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(ISSN: 1811-9506)
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Bioscience Research

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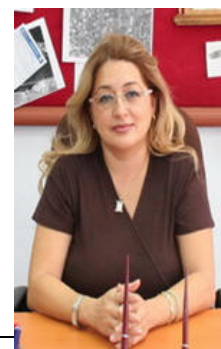
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Bioscience Research, volume 14, issue 4 (Oct.-Dec.), 2017

Sr. #	Titles, Authors & affiliation (s)	Down
<i>Research Articles</i>		
1	<p>RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4): 713-720. OPEN ACCESS</p> <p>Biodegradation of phenol by <i>Pseudomonas aeruginosa</i> isolated from soil contaminated with diesel fuel</p> <p>Kais Kassim Ghaima*, Basim Shamkhi Rahal , Mahir Mahmoud Mohamed Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq</p>	Free Fu [PD]
2	<p>RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4):721-730. OPEN ACCESS</p> <p>Efficacy of some essential oils as seed dressing against faba bean root rot incidence under field conditions</p> <p>Nehal Samy El-Mougy, Mokhtar Mohamed Abdel-Kader and Mahfouz Mohamed Mostafa Abd-Elgwad Plant Pathology Dept., National Research Centre, Dokki, 12622, Giza, Egypt</p>	Free Fu [PD]
3	<p>RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4): 731-740. OPEN ACCESS</p> <p>Molecular mechanism of synthesized chalcone as an anticancer agent in Leukemia Cell Line HL60</p> <p>Arina Novilla^{1*}, Mustofa Mustofa², Indwiani Astuti², Jumina Jumina³, Hery Suwito⁴ ¹Doctoral Program of Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia ²Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia ³Faculty of Chemistry, Gadjah Mada University, Yogyakarta, Indonesia ⁴Department of Chemistry, Faculty of Mathematics and Natural Science, Airlangga University, Surabaya, Indonesia.</p>	Free Fu [PD]
4	<p>RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4): 741-749. OPEN ACCESS</p> <p>Quality traits of some entomopathogenic nematodes and their energy reserves</p> <p>Hala Mohamed Sayed Metwally, Amany Ramadan Ebeid, Mohamed Ahmed Gesraha and Wafaa Lotfy Abdou Pests and Plant Protection Department, National Research Centre, Giza, Egypt</p>	Free Fu [PD]
5	<p>RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(4): 750-755. OPEN ACCESS</p> <p>Antifungal and antibacterial activity of black betel (<i>Piper betle</i> L. var <i>Nigra</i>) extract</p> <p>Junairiah^{1*}, Ni' matuzahroh¹, Nabilah Istighfari Zuraidassanaaz¹ and lilis Sulistryorini²</p>	Free Fu [PD]

¹Department of Biology, Faculty of Science and Technology, University of Airlangga, St. Mulyorejo, Sukolilo, Mulyorejo, Surabaya 60115, East Java, **Indonesia**.

