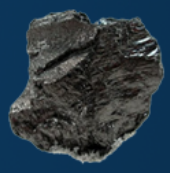
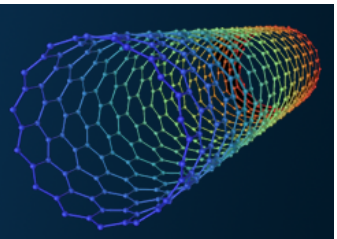
Moroccan Journal of Chemistry
ISSN : 2351-812X





Editorial Board

Editor in chief

Prof B. Hammouti

Editor

Prof. A. Chetouani, Mohammed Premier University, Oujda

National Committee

Akssira M., Hassan II University, Mohammadia
Amine A., Hassan II University, Mohammedia
Aouniti A., Mohammed Premier University, Oujda
Aride J., Mohammed Premier University, Rabat
Azzi M., Hassan II University, Casablanca
Bazzi L., Ibn Zohr University Agadir
Bellaouchou A., Mohammed V University, Rabat
Benchat N., Mohammed Premier University, Oujda
Ben Hadda T., Mohammed Premier University, Oujda
Bensitel M., Chouaib Doukali University El Jadida
Bentiss F., Chouaib Doukali University El Jadida
Berrabah M., Mohammed Premier University, Oujda
Berraho M., Cadi Ayyad University Marrakech
Bouhfid R., Mohamed V University Rabat
Bouachrine M., My Ismail University, Meknes
Bougrin K., Mohamed V University Rabat
Bouyanzer A., Mohammed Premier University, Oujda
Cherkaoui M., Ibn Tofail University Kenitra
Chetouani A., Mohammed Premier University, Oujda
Chtaini A., My Slimane University Béni Mellal
Ebn Touhami M., Ibn Tofail University Kenitra
El Ammari L., Mohamed V University Rabat
EL Haddad M., Cadi Ayyad University Safi
El Kadiri S., Mohammed Premier University, Oujda
Elmidaoui A., Ibn Tofail University Kenitra
Elidrissi A., Mohammed Premier University, Oujda

Essassi E.M., Mohamed V University Rabat
Guenbour A., Mohamed V University Rabat
Hamdani M., Ibn Zohr University Agadir
Hamidi M., My Ismail University Errachidia
Hlaibi M., Hassan II University, Casablanca
Irhzo A., Hassan II University, Casablanca
Kandri Rodi Y., USMBA University Fez
Kerbal A., USMBA University Fez
Kertit S., Mohamed V University Rabat
Khouili M., My Slimane University, Béni Mellal
Lachkar M., USMBA University Fez
Lazar S., Hassan II University, Mohammedia
Majidi L., My Ismail University Errachidia
Massaoui M., Mohamed V University Rabat
Mejdoubi E.M., Mohammed Premier University, Oujda
Oudda H., Ibn Tofail University Kenitra

Radi S., Mohammed Premier University, Oujda
Raihane M., Cadi Ayyad University Marrakech
Rakib E.M., My Slimane University Béni Mellal
Ramli Y., Mohamed V University Rabat
Romane A., Cadi Ayyad University Marrakech
Saadi M., Mohamed V University Rabat
Salghi R., Ibn Zohr University Agadir
Sfaira M., USMBA University Fez
Taibi M., Mohamed V University Rabat
Taleb M., USMBA University Fez
Touir R., Mohamed V University Rabat
Touzani R., Mohammed Premier University, Oujda
Zarrouk A., Mohamed V University Rabat
Zouihri H., Ibn Tofail University Kenitra
Zgou H., Ibn Zohr University Ouarzazate

International Committee

Is open to all researchers who contributed in Chemistry

Abidi N., Texas Tech University, Lubbock, United States
Al-Warthan A., King Saud University College of Science, Riyadh, Saudi Arabia
Aouad M.R., Taibah University, Madinah, Saudi Arabia

Boukheddaden K., Université de Versailles, France
Costa J., Universita di Corsica Pascal Paoli, Corte, France

Ng S.W., The University of Nottingham Malaysia Campus, Semenyih, Malaysia

Obbade S., Laboratoire d'Electrochimie et de Physico-Chimie des Materiaux et des Interfaces, Saint Martin d'Heres, France

Oshio, Hiroki University of Tsukuba, Japan

Daran J.C., Université de Toulouse, Toulouse, France
El-Bindary A.A., Damietta University, Damietta, Egypt
Eddaoudi M., King Abdullah University of Science and Technology, Jeddah, Saudi Arabia

Fauconnier M.L., Universite de Liege, Liege, Belgium
Fouda A. S., Mansoura University, Mansoura, Egypt

Garcia Y., Université catholique de Louvain, ***Belgium***

Göksu, Süleyman Atatürk Üniversitesi, Erzurum, Turkey
Guillaumet G., Saint Francis Xavier University, Antigonish, Canada
Idrissi H., Matériaux : Ingénierie et science, Villeurbanne, France

Jama C. Université des Sciences et Technologies de Lille, Villeneuve-d'Ascq, France
Khadom A.A., University of Diyala, Baqubah, Iraq

Khaled K.F., Ain Shams University, Cairo, Egypt
Messali M., Taibah University, Madinah, Saudi Arabia

Oturan M.A., Université Paris-Est, Marne-la-Vallee, France

Özdemïr, İsmail Inönü Üniversitesi, Malatya, Turkey

Quraishi M. A., King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia
Rios A., Universidad de Castilla-La Mancha, Ciudad Real, Spain

Siaj M. Université du Québec à Montréal, Montreal, Canada

LF Tietze Universität Göttingen Germany
Visseaux M. Universite d'Artois, Arras, France
Warad I., An-Najah National University, Nablus, Palestine

Xiaodan, Zhao China Medical University Shenyang, China

Zougagh M., Universidad de Castilla-La Mancha, Ciudad Real, Spain

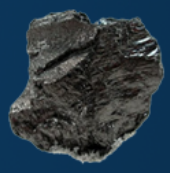
Kaya S., Cumhuriyet University, Turkey

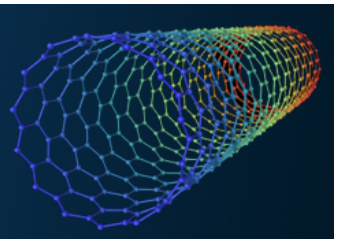
Nandiyanto A.B.D., Universitas Pendidikan Indonesia, Bandung, Indonesia

