

Biotechnology Reports



Editorial board

Editor in Chief



Professor Mingfeng Cao

Xiamen University, Department of Chemical and Biochemical Engineering, Xiamen, China

Synthetic biology, Metabolic engineering, Genome editing, Fermentation engineering, Microbial biotechnology

[View full biography](#)

Founding Editor

Professor Emeritus Bo Mattiasson

Lund University, Department of Biotechnology, Lund, Sweden

downstream processing, enzyme technology, environmental biotechnology, biosensors/bioanalysis

Editors



Dr. Karl Bayer, PhD

University of Natural Resources and Life Sciences Vienna, Wien, Austria

Upstream processing, Bioprocess monitoring, Model based prediction of non-measurable process variables, Recombinant protein production using microbial host vector systems



Professor Bauke W. Dijkstra, PhD

University of Groningen, Groningen, Netherlands

Structural biology, protein engineering, enzyme mechanisms, thermostability



Dr. Mohammad Faisal, PhD

King Saud University, Department of Botany and Microbiology, Riyadh, Saudi Arabia

Plant Biotechnology, in vitro morphogenesis, tissue culture, micropropagation, germplasm conservation, genetic transformation, molecular markers and environmental phytotoxicity of nanoparticles and bisphenols.



Dr. Jun Feng, PhD

DMC Biotechnologies, Inc., Boulder, Colorado, United States of America

Microbial synthesis and fermentation, Synthetic biology, Metabolic engineering, Biofuels and bioenergy, Microbial consortium, Molecular biology, Gut Microbe



Professor Mahmoud Labib, PhD

Northwestern University, Department of Chemistry, Evanston, Illinois, United States of America

Bioanalysis, Cancer Diagnostics, Drug Discovery, Immunotherapy, Biosensors, Electrochemistry



Prof. Dr. Xiaobo Liu, PhD

Nanjing University of Science and Technology, School of Environmental and Biological Engineering, Nanjing, China

Environmental microbiome, Biodeterioration, Biofouling, Biofilm, Biocatalysis and biosynthesis, Environmentally benign biocides, Food microbial contamination and sanitation



Dr. Raúl Muñoz Torre, PhD

University of Valladolid, Department of Chemical Engineering and Environment, Valladolid, Spain

Environmental biotechnology

Assoc. Professor Paola Paci, PhD in Physics

Sapienza University of Rome, Department of Computer, Control and Management Engineering, Roma, Italy

Bioinformatics

Editorial Board



Professor Edward A. Bayer, PhD

Weizmann Institute of Science, Department of Biomolecular Sciences, Rehovot, Israel

Avidin-biotin technology, Cellulosome research, Microbial biotechnology, Synthetic biology

Professor Thomas Bruck

Technical University of Munich, Department of Chemistry, Garching, Germany



Professor Yusuf Chisti, PhD

Massey University, Palmerston North, New Zealand

Biochemical engineering, Bioprocess engineering, Industrial biotechnology, Fermentation technology

Dr. Hussain Dahodwala

University of Delaware, Newark, Delaware, United States of America

Cell line development, CHO culture based genetic engineering, site-specific integration, cell line stability, bioprocess engineering, synthetic biology, CHO cell-derived glycosylation, cell nuclear proteomics



Dr. Hassan Etesami, PhD

University of Tehran, Department of Soil Science, Tehran, Iran

Microbial ecology, Plant-microbe interactions, Biofertilizers, Integrated management of abiotic stresses, and Bioremediation



Dr. Giulia Fiscon, PhD

Sapienza University of Rome, Rome, Italy

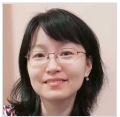
Bioinformatics, Computational Biology, Biomedical Engineering, Network Medicine



Dr. Vijai Kumar Gupta, PhD

Scotland's Rural College, Edinburgh, United Kingdom

Biobased biorefineries, Serectomics and bioprocessing technologies, Glycobiotechnology, Bioactive natural products, Microbial engineering biotechnologies, and Environmental sustainability, Functional Microbiome Interactions, Bioactive Natural Products



Dr. Linna Han, PhD

NC State University, Raleigh, North Carolina, United States of America

Synthetic biology, Metabolic engineering, Enzyme engineering, Genome editing



Prof. Dr. Alexander A. Kamnev, DSc

Institute of Biochemistry and Physiology of Plants and Microorganisms of the Russian Academy of Sciences, Saratov, Russian Federation

Molecular-level spectroscopic studies related to microbial ecology and agricultural biotechnology of plant-growth-promoting rhizobacteria, Biosynthesis, accumulation and properties of biopolyesters of bacterial origin, Biogenesis and properties of metal and chalcogen-containing nanoparticles

Professor Gopalakrishnan Kumar

University of Stavanger, Stavanger, Norway

Bioenergy production from waste /biomass, Biomass (lignocellulose/lignin) valorization to value added chemicals, Environmental biotechnology/remediation, Biosynthesis of nanoparticles and applications in energy& , medicine, Fermentation and process engineering, Immobilization, wastewater/waste treatment for energy production



Dr. Jiazhang Lian, PhD

Zhejiang University, Hangzhou, China

Synthetic biology, Genome engineering, Metabolic engineering, Natural product biosynthesis

All members of the Editorial Board have identified their affiliated institutions or organizations, along with the corresponding country or geographic region. Elsevier remains neutral with regard to any jurisdictional claims.



Copyright © 2023 Elsevier B.V. or its licensors or contributors.
ScienceDirect® is a registered trademark of Elsevier B.V.

RELX™



Biotechnology Reports

Open access

8
CiteScore

[Submit your article](#)

[Guide for authors](#)

[Menu](#)

[Search in this journal](#)

Volume 25

March 2020

[Download full issue](#)

[← Previous vol/issue](#)

[Next vol/issue >](#)

Receive an update when the latest issues in this journal are published

[Sign in to set up alerts](#)

Review Articles

Review article *Open access*

Metabolomic applications for understanding complex tripartite plant-microbes interactions: Strategies and perspectives

Adetomiwa Ayodele Adeniji, Olubukola Oluranti Babalola, Du Toit Loots

Article e00425



[View PDF](#)

[Article preview](#)

Research article *Open access*

Household aluminum foil matte and bright side reflectivity measurements: Application to a photobioreactor light concentrator design

Victor Pozzobon, Wendie Levasseur, Khanh-Van Do, Bruno Palpant, Patrick Perré

Article e00399



[View PDF](#)

[Article preview](#)

Research article *Open access*

Draft genomes and initial characterization of siderophore producing pseudomonads isolated from mine dump and mine drainage

Marika Hofmann, Thomas Heine, Vivian Schulz, Sarah Hofmann, Dirk Tischler

Article e00403



[View PDF](#)

[Article preview](#)

Research article *Open access*

Biogas production from recycled paper mill wastewater by UASB digester: Optimal and mesophilic conditions

Mohammed Bakraoui, Fadoua Karouach, Badr Ouhammou, Mohammed Aggour, ... Hassan El Bari

Article e00402



[View PDF](#)

[Article preview](#)

Research article *Open access*

Coproduction of lipids and extracellular polysaccharides from the novel green alga *Parachlorella* sp. BX1.5 depending on cultivation conditions

Mitsuki Sasaki, Akari Takagi, Shuhei Ota, Shigeyuki Kawano, ... Munehiko Asayama

Article e00392



[View PDF](#)

[Article preview](#)

Research article *Open access*

Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest

Francisco X. Nascimento, Anabel G. Hernández, Bernard R. Glick, Márcio J. Rossi

Article e00406



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Optimization of carotenoids production by *Rhodotorula mucilaginosa* (MTCC-1403) using agro-industrial waste in bioreactor: A statistical approach

Rajan Sharma, Gargi Ghoshal

Article e00407



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Controlling anthracnose by means of extracts, and their major constituents, from *Brosimum rubescens* Taub

Janio Martinez, Andrés Gomez, Cesar Ramirez, Jesús Gil, Diego Durango

Article e00405



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Investigation of antifouling properties of polypropylene/TiO₂ nanocomposite membrane under different aeration rate in membrane bioreactor system

Habib Etemadi, Milad Fonouni, Reza Yegani

Article e00414



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Impact of process parameters and plant polysaccharide hydrolysates in cellulase production by *Trichoderma reesei* and *Neurospora crassa* under wheat bran based solid state fermentation

Nitin Verma, Vivek Kumar

Article e00416



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Indirect regeneration and genetic fidelity analysis of acclimated plantlets through SCoT and ISSR markers in *Morus alba* L. cv. Chinese white

Gulab Khan Rohela, Phanikanth Jogam, Mohammad Yaseen Mir, Aftab Ahmad Shabnam, ... Azra Nahaid Kamili

Article e00417



[View PDF](#)

[Article preview](#)

Research article [Open access](#)

Phosphorus removal from landfill leachate by microalgae

Zareen T. Khanzada

Article e00419

 [View PDF](#) Article preview 

Research article [Open access](#)

Immobilisation of lipase enzyme onto bacterial magnetosomes for stain removal

Jobin John Jacob, K Suthindhiran

Article e00422

 [View PDF](#) Article preview 

Research article [Open access](#)

Generation of biparatopic antibody through two-step targeting of fragment antibodies on antigen using SpyTag and SpyCatcher

Hiroki Akiba, Kensuke Takayanagi, Osamu Kusano-Arai, Hiroko Iwanari, ... Kouhei Tsumoto