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- 6 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 756-767. **OPEN ACCESS** **Free Fu**
Late nitrogen application and late season irrigation increased **PD**
bulb yield but negatively affected quality and storability of onion.
Abdalla A. Ghoname; Gamal S. Riad*; Abd-Elmohsin M. El-Bassiony; Zakaria F. Fawzy and Maged
A. El-Nemr
Vegetable Research Department, Agricultural and Biological Research Division, National Research Centre, Cairo, **Egypt**.
- 7 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 768-775. **OPEN ACCESS** **Free Fu**
Ascorbic acid, carotenoid contents and antioxidant properties of **PD**
Australian summer carrot with different irrigation amounts on a
free-draining, sandy soil
Daniel Peter Mantilen Ludong¹, Song Ai Nio², Peter O'Malley³, Zora Singh⁴ and Mark Gibberd⁵
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⁵School of Science, Faculty of Science and Engineering, Curtin University of Technology, **Australia**.
- 8 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 776-787. **OPEN ACCESS** **Free Fu**
Interleukins and VEGF secretome of human wharton's Jelly **PD**
mesenchymal stem cells-conditioned medium (hwjmscs-CM) in
different passages and oxygen tensions
Wahyu Widowati^{1*}, Halida Widyastuti², Harry Murti², Dian Ratih Laksmiawati³, Hanna Sari W
Kusuma⁴, Rizal Rizal⁴, Ervi Afifah⁴, Sutiman B. Sumitro⁵, M. Aris Widodo⁶, Indra Bachtiar²
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- 9 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 788-800. **OPEN ACCESS** **Free Fu**
Solvents extraction efficiency for lycopene and β -carotene of **PD**
GAC fruit (*Momordica cochinchinensis*, Spreng) cultivated in
Iraq
Shurook Mohammad Kadhim Saadedin¹, Iqbal Harbi Mohammed Al-Zaidi^{1, 2} and
Salwa Jaber Abdullah AL-Awadi³
¹Institute of Genetic Engineering and Biotechnology, University of Baghdad, Baghdad, **Iraq**
²Ministry of Agriculture office –Horticulture, Baghdad, **Iraq**
³College of Applied Biotechnology – Al Nahrain University, Baghdad, **Iraq**
- 10 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 801-808. **OPEN ACCESS** **Free Fu**
Response of sunflower cultivars to partial replacement of **PD**
recommended nitrogen fertilizer by organic and biofertilizers
Hassanein, M.S.*, Nabila M. Zaki and Amal G. Ahmed
Field Crops Research Department, National Research Centre, 33 El-Bohouth St., (former El- Tahrir St.) Dokki, Giza, **Egypt**.
- 11 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 809-816. **OPEN ACCESS** **Free Fu**
Expression analysis of AtTPS1 gene coding for trehalose-6- **PD**
phosphate synthase in salt stressed olive (*Olea europaea* L.)
Eglal. M. Said
Department of Fruit and Ornamental Plants Breeding, Horticulture Research Institute, Agricultural Research Center, **Egypt**.
- 12 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 817-830. **OPEN ACCESS** **Free Fu**
Phenolic profile and antimicrobial activity of green synthesized **PD**
***Acalypha wilkesiana* seed's silver nanoparticles against some food**
borne pathogens
Diaa A. Marrez¹, Mohamed A. El Raey², Ali M. El-Hagrassi², Mohamed M. Seif¹
Tamer I.M. Ragab³, Sabry I. El Negoumy², Mahmoud Emam²
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- 13 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4):831-838. **OPEN ACCESS** **Free Fu**
Molecular analysis of somaclonal variations in chili pepper **PD**
(*Capsicum annuum* L).
Zahra Noori. Al Hattab¹, Ekhlas AbdulKareem El Kaaby¹ and Saadon Abdulhadi Al-Ajeel²
¹Department of Genetic Engineering, Biotechnology Center, Ministry of Science and Technology, Baghdad, **Iraq**

- 14 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 839-844. **OPEN ACCESS**
Spermatogenic cell count of mice (*Mus musculus*) after the treatment with polysaccharide-K from *Coriolus versicolor* extract Free Fu [PD]
Sandhy Kartikasari and Sri Puji Astuti Wahyuningsih*
Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia.
- 15 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 845-851. **OPEN ACCESS**
Resistance patterns associated with bacterial pathogens causing omphalitis in baby chicks Free Fu [PD]
Zeinab A Saad¹, Soad A. Nasef¹, Mahmoud Elhariri^{2*}, Rehab Elhelw², Nashwa Ezzeldeen³
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²Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
³Microbiology Departments, Faculty of Veterinary Medicine, Cairo University, Taif University, Kingdom of Saudi Arabia
- 16 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 852-859. **OPEN ACCESS**
Prevalence of *Hafnia alvei* (*H. alvei*) in hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) patients and food samples in Iraq Free Fu [PD]
Sahar H. Ali¹ Kamil M. AL-Jobori¹ Muna T. AL-Musawi² Hayder S. Kareem³
¹ Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.
² College of Science for Women , University of Baghdad, Iraq.
³ Central Public Health Laboratory, Ministry of Health, Baghdad, Iraq.
- 17 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 860-868. **OPEN ACCESS**
Influence of juvenile hormone analogue on respiratory metabolism during non-diapausing *Sesamia cretica* wandering Larvae (Lepidoptera :Noctuidae) Free Fu [PD]
Elham Ahmed Abdel-Hakim
Department of Pests and Plant Protection, National Research Centre, Dokki, Cairo, Egypt.
- 18 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 869-873. **OPEN ACCESS**
Influence of Silver Nitrate on the production of Astragalosides in hairy root cultures of *Astragalus membranaceus* Free Fu [PD]
Yun Ji Park¹ Jae Kwang Kim^{2*}, and Sang Un Park^{1*}
¹Department of Crop Science, Chungnam National University, 99 Daehak-Ro, Yuseong-Gu,Daejeon 305-764, Republic of Korea.
²Division of Life Sciences and Convergence Research Center for Insect Vectors, Incheon National University, Incheon 22012, Korea
- 19 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 874-878. **OPEN ACCESS**
Insecticidal effects of essential oils of *Mentha* against *Tetranychus urticae* Free Fu [PD]
Sally farouk. Allam¹, basem abdel-nasser, soudy², and ahmed .salah .hassan ¹
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²Applied Center of Entomonematodes, Faculty of Agriculture, Cairo University, Giza, Egypt
- 20 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 879-886. **OPEN ACCESS**
Population dynamics of *Tetranychid* mite and its predator on watermelon and muskmelon and effect of mite feeding on the phytochemical components of the host plants Free Fu [PD]
Badawi Abou-Awad, Sahar Ibrahim Afia and El-Sayed El-Saiedy
Pests and Plant Protection department, National Research Centre, 33 El Bohouth St., Dokki, Giza, 2, Egypt.
- 21 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 887-894. **OPEN ACCESS**
Screening of genetic diversity in pomegranate (*Punica granatum* L.) cultivars from Egypt and Kingdom of Saudi Arabia (KSA) Free Fu [PD]
Eldessoky S. Dessoky^{1,2*}; Attia O. Attia^{1,2}; Ismail A. Ismail^{1,2}; Hossam M. Zakaria²; Mohamed M. Hassan^{1,3}; Ahmed Gaber^{1,4}
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⁴Department of Genetics, Faculty of Agriculture, Cairo University, Egypt.
- 22 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 895-899. **OPEN ACCESS**
Plant regeneration from immature female inflorescence explants of date palm (*Phoenix dactylifera* L.) via direct somatic embryogenesis Free Fu [PD]
Fayek M.A.^{1*} Mohamed Helmy¹ Eman M.M Zayed² and Maha Rashed ahmed¹
¹Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
²Central Lab of Date Palm Researches and development, Agriculture Research Center, Giza, Egypt.
- 23 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 900-907. **OPEN ACCESS**
Quantitative assessment of genetic diversity among local chicken Free Fu [PD]

