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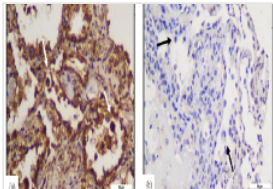


Pharmacognosy Journal Volume 14, Issue 6 (Suppl.), Nov-Dec 2022



**Medicinal Plants Adopted to Treat Children's Diseases by Traditional Pediatrics
"Women Healers" In The Souss Massa Region (Agadir Idaoutanan, Inzegane Ait
Meloul and Chtouka Ait Baha) Morocco (/article/1924)**

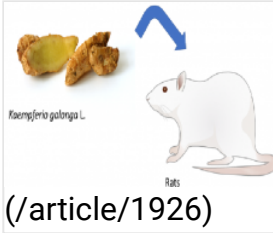
Jan 3, 2023 - 13:29



(/article/1925)

Differences in interleukin-6 and interleukin-17 expression in covid-19 post-mortem lung tissue biopsy compared with noncovid-19 (/article/1925)

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(/article/1926)

Kaempferia galanga L. Extract Administration Attenuate Aquaporin-4 Expression in Traumatic Brain Injury: An Experimental Study in Rats (/article/1926)

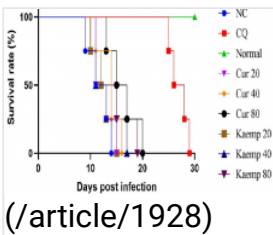
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(/article/1927)

Effect of Pomegranate Extract On N-Terminal Pro Brain Natriuretic Peptide and Asymmetric Dimethylarginine Levels in Children with Pulmonary Artery Hypertension in Acyanotic Congenital Heart Disease (/article/1927)

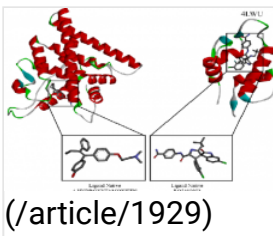
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Evaluation of Experimental Cerebral Malaria of Curcumin and Kaempferol in Plasmodium berghei ANKA-Infected Mice (/article/1928)

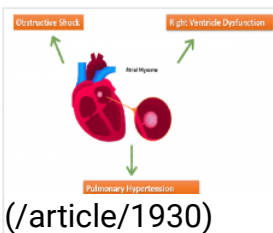
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Molecular Docking Estrogen Receptor Alpha Antagonist and P53- MDM2 Inhibitor, ADMET Prediction of Alkaloid Compound from Mitragyna speciosa for Breast Cancer Therapy (/article/1929)

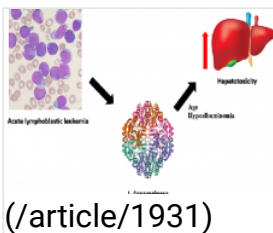
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(/article/1930)

Left Atrial Myxoma Presented with an Obstructive Shock, Right Ventricle Dysfunction and Pulmonary Hypertension (/article/1930)