Article e00418

 [View PDF](#) Article preview 

Research article [Open access](#)

Integrated control of dry root rot of chickpea caused by *Rhizoctonia bataticola* under the natural field condition

Abdul Khaliq, Sartaj Alam, Irfan Ullah Khan, Dilawar Khan, ... Assar Ali Shah

Article e00423

 [View PDF](#) Article preview 

Research article [Open access](#)

2-Phenylethanol and 2-phenylethylacetate production by nonconventional yeasts using tequila vinasses as a substrate

José de Jesús Rodríguez-Romero, César Arturo Aceves-Lara, Cristina Ferreira Silva, Anne Gschaedler, ... Javier Arrizon

Article e00420

 [View PDF](#) Article preview 

Research article [Open access](#)

Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution

Farid Rachidi, Redouane Benhima, Laila Sbabou, Hicham El Arroussi

Article e00426

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review

Anirudh Singh, Pavan Kumar Gautam, Arushi Verma, Vishal Singh, ... Sintu Kumar Samanta

Article e00427

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Manganese (II) removal from aqueous solutions by *Cladosporium halotolerans* and *Hypocrea jecorina*

Ester Alves Mota, Érica Barbosa Felestrino, Versiane Albis Leão, Renata Guerra-Sá

Article e00431

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

At-line high throughput site-specific glycan profiling using targeted mass spectrometry

Bertie Chi, Christel Veyssier, Toyin Kasali, Faisal Uddin, Christopher A. Sellick

Article e00424

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Characterization of Soluble Algal Products (SAPs) after electrocoagulation of a mixed algal culture

Poorya Rafiee, Sirous Ebrahimi, Maryam Hosseini, Yen Wah Tong

Article e00433

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Mesenchymal stem cells proliferation and remote manipulation upon exposure to magnetic semiconductor nanoparticles

Tudor Braniste, Vitalie Cobzac, Polina Ababii, Irina Plesco, ... Ion Tiginyanu

Article e00435


 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage

Hadi Pourjafar, Negin Noori, Hasan Gandomi, Afshin Akhondzadeh Basti, Fereshteh Ansari

Article e00432

 [View PDF](#) Article preview 

Research article [Open access](#)

Optimization of recombinant Zika virus NS1 protein secretion from HEK293 cells

Julieta S. Roldán, Alejandro Cassola, Daniela S. Castillo

Article e00434

 [View PDF](#) Article preview 

Research article [Open access](#)

Identification of a novel 1,2 oxazine that can induce apoptosis by targeting NF-κB in hepatocellular carcinoma cells

Chaithanya Somu, Chakrabhavi Dhananjaya Mohan, Sachin Ambekar, Dukanya, ... Kanchugarakoppal S. Rangappa

Article e00438

 [View PDF](#) Article preview 

Research article [Open access](#)

Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of *Melia azedarach* L. wild type leaves

Martha Ervina, Hadi Poerwono, **Retno Widyowati**, Katsuyoshi Matsunami, Sukardiman

Article e00437

 [View PDF](#) Article preview 

Research article [Open access](#)

Removal of Pb (II) from aqueous solution using nanoadsorbent of *Oryza sativa* husk: Isotherm, kinetic and thermodynamic studies

Mandeep Kaur, Santosh Kumari, Praveen Sharma

Article e00410

 [View PDF](#) Article preview 

Research article [Open access](#)

Study of antioxidant activity and some herbal compounds of *Dracocephalum kotschyi* Boiss. in different ages of growth

Hossein Moradi, Mansureh Ghavam, Ali Tavili

Article e00408



[View PDF](#)

[Article preview](#)

Short Communications

Short communication [Open access](#)

Selective and sensitive colorimetric detection of platinum using *Pseudomonas stutzeri* mediated optimally synthesized antibacterial silver nanoparticles

Megha P. Desai, Reshma V. Patil, Kiran D. Pawar

Article e00404



[View PDF](#)

[Article preview](#)

Short communication [Open access](#)

Draft genome sequence and nomenclature adjustment of *Rhodococcus qingshengii* CS98, a cesium-accumulating strain isolated in Japan

Candice Raeburn, Shin Kasahara, Toshi Komoda, Catherine Abbott, Peter M. Smooker

Article e00415



[View PDF](#)

[Article preview](#)

Short communication [Open access](#)

Porous starch citrate biopolymer for controlled release of carbofuran in the management of root knot nematode *Meloidogyne incognita*

Oluwatoyin Adenike Fabiyi, Oluwaseyi Damilare Saliu, Abiodun Olufunmilayo Claudius-cole, Israel Olayiwola Olaniyi, ... Gabriel Ademola Olatunji

Article e00428



[View PDF](#)

[Article preview](#)

Short communication [Open access](#)

Composition and functional profiling of the microbiota in the casts of *Eisenia fetida* during vermicomposting of brewers' spent grains

Marilena Budroni, Ilaria Mannazzu, Severino Zara, Sara Saba, ... Giacomo Zara

Article e00439



[View PDF](#)

[Article preview](#)

Articles from the Special Issue on Microbial technology for the development of sustainable energy and environment; Edited by Xiaobo Liu

Research article [Open access](#)

The current strategies and parameters for the enhanced microbial production of 2,3-butanediol

Olivier Hakizimana, Emmanuel Matabaro, Byong H. Lee

Article e00397

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Sustainability potentials of novel laccase tinctures from *Stenotrophomonas maltophilia* BIJ16 and *Bordetella bronchiseptica* HSO16: From dye decolourization to denim bioscouring

John O. Unuofin

Article e00409

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Isolation and characterization of an alkali and thermostable laccase from a novel *Alcaligenes faecalis* and its application in decolorization of synthetic dyes

Seema Mehandia, S.C. Sharma, Shailendra Kumar Arya

Article e00413

 [View PDF](#) [Article preview](#) 

Research article [Open access](#)

Sequential degradation of raw vinasse by a laccase enzyme producing fungus *Pleurotus sajor-caju* and its ATPS purification

Joberson Alves Junior, Yago Araujo Vieira, Ianny Andrade Cruz, Débora da Silva Vilar, ... Luiz Fernando Romanholo Ferreira

Article e00411

 [View PDF](#) [Article preview](#) 

Review article [Open access](#)

Relevance of biogas technology to Vhembe district of the Limpopo province in South Africa

T.E Rasimphi, D Tinarwo

Article e00412

 [View PDF](#) [Article preview](#) 

Short communication [Open access](#)

The complete genome sequence of the algicidal bacterium *Bacillus subtilis* strain JA and the use of quorum sensing to evaluate its antialgal ability

Sheng-Jie Zhang, Xiao-Peng Du, Jian-Ming Zhu, Chen-Xu Meng, ... Ping Zuo

Article e00421



[View PDF](#)

[Article preview](#)

Corrigendum

[Erratum](#) [Open access](#)

Corrigendum to “Inhibitory effects of leaf extract of *Lawsonia inermis* on *Curvularia lunata* and characterization of novel inhibitory compounds by GC–MS analysis” [Biotechnol. Rep. 23 (2019) e00335]

Tansukh Barupal, Mukesh Meena, Kanika Sharma

Article e00430



[View PDF](#)

[< Previous vol/issue](#)

[Next vol/issue >](#)

ISSN: 2215-017X

Copyright © 2023 Elsevier B.V. All rights reserved



Copyright © 2023 Elsevier B.V. or its licensors or contributors.
ScienceDirect® is a registered trademark of Elsevier B.V.

RELX™



Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of *Melia azedarach* L. wild type leaves

Martha Ervina^{a,b}, Hadi Poerwono^c, Retno Widyowati^d, Katsuyoshi Matsunami^e, Sukardiman^{d,*}

^a Doctoral Program of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry Faculty of Pharmacy, Universitas Airlangga, Indonesia

^b Department of Pharmaceutical Biology, Faculty of Pharmacy, Widya Mandala Catholic University, Indonesia

^c Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Indonesia

^d Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Indonesia

^e Department of Pharmacognosy, Graduate School of Biomedical & Health Sciences, Hiroshima University, Japan

ARTICLE INFO

Article history:

Received 2 November 2019

Received in revised form 30 January 2020

Accepted 16 February 2020

Keywords:

Antioxidants

DPPH

FRAP

LCMS

Melia azedarach

TPC

T47D cytotoxic

ABSTRACT

Melia azedarach L. is used widely in traditional medicine for local or systemic ailments. Although studies exist on phytochemicals and potencies of Chinese and Indian cultivars of *Melia*, the present study investigated *in vitro* antioxidant properties of *Melia* wild type and its cytotoxicity against T47D cell. The ethanolic extract of the *Melia* leaves was fractionated with n-hexane, ethyl acetate and water, and the secondary metabolites were obtained. The antioxidant properties were determined with IC₅₀ DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and FRAP (ferric reducing antioxidant power), while the cytotoxicity was determined with the MTT method. The total phenolic (TPC) and β-sitosterol (SC) contents were also measured. The results showed that the ethyl acetate fraction had higher antioxidant and cytotoxic activities (IC₅₀ 211.89 ± 10.86 and 147.90 ± 8.49 μg/mL, respectively) than others. Significant (p < 0.05) correlations were observed between TPC, IC₅₀DPPH, FRAP and IC₅₀T47D. LC-EI MS analysis of the ethyl acetate fraction revealed the steroid and triterpenoid saponins, limonoids and quercetin glycosides, which influenced the medicinal properties of the *Melia* leaves. *Melia azedarach* L. wild type leaf extracts are a promising natural resource for managing breast cancer.