breeds detected by microsatellite markers

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Effects of exercise on bilateral transfer of function and kinematic parameters in short servicing badminton skill [Free Fu](#) [PD](#)
Hamideh Abdolzadeh
Faculty of Sport Sciences and Physical Education, Vali-e-Asr University, Rafsanjan, **Iran**.
- 25 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 915-923. **OPEN ACCESS**
Phenotypic and phylogenetic diversity of mycorrhizae isolated from Al-Jabal Al- Akhdar, Libya [Free Fu](#) [PD](#)
Salma O. Jadallah¹, Manal Farouk M. Abdelal², O. S. Elkobeisy³ and M. A. Ali⁴
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⁴Agric., Microbiology Dept., Faculty of Agric., Cairo Univ., Giza 12613, **Egypt**.
- 26 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 924-933. **OPEN ACCESS**
Genetic diversity and *in vitro* propagation of some *Acacia spp.* trees grown in Taif governorate. [Free Fu](#) [PD](#)
Attia O. Attia^{1,3*}, El Dessoky S. Dessoky^{1, 3} and Yassin M. Al-Sodany^{2, 4}
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⁴Botany Department, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, **Egypt**
- 27 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 934-941. **OPEN ACCESS**
Biomass and Flavonoid production of *Gynura procumbens* adventitious roots induced by sucrose, Phenylalanine and Tyrosine [Free Fu](#) [PD](#)
Rine Noviyanti¹, Rafika Lailiyatul Kurnia Sari¹, Alfinda Novi Kristanti², Arif Yachya³, Yosephine Sri Wulan Manuhara^{1*}
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³Biology Department, Faculty of Mathematics and Natural Sciences, PGRI Adi Buana University, Surabaya, **Indonesia**
- 28 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4):942-948. **OPEN ACCESS**
The potential role of iron molecule in some insect's guidance and navigation [Free Fu](#) [PD](#)
Mohamed Ragaie, Al-kazafy Hassan sabry*, Atef Abd-El Rahman, Huda Hassan Elbehery, and Nagy Abd Ellatif Farag
Pests and Plant Protection Department, National Research Centre, Cairo, **Egypt**.
- 29 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 949-954. **OPEN ACCESS**
Effect of Rhizosphere Bacteria application on saline tolerant to Proline and Chlorophyll contents of Cucumber [Free Fu](#) [PD](#)
Rikza Alfya Anugrah Cahyaty^{1*}, Nurul Aini² and Titin Sumarni²
¹Postgraduate Program, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65145, **Indonesia**
²Department of Agronomy, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65145, **Indonesia**
- 30 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 955-965. **OPEN ACCESS**
Characterization of biochar combination with organic fertilizer: the effects on physical properties of some soil types [Free Fu](#) [PD](#)
Widowati, Sutoyo, Taufik Iskandar, and Hidayati Karamina
Agrotechnology Department, Agriculture Faculty, Univerisity of Tribhuwana Tunggaladewi Malang, East Java, **Indonesia**
- 31 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 966-975. **OPEN ACCESS**
Effects of the Juvenoid (Fenoxycarb) on Entomopathogenic Nematode *Steinernema carpocapsae* S2 strain. [Free Fu](#) [PD](#)
Hala Mohamed Sayed Metwally*, Elham Abdel-Hakim and Wafaa Lotfy Abdou,
Pests and Plant Protection Department, National Research Centre, 33 El Buhouth St., Dokki, Cairo, **Egypt**.
- 32 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4):976-981. **OPEN ACCESS**
Evaluation of Different Methods in Detection of *Campylobacter* [Free Fu](#) [PD](#)