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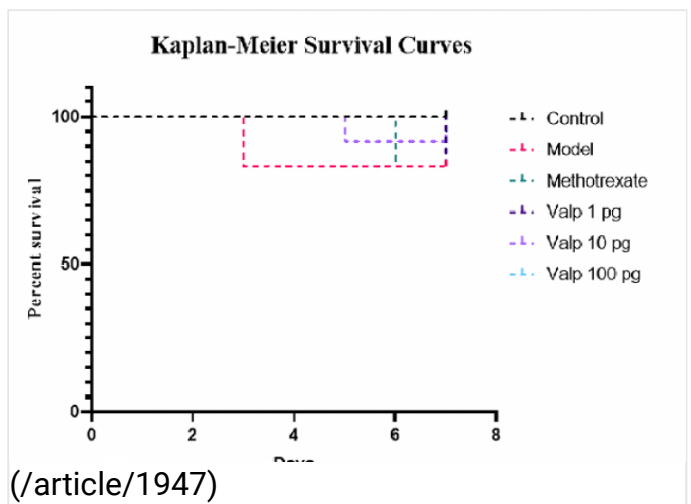
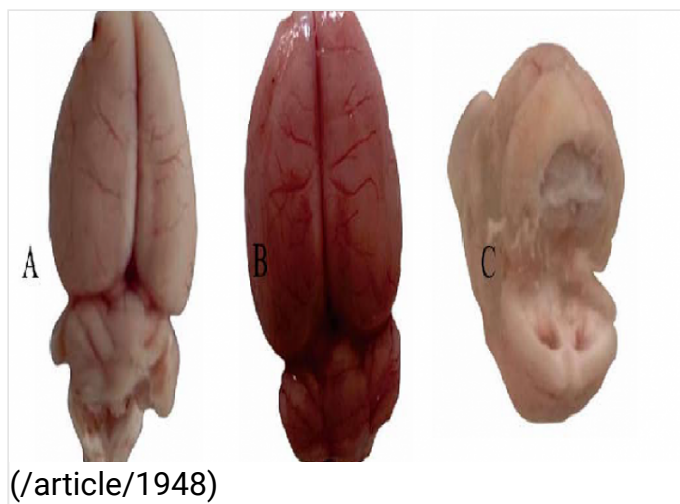
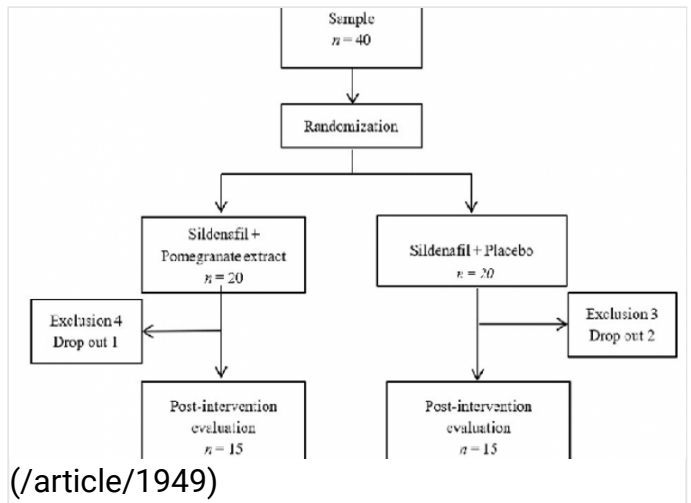
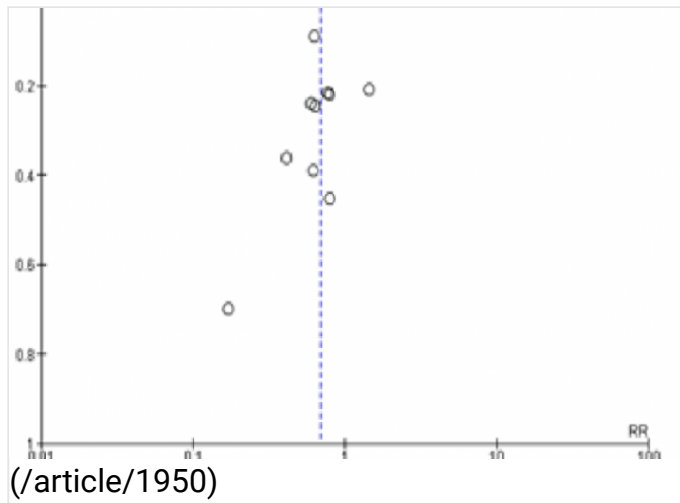
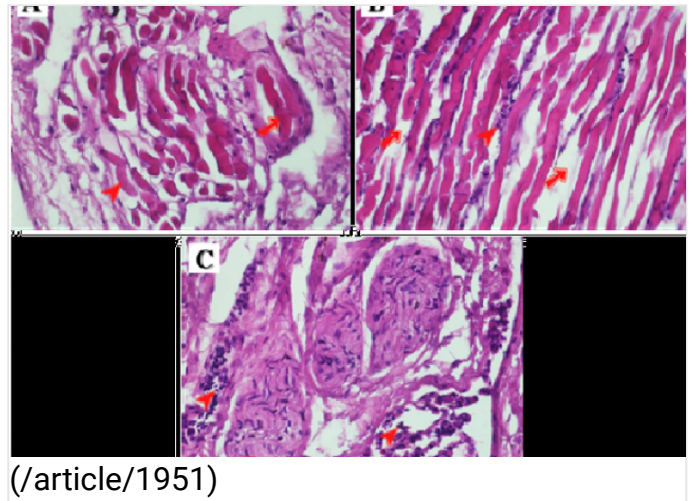
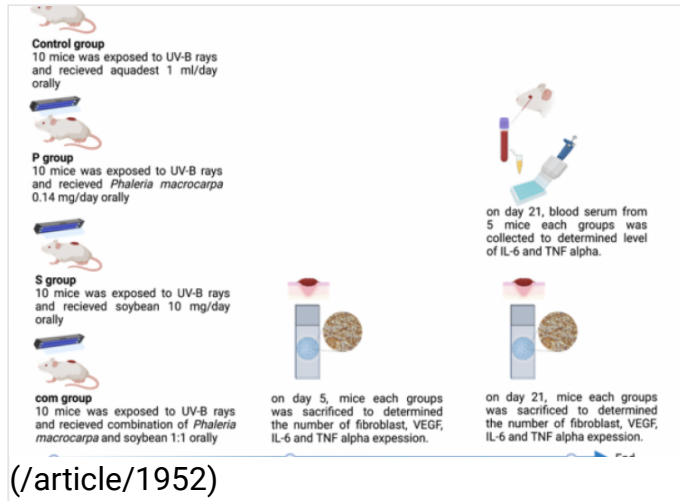
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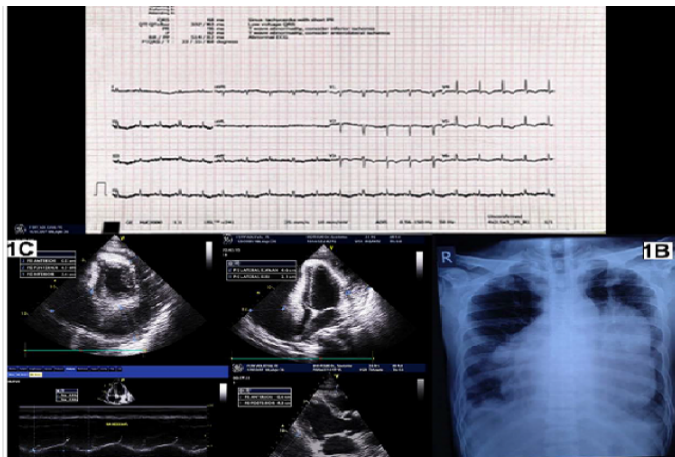
Risk Factors for Hepatotoxicity From L-Asparaginase Chemotherapy In Children With Acute Lymphoblastic Leukemia (/article/1931)