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cancer is a global burden due to its mortality and morbidity. Its incidence and prevalence are rapidly growing worldwide. It has become a major cause of death on productive ages in all countries. Among cancer types, breast cancer is the most diagnosed and the major cause of death in females (11.6%). The uncontrolled growth of cancer cells, resulting from genetics, infections and life styles, are complex [1]. Mutagenesis by DNA and free radical damages are related to the cancer cell initiation phase. Chemotherapies are the most widely used management technique, but they naturally kill normal and neoplastic cells. Although reactive oxygen species (ROS) play a role in cancer cell death mechanisms, they generally affect human bodies adversely. Some of the adverse ROS chemotherapies include alkyl sulfonates, ethyleneamines and hydrazines (alkylating agents), doxorubicin (anthracyclines), cisplatin and carboplatin (platinum coordination complexes), etoposides

(podophyllin derivatives), and irinotecan and topotecan (camptothecins) [2]. However, intakes of antioxidant supplements, as primary and secondary steps to cancer preventions, eliminate the ROS side effects, improve tumor responses and increase patient survival rates [3].

With advances in pharmaceutical science, chemotherapy synthetic drugs are also being developed and improved to minimise these ROS side effects. Nevertheless, the array of plants available and their different phytochemicals with anticancer activities have opened research into medicinal plants to complement chemotherapy synthetic drugs for managing all types of cancer. For example, taxol from *Taxus brevifolia* (Taxaceae), vinblastine and vincristine from *Catharanthus roseus* L. G. Don (Apocynaceae), and etoposide and teniposide from *Podophyllum* sp. Linn (Berberidaceae), camptothecin, paclitaxel, homoharringtonine and many natural-derived compounds have been studied [4]. Their contents of terpenes, phenolics and alkaloids are examples of secondary metabolites with combined cytotoxic and antioxidant activities.

There are many other plants, however, that have not been studied to a comparable extent, and their medicinal potential is yet to be fully

* Corresponding author.

E-mail address: sukardiman@ff.unair.ac.id (Sukardiman).

understood. One of these plants is a mahogany plant Chinaberry (*Melia azedarach* L.) that originates from Asia but now have a global spread. Three types (wild, Chinese and Indian) of *M. azedarach* are known based on the average size of the plants [5]. Almost all the parts of the Chinese and Indian types are used in traditional medicine, for example, their bark, seed, root and leaves are reported [6–8] to have antiparasitic, antifungal, diuretic, emmenagogue, antibacterial, antimalarial, hepatoprotection, antioxidant, antifertility and antipyretic properties or activities. Even though the wild type is mostly utilized for wood and ecological purposes, relatively, the cytotoxicity of the plant and its medicinal properties are yet to be fully established and understood, more so the efficacies of its extracts in different solvents on different cancer cells.

Zahoor et al. found the influence of different solvents (chloroform, butanol, hexane water and ethyl acetate) on the antibacterial, antioxidant and brine shrimp cytotoxicity of *M. azedarach* bark. It was found that different solvents has difference antibacterial activity, although it can be observed that ethyl acetate extract had better antioxidant and cytotoxic compare to others [8].

Furthermore, a research revealed the influence of different solvents (ethanol, petroleum ether and water) on the phytochemical content, total phenolic content (TPC) and DPPH antioxidant activity (AA) of *M. azedarach* leaves [9]. The ethanolic extract had the highest amount of phenolic compounds and exhibited the strongest antioxidant activity compared to petroleum ether and aqueous extracts. The toxicity of an ethanolic extract of the plant's leaves against vero cells had an $IC_{50} > 1000 \mu\text{g/mL}$ [10]. Another study, against HT-29, A-549, MCF-7, HepG-2 and MDBK cell lines, observed that the methanolic extract of the plant's leaves safer in term of cytotoxic activity compared to the extracts of its pulps and seeds [11]. Cytotoxic activities of the bark and root bark of *M. azedarach* against some cells have been reported and related to their steroid and limonoid tirucallane contents [12–16].

Based on the chemotaxonomy point of view, however, we are not aware of any in-depth study on the selective hormones of cytotoxic cells and antioxidant activities of *M. azedarach* leaves' extracts and fractions, specifically on breast cancer cells. The T47D and MCF7 are hormone dependent cell lines, which are mostly used *in vitro* breast cancer cell line research. The T47D reveals more in screening breast cancer phytochemical targeting compounds though. It's an ideal object for experimental progesterone-specific effects of breast cancer, as it is susceptible to progesterone in the presence of estrogen, while the MCF7 is not [17]. Using the wild type of *M. azedarach*, and to understand the potential activity of the plant on selective progesterone–estrogen receptor targeting breast cancer, the objectives of the study were, therefore, to investigate the cytotoxic activity against T47D cell line, antioxidant activities using DPPH and FRAP of the extracts and fractions; and examine correlations among the cytotoxic and antioxidant parameters.

2. Materials and methods

2.1. Plant material

M. azedarach dried leaves were obtained on dried season from Materia Medica Batu, which is an Indonesian government office on medicinal plants. Upon identification (No. 074/346/102.7/2017), the dried leaves were stored (herbarium No. Ma011017) prior to analysis [18] for drying shrinkage and ash, moisture and ethanol-soluble contents, and phytochemical screening [19] using standard procedures.

2.2. Preparation of the extract

The dried leaves were ground and extracted with 96 % ethanol (1:10) three times each for 24 h at room temperature. The solvent

was removed in a rotary vacuum evaporator to result of the ethanolic extract (E). It was dispersed in water (1:10) and fractionated in a separating funnel with n-hexane (1:1) and ethyl acetate (1:1) to obtain n-hexane (FH), ethyl acetate (FE) and water (FW) fractions. Three replicates of the extract and fractions were done before their phytochemistry screening and analyses for cytotoxic and antioxidant activities.

2.3. MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) Cytotoxic test of T47D cell

The T47D cell lines (epithelial cells of human ductile pleural effusion from a 54-year-old mammary gland tissue) were kindly provided by Parasitology Laboratories, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. The cytotoxicity test, based on CCRC method [20], was conducted with the University's ethics approval No. KE/FK/0310/EC/2018. The T47D cell was grown on RPMI with 10 % (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) penicillin-streptomycin into 96-well plates. The optimized cell growth (70–80 % confluent) was treated with 100 μl of different concentrations of each of the extract and fractions (1000 to 10 $\mu\text{g/mL}$) and doxorubicin (100 to 0.1 $\mu\text{g/mL}$) before incubation for 24 h at 37 °C. MTT (10 %) was added to differentiate viable cells metabolism when purple formazan crystals were observed after 4 h in the dark before stopping the reactions and dissolving the crystals in 10 % dodecyl sulphate in a sulphuric acid solution. The cells were shaken (MRK-Retac) for 10 min and their absorbances were read (Elisa reader, Bio-Rad microplate reader Benchmark serial no.11565, Japan) at a wavelength of 595 nm. The absorbances were converted to percentage of viable cells as the following formula and graphed to obtain the IC_{50} (the sample concentration that inhibited 50 % of the cell growth) with linear regression analysis. Blank control (media/ A_b) experiment was conducted. A_0 is absorbance of cell growth without sample, while A_s is absorbance of cell growth treatment with sample.

$$\% \text{ viable cells} = (A_s - A_b) / (A_0 - A_b) \times 100$$

2.4. DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity assay

The DPPH antioxidant activity was assayed with a Microplate reader UV/VIS spectrophotometer (Multiskan Go, Thermo Scientific, Finland) using a published procedure on the concentration range sample (100–1000 $\mu\text{g/mL}$ for extract and fractions; while 1,0–30 $\mu\text{g/mL}$ for rutin), and the absorbances were read at a wavelength of 517 nm [21] with a solvent blank. The IC_{50} antioxidant activity (IC_{50AA}) was estimated using a linear regression analysis. Rutin was used as the reference. The formula to obtain % inhibition as A_0 (absorbance of DPPH without sample), and A_s (absorbance of DPPH with samples)

$$\% \text{ inhibition} = ((A_0 - A_s) / A_0) \times 103$$

2.5. Ferric reducing antioxidant power (FRAP)

The FRAP was carried out according to [22], in which the colored complexes of the antioxidant samples reacted with potassium ferricyanide, trichloroacetic acid and ferric chloride, and measured at 700 nm wavelength with the spectrophotometer. The samples were prepared at 100 $\mu\text{g/mL}$, while for rutin was made on concentrations range (50–200 $\mu\text{g/mL}$). A high absorbance reflects a high antioxidant potency, and this was calculated equivalent to rutin, a natural well-known glycoside flavonoid antioxidant. Solvent and reagent blanks were also analyzed.

2.6. Determination of total phenolic content (TPC)

The TPC was determined with the Folin-Ciocalteu (FC) reagent with slight modifications to the micro-plate preparation [21]. The samples were prepared at 100 µg/mL; while for gallic acid was made base on pre-experiments concentrations range (10–200 µg/mL) and absorbances were measured with the reader at 765 nm wavelength using a solvent blank. The TPC (µg GAE/ mg extract or fraction) was reported as gallic acid equivalents.