Infection in Poultry

Mai M. Morsy¹, Engy A. Hamed¹, Osama Mahna¹, Soad² A. Nasef, El-Enbaawy².M.I

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- 33 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 982-992. **OPEN ACCESS** **Free Fu [PD]**
Plants formation from protoplast-derived from date palm callus
Awatef Badr-Elden¹, Ibrahim Ibrahim¹, Hamdy Emara¹, Mahdia Gabr², Ahmed Nower¹, and Tamer Abd Elaziem²
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²Department of Plant Genetic Resources, Desert Research Center, Cairo, **Egypt**
- 34 **RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2017 14(4): 993-1004. **OPEN ACCESS** **Free Fu [PD]**
Potential renoprotective effect of Silymarin against Amikacin-Induced acute nephrotoxicity in rats
Abeer A. Abd El-Baky^{1*}, Shaymaa I. Salem¹ and Faten F. Mohammed²
¹Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, **Egypt**.
²Department of Pathology, Faculty of Veterinary Medicine, Cairo University, **Egypt**.
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¹Department of Forestry, Shaheed Benazir Bhutto University Sheringal, Dir (U), Khyber Pakhtunkhwa, 18000, **Pakistan**

²Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir (U), Khyber Pakhtunkhwa, 18000, **Pakistan**.

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¹Medical Faculty, Sam Ratulangi University; **Indonesia**

²Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University **Indonesia**.