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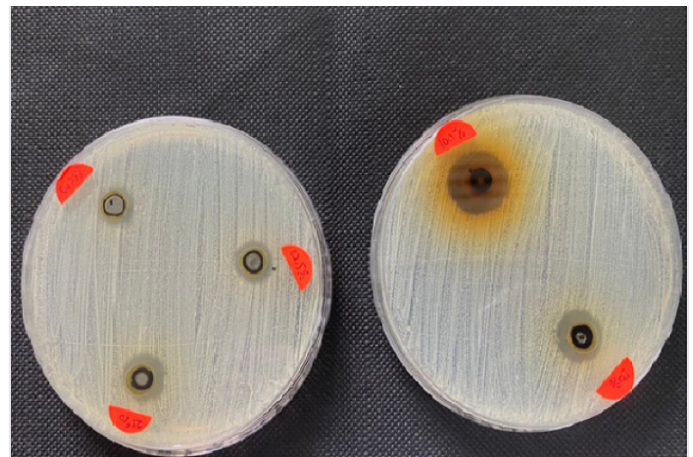
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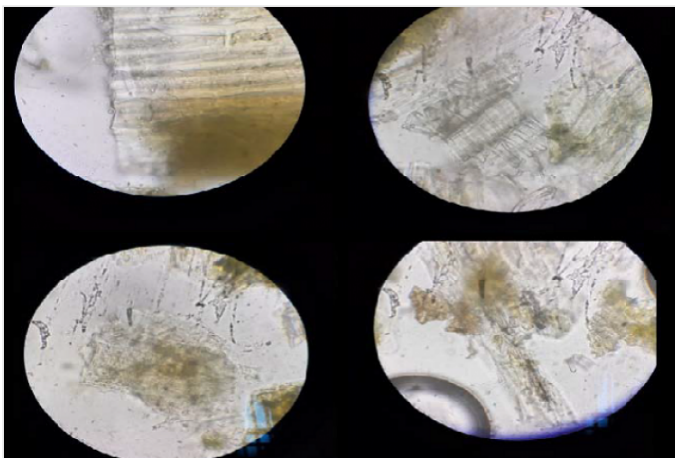




