PROPAGASI DAN BIOSINTESIS KANDUNGAN *GINGEROL, SHOGAOL* DAN *ZINGERONE* (*GINGER OIL*) DARI KALUS JAHE EMPRIT (*Zingiber majus* R.) DENGAN PERLAKUAN JENIS MEDIA DAN MACAM KARBOHIDRAT

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Abstrak

Jahe Emprit (Zingiber majus R.) adalah salah satu diantara tanaman rempah-rempah yang memiliki manfaat ekonomis yang tinggi sebagai rempah, minyak atsiri, pemberi aroma, ataupun sebagai obat . Kandungan komponen kimia jahe adalah Gingerol, Shogaol dan Zingerone (Ginger oil). Kandungan tersebut mempunyai efek farmakologi dan fisiologi seperti antioksidan, anti imflammasi, analgesik, antikarsinogenik non-toksik dan mutagenik Tujuan khusus penelitian ini sebagai berikut (1) Mengetahui kualitas kalus yang dihasilkan (friabel atau kompak) sesuai untuk mikro propagasi jahe Emprit (2)Mengetahui jenis media dan karbohidrat yang ditambahkan sesuai untuk meningkatkan biosintesis pada kalus jahe Emprit dalam menghasilkan ginger oil . Metode penelitian ini menggunakan Rancangan Acak Lengkap Berfaktorial dengan dua faktor. Faktor I : Media dasar MS dan VW dan Faktor II: Macam – macam karbohidrat yaitu glukosa fruktosa dan sukrosa. Terdapat enam kombinasi perlakuan diulang 5x dengan 4 sampel; kemudian dicari produksi kalus friabel dan kandungan ginger oil yang terbaik. Hasil Penelitian ini adalah (1) kuantitas kalus terbanyak ada pada kalus jahe Emprit dengan perlakuan media MS dan perlakuan fruktosa (2) kualitas jahe Emprit dengan media MS dan fruktosa adalah friabel, kalus ini dapat digunakan sebagai bahan tanam mikro - propagasi jahe Emprir ;pada perlakuan lainnya cenderung kompak kalus ini lebih sesuai untuk peningkatan ginger oil

Kata Kunci : *Biosintesis Gingerol, Shogaol dan Zingerone,Eksplan ;Propagasi ;Kalus jahe Emprit.*

PROPAGATION AND BIOSINTESIS CONTENT GINGEROL, SHOGAOL AND ZINGERONE (GINGER OIL) FROM EMPRIT GINGER (Zingiber Majus R.) WITH MEDIATYPES OF TREATMENT AND CARBOHYDRATES

Abstract

Emprit Ginger (Zingiber majus R) is one of the spice plants that has high economic benefits as spices, essential oils, fragrance, or as medicine. The chemical components of ginger are Gingerol, Shogaol and Zingcrone (Ginger oil). The content has pharmacological and physiological effects such as antioxidant, anti-inflammatory, analgesic. anti-carcinogenic non-toxic and mutagenic. The specific objectives of this study arc as follows (I) Knowing the quality of the produced (friable or compact), callus for micro-propagation of ginger Emprit (2) types of media and carbohydrates are added according to increase biosynthesis in Emprit ginger callus in producing ginger oil .This research method uses a completely randomized factorial design with two factors. Factor I: Basic media MS and VW and Factor II Types, of carbohydrates, namely glucose fructose and sucrose There are six combinations of treatments repeated 5 times with 4 samples; then friabcl callus production and the higest ginger oil content were sought. The results of this study are (I) the most callus quantity is in Emprit ginger callus with MS media treatment and fructose treatment (2) the quality of Emprit ginger with MS media and fructose is fnabel, this callus can be used as ginger Emprit micro-propagation planting material, other treatments tend to be compact, this callus is more suitable for increasing ginger oil

Key Words *: Biosynthesis Ginger oil; Callus; Emprit ginger.karbohidra;, secondary Metabolite*

INTRODUCTION

Ginger (Zingiber officinale R.) is one of the spices that has long been cultivated in Indonesia and has high economic value as a spice, essential oil, aromatic scent and medicinal herb (Bartley and Jacobs, 2000). Ginger is one of the important export commodities and ingredients of traditional medicine as well as of phytopharmacy that has been commonly used in the herbal medicine industry in Indonesia (Rostiana, 2017). *Ginger oil* contains *Gingerol*, *Shogaol* and *Zingerone*. These compounds have pharmacological and physiological effects like anti-oxidant, anti-inflammatory, analgesic, anti-carcinogenic, non-toxic and mutagenic effects (Pradeep, 2016).

