%) (Table1). This followed with trimethylbicyclo heptene (2.06%), dodecatrien-ol (1.87%) and ocimene (1.83%) in gynoecia. Differ with stamen cis-furanmethanol (1.85%) and 3,7-octadien-2,6-diol (1.36%) while the rest of the compounds are not more than 1%. All together there are 27 compounds were identified in stamen and 30 compounds were identified in gynoecia.

Table 1. Major chemical compounds present in essential oils of gynoecium and stamen of *M. alba* flower.

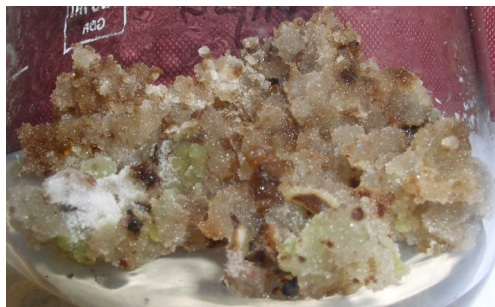
Chemical compound	Composition (%)	
	Stamen	Gynoecia
Linalool	74.41	74.56
2-methyl-methyl-ester butanoic acid	0.52	0.85
2-methyl butanoic acid	0.58	
β-elemen	4.78	5.17
Dodecatrien-1-ol		1.87
Ocimene	0.28	1.83
Phenylethyl alcohol	0.51	0.26
3,7-octadien-2,6-diol	1.36	
trimethylbicyclo heptene		2.06
cis-furanmethanol	1.85	
α-caryophyllene		0.7
Germacrene D		1.1
Caryophyllene		0.32

Table 2. Major chemical compounds present in essential oils of callus cultures from gynoecium and stamen of *M. alba* flower

Chemical compound	Composition (%)	
	Stamen	Gynoecia
Linalool	28.80	49.98
β -elemen	4.87	10.0
caryophyllene oxide	2.88	8.43
caryophyllene	3.43	6.39
Germacrene D	3.95	4.20
δ -cadinene	4.76	5.63
eugenol methyl ether		2.27
α -bergamotene	12.52	

Cultures performed nodular, fragile and quite compact callus. The callus is brownish in colour with slight green nodular spot because of the illumination. The compact callus produce aromatic odour when squeezed the tissue.

Fig. 1. Callus cultures of gynoecium incubated under illumination



Discussion

The composition of essential oil from callus cultures of gynoecium and stamen were almost similar with the fresh gynoecium and stamen of *M. alba* flower. As expected, some of the compounds in fresh flower were not present *in vitro* and the composition of chemical compounds in essential oil was least than *in vivo* production. Both chemical compositions *in vivo* and *in vitro* were having linalool as the most major compound with 49.978% in gynoecium oil and 28.803% in stamen oil for 0.1g of dry weight callus. The other abundant chemical components of gynoecium oil were found as β -elemen (10.0%), caryophyllene oxide (8.43%), caryophyllene (6.39%), germacrene D (4.20%), δ -cadinene (5.63%) and eugenol methyl ether (2.27%) The other abundant chemical constituents of stamen oil were α -bergamotene (12.52%), β -elemen (4.87%), germacrene-D (3.95%), δ -cadinene (4.76%), caryophyllene (3.43%) and caryophyllene oxide (2.88%). The rest of the compounds were present less than 2%. Interestingly, α -bergamotene was also the new existence *in vitro* produced compound, that presence in fresh flower and leaf of *M. alba* reported earlier (Ueyema et al., 1992) The odour in 100% was

described as woody warm tea which can be also found in basil (Abdullah et al., 2008), sandalwood (Christopher et al., 2006) and bergamot oil (Figolia et al. 2006).

Production of aromatic compound from natural sources is not exactly the same every time of extraction and is highly variable. The production may differ between each batch may be due to factors such as the genetic of plant, climate changes, part of the plant used or time of plant collected and extraction process. Through tissue culture technique some of the factors can be controlled especially the illumination, temperature, nutrient, pH, uniformity of the tissues. This study showed that compact mass of non- morphogenic cells was proliferated from the explant. This non-morphogenic cell mass can be established as a good source of the metabolic potential (Shrivastava et al., 2006).

Conclusion

The *in vitro* cultures have potential in fragrance production from *M. alba* flower as a solution for low flower production in dry season. Through this technique fragrance compound can be stimulated through cell cultures or from other plant parts. The variation in the chemical composition compared to the fresh flower showed that appropriate chemical composition of nutrient medium, plant growth regulators and physical components of the axenic culture play an important role in the biosynthesis of the chemical constituents in the *in vitro* grown cells (Han et al., 2001). Production of new chemicals derived besides of common compounds of *M. alba* may also be beneficial as new fragrance in fragrance industry.

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