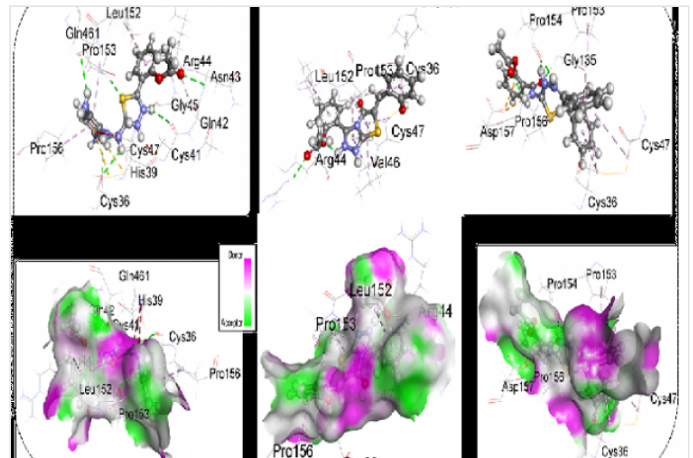
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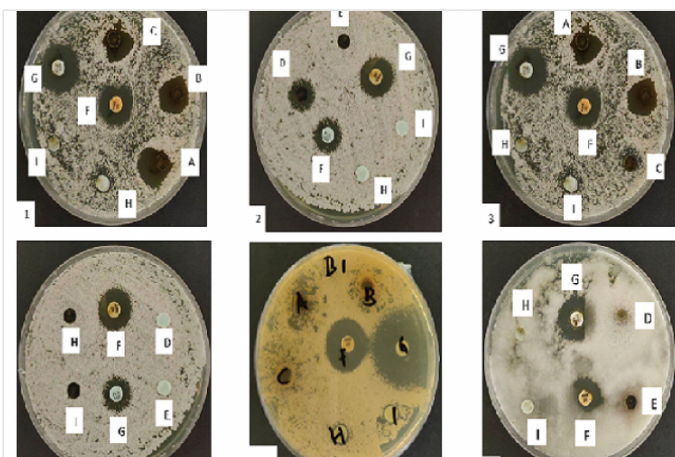
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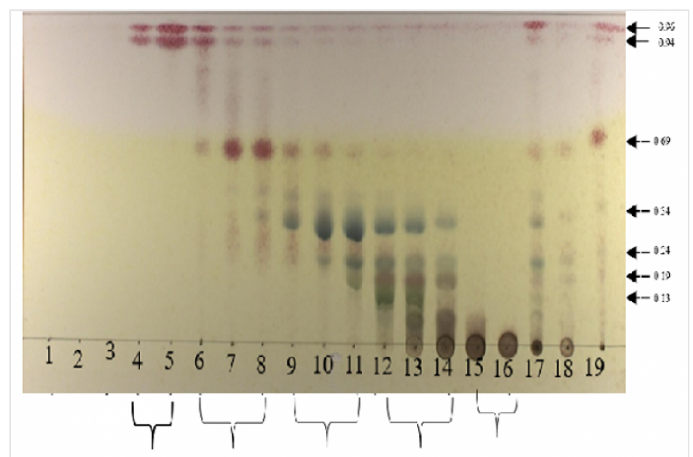
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The Role of Breast Milk on Reducing the Risk of Neonatal Sepsis in Preterm and Low Birth Weight Infants: A Systematic Review and Meta-Analysis (/article/1950)