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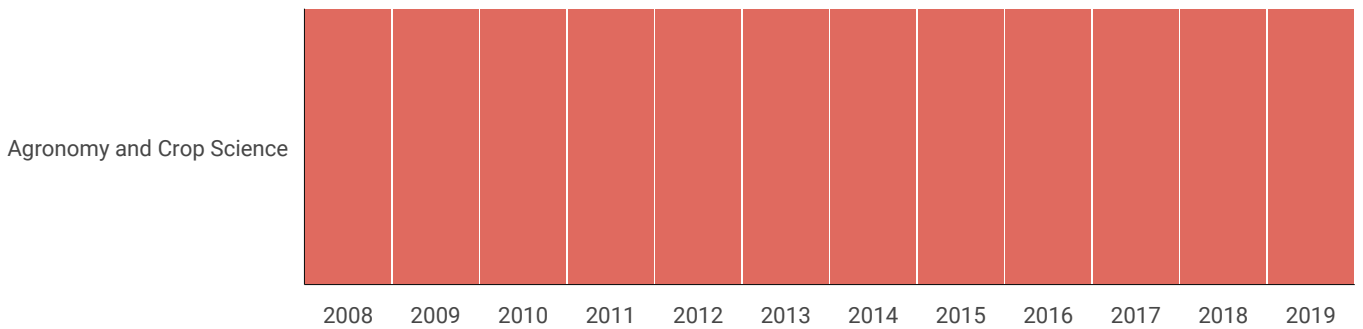
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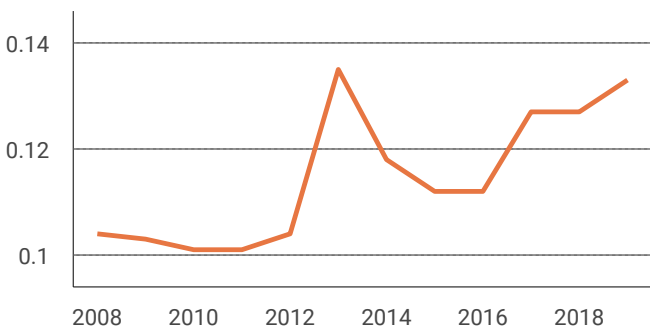
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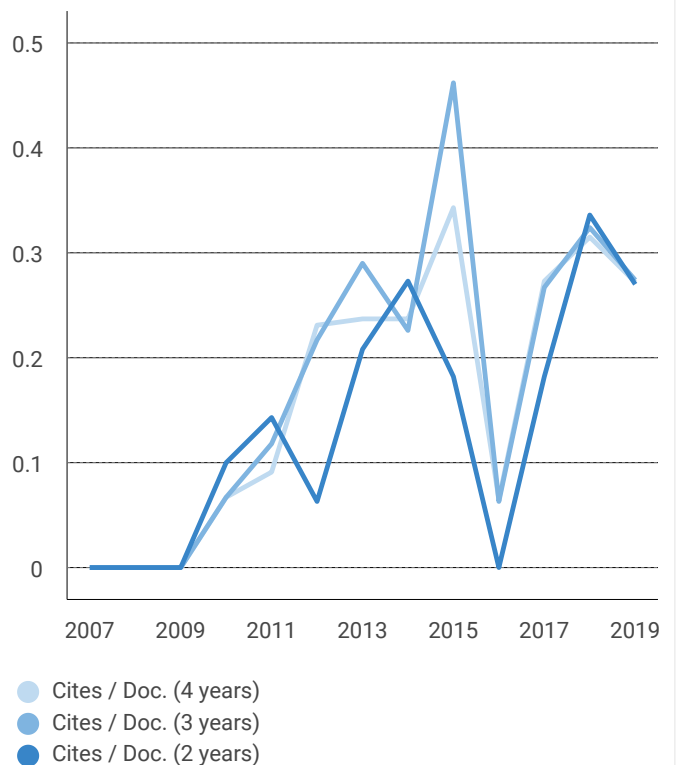
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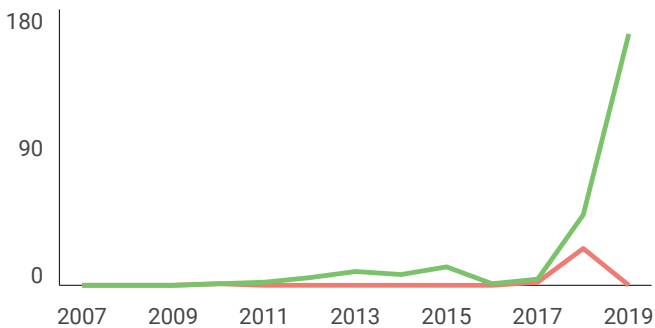
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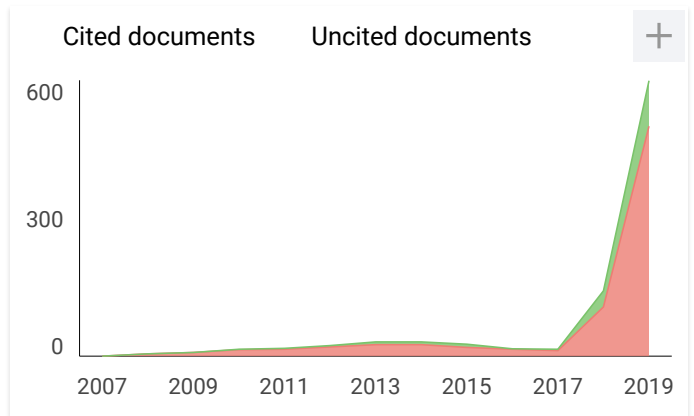
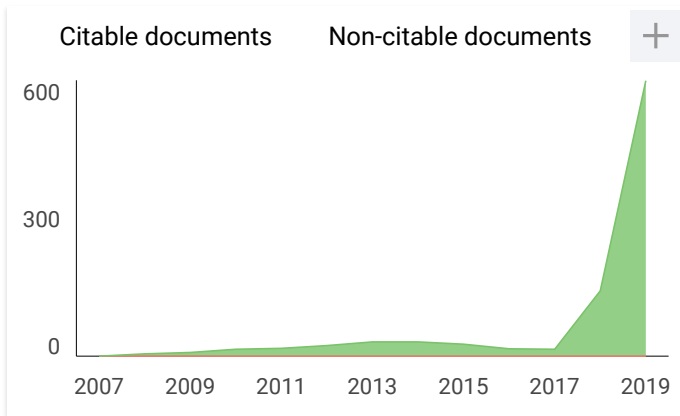


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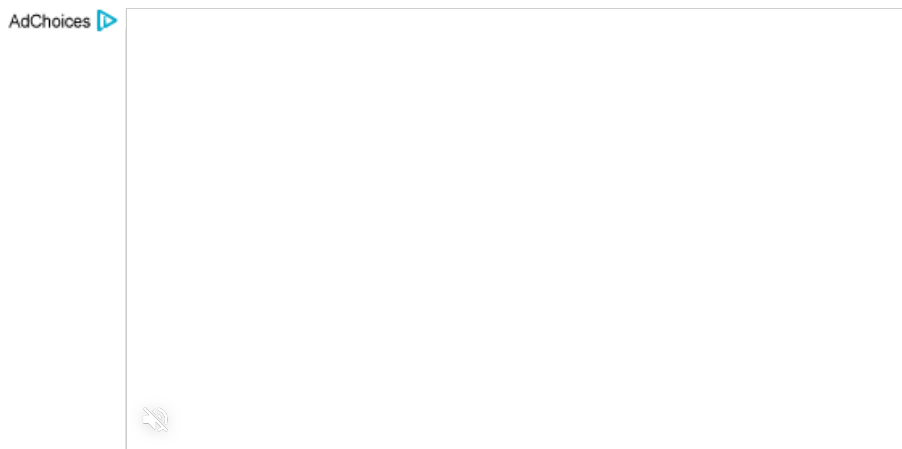
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Antifungal and antibacterial activity of black betel (*Piper betle* L. var Nigra) extract