The conventional process for extracting *Ginger oil* is not efficient; it requires a large quantity of rhizomes and solvents, as well as vast agricultural fields and a lot of time. Rhizome production is highly influenced by climate and also influenced by the age of ginger.

One alternative to fulfill the needs of phytopharmacy for medicinal herbs and spices is by developing tissue culture techniques through metabolite biosynthesis processes (Kamaliroosta, 2012). The production of secondary metabolites through plant tissue culture processes is a more effective way to improve these contents than conventional methods (Anasori, 2008).

Some of the advantages of using plant tissue culture techniques for the production of secondary metabolites include: not depending on environmental factors such as climate, pest, geographic and seasonal constraints; the production system can be regulated, where production is carried out when needed and in desired amount; quality and production results are more consistent and reduce land use (Marlina, 2004).

To produce secondary metabolite compounds in ginger, a profiling process was done to investigate the content of *Gingerol*, *Shogaol* and *Zingerone* in jahe Emprit that were grown on Murashige and Skoog and VW medium;that has added bye glukosa fruktosa and sukrosa . The purposes of this research are: to investigate the quantity and quality of callus of jahe Emprit ; and to obtain callus from the jahe Emprit that can produce the best composition of ginger oil.

METHOD OF RESEARCH

Time and Location of Research

The research was done in the Laboratory of Tissue Cultures in the Department of Agriculture of Wijaya Kusuma University, Surabaya. This research was carried out from early January 2019 to December 2019.

Experimental Design

The method used a factorial complete randomized design on the firast factor are two medium MS and VW; and thec second factor are glukosa fruktosa and sukrosa within 5 repetition, with four test samples.

Fisrt Factor :
M1 =MS
M2 =VW
Second Factor :
C1=glukosa
C2=fruktosa
C3=sukrosa

After incubation of callus on MS medium for 3rd months, profiling was done to determine the composition of *Gingerol*, *Shogaol* and *Zingerone* in the callus.

Media and Planting

The MS and VW medium added bye glukosa fruktosa and sukrosa used was a medium package created by the Bunga Harapan florist shop that produces various tissue culture media according to the researchers' orders. This medium was not made in the research laboratory. In this study, the researchers modified it by only adding 5ppm NAA and 3.5ppm BAP.

The explants used were the rhizomes of jahe Emprit plants from the start of planting until they grow rhizomes at around 12-14months. After that time, at around 2months, rhizomes were taken as planting material. The rhizomes used were sliced or cut near the buds. Explants were sterilized with 0.1% HgCI for 1min, and 20% Chlorox was added with 1 drop of Tween for 5min; the process continued with 10% Chlorox + 1 drop of Tween for 10min and 5% Chlorox + 1 drop of Tween for 20min, then rhizomes were rinsed three times with sterilized water. Afterwards, the explants were cut to approximately 0.5cm2 and were planted in culture tubes in the prepared medium.

Data Observation

Observations were done every week by visually observing the growth of callus:

Quantity of Callus

Visually observed once a week by scoring of:

1 = score when there is no callus on explants measuring 0.5cm^2
2 = score when the explant has started to grow callus that is smaller than the explant
3 = score when the explant grows callus which is around one to two times the size of the explant
4 = score when the explant has grown callus more than twice the size of the explant

Quality of Callus

Visually observed every week by scoring of:

1 = no callus
1-2 = compact callus
2-3 = friable callus

A callus with a score between 1 and 2 is a compact callus, which is dense and slow growing and which contains more secondary metabolites. A callus with a score between 2 and 3 is callus that is friable, tends to grow faster and is better used for vegetative propagation (Rahmawati, 2006).

Composition of Secondary Metabolites

Extraction of *Gingerol*, *Shogaol* and *Zingerone* from ginger callus was carried out by the Industrial Research and Consultation Agency (BPKI) of the Research Laboratory and Research and Consultation Center, JI Ketintang Baru XVII NO 14, Surabaya, Jawa Timur. Compounds were extracted and visually observed spectrophotometer.

***Gingerol* analysis**

Callus was weighed and dried until the maximum moisture content was 10%. The callus was distilled, the steam flowing through the cooler so that the vapor melted; this vapor was stored in a separating flask. The distillation was carried out until the aroma of *Ginger oil* was dissappear. The obtained distillate was put into a separator flask in which *Gingerol* was obtained.