2.7. Determination of β-sitosterol content (SC)

The determination of β-sitosterol content was done according to Sutar et al. (2014) [23] with slight modifications. The extract and fractions were made at 100 µg/mL; while for β-sitosterol were prepared on concentrations range (100–1500 µg/mL) and spotted 10 µL each on TLC silica gel plates. The plates were developed with n-hexane:ethyl acetate (7:3) and sprayed with anisaldehyde-SO₄ to determine the areas of β-sitosterol purple spots at 517 nm wavelength (TLC Scanner Camag 3, Switzerland). The SC is reported as µg β-sitosterol equivalent/mg of extract or fraction and calculated from a β-sitosterol calibration curve.

2.8. LCMS (Liquid Chromatography-Mass Spectrophotometry) of the ethyl acetate fraction

The ethyl acetate fraction was pre-treated in a solid-phase extraction (Qasis[®] HLB solvents, Waters, Milford, USA), dissolved in methanol and filtered through a 0.2 µm syringe membrane filter before chromatographic analysis [24]. The C-18 (1.8 µm, 2.1 × 100 mm) ultra-performance liquid chromatography (Acquity UPLC[®]-H class system, Waters, Milford, USA) was HSS column at 50 °C). The mobile phase and flow rate were followed as described [21] so was with the electrospray ionization mass spectrometry (MSsystem (Xevo G2-S QToF, Waters, Milford, USA). The results were analyzed with MassLynx 4.1 program to determine the Rt, m/z fragments and molecular formula. The predictive compounds were obtained from similarities of literature of Melia's compounds, mass bank (Fiehnlab), pubchem or chemspider.

2.9. Statistical analysis

The data was presented as average values and standard deviations of triplicates. The one-way ANOVA was used for mean comparisons at a 5% significant level with SPSS version 24 and the Pearson correlation analysis was also performed.

3. Results and discussion

3.1. Physical and phytochemical profile of the extract

The *M. azedarach* used was the wild type, and it usually has big trees that can be up to 40 m high. This wild type is usually different from the medium-sized Chinese and Indian types [5]. Table 1 presents the physical and phytochemical quality of the extract and the fractions of the leaves. This is the first report on the quality of *M. azedarach* dried leaves' extracts and fractions, while earlier studies were on the plant's bark [6]. The drying shrinkage was low (1.67 ± 0.12 %), and it showed that the leaves was dried enough for further extraction processes. The ash content (6.77 ± 0.28 %) was compared to the data of the plant's bark. Interestingly, the ethanolic soluble content (27.28 ± 1.30 %) is much higher than about 6% reported for the plant's bark. The extract yield was about 30 %, while the yields of the fractions ranged from 8 to 65%, and there were differences in their physical colors. The extract yield in this study that used a semi-continuous repeated percolation method, is higher than the yields of 11 % [9] and 27 % [25] reported when a maceration procedure was used. However, the extract yield is comparable to a yield of about 35 % earlier reported for dried *M. azedarach* fruits [26]. Apart from differences in extractions, plant parts, and plant growth conditions (e.g. environments) will affect extract yields.

With respect to the yields of the fractions, ethyl acetate yielded the least, possibly because of the semi polarity characteristic of the solvent, while water yielded the highest. Both the extract and the fractions contained tannins, flavonoids, saponins, steroids, alkaloids and glycosides (Table 1), and these compounds had also been reported by Ahmad et al. [9]. It can be observed in Table 1 that the fractions showed differences in the phytochemical profiles.

Table 1

The quality parameters of *M. azedarach* dried leaves, extract and fractions*.

Parameter	Dried leaves			
Identity	<i>Melia azedarach</i> leaves			
Macroscopic appearance	Medium and deciduous tree, leaves is opposite non-decussate phyllotaxis with specific inipinnate (imparipinnate) compound leaves, 3–8 cm long, serrated edge, typical smelling when squeezed, dark green to pale green colour			
Ethanol soluble extract content (%)				27.28 ± 1.30
Water soluble extract (%)				33.85 ± 0.56
Total ash content (%)				6.77 ± 0.28
Drying shrinkage (%)				1.67 ± 0.12
Parameter	Ethanolic extract (E)	Fractions		
		Hexane (FH)	Ethyl acetate (FE)	Water (FW)
Appearance	Greenish black thick extract	Dark green, oily extract	Dark green thick extract	Brownish viscous extract
Yield (%)	30.1±0.42	27.30±1.03	8.38±0.55	64.71±2.54
Tannin	+	–	–	+
Flavonoid	+	–	+	+
Saponin	+	–	+	+
Steroid	+	+	+	–
Alkaloid	+	+	+	+
Glycoside	+	–	+	+
Anthraquinone	–	–	–	–

For the yields, values with different superscripts are significantly different (p < 0.05).

+ = present, - = absent.

* E = ethanolic extract, FH = hexane fraction, FE = ethyl acetate fraction and FW = water fraction.

Table 2
IC₅₀T47D, IC₅₀AA TPC and SC of the *M. azedarach* extract and fractions*.

Samples	(μg/mL)		(μg/mg)		
	IC ₅₀ T47D	IC ₅₀ AA	FRAP (RE/ sample)	TPC (GAE/ sample)	SC (βSE/ sample)
E	628.05 ± 35.69 ^c	232.00 ± 11.00 ^b	106.20 ± 1.53 ^b	102.18 ± 2.59 ^c	5.49 ± 0.37 ^b
FH	757.09 ± 21.57 ^d	574.25 ± 29.71 ^c	55.08 ± 2.64 ^a	16.33 ± 0.23 ^a	17.38 ± 2.47 ^c
FE	147.90 ± 8.49 ^b	211.89 ± 10.86 ^b	157.75 ± 2.51 ^d	109.43 ± 3.54 ^d	5.04 ± 0.12 ^b
FW	820.26 ± 8.25 ^e	229.32 ± 8.10 ^b	144.84 ± 2.67 ^c	55.72 ± 1.36 ^b	nd ^a
R	–	11.78 ± 0.74 ^a	–	–	–
Doxo	3.23 ± 0.22 ^a	–	–	–	–

* IC₅₀ = inhibition concentration, T47D = T47D cell line, AA = DPPH antioxidant activity, FRAP = ferric reducing antioxidant potency, RE = rutin equivalence, TPC = total phenolic content, GAE = gallic acid equivalence, SC = equivalent to β-sitosterol content, βSE = β-sitosterol equivalence, R = rutin, Doxo = doxorubicin, nd = not detectable, – = not tested. TPC linear regression equation $y = 0.0020x + 0.0144$, $R^2 = 0.9993$; SC $y = 624.32x + 331.10$, $R^2 = 0.9982$; while rutin linearity $y = 0.0009x + 0.0048$, $R^2 = 0.9803$). Different superscript letters between the means in the same column, are statistically different significantly ($p < 0.05$).

3.2. Cytotoxicity and antioxidant activities

Table 2 presents the cytotoxicity (IC₅₀T47D) of the samples that ranged from 148 (FE) to 820 μg/ml (FW). The IC₅₀ antioxidant of the samples ranged from 212 (E) to 574 μg/ml (FH), while their FRAP was from 55 (FH) to 158 (FE) μg/mg rutin equivalent. The sequence of potential cytotoxicity was FE > E > FH and FW, while for both (DPPH and FRAP) antioxidant activities were FE > FW > E and FH (Fig. 1). The FE was effective on the T47D cells at a lower IC₅₀ compared to vero cells (>1000 μg/mL) [10], though it was not as strong as doxorubicin (3.23 ± 0.22 μg/mL), the chemotherapy drug positive control. Wu et al. [27] found that three out of six cytotoxic steroids from ethyl acetate fractions of an ethanolic extract of *M. azedarach* leaves were effective against human cancer cell lines A549, H460 and U251. The three cytotoxic steroids were (20S)-5,24(28)-ergostadiene-3β,7α,16β,20-tetrol, (20S)-5-ergostene-3β,7α,16β,20-tetrol and 5-stigmastene-3,7,20-triol with an IC₅₀ of about 12 to >80 μg/ml compared to IC₅₀ of about 7–15 μg/mL of 5-fluorouracil. Other reported cytotoxic compounds from the plant are tirucallane triterpenes from dichloromethane and trichilin from chloroform solubles of methanolic extracts of the plant's fruits and root barks [28,29]. Furthermore, melianones exhibited high cytotoxic effects (IC₅₀ 3.6 μg/mL), while 21-β-acetoxymelianone and 3-α-tigloylmelianol were classified as having moderate antiproliferative effects (IC₅₀ of 100 and 91.8 μg/mL), whereas there were no reports on cytotoxic or antiproliferative effects of methyl kulonates. Among limonoids isolated from *Melia* root bark, it was 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin that showed significant cytotoxic activities against P388 lymphocytic leukemia (1.5 μg/mL). The structure activity revealed the influence of C-3 and C-20 acetate substituents, though 1-deoxy structures decreased the cytotoxicity. Furthermore, the trichillins had strong cytotoxic activities in a range of 0.011–5.4 μg/mL, with 12-deacetyltrichilin being the most cytotoxic substance against P388 cells [29]. These results showed the importance of purification and isolation of potential substances to enhance cytotoxic activities, especially to selected breast cancer cell lines.