ISSN: 2351-812X

Copyright©2012-2018 IMIST
CNRST-IMIST Angle Allal Al Fassi et Avenue des FAR, Hay Ryad, BP 8027 10102 Rabat, Maroc
Tél:(+212) 05 37.56.98.00 Fax: (+212) 05 37.56.98.34
Site Web: http://www.imist.ma

Moroccan Journal of Chemistry
ISSN : 2351-812X





USER

Username
Password
Remember me
Login

JOURNAL CONTENT

Search
Search Scope
All
Search

Browse

- By Issue
- By Author
- By Title
- Other Journals
- Categories

FONT SIZE

CURRENT ISSUE

ATOM 1.0
RSS 2.0
RSS 1.0

Special Volume 8 S1 (2020) : The 1st International Conference on Science, Applied Science, (ICoSASTE) 2019

Table of Contents

Improvement of Reaction Selectivity Performance with Alkali Charge and Reaction Time on Oxygen Delignification Process — PDF
Bambang Irawan, Aria Darmawan, Achmad Roesyadi, Danawati Hari Prajitno
Mor. J. Chem. 8 S1 (2020) 001-011

Models, kinetics, and thermodynamics for the adsorption of Pb2+ and Cd2+ metal ions by solid tofu waste immobilized on silica's SURFAC — PDF
Handoko Darmokoesoemo, Eko Prasetyo Kuncoro, Ganden Supriyanto,, Yosephine Sri Wulan Manuharab
Mor. J. Chem. 8 S1 (2020) 012-023

Molarity optimization of calcium hydroxide in the forming of bioceramic hydroxyapatite from nano coral by precipitation method — PDF
Siswanto, Dyah Hikmawati, Aminatun, Adri Supardi
Mor. J. Chem. 8 S1 (2020) 024-031

Identification of phytocemical compounds in ethanol and n-hexane leaf extracts of Piper retrofractum vahl. by gas chromatography mass spectrometry — PDF
Junairiah, Sibyandhita Erhaa Amalia, Ni'matuzahroh, Tri Nurhariyati
Mor. J. Chem. 8 S1 (2020) 032-037

Synthesis of resorcinic acid and its Staphylococcus aureus antibacterial activity — PDF
I Gusti Made Ngurah Budiana, Ni Nyoman Yuliani
Mor. J. Chem. 8 S1 (2020) 038-043

Synthesis of silica coated on iron sand magnetic materials modified with 2- mercaptobenzimidazole through sol-gel — PDF
Martasiana Karbeka, Nuryono, Suyanta
Mor. J. Chem. 8 S1 (2020) 044-052

Adsorption of carbon dioxide with nanofibers based on alginate/polyethylene oxide/triton X-100 — PDF
Faryda Veronica Lamma Koly, Adhitasari Suratman, Suyanta
Mor. J. Chem. 8 S1 (2020) 053-063

Kinetic Study in the Extraction of Essential Oil from Clove (Syzgium aromaticum) Stem using Microwave Hydrodistillation — PDF
Haqqyana Haqqyana, Verycha Finish Wiya Tania, Ayu Mardinah Suyadi, Heri Septya Kusuma, Ali Altway, Mahfud Mahfud
Mor. J. Chem. 8 S1 (2020) 064-071

Development of the Analytical Method of Iron through Optimization Reducing Agent Ability of Iron (III) to Iron(II) — PDF
R. Djarot Sugiarso, Nadya Zahiroh, AnnisaRahmayanti, Herdayanto Sulistyo Putro, Ratna Ediati
Mor. J. Chem. 8 S1 (2020) 072-077

Models, Kinetics, and Thermodynamics for The Adsorption of Ni2 + Metal Ions by Solid Tofu Waste Immobilized on Silica's Surface — PDF
Handoko Darmokoesoemo, Eko Prasetyo Kuncoro, Ganden Supriyanto, Yosephine Sri Wulan Manuhara
Mor. J. Chem. 8 S1 (2020) 078-087

Biomass and terpenoids profile of callus extract of Piper betle L. var. Nigra with abiotic elicitor cobalt (II) chloride — PDF
Junairiah, Nabilah Istighfari Zuraidassanaaz, Sri Wulan Manuhara, Ni'matuzahroh, Lilis Sulistyorini
Mor. J. Chem. 8 S1 (2020) 088-098

The detection of lead ion level in distilled water using 405 nm laser light and optical probe based on the Directional coupler — PDF
Pujiyanto, Samian, Moh Yasin, Herry Trilaksana, Supadi
Mor. J. Chem. 8 S1 (2020) 099-104

Effect of hydrophilic coating for membrane assisted air diffuser for Chlorella vulgaris cultivation — PDF
A. S. Ishak, N. A. H. M. Nordin, M. R. Bilad, M. K. Lam
Mor. J. Chem. 8 S1 (2020)105-112

also developed by scimago: SCIMAGO INSTITUTIONS RANKINGS

SJR Scimago Journal & Country Rank Enter Journal Title, ISSN or Publisher Name

Home Journal Rankings Country Rankings Viz Tools Help About Us

Moroccan Journal of Chemistry

Country	Morocco - SCIMAGO INSTITUTIONS RANKINGS
Subject Area and Category	Chemistry Chemistry (miscellaneous)
Publisher	University Mohammed Premier Oujda
Publication type	Journals
ISSN	2351812X
Coverage	2018-2020
Scope	Moroccan Journal of Chemistry (Mor. J. Chem.) is a free of charge open access journal that publishes original research articles as well as review articles in all areas of chemistry. It provides a platform for rapid publication of quality research papers, reviews and chemistry letters. Moroccan Journal of Chemistry was founded in 2013 by Professor B. Hammouti, University Mohammed Premier, Oujda Morocco. Moroccan Journal of Chemistry is published by the University Mohammed Premier, Oujda Morocco in partenarship with the Association CEMADES : Centre Marocain du developpement et des Sciences (Moroccan Center od Development and Science).