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Black betel (*Piper betle* L. var Nigra) belong to Piperaceae family and one of endemic species of Indonesia. This study was aimed to determine antifungal and antibacterial activity of n-hexane, ethyl acetate, and methanol extracts of *Piper betle* L. var Nigra toward pathogenic microbes *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. Leaf powder of black betel was macerated using n-hexane, ethyl acetate, and methanol solvents. Antifungal and antibacterial activity was determined using diffusion and dilution tests. Data collected from both tests including diameter of inhibition zone (mm) and Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC), Minimal Fungicidal Concentration. Data was then analyzed statistically. Result showed that methanol extract of black betel had the highest inhibition activity compared to n-hexane and ethyl acetate extract.

Keywords: antifungal, antibacterial, *Piper betle* L. var Nigra

INTRODUCTION

Piperaceae belong to Magnoliopsida class, sub ordo Nymphaeiflorae and Piperales ordo. This plant family have been widely known as source of bioactive compound (Santhakumari et al. 2003; Lei et al. 2003; Majumdar et al. 2002; Kato and Furlan et al. 2007; Regasini et al. 2009). Piperaceae consisted of 10 genera and 2000 species. *Piper* genus is known to contain secondary metabolites of polyphenol, alkaloid, steroid, saponin, and tannin (Navickiene et al. 2006; Regasini et al. 2008).

Water, methanol, ethyl acetate, and ether extracts of *Piper betle* L. were found to be able to inhibit growth of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli* (Chakraborty and Shah, 2011), while ethanol, hexane, ethyl acetate,

hydromethanol extracts of leaf and stem from both *Piper arboretum* and *Piper tuberculatum* could inhibit growth of *Candida albicans*, *Candida krusei*, *Candida parasilopsis*, and *Cryptococcus neoformans* (Regasini et al. 2009). In addition, ethanol and chloroform extracts of *Piper nigrum* were also able to inhibit *Escherichia coli*, *Salmonella typhi*, *Pseudomonas sp.*, and *Staphylococcus aureus* (Ganesh et al. 2014). Ethanol, hexane, chloroform, ethyl acetate, methanol, and water extracts of *Piper hayneanum* could suppress growth of *Staphylococcus aureus* and *Candida albicans* (Bastos et al. 2011), while ethanol extract of black pepper (*Piper nigrum* L.) was able to suppress *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium*

oxysporum (Rani et al. 2013).

Betel variety currently recorded including green, red, and black betel. Black betel (*Piper betle* L. var *Nigra*) is one of Piperaceae family contained various secondary metabolites, such as alkaloid, flavonoid, saponin, terpenoid, and steroid. Thus, this plant has potential as antibacterial, antifungal, anti-diabetic, antiulcer, antiplatelet, antifertility, antitumor, anti-mutagenic, and anti-helminthic agents (Lei et al. 2003; Majumdar et al. 2002). Based on previous study, few study has been conducted to determine the activities of n-hexane, ethyl acetate, and methanol extracts of *Piper betle* L. var *Nigra* towards pathogenic microbes *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922.

MATERIALS AND METHODS

Plant Collection

Black betel (*Piper betle* L. var *Nigra*) was obtained from Kayon Flower Mart, Surabaya, and East Java, Indonesia. This plant was identified in Plant Physiology Laboratory, Faculty of Science and Technology, University of Airlangga.

Pathogenic Microbes

Pathogenic microbes used were *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. The three microbes were collection of Microbiology Laboratory, Department of Biology, Faculty of Science and Technology, University of Airlangga.

Extraction Procedure

Leaves of *Piper betle* L. var *Nigra* were firstly washed and air-dried, then crushed into powder. Betel powder weighed to 29.5 grams then extracted using n-hexane, ethyl acetate, and methanol solvents respectively. Extraction was performed using maceration method for three days and repeated for three times. Crude extract

filtered using filter paper, then extract was made into a series of concentration; 0 ppm; 250 ppm; 500 ppm; 750 ppm; and 1000 ppm.

Microbe Culture

Examined microbe was made into stock solution. Microbe suspension was produced by mixing several ose of bacteria from slanted agar into 20 mL saline water. Suspension was homogenized, then taken several mL for measuring its optical density using spectrophotometer. Stock used had OD 0.1 at 600 nm wavelength for fungi and 625 nm for bacteria.