The IC₅₀AA and FRAP values of FE were the lowest (Table 2). Previous results showed young leaves exhibited higher DPPH antioxidant activities than old leaves [14], while Orhan et al. [30] found ethyl acetate extracts of the plant's leaves and fruits to be most notable in iron and ferrous metal-chelating assays. The flavonoid quercetin-3-O-neohesperidoside, rutin, kaempferol-3-O-rutinoside and kaempferol-O-dihexoside were found as the main radical scavengers [14]. Other studies also found that rutin, quercetin-3-O-neohesperidoside and kaempferol had potent DPPH radical scavenging activities (IC₅₀ of 4–6 μM) [31,32]. The chemical structures of rutin and quercetin-3-rutinoside-7-rhamnoside had glycosylation position, esterified sugar type, 2,3-double bond in

conjugation with 4-oxo function in the C-ring and the twist angles of the B-ring compared to the A- and C-rings that determine its ability to scavenge free radicals [33]. It was also found that kaempferol-3-O-rutinoside exhibited strong antioxidant activities. Jafari et al. [11] isolated rutin, kaempferol-3-O-robinobioside, kaempferol-3-O-rutinoside, and isoquercetin (flavonol 3-O-glycosides) also from methanolic extracts of *M. azedarach* leaves. The flavonols in the leaves were highly associated with their medicinal effects.

3.3. Total phenolic and β-sitosterol contents

The results of TPC and βSE linear regression equations were $y = 0.0020x + 0.0144$, $R^2 = 0.9993$ and $y = 624.3201x + 331.1001$, $R^2 = 0.9982$). The purple spot of TLC β-sitosterol was in a good separation (R_f 0.67 and R_s 0.98–1.2) from other compounds. Table 2 also presents the TPC (16–109 μg GAE/mg) and SC (5–17 μg βSE/mg) of the extract and fractions, with the latter was not detected in the FW sample. Ethanol had been shown to better extract phenolic compounds from *M. azedarach* than water and petroleum ether [9]. Other studies have shown that ethanol, ethyl acetate and water also solubilize phenolic compounds, while β-sitosterol is more soluble in ethyl acetate and hexane [34]. Nampoothiri et al. [35] also found that ethyl acetate fractions exhibited higher antioxidant, DPPH, and others radical (superoxide and hydroxyl) scavenging activity than hexane and water fractions.

3.4. The LC-MS of the ethyl acetate fraction

Being the most cytotoxic (Table 2), the FE sample was processed on to understand and identify its constituents. The LCMS had been

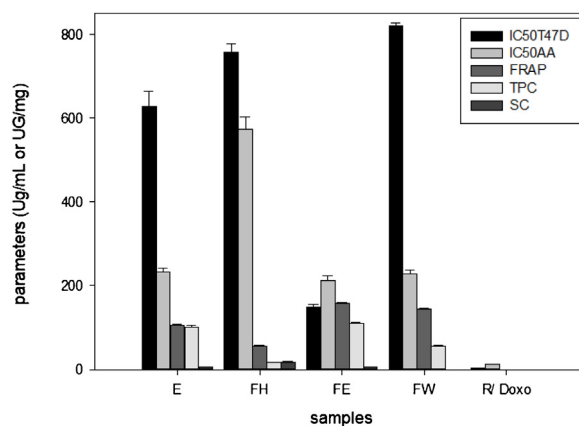


Fig. 1. Parameters histogram of the samples result. The lower IC₅₀AA and IC₅₀T47D of FE showed the potent of its antioxidant and cytotoxic than others samples.

used to predictive *Melia's* FE fraction compounds. Though not ascertain as isolate identification, it provides more specific screening of phytochemical compounds compare to conventional method. Fig. 2 shows 32 peaks of LC separation of the FE fraction. They revealed 15 peaks with percentage area above 1% and ion fragments of the FE fraction phytocontents. The peaks were predictive as saponins (steroid glycoside), limonoids, triterpenoids and polyphenolics compounds (Table 3). The highest percentage was saponin with 40.80 % on Rt 18.438 min, while the lowest was the flavonoids with 4.68 % on Rt 5.364 and 8.841 min.

The quercetin was obtained with m/z [M+H]⁺ 303. Flavonoid quercetin-7-O- β -D-glucopyranoside was obtained with ion parents 465 compare to its data on [M] 464.6 [36]. The compounds on Rt 8.841 min had similar fragmentation pattern, but higher m/z value on 763 which was assumed as quercetin-3-O-(2'',6''-digalloyl)- β -D-galactopyranoside [M] 762.7 [36–38]. The MS result can not differentiate C3 or C7 bond glycoside on the aglycone flavonoid, accordingly though. Two peaks revealed toosendanin and its derivate, which have m/z at 557 (R_t 11.032 min, 6.53 % and 12.169 min, 1.62 %) [15,39]. It was also observed the ions fragmentation at m/z 497, 479, 437, and 419; which were identified as existence of two acetoxy and two hydroxyl groups in toosendanin as the fragments of [M+H-H₂O-CH₃COOH]⁺, [M+H-2H₂O-CH₃COOH]⁺, [M+H-H₂O-2CH₃COOH]⁺, and [M+H-2H₂O-2CH₃COOH]⁺, respectively [39]. The 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin with m/z [M+H]⁺ 697 was observed on Rt 11.474 min with 6.53 % [37,38]. The 12-hydroxyamoorastatin and its acetyl derivate were on Rt 16.942 and 16.521min with 4.16 % and 9.39 % respectively [40]. Highly percentage of steroids and terpen saponins compounds were detected on 18.438 min (40.80 %) and Rt 12.802 (2.50 %), while triterpenoids aglycone was on Rt 17.68 min (4.90 %). Sterol fragment was observed at R_t 18.438 min (m/z of 275 with (M+-C₃H₇O)18-CH₃+side chain) with 16-ketosteroids cleavage, which was identified as β -sitosterol glucoside [M+H]⁺ 577 compound [12,36,37]. Others compounds were analyzed as meliarachin, salannin, and salannal as listed in Table 3. Salannin and salannal (R_t 13.265 min,

1.14 %; 7.987 min, 1.31 %) [41], and meliacarpinin derivatives (R_t 11.474 min, 6.35 %) [37,42,43]. The identification process based on comparison with the most identical fragments to available references data, though others may have similarity only some part to quite different. The varieties on the technical analysis method and the limitation of the data were leading to the used of references available on the experiment or the importance of isolation to identification of the substances further. The environment influence and local variety of the *M. azedarach* wild type provide opportunity to obtain new or modified of its chemical entities. For example quercetin glycoside at Rt 8.841 min was found have longer glycoside and not identical with the references available. Others of saponin and triterpen at Rt 18.438, 17.680 and 12.802 min were identical with references data [36–38], but had not been reported on the *Melia's* content yet. Some compounds resulted specific R_t and fragmentations ion pattern such as meliatoxin, meliarachin, β -sitosterol and flavonoids, which were suggested to be used as biomarker for *Melia* extracts [12].

3.5. Correlation analysis among activities and contents

Correlation analysis between the activity parameters revealed negative and positive significant outcomes. TPC significantly ($p < 0.05$) correlated with parameters IC₅₀AA (-0.845), FRAP (0.695) and IC₅₀T47D (-0.709), while IC₅₀T47D significantly correlated with IC₅₀AA (0.671), but there were no significant correlations between SC and others. Though they have small percentage in the fraction, phenolic compounds play important roles in cytotoxicity and antioxidant activities. The significant correlations reported were consistent with these relationships. Positive correlations had been reported for pomegranate cytotoxicity and TPC on MDA-MB-231 (0.980); and total flavonoid content to HT-29 cell line cytotoxic (0.864) [44]. Grigalius and Petrikaite [45] suggested the structure-activity relationships of both antioxidant and anticancer activities were due to ortho-dihydroxy group in ring C of flavonoids. The ortho-dihydroxy was obtained (about 4.68 %) in FE as quercetin glycoside (Table 3). Quercetin was

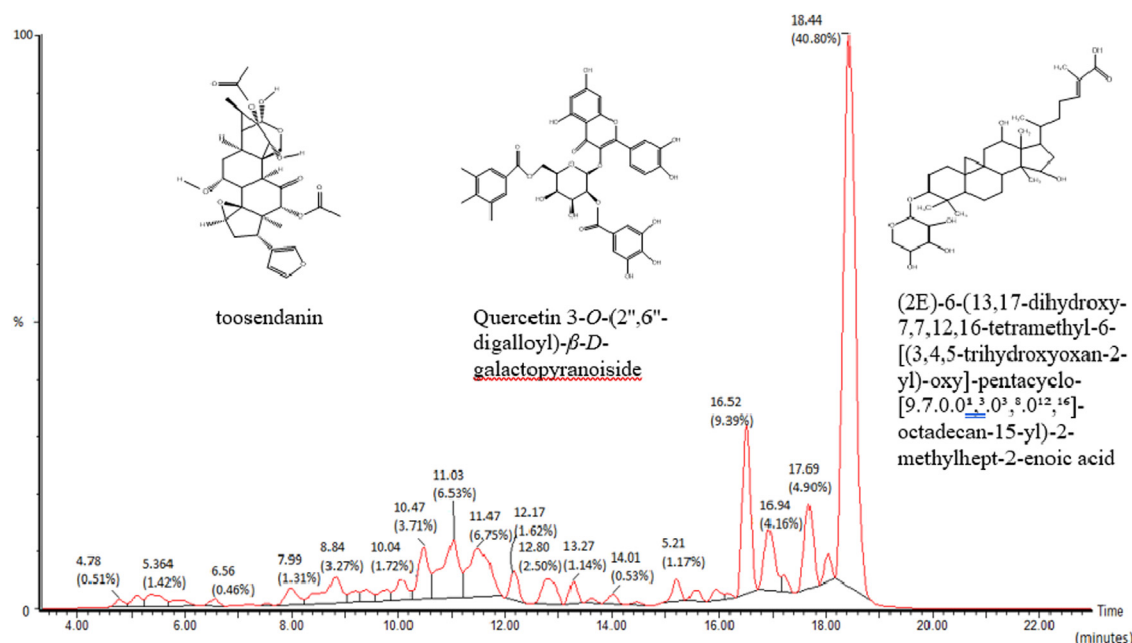


Fig. 2. LC- EI MS chromatogram results analysis and predictive phytochemicals content from the *M. azedarach* leaves ethyl acetate fraction. It contents of limonoid (toosendanin, meliacarpinin, 12-hydroxyamoorastatin and their derivatives, meliarachin, salannin and salannal), flavonoid glycosides (quercetin-7-O- β -D-glucopyranoside and quercetin 3-O-(2'',6''-digalloyl)- β -D-galactopyranoside), saponins (triterpene and steroid glycosides), and triterpene aglycone.