3

H Index

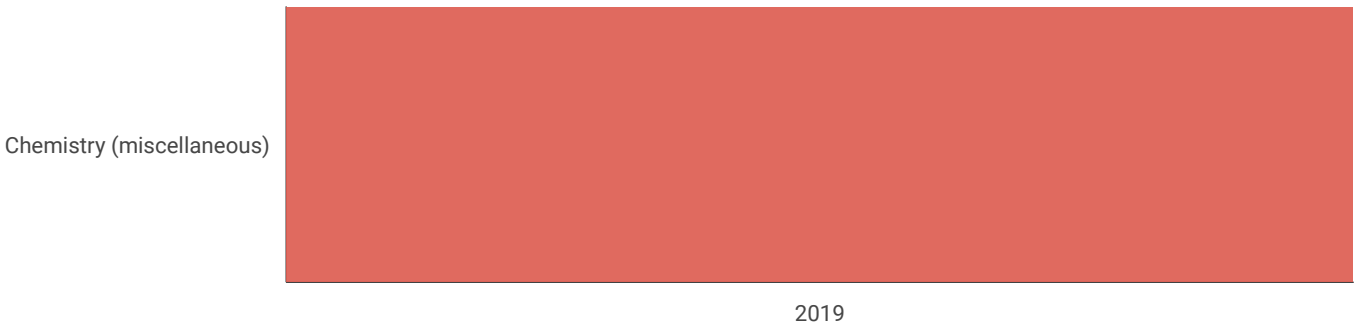
Homepage

How to publish in this journal

Contact

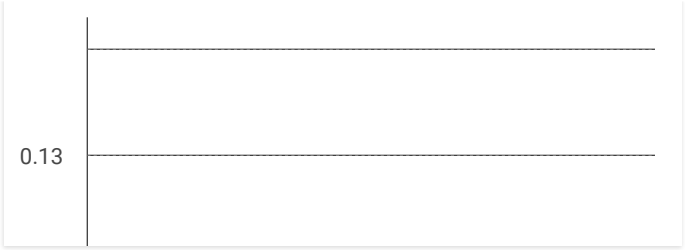
Join the conversation about this journal