Diffusion Test

Medium used for diffusion test was Mueller Hinton agar (MHA). 1 mL microbe suspension was placed in petri dish, then 15 mL MHA medium was added, mixture was homogenized and left to be solidified. On the surface of medium, 3 paper discs (6 mm diameter) saturated with 20 µl extract of respective solvent at each concentration of 0, 250, 500, 750, and 1000 ppm. Diameter of inhibition zone was measured using caliper.

Dilution test

1 mL microbe suspension in Mueller Hinton Broth (MHB) medium was placed into reaction tube filled with 1 mL betel extract of each solvent type and concentration. Each culture was incubated for 24 hours. Next, 0.1 mL of each culture was placed into petri dish and added 15 mL medium, then incubated for 24 hours for bacteria and 48 hours for fungi. If microbe growth was observed in medium, then concentration used was MIC value. If microbe growth was not observed, then concentration used was MBC/MFC value.

RESULTS

Diffusion test was a method commonly used for determining microbe activity, due to its inexpensive cost. Result of diffusion test was presented in Table 1.

Table 1. Antifungal and antibacterial activities of three extracts from *Piper betle* L. var *Nigra*

Microbe strain	Diameter of inhibition zone (mm)														
	n-hexane (µg/disc)					ethyl acetate (µg/disc)					methanol (µg/disc)				
	2	5	10	15	20	2	5	10	15	20	2	5	10	15	20
<i>E. coli</i> ATCC 25922	8,88	8,92	7,20	7,53	6,00	9,57	9,56	6,00	6,00	6,00	7,02	10,20	9,80	7,47	8,22
<i>S. aureus</i> ATCC 25923	6,00	6,00	6,00	7,00	6,00	6,00	6,00	10,63	7,53	10,03	6,00	6,00	12,69	12,00	8,52
<i>C. albicans</i> ATCC 10231	6,00	6,00	6,00	6,00	6,00	6,00	7,42	6,00	6,00	6,00	6,82	7,87	7,68	6,00	6,00

Table 2. MIC and MBC of each extract from *Piper betle* var *Nigra* towards *E. coli*, *S. aureus* and *C. albicans*.

Microbe strain	Extract solvent					
	n-hexane		ethyl acetate		methanol	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>E. coli</i> ATCC 25922	0,05	0,1	0,015	0,02	0,0275	0,03
<i>S. aureus</i> ATCC 25923	0,05	0,1	0,02	0,04	0,035	0,02
<i>C. albicans</i> ATCC 10231	-	-	0,02	0,015	0,0275	0,03

It was showed that n-hexane, ethyl acetate, and methanol extracts was able to inhibit the three species of pathogenic microbes. However, each extract was found to have different sensitivity towards specific microbe. N-hexane extract of black betel was found to inhibit *E. coli* ATCC 25922 better at 5 µg/disc, with inhibition zone diameter of 8.92 mm. Extract of ethyl acetate inhibited *S. aureus* ATCC 25923 at 10 µg/disc, with zone diameter of 10.63 mm, while methanol extract was able to inhibit *S. aureus* ATCC 25923 at 10 µg/disc concentration, with zone diameter of 12.69 mm.

For determining MIC and MBC, dilution test was performed for each solvent and concentration used. MIC and MBC was presented in Table 2.

DISCUSSION

Based on result of current study, methanol extract was found as the best extract solvent to be used compared to other solvent n-hexane and ethyl acetate. This was due to methanol as universal solvent, thus was able to bound various compound or secondary metabolites. Methanol extract was found to contain terpenoid, steroid, flavonoid, polyphenol, tannin, and alkaloid. Steroid was found to have function as antibacterial agent (Silvia et al. 2003). Steroid was able to act as antibacterial by preventing spore germination process (Subisha and Subramoniam, 2005). Other than them, alkaloid also had cytotoxic property against bacteria (Ezekiel et al. 2009). Indoquinolone alkaloid specifically previously found to induce cell lysis and morphological alteration on *S. aureus* (Sawer et al. 2005). Flavonoid inhibited function of cytoplasm membrane and energy metabolism (Cushnie and Lamb, 2009). Flavonoid possessed ability as anti-microbe (Ogundipe et al. 2001; Silvia et al. 2003) and was able to suppress growth of *S. aureus* and

E. coli (Chattopadhyay et al. 2001). Flavonoid was currently applied as new compound in antimicrobial therapy (Ozcelik et al. 2008). Polyphenol had antibacterial activity (Taguri et al. 2004), while tannin had previously hypothesized as antibacterial agent (Akiyama, 2001; Futanogawa, 2004; Guittat et al. 2003; Lisgarten et al. 2002).