Table 3
Predictive compounds of FE *M. azedarach*.

Peak No	Rt (minutes)	% area	m/z [M+H]	Predictive compounds [molecular formula, M]	References
4	5.364	1.42	465	Quercetin-7-O- β -D-glucopyranoside [C ₂₁ H ₂₀ O ₁₂ , 464.4]	[37,38]
8	7.987	1.31	613	Salannal [C ₃₄ H ₄₄ O ₁₀ , 612.716]	[37]
9	8.841	3.27	763	Quercetin 3-O-(2'',6''-digalloyl)-Beta-D-galactopyranoside [C ₃₈ H ₃₄ O ₁₇ , 762.7]	[37]
13	10.041	1.72	573	Meliarachin B [C ₃₀ H ₃₆ O ₁₁ , 572.6]	[37]
14	10.474	3.71	683	(2R,3S,4S,5R,6S)-3,4-dihydroxy-6-[3,7,8-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxochromen-5-yl]-oxy-5-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]-oxyoxan-2-yl]-methyl acetate [C ₃₀ H ₃₄ O ₁₈ , 682.1745]	[36]
15	11.032	6.53	575	Toosendanin [C ₃₀ H ₃₈ O ₁₁ , 574.616]	[15,39]
16	11.474	6.75	697	Methyl (23S)-7,14,23-trihydroxy-4-methoxy-6,16,22-trimethyl-25-[(E)-3-phenylprop-2-enoyl]-oxy-3,9,11,17,20-pentaoxaocyclo [17.6.1.18,15.01,5.06,18.07,16.010,14.022,26]-heptacos-12-ene-4-carboxylate (1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin) [C ₃₀ H ₄₉ O ₁₈ , 696.7]	[37]
17	12.169	1.62	557,497	toosendanin derivatives [M-H ₂ O+H] ⁺ at 557.4 and [M-CH ₃ COO+H] ⁺ at 497.2	[15,39]
18	12.802	2.50	537	Cyclopenta[c]pyran-4-carboxylic acid, 1-(β -D-glucopyranosyloxy)-1,4a,5,6,7,7a-hexahydro-5-hydroxy-7-methyl-6-[(E)-1-oxo-3-phenyl-2-propen-1-yl]-oxy- methyl ester, [C ₂₆ H ₃₂ O ₁₂ , 536.1894]	[36]
19	13.265	1.14	597	Salannin [C ₃₄ H ₄₄ O ₉ , 596.7]	[37,41]
23	15.214	1.17	547	Meliarachin H/I [C ₂₉ H ₃₈ O ₁₀ , 546.6]	[37]
27	16.521	9.39	593, 533	12-hydroxyamoorastatin - acetyl derivate [C ₃₀ H ₄₀ O ₁₂ , 592.23]	[37,40]
28	16.942	4.16	533	12-hydroxyamoorastatin [C ₂₈ H ₃₆ O ₁₀ , 532.2308]	[37,40]
30	17.68	4.90	637	5-[17-(5,6-dihydroxy-6-methylheptan-2-yl)-3,12-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-2-yl]-oxy-3-hydroxy-3-methyl-5-oxopentanoic acid [C ₃₆ H ₆₀ O ₉ , 636.4237]	[36]
32	18.438	40.80	621	(2E)-6-(13,17-dihydroxy-7,7,12,16-tetramethyl-6-[(3,4,5-trihydroxyoxan-2-yl)-oxy]-pentacyclo-[9.7.0.0 ^{1,3} .0 ^{3,8} .0 ^{12,16}]-octadecan-15-yl)-2-methylhept-2-enoic acid [C ₃₅ H ₅₆ O ₉ , 620.3924]	[36]

found as powerful hypochlorous acid, chloramines, nitric oxide, and superoxide scavengers; and also cytotoxic to red blood cell haemolysis, compared than that of kaempferol and isoquercitrin [46]. Quercetin significantly inhibited human breast cancer cells (MCF-7 and MDA-MB231), and moreover it mentioned has the cytoprotective role against oxidative stress through antioxidant effect, motivating apoptotic cell death via prooxidant activity, and inhibiting tumourigenesis [47].

Furthermore, Ashraf et al. [48] found that steroids and triterpene saponins were cytotoxic against MCF-7. Podolak et al. [49] highlighted the potential of saponins as anticancer. He found the important factors responsible for improving the cytotoxicity including structural feature, number and the sequence of sugar residues in a carbohydrate chain, also the position of sugar attachment to the aglycone. The cytotoxicity was enhance with the prolonged of the sugars chain. The saponins were stimulate apoptotic process in tumor cells, in intrinsic pathway mostly. Non apoptotic processes were also involved as cell cycle arrestment, autophagic cell, death stimulation, inhibiting of metastasis and cytoskeleton, including disintegration of the cell. Saponins are also promising as inhibiting tumor cells angiogenesis and recombinant protein toxins. Furthermore saponins have physiologically binding to nuclear receptors activity, including to conventional steroid hormone receptors (estrogen receptor, glucocorticoid receptor, mineralocorticoid receptor, and androgen receptor) and the orphan receptors (peroxisome proliferatoractivated receptor (PPAR), liver X receptor (LXR), farnesoid X receptor (FXR), and Pregnane X receptor (PXR)) [50].

Akhihisa et al. [51] revealed limonoid trichillin type of meliarachin C and salannin type of 3-O-deacetyl-40-demethyl-28-oxosalannin are cytotoxic to HL-60 cells by inducing apoptotic cell death. Zhou et al. [43] observed limonoids tetracyclic sendanin, trichillin and C-*seco* limonoid types with 14,15-epoxide ring and a C-19/C-29 acetal bridge exhibit very strong and strong cytotoxicity antiproliferation against Hela S3 (human epithelial cancer) cell line and against P388 cells. Yadav et al. [52] found the role of neem

limonoids in mitochondria oxidative phosphorylation complexes, though not effective to p53 and Bax-independent. Neem limonoids are, however, useful for multiple cancers, including cancer-drug-resistant ones and as a novel for solid cancer therapy.

4. Conclusions

The ethyl acetate fraction from the *M. azedarach* L. wild type leaves ethanolic extract showed the most T47D bio-selective hormonal cytotoxicity and antioxidant activities. Significant correlations among TPC, IC₅₀T47D, IC₅₀AA and FRAP activities were obtained to lend credence to compound-antioxidant-cytotoxicity relationships. The ethyl acetate fraction contain flavonoid and triterpenoid saponins, triterpenoid, ortho-dihydroxy flavonols of quercetin glycosides, limonoid toosendanin and its derivate, 12-hydroxyamoorastatin and its acetyl derivate, 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin, meliarachin, salannin and salannal. The compounds were potent against the breast cancer cells and showed promises in breast cancer managements.

Ethical statement for biotechnology reports

Hereby, I /insert author name/ consciously assure that for the manuscript /insert title/ the following is fulfilled:

- 1) This material is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.

- 6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- 7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

To verify originality, your article may be checked by the originality detection software iThenticate. See also <http://www.elsevier.com/editors/plagdetect>.

I agree with the above statements and declare that this submission follows the policies of Solid State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Acknowledgments

The authors are grateful to the Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia for PUPT Ristek DIKTI 2019 and PKPI-sandwich like program 2019.

Appendix A. Supplementary data

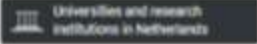
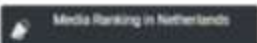
Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.btre.2020.e00437>.