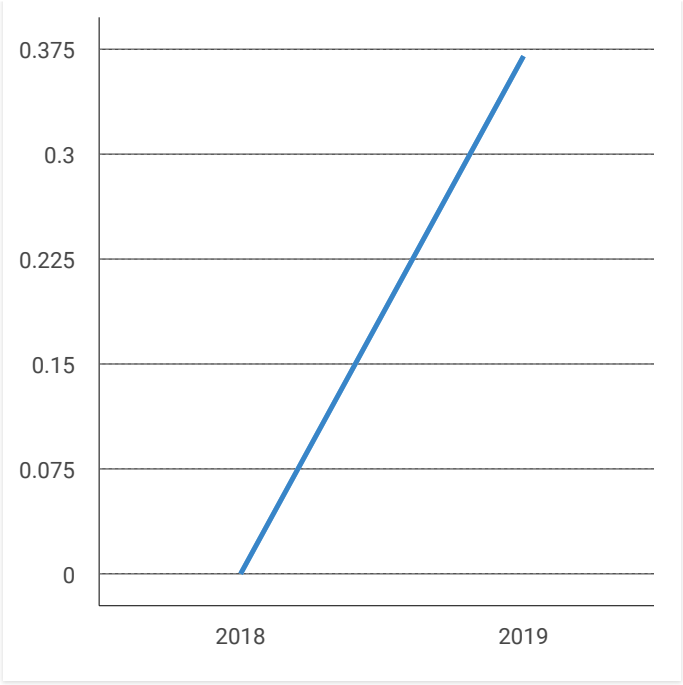
Quartiles

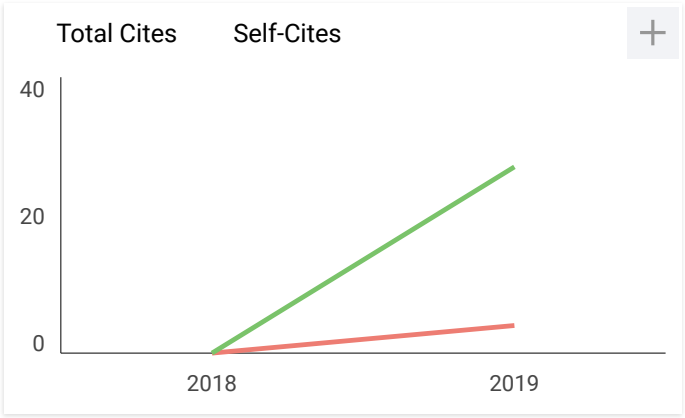


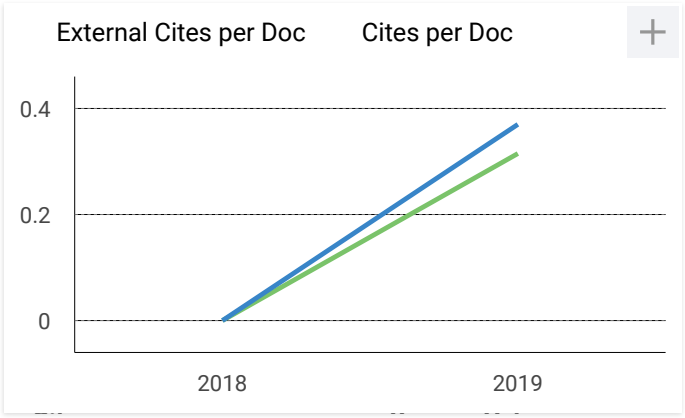
SJR

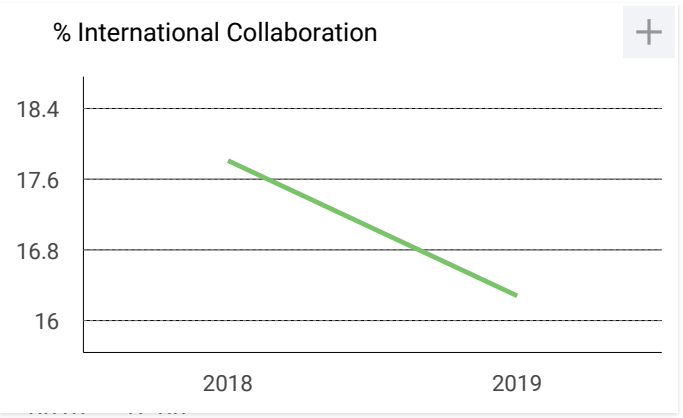
Citations per document

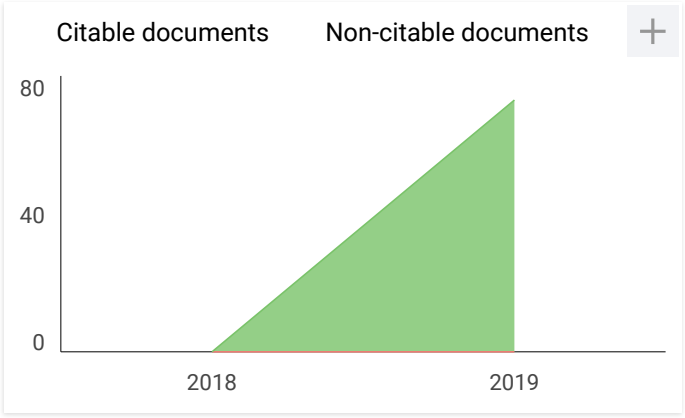


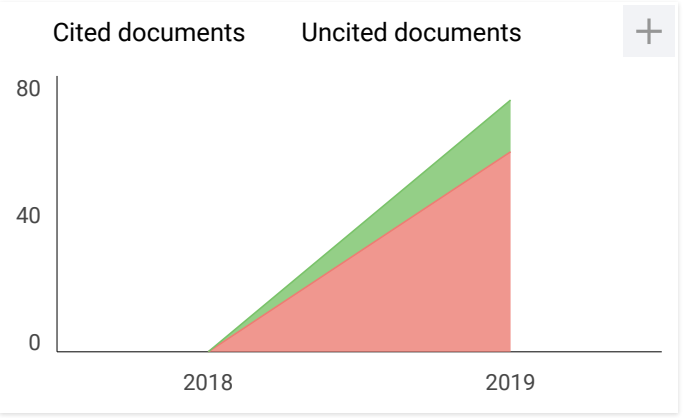












← Show this widget in your own website

Just copy the code below and paste within your html code:

<a href="https://www.scimag

Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Junairiah& al. / Mor. J. Chem. 8 S1 (2020) 088-098

Biomass and terpenoids profile of callus extract of *Piper betle* L. var. Nigra with abiotic elicitor cobalt (II) chloride

Junairiah[a], Nabilah Istighfari Zuraidassanaaz[a], Y. Sri Wulan Manuhara[a], Ni'matuzahroh[a], Lilis Sulistyorini[b]*

[a]Departement of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia, 60115
[b]Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia, 60115

** Corresponding author:*
alip.jun1@gmail.com
Received 22 Dec 2019,
Revised 02 Jan 2020,
Accepted 09 Jan 2020

Abstract

Black betel (*Piper betle* L. var. Nigra) is traditional medicinal plant potentially produce secondary metabolites. Its secondary metabolite content can be promoted using callus culture with abiotic elicitor. This study was aimed to determine the effect of cobalt (II) chloride ($CoCl_2$) concentration towards biomass and terpenoids profile of leaf callus of *Piper betle* L. var. Nigra. Leaf explants of *Piper betle* L. var. Nigra were cultured in solid Murashige and Skoog (MS) medium supplemented with growth regulators 2,4-D (0.5 mg/L) and BAP (2.0 mg/L) to induce callus formation. Elicitation was performed after callus reached five weeks of age by performing subculture on MS medium added with $CoCl_2$ (0.5 mg/L; 1.0 mg/L; 2.5 mg/L). Calli of *Piper betle* L. var. Nigra were harvested after one, two, or three weeks before they were extracted using methanol and analyzed of its terpenoids content using Gas Chromatography-Mass Spectrometry (GC-MS) method. Results showed that calli had compact and crumb texture with green, white, brown, and white color. Callus added 1.0 mg/L $CoCl_2$ at two weeks age produce terpenoids at 5.95%. Callus with 1.0 mg/L $CoCl_2$ at three weeks of age produced higher fresh and dry weight at 916.4±162.3 mg and 93.3±11.7 mg respectively. Callus supplemented with 1.0 mg/L $CoCl_2$ at two weeks of age had higher terpenoids percentage compared to control. Two of the highest terpenoid types found in the callus of black betel leaf explant were 1,2-epoxy-1-vinylcyclododecene and hexadecanoic acid.

Keywords: *biomass; $CoCl_2$; Piper betle L. var. Nigra callus; terpenoids profile*

1. Introduction

One of the plants which have potential as secondary metabolites-producing medicinal plant is black betel (*Piper betle* L. var. Nigra). Secondary metabolites content of black betel including alkaloids, flavonoids, saponins, tannins,

steroids, triterpenoids, and polyphenolates [1]. Terpenoids are group of secondary metabolites with the highest number of compound types (±25,000 types) compared to alkaloids (±12,000 types) and phenols (±8,000 types). Due to its high number of types, terpenoids have high potential for utilization in pharmacology and industry [2–5]. Terpenoids are useful for fragrance and coloring additives, insecticides in farming, and medicine industry [6]. Several studies stated that terpenoids were able to combat various diseases, such as cancer, malaria, and HIV [7–9]. Essential oil in betel leaves had antibacterial activity against *Streptococcus mutans*, *Streptococcus sanguis*, and *Actinomyces viscosus* which caused periodontal diseases, and *Streptococcus viridans* and *Staphylococcus aureus* which caused respiratory tract disease in human [10]. In addition, ethanol extract of black betel leaves could also inhibit the growth of *Candida albicans* [11]. Based on these, more intensive effort is needed using the right alternative technique to fulfill demand of plant raw materials to produce secondary metabolites, one of them via callus culture [12–15]

Callus culture is one of the initial effective biotechnology methods that can suppress plant exploitation in its original habitat, produce biomass in large scale, and accumulate secondary metabolites component [16,17]. Resulting secondary metabolites from optimum callus culture can be increased using elicitation strategy, which is based on natural phenomenon of host cells defense mechanism against pathogen by producing secondary metabolites [18–21]. The quality and type of secondary metabolite compounds is affected by environmental, physical, pathogenic, and insect irritant factors entering plant cells which affecting biosynthesis of plant secondary metabolites [22]. Secondary metabolites commonly produce in plant cells as a response from various biotic and abiotic environmental stresses, known as elicitor [23–25]. Elicitor is a signal inducing stress response in defense mechanism of plant cells [26]. Plants will respond by inducing certain changes that cause stress and trigger a series of morphological, physiological, biochemical, and molecular alteration in plants [27].