Previous study had shown that water, methanol, petroleum ether, diethyl acetate, chloroform, and methanol extract of *Tamarindus indica* leaves at 100 mg/mL concentration were found to be able to inhibit growth of various Gram positive (*Bacillus subtilis* ATCC 11774, MRSA ATCC 977, *Staphylococcus aureus* ATCC 29213, and *Micrococcus luteus* ATCC 4698) and Gram negative bacteria species (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853). Lowest inhibition was found from petroleum ether extract towards *Bacillus subtilis* with 11.67 mm zone diameter, while highest inhibition was recorded from methanol extract against *Micrococcus luteus*, with zone diameter of 43.33 mm. In addition, ethanol, petroleum ether, diethyl acetate, ethyl acetate, and chloroform extracts were also found to be able to inhibit fungi species *Aspergillus flavus* ATCC 2000226, *Aspergillus fumigatus* ATCC 204305, *Aspergillus niger* ATCC 1015, and *Candida albicans* ATCC 10231. Highest inhibition was reported from ethanol extract of *Tamarindus indica* towards *Aspergillus fumigatus*, while lowest inhibition was reported from diethyl acetate extract towards *Aspergillus niger* (Gumgumjee et al. 2012).

Piperin isolated from *Piper nigrum* at 25 µl volume was found to be able to inhibit *S. aureus* with zone diameter of 4 mm, however was not able to inhibit *Escherichia coli* (Rani et al. 2013). Water, methanol, ethyl acetate, and ether extracts of *Piper betle* leaves at 5 mg/ml

concentration was able to inhibit growth of *S. aureus* and *E. coli* (Chakraborty and Shah, 2011). N-hexane, ethyl acetate, and methanol extract was found unable to suppress *S. aureus*, but able to inhibit *Candida albicans* with inhibition zone diameter of 1.0 up to concentration of 350 µg/disc. MIC of n-hexane, ethyl acetate, and methanol extract were 350 µg/disc, 350 µg/disc, and 1.0 µg/disc respectively (Bastos et al. 2011). Black pepper extract at 1 mg/mL up to 4 mg/mL concentration could suppress *E. coli*, *S. typhi*, *Pseudomonas sp.*, and *Proteus sp.* (Ganesh et al. 2014).

MIC of hexane extract from fruits and leaves of *Piper arboretum* was 250 µg/mL, while MIC of hexane and ethyl acetate extracts of *Piper tuberculatum* was >1000 µg/mL (Regasini et al. 2009). Ethanol extract of *Tamarindus indica* fruits was able to suppress *S. aureus* and *E. coli* have inhibition zone of 18.0 mm and 13.0 mm. MIC of ethanol extract against both bacteria were 500 mg/mL (Gupta et al. 2014). Methanol extract of *Origanum vulgare* could suppress *E. coli* and *S. aureus* at 250 µg/ml concentration (Ashraf et al. 2011). Ethanol extract of leaf, stem, and fruit of *Tamarindus indica* were able to inhibit *E. coli* and *S. aureus* (Nwodo et al. 2011). Water, acetone, and ethanol extract of stem and leaf of *Tamarindus indica* could inhibit *E. coli* and *S. aureus*, but unable to suppress *C. albicans*. MIC of ethanol extract against *E. coli* and *S. aureus* were 15.5 mg/mL and 8 mg/mL respectively. MBC of both extract against *E. coli* and *S. aureus* were 18 mg/mL and 20 mg/mL (Doughari, 2006).

MIC and MBC of ethanol leaf extract from *Tamarindus indica* against *E. coli* were 15.5 mg/mL and 18 mg/mL respectively, while against *S. aureus* was 8 mg/mL and 20 mg/mL (Doughari, 2006). MIC and MBC of hexane and ethyl acetate leaf extracts of *Piper arboretum* against *C. albicans* were respectively 250 µg/mL and >1000 µg/mL. MIC and MBC of leaf extract from *Piper tuberculatum* against *C. albicans* was >1000 µg/mL (Regasini et al. 2009). MIC and MBC ethanol and water extract of *Tamarindus indica* towards *E. coli* ATCC 11775 were 31.25 and 125 mg/mL respectively (Nwodo et al. 2011). Based on data in Table 2, MIC and MBC of ethyl acetate and methanol extracts of black betel was higher compared to extracts from *Tamarindus indica*, *Piper arboretum*, and *Piper tuberculatum*. Based on result of current study, three types of extract using n-hexane, ethyl acetate, and methanol extracts had antibacterial and antifungal

activities. From those varieties of extracts examined, methanol extract had highest antibacterial and antifungal activities.

CONCLUSION

Methanol extract of black betel had the highest inhibition activity compared to n-hexane and ethyl acetate extract.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Junairiah and Ni'matuzahroh designed and performed the experiments and also wrote the manuscript. Nabilah Istighfari Zuraidassanaaz performed plant collection, diffusion and dilution test, data analysis. Lilis Sulistyorini designed experiments and reviewed the manuscript. All authors read and approved the final version. All authors read and approved the final version.

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