***Shogaol* analysis**

Dried callus was put into a separating flask with alcohol and benzene solvents (1:50) for 24 h at a temperature of 40-60°C. Liquid was separated from callus residue with Whatman 40 filter paper so that a clear filtrate was obtained. The absorbance value of filtrate was measured at a wavelength of 212nm; *Shogaol* was measured using a calibration curve.

***Zingerone* analysis**

To analyze *Zingerone*, the same stages were used as for *Shogaol* analysis, except that the solvent was benzene, ether and ethanol (1:1:1), and the wave length was 240.5nm.

Data Analysis

Univariate data for callus quantity and quality was analyzed using One-Way ANOVA with SPSS 18; if significant differences were found, analysis continued by least significant difference (LSD) test at 5%.

RESULTS OF RESEARCH

Quantity of Callus

Tabel 1. Quantity of ginger callus for jahe Emprit bye treatment varity of karbohidrate and medium

Perlakuan	Minggu Setelah Tanam (MST)											
	1	2	3	4	5	6	7	8	9	10	11	12
C1M1	1.00	1.00	1.00	1.00	1.20	1.20 B	1.30 B	1.30 B	1.60 B	1.80 B	1.70 B	1.90 B
C1M2	1.00	1.00	1.00	1.00	1.20	1.20 B	1.30 B	1.30 B	1.50 B	1.80 B	1.60 B	2.00 B
C2M1	1.00	1.00	1.00	1.00	1.70	1.80 A	1.90 A	1.90 A	2.50 A	3.00 A	2.80 A	3.30 A
C2M2	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.40 B	1.60 B	2.00 B	1.90 B
C3M1	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.60 B	1.70 B	1.80 B	2.20 B
C3M2	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.50 B	1.60 B	1.60 B	1.90 B
BNT 5 %	TN	TN	TN	TN	TN	0,44	0,44	0,44	0,56	0,51	0,46	0,36

Note: Average values followed by the same letter in the same column indicate significant indifference based on LSD 5% test. NS = non-significant.

In Table 1 above, the results show that there was a significant difference in the growth of callus from the six week. It seems that callus C2M1 growth on is faster than the other.

Quality of Callus

Tabel 2. Quality of ginger callus for jahe Emprit by treatment varity of karbohidrate and medium

Perlakuan	Minggu Setelah Tanam (MST)											
	1	2	3	4	5	6	7	8	9	10	11	12
C1M1	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.30 B	1.50 B	1.70 B	2.10 B
C1M2	1.00	1.00	1.00	1.00	1.20	1.20 B	12.0 B	1.20 B	1.30 B	1.50 B	1.60 B	1.80 B
C2M1	1.00	1.00	1.00	1.00	1.70	1.90 A	1.90 A	1.90 A	2.60 A	2.70 A	2.80 A	2.90 A
C2M2	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.20 B	1.60 B	2.00 B	2.00 B
C3M1	1.00	1.00	1.00	1.00	1.20	1.30 B	1.30 B	1.30 B	1.60 B	1.80 B	1.80 B	2.20 B
C3M2	1.00	1.00	1.00	1.00	1.20	1.20 B	1.20 B	1.20 B	1.40 B	1.50 B	1.80 B	1.90 B
BNT 5 %	TN	TN	TN	TN	TN	0,43	0,43	0,43	0,53	0,53	0,46	0,41

Note: Average values followed by the same letter in the same column indicate significant indifference based on LSD 5% test. NS = non-significant; LSD = least significant difference (method of Ronald Fisher).

Composition of Secondary Metabolites

Extraction of gingerol, shogaol and zingeronefrom ginger callus was carried out by the Industrial Research and Consultation Agency (BPKI) of the Research Laboratory and Research and Consultation Center, JI Ketintang Baru XVII NO 14, Surabaya, Jawa Timur. Compounds were extracted and visu ally observed using a spectrophotometer

Tabel 3. Content of Ginger oil

CODE	GINGER OIL (%)				
	I	II	III	IV	V
VW gluk	**0,122**	0,121	0,120	0,121	0,120
VW frukt	0,142	0,144	**0,145**	0,143	0,142
VW sukr	0,101	**0,105**	0,103	0,104	0,102
MS gluk	0,110	0,111	0,113	0,111	**0,114**
MS frukt	0,119	0,118	**0,121**	0,117	0,115
MS sukr	0,109	**0,112**	0,108	0,107	0,106

The greatest quantity of Ginger oil were produced by callus of jahe Emprit were grown on VW medium and fruktosa

DISCUSSION

Quantity of Callus

Table 1 above shows that callus growth started in observation week 6; there were significant differences in the quantity of callus grown. Growth on C2M1 was faster than the other. Explants as a plant commodity and their compatibility with the medium are factors that support the growth of callus.