Studies using abiotic elicitors had been conducted extensively, such as in leaf callus of *Cordia myxa* L. which showed increase of robinin content when 1 mg/L $CoCl_2$ was added into solid medium [28]. In-vitro propagation of *Phoenix dactylifera* L. cv. Ashgar with $CoCl_2$ addition at 0.5 µM could increase phenols contained in the callus [29].

Abiotic elicitor used in the current study was $CoCl_2$ (Cobalt (II) Chloride), one of essential micronutrients that can activate ATPase enzyme using Co^{2+} ions during secondary metabolites production. The addition of $CoCl_2$ in high level also caused plant cells to produce secondary metabolites as response of defense mechanism against its toxicity [28,30]. Abiotic elicitor was added to MS medium separately to compare the effect of elicitor concentration in producing biomass and terpenoids profile of black betel leaf callus. Related study on the effect of different $CoCl_2$ elicitor concentrations on biomass production and terpenoids profile of black betel leaf callus has never been conducted previously. This study was aimed to determine the effect of $CoCl_2$ elicitor on biomass and terpenoids profile of black betel leaf callus.

2. Methods

2.1. Materials

Materials used in this study were black betel leaves (*Piper betle* L. var. Nigra) obtained from Kayon Flower Market, Surabaya, Murashige and Skoog medium, chlorox, 70% alcohol, 2,4-Diclorophenoxyacetic acid (2,4-D), Benzylaminopurine (BAP), $CoCl_2$, distilled water, methanol, filter paper, aluminum foil, liquid detergent, spiritus, pH indicator strips, 1 N KOH, 1 N HCl.

2.2. Black betel callus induction

1. Explants preparation

Explants used were meristematic black betel (*Piper betle* L. var. Nigra) leaves, taken from third and fourth leaves from tip of plants.

2. Preparation of MS medium added with growth regulators

About 1 L MS medium was prepared with macronutrient stock (10 mL of macro I, macro II, and macro III respectively), 1 mL micronutrient stock, 5 mL iron, 4 mL vitamin, 0.5 mg/L 2,4-D and 2 mg/L BAP growth regulators which were homogenized in 500 mL distilled water one by one. Then, myoinositol and sucrose were added into mixture before distilled water was added until total volume of 1 L and mixture was properly homogenized. Acidity of solution was recorded using pH indicator and adjusted accordingly until pH was 5.5-5.6. Agar powder was added to solidify solution, then medium was placed into 1000 mL Erlenmeyer glass, covered with aluminum foil, and labelled accordingly. Medium was sterilized using autoclave at pressure of 1.2 atm and temperature of 121^{o}C for 15 minutes (Manuhara, 2014).

3. Black betel explants planting

Black betel leaves were washed with liquid detergent for five minutes and rinsed three times using tap water, before being submerged in 70% alcohol for 6 minutes. Explants were rewashed three times using sterile distilled water, then soaked in 20% chlorox for 10 minutes. Leaves were then cut into ±1 cm^2 pieces and planted on MS medium, then cultured for five weeks in temperature of 25±2^{o}C, lighting of 3000-3500 lux for 24 hours.

2.3. Elicitation using $CoCl_2$

1. Preparation of $CoCl_2$ stock solution

Stock solution of $CoCl_2$ was prepared by dissolving 0.1 g $CoCl_2$ into 50 mL distilled water, then it was homogenized and added distilled water up to final volume of 100 mL. Stock was stored in refrigerator. Preparation of MS medium with addition of $CoCl_2$ at various concentration

$CoCl_2$ was added into 1 L MS medium at various concentration of 0.5 mg/L, 1.0 mg/L, and 2.5 mg/L respectively (±20 mL/bottle). Bottle mouth was covered with aluminum foil and labelled accordingly. Medium filled into bottle was sterilized using autoclave for 15 minutes at 121^{o}C and 1.2 atm, then stored in sterile room. Abiotic and biotic elicitors used in the current study were applied into medium separately.

2. Elicitation of black betel leaf callus

Callus from black betel leaf was moved onto MS medium contained 0.5 g $CoCl_2$ at five weeks of age. Callus was harvested after one, two, or three weeks culture period (Ravi *et al.*, 2011).

3. Black betel callus harvest

Harvested callus was weighed its fresh weight, dried in oven at 60^{o}C for 72 hours, then weighted its constant dry weight using analytical balance. Dried callus was stored and labelled accordingly for then to be extracted.

2.4. Analysis of terpenoids content profile

1. Terpenoids extraction

Dried callus was weighed at about 0.5 g, then crushed into powder using mortar. Powder was macerated by adding 5 mL methanol. Extraction was performed on water heater at 60^{0}C for 5 minutes, then solution was filtered using filter paper. Extract was concentrated until volume left was 2 mL. Extraction was also performed on dried black betel leaves with the same method as callus extraction to compare compounds contained in mother plant and callus.

2. Analysis of terpenoids content profile

About 1 µL sample from resulting callus extraction of each treatment was taken and injected into column type HP-5 MS (60 m x 200 µm x 0.33 µm) to be analyzed its content compounds and areal percentage (%) of terpenoids content using GC-MS Agilent Technologies 7890A and Willy Mass Spectral Data Base. Oven temperature was set at 40^0C for 1 minute, then raised to 160^0C for 1 minute, and increased for second time to 270^0C for 5 minutes. Detector temperature was set at 300^0C and injector at 230^0C. Running time of each test extract was ±41 minutes [31].

Result of GC-MS chromatogram showed peaks indicating generally identified compounds. Compounds then categorized into terpenoids group by comparing compound name and molecular formula to secondary data from *Terpenoid Library List* based on its chemical structure and *U.S National library of medicine*).