Effects of Pomegranate Extract Supplementation (Punica granatum L.) on Clinical Manifestations of Pulmonary Arterial Hypertension in Children with Acyanotic Congenital Heart Disease (/article/1949)



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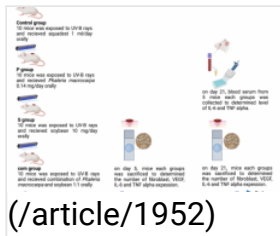
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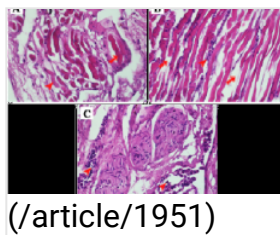
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(/article/1952)

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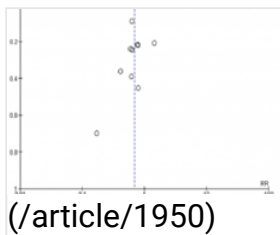
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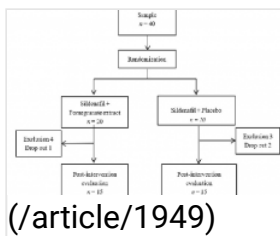
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(/article/1950)

The Role of Breast Milk on Reducing the Risk of Neonatal Sepsis in Preterm and Low Birth Weight Infants: A Systematic Review and Meta-Analysis (/article/1950)

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(/article/1949)

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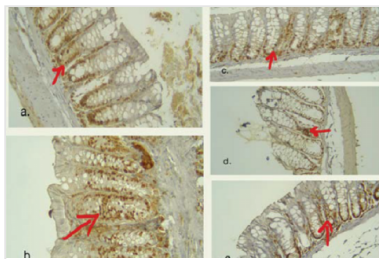
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Original Article

The Effect of Pomegranate Peel Ethanol Extract to TNF- α Expression of Mice Colonic Epithelial Cells Induced Using Dextran Sodium Sulfate (DSS) (/article/1793)

**Kusmardi Kusmardi, Raja Yasmin Khalilah, E Zuraidah, Ari
Estuningtyas, Aryo Tedjo**

Pharmacognosy Journal, 14(3):480-488

DOI: 10.5530/pj.2022.14.61

Published: Wed, 29-Jun-2022

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Original Article

Azadirachta indica Hexane Extract: Potent Antibacterial Activity Against Propionibacterium acne and Identification of its Chemicals Content (/article/1794)

Annysa Ellycornia Silvyana,Ratika Rahmasari,Berna Elya



Pharmacognosy Journal,14(3):489-496

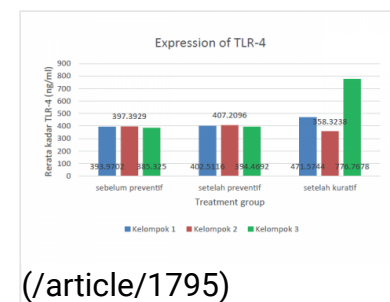
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Original Article