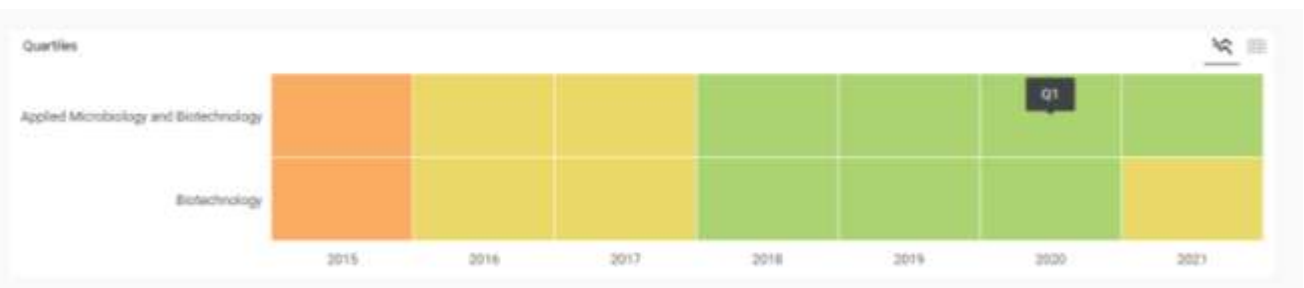
References

- [1] F. Bray, J. Ferlay, I. Soerjomataram, Global Cancer Statistics 2018 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, (2018), pp. 394–424, doi:<http://dx.doi.org/10.3322/caac.21492>.
- [2] H. Joensuu, Systemic chemotherapy for cancer: from weapon to treatment, *Lancet Oncol.* 9 (2008) 304.
- [3] K.A. Conklin, Chemotherapy associated oxidative stress: impact on chemotherapeutic effectiveness, *Integr. Cancer Ther.* 3 (2004) 294–300.
- [4] A. Prakash, O. Kumar, A.P. Kumar, Anticancer potential of plants and natural products: a review, *Am. J. Pharmacol. Sci.* 1 (2013) 104–115.
- [5] D.J. Mabberley, A monograph of Melia in Asia and the Pacific. The history of white cedar and Persian lilac, *Gard. Bull.* 37 (1984) 49–64. <http://www.cabi.org/ISC/abstract/19840326280>.
- [6] D. Sharma, Y.P. Singla, Preliminary and pharmacological profile of *Melia azedarach* L.: an overview, *Int. J. Appl. Pharm. Sci. Res.* 3 (2013) 133–138, doi:<http://dx.doi.org/10.7324/IJAPS.2013.31224>.
- [7] M. Ervina, Sukardiman, A review: *Melia azedarach* L. as a potent anticancer drug, *Pharmacogn. Rev.* 12 (2018) 94–102, doi:http://dx.doi.org/10.4103/phrev.phrev_41_17.
- [8] M. Zahoor, M. Ahmed, S. Naz, M. Ayaz, Cytotoxic, antibacterial and antioxidant activities of extracts of the bark of *Melia azedarach* (China Berry), *J. Nat. Prod. Res.* 29 (12) (2015) 1170–1172, doi:<http://dx.doi.org/10.1080/14786419.2014.982649>.
- [9] M.F. Ahmad, A.S. Rao, S.R. Ahemad, M. Ibrahim, Phytochemical studies and antioxidant activity of *Melia azedarach* Linn. Leaves by DPPH scavenging assay, *Int. J. Pharm. App.* 3 (2012) 271–276.
- [10] V. Srinivasan, R. Panneerselvam, S. Gunasekaran, S. Palani, Nephro-protective activity of ethanolic extract of *Melia azadirach* against H₂O₂ induced toxicity in vero cell line, *Int. J. App. Biol. Pharm. Tech.* 6 (2015) 44–49.
- [11] M. Jafari, S. Saeidnia, S. Hajimehdipoor, H. Ardekani, M.R.S. Faramarzi, M.A. Hadjiakhoondi, A. Khanavi, Cytotoxic evaluation of *Melia azedarach* in comparison with, *Azadirachta indica* and its phytochemical investigation, *DARU, J. Pharm. Sci.* 21 (2013), doi:<http://dx.doi.org/10.1186/2008-2231-21-37>.
- [12] A. Inada, M. Kobayashi, T. Nakanishi, Phytochemical studies on Meliaceae plants. III structures of two new pregnane steroids, toosendansterols A and B, from leaves of *Melia toosendan* et Zucc, *Chem. Pharm. Bull.* 36 (1998) 609–612.
- [13] V.E. Cornelio, M.R. Forim, B. Perlati, J.B. Fernandes, P.C. Vieira, M.P. Napolitano, R.A. Yost, M.F.G.F. Da Silva, Identification of meliatoxins in *Melia azedarach* extracts using mass spectrometry for quality control, *Planta Med.* 83 (2017) 312–317, doi:<http://dx.doi.org/10.1055/s-0042-115773>.
- [14] Y. Mrabet, N. Rokbeni, S. Cluzet, A. Boulila, T. Richard, S. Krisa, L. Marzouki, H. Casabianca, K. Hosni, Profiling of phenolic compounds and antioxidant activity of *Melia azedarach* L. leaves and fruits at two stages of maturity, *Ind. Crops Prod.* 107 (2017) 232–243, doi:<http://dx.doi.org/10.1016/j.indcrop.2017.05.048>.
- [15] E.S. Ong, C.M. Ong, Qualitative and quantitative analysis of toosendanin in *Melia toosendan* Sieb. et Zucc (Meliaceae) with liquid chromatography/ tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 21 (2007) 589–598.
- [16] K. Takeya, Z.S. Qiao, C. Hirobe, H. Itokawa, Cytotoxic azadirachtin-type limonoids from *Melia azedarach*, *Phytochemistry* 42 (1996) 709–712, doi:[http://dx.doi.org/10.1016/0031-9422\(96\)00044-1](http://dx.doi.org/10.1016/0031-9422(96)00044-1).
- [17] S. Yu, T. Kim, K.H. Yoo, K. Kang, The T47D cell line is an ideal experimental model to elucidate the progesterone-specific effects of a luminal subtype of breast cancer, *Biochem. Biophys. Res. Commun.* 486 (2017) 752–758, doi:<http://dx.doi.org/10.1016/j.bbrc.2017.03.114>.
- [18] WHO, Quality Control Methods for Herbal Materials, (2011) .
- [19] N.R. Farnsworth, Biological and phytochemical screening of plants, *J. Pharm. Sci.* 69 (1966) 225–268.
- [20] Cancer Chemoprevention Research Center (CCRC), Cytotoxic Test With MTT Method, Faculty of Pharmacy Gajah Mada University, 2013.
- [21] M. Ervina, H.S. Lie, J. Diva, Caroline, S. Tewfik, I. Tewfik, Optimization of water extract of *Cinnamomum burmannii* bark to ascertain its *in vitro* antidiabetic and antioxidant activities, *Biocatal. Agric. Biotechnol.* 19 (2019), doi:<http://dx.doi.org/10.1016/j.bcab.2019.101152>.
- [22] M. Vijayalakshmi, K. Ruckmani, Ferric Reducing Anti-oxidant Power Assay in Plant Extract, (2016), pp. 570–572, doi:<http://dx.doi.org/10.3329/bjp.v11i13.27663>.
- [23] R.C. Sutar, S.B. Kasture, V.K. Kalaichelvan, Identification, quantification and validation of β -sitosterol from *Holoptelea integrifolia* (roxb.) planch using high performance thin layer chromatography method, *Int. J. Pharm. Pharm. Sci.* 6 (2014) 249–252.
- [24] J. Danaceau, Successful Sample Preparation Strategies for LC/MS/MS Analysis of Drugs in Complex Technical Biological Matrices for Forensic Toxicology Applications, (2013) http://www.waters.com/webassets/cms/events/docs/FINALSamplePrepStrategiesClinical_Forensic_11_5_13.pdf, accessed 10 September 2018.
- [25] Y. Irshan, I.H. Suparto, S. Sulistiyani, Cytotoxic and antiproliferative activities of *Melia azedarach* leaves ethanolic extract on A549 human lung cancer cells, *J. Jamu Indone.* 2 (2017) 17–24, doi:<http://dx.doi.org/10.29244/jji.v2i1.26>.
- [26] M.C. Carpinella, G.G. Herrero, R.A. Alonso, S.M. Palacios, Antifungal activity of *Melia azedarach* fruit extract, *Fitoterapia* 70 (1999) 296–298, doi:[http://dx.doi.org/10.1016/S0367-326X\(99\)00009-X](http://dx.doi.org/10.1016/S0367-326X(99)00009-X).
- [27] S.B. Wu, Y.P. Ji, J.J. Zhu, Y. Zhao, G. Xia, Y.H. Hu, J.F. Hu, Steroids from the leaves of Chinese *Melia azedarach* and their cytotoxic effects on human cancer cell lines, *Steroids* 74 (2009) 761–765, doi:<http://dx.doi.org/10.1016/j.steroids.2009.04.005>.
- [28] N.G. Ntalli, F. Cottiglia, C.A. Bueno, L.E. Alché, M. Leonti, S. Vargiu, E. Bifulco, U. Menkissoglu-Spiroudi, P. Caboni, Cytotoxic tirucallane triterpenoids from *Melia azedarach* fruits, *Molecules* 15 (2010) 5866–5877, doi:<http://dx.doi.org/10.3390/molecules15095866>.
- [29] K. Takeya, Z.S. Qiao, C. Hirobe, H. Itokawa, Cytotoxic trichilin-type limonoids from *Melia azedarach*, *Bioorg. Med. Chem. Lett.* 4 (1996) 1355–1359, doi:[http://dx.doi.org/10.1016/0968-0896\(96\)00128-9](http://dx.doi.org/10.1016/0968-0896(96)00128-9).
- [30] I.E. Orhan, E. Guner, N. Ozturk, F.S. Senol, S.A. Erdem, M. Kartal, B. Sener, Enzyme inhibitory and antioxidant activity of *Melia azedarach* L. naturalized in Anatolia and its phenolic acid and fatty acid composition, *Ind. Crop. Prod.* 37 (2012) 213–218, doi:<http://dx.doi.org/10.1016/j.indcrop.2011.11.028>.
- [31] H. Matsuda, T. Morikawa, T. Ishiwada, H. Managi, M. Kagawa, Y. Higashi, M. Yoshikawa, et al., Medicinal flowers:VIII. Radical scavenging constituents from the flowers of *Prunus mume*: structure of prunose III, *Chem. Pharm. Bull.* 51 (2003) 440–443.
- [32] R. Zhang, B.L. Zhang, T. He, T. Yi, J.P. Yang, B. He, Increase of rutin antioxidant activity by generating maillard reaction products with lysine, *Bioorg. Med. Chem. Lett.* 26 (2016) 2680–2684.
- [33] D. Su, H. Ti, R. Zhang, M. Zhang, D. Wei, Y. Deng, J. Guo, Structural elucidation and cellular antioxidant activity evaluation of major antioxidant phenolics in lychee pulp, *Food Chem.* 158 (2014) 385–391.
- [34] D. Wei, L. Wang, C. Liu, B. Wang, β -Sitosterol solubility in selected organic solvents, *J. Chem. Eng. Data* 55 (2010) 2917–2919, doi:<http://dx.doi.org/10.1021/je9009909>.
- [35] S.V. Nampoothiri, S.S. Binil Raj, A. Prathapan, P.A. Abhilash, C. Arumughan, A. Sundaresan, *In vitro* antioxidant activities of the methanol extract and its different solvent fractions obtained from the fruit pericarp of *Terminalia bellerica*, *Nat. Prod. Res.* 25 (2011) 277–287, doi:<http://dx.doi.org/10.1080/14786419.2010.482053>.
- [36] Mona-Mass Bank of North America, (2020) . VF-NPL-QEHF023440, VF-NPL-QEHF023122, CCMSLIB00000855190 <https://mona.fiehnlab.ucdavis.edu/spectra/display/VF-NPL-QEHF010860>.
- [37] U.S. National Library of Medicine, National Center for Biotechnology Information, Pubchem, (2020) . <https://pubchem.ncbi.nlm.nih.gov/>.