3. Results and Discussion

Black betel callus given abiotic elicitor $CoCl_2$ at 0.5 mg/L, 1.0 mg/L and 2.5 mg/L concentration showed varying fresh and dry weight. Analysis indicated significant difference of mean fresh weight between control callus (1066.4±45.3 mg) and the three $CoCl_2$ treatment with recorded fresh weight of 916.4±162.3 mg (1.0 mg/L $CoCl_2$), 772.7±104.6 mg (0,5 mg/L $CoCl_2$), and 804.4±150.8 mg (2.5 mg/L $CoCl_2$) respectively, at the same age of three weeks. In the other hand, mean dry weight of callus at three weeks of age showed no difference between control (112.0±20.7 mg) and 1.0 mg/L $CoCl_2$ (93.3±11.7 mg), but significant difference was found in 0.5 mg/L $CoCl_2$ (77.4±7.7 mg) and 2.5 mg/L $CoCl_2$ (84.6±17.3 mg) (Table 1).

All callus given elicitor $CoCl_2$ showed lower fresh and dry weight compared to control at three weeks of age. Control callus after being subcultured had the highest fresh and dry weight with significant difference compared to $CoCl_2$ callus, at mean of 1066.4±45.3 mg and 112.0±20.7 mg, respectively. Compared to biotic elicitor (*S. cerevisiae* extract), callus given 0.5, 1.0 and 2.5 mg/L $CoCl_2$ also showed lower fresh and dry weight. Callus grown on MS medium added with 1.0 mg/L $CoCl_2$ at three weeks of age had higher fresh (916.4±162.3 mg) and dry weight (93.3±11.7 mg) compared to other $CoCl_2$ concentration.

This is in line with previous study, which found that fresh and dry weight of *Cordia myxa* leaf callus were relatively lower than of control [28]. This was due to different response of each plant even though they were given the same concentration. Taha (2017) also explained that the addition of heavy metals to culture medium caused reduce of callus weight, due to excessive toxicity towards plant cells, thus heavy metals was only applied at low concentration [28].

Table 1: Fresh and dry weight of black betel leaf callus given various $CoCl_2$ concentration

Treatment	Concentration	Callus age (weeks)	Fresh weight (mg)	Dry weight (mg)
Control		1	470.9±19.9^{f}	51.7±2.5^{efg}
		2	674.6±94.9^{d}	69.5±21.5^{cde}
		3	1066.4±45.3^{a}	112.0±20.7^{a}
CoCl2	0.5 mg/L	1	488.3±86.9^{ef}	60.3±16.9^{def}
		2	459.7±69.5^{f}	49.4±4.2^{fg}
		3	772.7±104.6^{c}	77.4±7.7^{c}
	1.0 mg/L	1	480.2±53.6^{f}	45.1±4.0^{h}
		2	548.8±49.7^{e}	60.0±9.6^{e}
		3	916.4±162.3^{b}	93.3±11.7^{ab}

2.5 mg/L	1	460.4±13.6^{f}	45.8±11.3^{gh}
	2	734.2±82.5b^{cd}	73.7±6.8^{cd}
	3	804.4±150.8^{c}	84.6±17.3^{bc}

**) Different letters indicate significant difference based on result of Mann-Whitney test ($\alpha = 0,05$).*

The same case was also found in *Catharanthus roseus* callus with heavy metals $HgCl_2$ added into its medium; callus weight was lowered [32]. However, *T. paniculatum* callus given 5 mg/L $CuSO_4$ had increase of weight compared to control instead, because the callus was still tolerant towards heavy metals due to specific mechanism developed by plant cells [33].

Callus grown during current study showed compact (1.0 mg/L $CoCl_2$ at three weeks of age) and crumb (0.5 mg/L $CoCl_2$ at two weeks of age) texture with various color, such as green, white, brown, and black. Generally, callus given $CoCl_2$ had green color. Callus colored green indicated that callus cells contained high amount of chlorophyll, while white color indicated starch content in the cells with chlorophyll had yet to be developed [34].

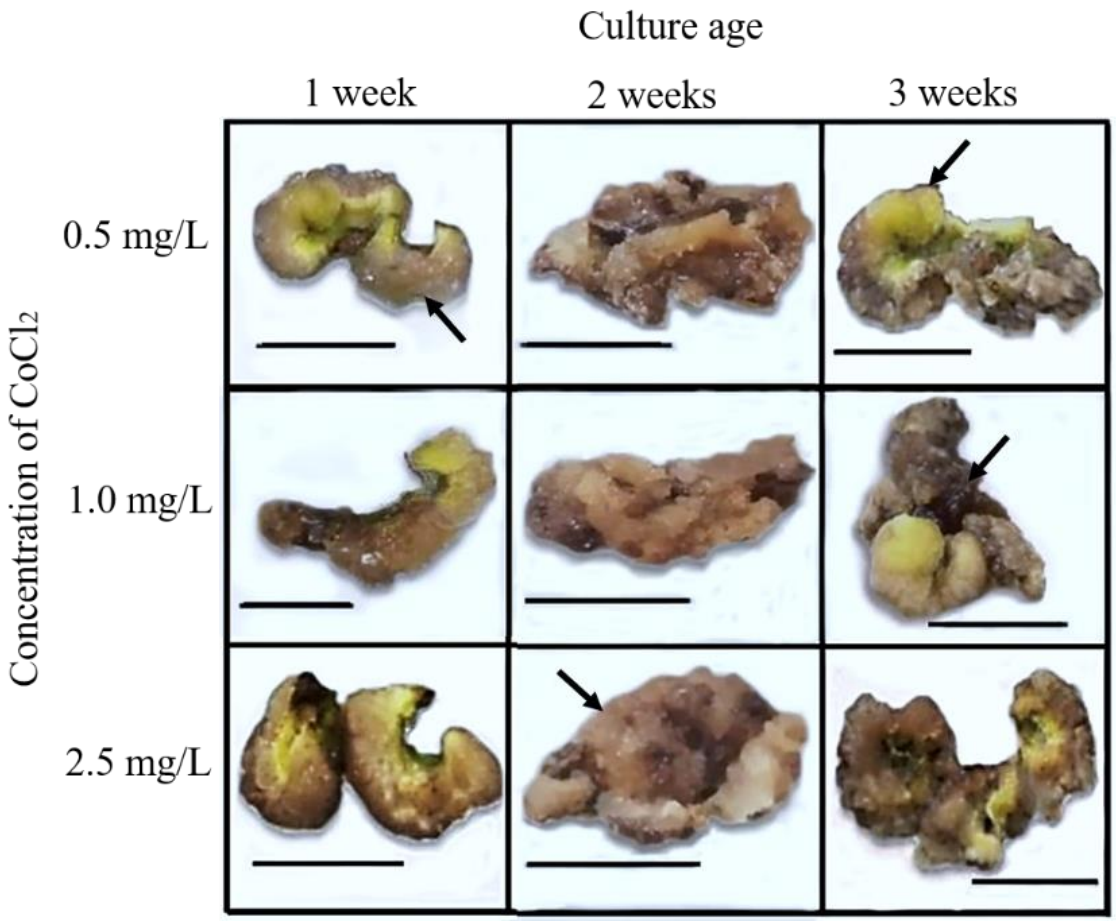


Figure 1: Morphological difference of texture and color found in black betel callus at various $CoCl_2$ level. Arrow indicates difference of callus color. Scale bar = 1 cm