Toll Like Receptor 4 Expression Profile in Mice Infected Mycobacterium Tuberculosis Given with Miana Leaves Extract (Coleus scutellarioides (L.) Benth) (Tuberculosis Preventive and Curative Mechanisms) (/article/1795)

Sesilia Rante Pakadang,St. Ratnah,Alfrida Monica Salasa,
,Mochammad Hatta[\(/article/1795\)](#)

Pharmacognosy Journal,14(3):497-505

DOI: 10.5530/pj.2022.14.63

Published: Mon, 27-Jun-2022

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Original Article

Effective Enzyme-Producing Bacteria Isolated from Diversified Thai Rice and Native Thai Bees (Cavity Nesting Honey Bees) and their Potential for Production of Protease Enzymes (/article/1796)

Thapakorn Chumphon,Yaowanoot Promnuan,Sujinan Meelai,Saran Promsai



Pharmacognosy Journal,14(3):506-517

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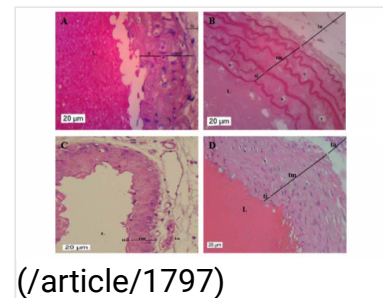
Antihypercholesterolemic Power of Red Dragon Fruit (*Hylocereus polyrhizus*) Peel Extract (/article/1797)

Ruqiah Ganda Putr Panjaitan, Syafira Amelia

Pharmacognosy Journal, 14(3):518-523

DOI: 10.5530/pj.2022.14.65

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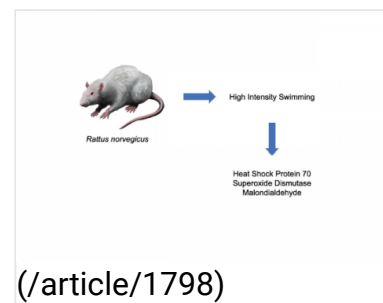
Effects of High Intensity Swimming on Heat Shock Protein 70, Superoxide Dismutase and Malondialdehyde of *Rattus norvegicus* Male Rats (/article/1798)

Dody Taruna, Bambang Purwanto, Harianto Notopuro, Budi Utomo, Lilik Herawati, Reny I'tishom,

Pharmacognosy Journal, 14(3):524-530

DOI: 10.5530/pj.2022.14.66

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Subchronic Toxicity Studies of a Combined *Andrographis paniculata* (Burm.f.) Nees, *Syzygium cumini* (L) Skeels, and *Caesalpinia sappan* L Extract in Sprague-Dawley Rats (/article/1799)

Atini Solawati, Berna Elya, Heri Setiawan, Raysa Yunda Pratiwi

Pharmacognosy Journal, 14(3):531-535

DOI: 10.5530/pj.2022.14.67

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Utilization of Secondary Metabolites in Algae *Kappaphycus alvarezii* as a Breast Cancer Drug with a Computational Method (/article/1800)

AF Dibha,S Wahyuningsih,ANM Ansori,VD Kharisma,MH Widyananda,AA Parikesit,MT Sibero,RT Probojati,AAA Murtadlo,JP Trinugroho,TH Sucipto,DDR Turista,I Rosadi,ME Ullah,V Jakhmola,R Zainul



Pharmacognosy Journal,14(3):536-543

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Phytochemical Evaluation and Antioxidant Activity of Virginia tobacco Leaves (*Nicotiana tabacum* L. var *virginia*) Fractions with DPPH and FTC Methods (/article/1791)