The composition of the medium also affects growth, along with the research treatments (Hendaryono and Wijayanti, 2006). Therefore, in this research, callus quantities were determined by the treatment of variety medium and variety of karbohidrate. There was more callus on MS medium and Fruktosa .. Also, it obtained that the growth of callus on VW medium was slower because the MS medium is more completly nutrient (Rahmavati, 2006). Arijanti (2008) stated that a plant medium should contain all nutrients .needed to ensure the growth of explants.

Quality of Callus

The quality of callus was determined by the characteristics of callus produced by the explants. According to Wattimena (1991), callus can be divided into two types: compact callus and friable callus. Compact callus is compact and indestructible if pinned by a needle, while friable callus tends to grow into new individuals. In this research, the callus of C2M1 was friable, while the other callus was compact. The growth was different because of the specific responses to the media and treatments (Arijanti, 2008).

In Table 2, it can be seen that by week 6 there was a significant difference in the quality parameters of the calluses. In week 12, C2M1 callus tended to be friable, while the other callus was compact.According to Prasetyo (2006), the yellow-brown calluses with noduls are the embrionic calluses. The embrionik calluses can growth and produce the young individu plant. and the other treatment produced compact callus (organogenic).

Composition of Secondary Metabolites

Compounds were extracted and visually observed using a spectrophotometer.

1/ Gingerol analysis

Callus was weighed and dried until the maximum moisture content was 10%. The callus was distilled, the steam flowing through the cooler so that the vapor melted; this vapor was stored in a separating flask. The distillation was carried out until the aroma of ginger oil was dissappear. The obtained distillate was put into a separator flask in which gingerol was obtained.

2/ Shogaol analysis

Dried callus was put into a separating flask with alcohol and benzene solvents (1:50) for 24h at a temperature of 40-60°C. Liquid was separated from callus residue with Whatman 40 filter paper so that a clear filtrate was obtained. The absorbance value of filtrate was measured at a wavelength of 212nm; shogaol was measured using a calibration curve.

3/ Zingerone analysis

To analyze zingerone, the same stages were used as for shogaol analysis, except that the solvent was benzene, ether and ethanol (1:1:1), and the wavelength was 240.5nm.

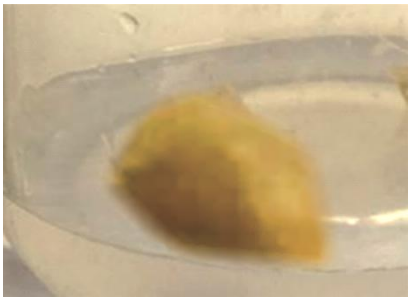
In VW medium can be produced better ginger oil because this medium is more suitable than media MS specific responses to the media and treatments (Arijanti, 2008) Addition of fructose also increases the production of ginger oil,becouse fructose has a higher degree of sweetness than the other carbohydratest here

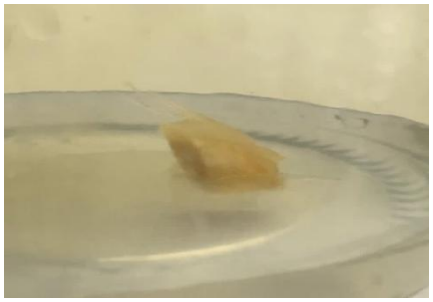
is more enegie to produce ginger oil (Arijanti, 2014) so in this study the use of VW and fructose medium produced the best ginger oil . In Table 3, it can be seen that the greatest quantity of Ginger oil were produced by callus of jahe Emprit were grown on VW medium and fruktosa (0,145%)

CONCLUSION

The greatest quantity of callus was produced by 2M1(fruktosa and MS medium). C2M1(fruktosa and MS medium) and have friable callus .the yellow-brown calluses with noduls are the embrionic calluses. The embrionik calluses can growth and produce the young individu plant. And the other has the compact callus. Greatest quantity of Ginger oil were produced by compact callus of jahe Emprit were grown on VW medium and fruktosa (C2M2)

Picture of callus





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