Methanol extract of black betel leaves contained higher level of terpenoids (20.59%) compared to callus given $CoCl_2$ at various level. This was due to callus harvested at three weeks of age; thus, it was still in exponential phase (growth stage), causing terpenoids level of callus given $CoCl_2$ far lower compared to leaf of mother plant. Several calli from control at one week, 1.0 mg/L $CoCl_2$ at one week, and 2.5 mg/L $CoCl_2$ at one and two weeks of age were not

identified of their terpenoids content, but contained other compounds instead such as alkaloids, flavonoids, saponins, and various fatty acids.

Extract of callus given 1.0 mg/L $CoCl_2$ at two weeks of age showed the highest areal percentage compared to other treatment, at 5.95% (Table 2). Selection of abiotic elicitor was thought to be one of the effective strategies to raise secondary metabolites production in in-vivo culture (Al-Khayri and Naik, 2016). Increase of abiotic elicitor concentration in MS medium was able to promote secondary metabolites biosynthesis. However, no further information present on elicitation mechanism of abiotic factor in enhancing secondary metabolites content.

The improvement of secondary metabolites biosynthesis is affected by several factors, for example determination of explant type and age, culture photoperiod, callus subculture time, elicitor type and concentration, and precursor (Bosila *et al.*, 2003; Karuppusamy, 2009). Based on that, it was hypothesized that unidentified terpenoids content of calli at several $CoCl_2$ concentration was caused by $CoCl_2$ level added into MS medium at level too low to induce terpenoids formation within black betel callus, in addition of early harvest after subculture. These affected terpenoids content, as secondary metabolites generally started to be synthesized at the end of growth phase. In the other hand, study previously performed showed that 1.0 mg/L $CoCl_2$ was able to induce higher robinin content in *Cordia myxa* leaf callus (Taha, 2017). Other study of callus culture at *Phoenix dactylifera* L. cv. Ashgar found that addition of 0.5 µM $CoCl_2$ could also promote higher phenols content (Al-Mayahi, 2014).

Table 2: Terpenoids profile of methanol extract of black betel leaf callus with various $CoCl_2$ level in solid MS medium

Group	Concentration (mg/L)	Culture age (weeks)	Number of Terpenoids type	Terpenoids type	Area of terpenoids (%)	Total (%)
Leaf of black betel mother plant			12	Caryophyllene	0.19	20.59
				β-bisabolene	0.28	
				1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-	0.28	
				Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1r-(1α,2α,3β,6α)]-	0.23	
				4-[(1E)-1,5-Dimethyl-1,4-hexadienyl]-1-methyl-1-cyclohexene	0.25	
				1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	2.32	
				1,2-Epoxy-1-vinylcyclododecene	0.10	
				Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)	0.32	
				α-Cadinol	0.29	
				2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R,R-(E)]]-phytol	0.54	
				Neophytadiene	15.34	
				Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	0.45	
Control	0	2	2	β-bisabolene	2.26	4.99
				(E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene) cyclohex-1-ene	2.73	
		3	9	Bicylo[7.2.0]undec-4-ene. 4. 11.11-trimethyl-8-methylene-. (E)-(1R. 9S)-(-)-	0.65	5.81
				β-bisabolene	0.71	
				Cyclopentadecanone, 2-hydroxy-	0.14	
				D-Campholic acid	0.33	
				(E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene) cyclohex-1-ene	1.02	
				trans-Geranylgeraniol	0.26	

Group	Concentration (mg/L)	Culture age (weeks)	Number of Terpenoids type	Terpenoids type	Area of terpenoids (%)	Total (%)
				Octadecanoic acid	0.06	
				Myristic acid	0.15	
				Ethyl (9Z,12Z)-9, 12-octadecadienoa	2.49	
$CoCl_2$	0.5	1	7	Longifolene	0.31	5.94
				Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1r-(1α,2α,3β,6α)]-	0.26	
				trans-γ-bisabolene	0.36	
				(1R,2S,4R,6S)-4-Isopropenyl-1-methylbicyclo[4.1.0]heptan-2-ol	0.32	
				Cyclopentadecanone, 2-hydroxy-	0.83	
				Octadecanoic acid	1.66	
				Squalene	2.20	
		2	3	Butanal, 3-methyl-	0.16	4.60
				[1,1`-Bicyclopentyl]-2-one	0.10	
				1,2-Epoxy-1-vinylcyclododecene	4.34	
		3	3	Butanal, 3-methyl-	0.20	3.39
				2-Pinen-4-one	0.14	
				Cyclopentadecanone, 2-hydroxy-	3.05	
$CoCl_2$	1.0	2	7	Copaene	0.23	5.95
				Bicylo[7.2.0]undec-4-ene, 4, 11,11-trimethyl-8-methylene-, (E)-(1R, 9S)-(-)-	1.17	
				Longifolene	0.79	
				trans-γ-bisabolene	0.59	
				Spathulenol	0.19	