- [38] Royal Society of Chemistry, Chemspider, 2020. <http://www.chemspider.com/>.
- [39] J.L. Wu, E.L.H. Leung, H. Zhou, L. Liu, N. Li, Metabolite analysis of toosendanin by an ultra-high performance liquid chromatography-quadrupole-time of flight mass spectrometry technique, *Molecules* 18 (2013) 12144–12153, doi: <http://dx.doi.org/10.3390/molecules181012144>.
- [40] J. Polonsky, Z. Varon, C. Marazano, B. Arnoux, G.R. Pettit, J.M. Schmid, M. Ochi, H. Kotsuki, The structure of amoorastatone and the cytotoxic limonoid 12-hydroxyamoorastatin, *Experientia* 35 (1979) 987–1130, doi: <http://dx.doi.org/10.1007/BF01949897>.
- [41] J. Zhu, J. Lu, X. Fan, X. Wu, R. Diao, H. Yua, R. Xu, H. Zi, A new cytotoxic salannin-class limonoid alkaloid from seeds of *Azadirachta indica* A. Juss, *Chin. Chem. Lett.* 29 (2018) 1261–1263, doi: <http://dx.doi.org/10.1016/j.ccllet.2017.11.042>.
- [42] J.B. Zhou, K. Tadera, Y. Minami, F. Yagi, J. Kurawaki, K. Takezaki, M. Nakatani, New limonoids from *Melia toosendan*, *Biosci. Biotechnol. Biochem.* 62 (1998) 496–500, doi: <http://dx.doi.org/10.1271/bbb.62.496>.
- [43] H. Zhou, A. Hamazaki, J.D. Fontana, H. Takahashi, C.B. Wandscheer, Y. Fukuyama, Cytotoxic limonoids from Brazilian *Melia azedarach*, *Chem. Pharm. Bull.* 53 (2005) 1362–1365, doi: <http://dx.doi.org/10.1248/cpb.53.1362>.
- [44] M. Eddebbagh, M. Massoudi, A. Abourriche, M. Berrada, M. Attaleb, L. Benbacher, A. Bennamara, Correlation of the cytotoxic and antioxidant activities of Moroccan pomegranate (*Punica granatum*) with phenolic and flavonoid contents, *J. Pharm. Pharmacol.* 4 (2016) 511–519, doi: <http://dx.doi.org/10.17265/2328-2150/2016.09.006>.
- [45] I. Grigalius, V. Petrikaite, Relationship between antioxidant and anticancer activity of trihydroxyflavones, *Molecules* 22 (2017), doi: <http://dx.doi.org/10.3390/molecules22122169>.
- [46] J.C.R. Velloso, L.O. Regasini, N.M. Khalil, V.S. Bolzani, O.A.K. Khalil, F.A. Manente, H.P. Netto, O.M.M.F. Oliveira, Antioxidant and cytotoxic studies for kaempferol, quercetin and isoquercitrin, *Ecl. Quím.* 36 (2011) 7–20.
- [47] S. Ranganathan, D. Halagowder, N.D. Sivasithambaram, Quercetin suppresses twist to induce apoptosis in MCF-7 breast cancer cells, *PLoS One* (2015), doi: <http://dx.doi.org/10.1371/journal.pone.0141370>.
- [48] M.F. Ashraf, M.A. Aziz, J. Stanslas, I. Ismail, M.A. Kadir, Assessment of antioxidant and cytotoxicity activities of saponin and crude extracts of *Chlorophytum borivilianum*, *The Sci.World J.* 216894 (2013) 7, doi: <http://dx.doi.org/10.1155/2013/216894>.
- [49] I. Podolak, A. Galanty, D. Sobolewska, Saponins as cytotoxic agents: a review, *Phytochem. Rev.* 9 (2010) 425–474, doi: <http://dx.doi.org/10.1007/s11101-010-9183-z>.
- [50] T. Zhang, S. Zhong, T. Li, J. Zhang, Saponins as modulators of nuclear receptors, *Cri. Rev. Food Sci. & Nut.* (2018), doi: <http://dx.doi.org/10.1080/10408398.2018.1514580>.
- [51] T. Akihisa, X. Pan, Y. Nakamura, T. Kikuchi, N. Takahashi, M. Matsumoto, E. Ogiwara, M. Fukatsu, K. Koike, H. Tokuda, Limonoids from the fruits of *Melia azedarach* and their cytotoxic activities, *Phytochemistry* 89 (2013) 59–70, doi: <http://dx.doi.org/10.1016/j.phytochem.2013.01.015>.
- [52] N. Yadav, S. Kumar, R. Kumar, P. Srivastava, L. Sun, P. Rapali, et al., Mechanism of neem limonoids-induced cell death in cancer: role of oxidative phosphorylation, *Free Radic. Biol. Med.* 90 (2016) 261–271, doi: <http://dx.doi.org/10.1016/j.freeradbiomed.2015.11.028>.

Biotechnology Reports

COUNTRY Netherlands  	SUBJECT AREA AND CATEGORY Biochemistry, Genetics and Molecular Biology └ Biotechnology Immunology and Microbiology └ Applied Microbiology and Biotechnology	PUBLISHER Elsevier BV	H-INDEX 43
PUBLICATION TYPE Journals	ISSN 2215017X	COVERAGE 2014-2021	INFORMATION Homepage How to publish in this journal



SJR

Year	SJR
2015	0.337
2016	0.625
2017	0.754
2018	0.967
2019	0.965
2020	0.932
2021	0.854





Source details

Biotechnology Reports

Open Access ⓘ

Scopus coverage years: from 2014 to Present

Publisher: Elsevier

E-ISSN: 2215-017X

Subject area: Immunology and Microbiology: Applied Microbiology and Biotechnology

Biochemistry, Genetics and Molecular Biology: Biotechnology

Source type: Journal

CiteScore 2021

8.0



SJR 2021

0.854



SNIP 2021

1.582



[View all documents >](#)

[Set document alert](#)

[Save to source list](#) [Source Homepage](#)

[CiteScore](#) [CiteScore rank & trend](#) [Scopus content coverage](#)

Improved CiteScore methodology

CiteScore 2021 counts the citations received in 2018-2021 to articles, reviews, conference papers, book chapters and data papers published in 2018-2021, and divides this by the number of publications published in 2018-2021. [Learn more >](#)

CiteScore 2021 ▾

$$8.0 = \frac{3,595 \text{ Citations 2018 - 2021}}{451 \text{ Documents 2018 - 2021}}$$

Calculated on 05 May, 2022

CiteScoreTracker 2022 ⓘ

$$12.6 = \frac{5,730 \text{ Citations to date}}{456 \text{ Documents to date}}$$

Last updated on 05 March, 2023 • Updated monthly

CiteScore rank 2021 ⓘ

Category	Rank	Percentile
Immunology and Microbiology	#19/118	84th
Applied Microbiology and Biotechnology		
Biochemistry, Genetics and Molecular Biology	#50/293	83rd
Biotechnology		

[View CiteScore methodology >](#) [CiteScore FAQ >](#) [Add CiteScore to your site &](#)