Group	Concentration (mg/L)	Culture age (weeks)	Number of Terpenoids type	Terpenoids type	Area of terpenoids (%)	Total (%)
				Cyclopentadecanone, 2-hydroxy-	0.31	
				Hexadecanoic acid	2.67	
		3	6	Butanal, 3-methyl-	0.44	2.51
				β-bisabolene	0.29	
				trans-γ-bisabolene	0.34	
				2,6,10-Dodecatrienal,3,7,11-trimethyl-	0.09	
				1,2-Epoxy-1-vinylcyclododecene	1.34	
				(-)-5-Oxatricyclo [8.2.0.0(4,6)]dodecane,12-trimethyl-9-methylene-,	0.01	
	2.5	3	4	Butanal, 3-methyl-	0.20	2.91
				1,2-Epoxy-1-vinylcyclododecene	2.12	
				Cyclopentadecanone, 2-hydroxy-	0.15	
				Octadecanoic acid	0.44	

4. Conclusion

The difference in $CoCl_2$ concentration affects the biomass and callus terpenoid profile of *Piper betle* L. var leaves. Nigra. Treatment of 1.0 mg/L $CoCl_2$ concentration in two weeks old culture was able to produce higher types of terpenoid compounds but callus biomass of *Piper betle* leaf L. var. Nigra produced less than control. In the callus culture of *Piper betle* L. var leaves. Nigra produces the two largest types of compounds namely Hexadecanoic acid and 1,2-Epoxy-1-vinylcyclododecene. Higher percentages of total types of terpenoid compounds were found at concentrations of 1.0 mg/L $CoCl_2$.

References

[1] A.J. Rija'i, Telaah Fitokimia Kandungan Metabolit Sekunder dalam Ekstrak Daun Sirih Hitam (Piper betle L.) dan Uji Bioaktivitasnya Terhadap Larva Udang (Artemia salina Leach), Bandung Univ. Islam Bandung. (2015).

[2] R. Croteau, T.M. Kutchan, N.G. Lewis, Biochem. Mol. Biol. Plants. 24 (2000) 1250–1319.

[3] S. Zwenger, C. Basu, Biotechnol. Mol. Biol. Rev. 3 (2008) 1.

[4] D. Tholl, Springer, (2015) 63–106.

[5] V. V Bhargava, S.C. Patel, K.S. Int. J. Herb. Med. 1 (2013) 14–21.

[6] S.C. Roberts, Nat. Chem. Biol. 3 (2007) 387.

[7] G.M. Cragg, D.J. Newman, Ann. Appl. Biol. 143 (2003) 127–133.

[8] G.M. Cragg, D.J. Newman, J. Ethnopharmacol. 100 (2005) 72–79.

[9] V. Srivastava, A.S. Negi, J.K. Kumar, M.M. Gupta, S.P.S. Khanuja, Bioorg. Med. Chem. 13 (2005) 5892–5908.

[10] A. Hermawan, W. Hana, T. Wiwiek, Pengaruh ekstrak daun sirih (Piper betle) terhadap pertumbuhan Staphylococcus aureus dan E. Coli dengan Metode Difusi Disk, (2007).

[11] Y.P.I. Ummah, Uji Daya Antifungal Ekstrak Etanol Daun Sirih Hitam (Piper betle var Nigra) dan Daun Sirih Hijau (Piper betle L.) terhadap Penghambatan Pertumbuhan Candida albicans Secara In Vitro., SKRIPSI Jur. Biol. MIPA UM. (2014).

[12] M.E. Kolewe, V. Gaurav, S.C. Roberts, Mol. Pharm. 5 (2008) 243–256.

[13] B. Janarthanam, M. Gopalakrishnan, T. Sekar, Bangladesh J. Sci. Ind. Res. 45 (2010) 243–248.

[14] S. Rao, K. Usha, Arjun, Ann. Phytomedicine-AN Int. J. 4 (2015) 74–78.

[15] M.K. Tripathi, N. Mishra, S. Tiwari, C. Shyam, S. Singh, A. Ahuja, Int. J. Curr. Microbiol. Appl. Sci. 8 (2019) 1002–1010.

[16] M. Sharma, A. Sharma, A. Kumar, S.K. Basu, Am J Plant Physiol. 6 (2011) 50–71.

[17] S.A. Khalil, N. Ahmad, R. Zamir, Plant Sci. 1 (2015) 1–5.

[18] A. Vasconsuelo, R. Boland, Plant Sci. 172 (2007) 861–875.

[19] P.M. Naik, J.M. Al-Khayri, J Adv Res Biotech. 1 (2016) 7.

[20] E.A. Hussein, M.S. Aref, M.M. Ramadan, Int. J. Innov. Sci. Eng. Technol. 4 (2016) 238–247.

[21] R. Javed, B. Yucesan, M. Zia, E. Gurel, Sugar Tech. 20 (2018) 194–201.

[22] S.J. Murch, P.K. Saxena, Can. J. Plant Sci. 86 (2006) 765–771.

[23] R. Akula, G.A. Ravishankar, Behav. 6 (2011) 1720–1731.

[24] T.S. Anusha, M. V Joseph, K.K. Elyas, Pharmacogn. J. 8 (2016).

[25] M.L. Krishnan, A. Roy, N. Bharadvaja, J. Appl. Pharm. Sci. 9 (2019) 67–74.

[26] P.J. Weathers, M.J. Towler, J. Xu, Appl. Microbiol. Biotechnol. 85 (2010) 1339–1351.
[27] P. Ahmad, A. Kumar, A. Gupta, X. Hu, M.M. Azooz, S. Sharma, Agric. Improv., Springer, 2012: pp. 491–512.
[28] A. Taha, IOSR J. Pharm. Biol. Sci. 6 (2016) 2250–3013.
[29] A.M.W. Al-Mayahi, Am. J. Agric. Biol. Sci. 9 (2014) 6.
[30] D.J. Pilbeam, A. V Barker, Handbook of plant nutrition, Florida, US: CRC Press, 2007.
[31] S. Abdel-Shafi, F.M. Reda, M. Ismail, Adv Tech Clin Microbiol. 1 (2017) 18.
[32] M.M. Fathalla, A.M. Abd-El-Kawy, H.S. Taha, Appl Sci Res. 7 (2011) 542–549.
[33] A.M. Santoso, Pengaruh Elisitor Ekstrak Saccharomyces cerevisiae dan CuSO4 terhadap Biomassa, Profil Protein, dan Kadar Saponin Kalus Talinum paniculatum (Jacq) Gaertn, (2012).
[34] S.N. Ariati, W. Waeniati, M. Muslimin, I.N. Suwastika, Nat. Sci. J. Sci. Technol. 1 (2012).