Kori Yati,Misri Gozan, ,Vivi Anggia,Rini Prastiwi,Mahdi Jufri



Pharmacognosy Journal,14(3):544-548

DOI: 10.5530/pj.2022.14.69

Published: Wed, 29-Jun-2022

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In silico Analysis of the Polyphenolic Metabolites of *Zea mays* L. "Purple Corn" on HMG-CoA Reductase (/article/1813)

Horna-Rodriguez Aleksandra M,López-Gamboa July A,Silva-Correa Carmen R,Sagástegui-Guarniz William Antonio,Gamarra-Sánchez César D,Villarreal-La Torre Víc E



Pharmacognosy Journal,14(3):549-558



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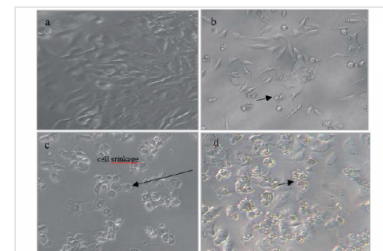
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Antioxidant and Cytotoxic Activities of Melinjo (Gnetum gnemon L.) Seed Fractions on HeLa Cell Line an In Vitro (/article/1801)

Asep Sukohar, , Hendri Busman, Nuning Nurcahyani, Evi Kurniawaty



[\(/article/1801\)](#)

Pharmacognosy Journal,14(3):559-564

DOI: 10.5530/pj.2022.14.71

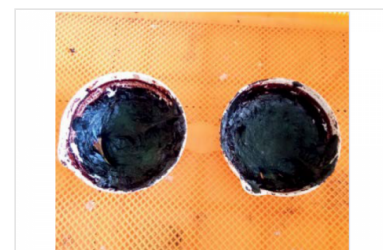
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Peel-off Mask Formulation from Stem of Sempeng (Nepenthes gracilis) as Anti Acne Against Propionibacterium acnes Bacteria (/article/1802)

Rezqi Handayani, Nurul Qamariah



[\(/article/1802\)](#)

Pharmacognosy Journal,14(3):565-570

DOI: 10.5530/pj.2022.14.72

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Azasterol Inhibition and Pharmacokinetic Effects on Thymidylate Synthase-Dihydrofolate Reductase from T. gondii: In Silico Study (/article/1803)

Alan Dharmasaputra, Annisa Ullya Rasyida



[\(/article/1803\)](#)

Pharmacognosy Journal,14(3):571-575

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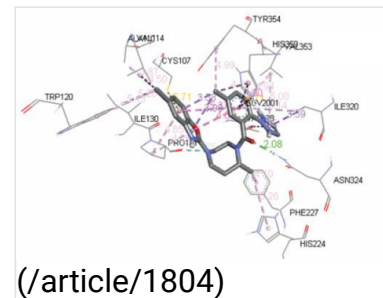


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Molecule Attachment and Prediction of ADMET Compounds in Cinnamomum burmannii on Orexin Receptor as Anti-insomnia (/article/1804)

Resmi Mustarichie, Nyi Mekar Saptarini, Sandra Megantara



Pharmacognosy Journal, 14(3):576-583

DOI: 10.5530/pj.2022.14.74

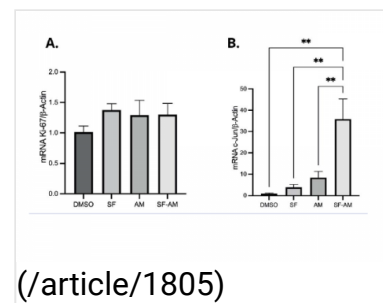
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Alpha-Mangostin Enhances Proliferation in Sorafenib-Surviving HepG2 Liver Cancer Cells by Increasing Anti-Apoptosis and Antioxidant Markers Expressions (/article/1805)

Melva Louisa, Meuthia Faralita Annisa, Pamela Basuki, Brigitta Cindy Lauren, Syarinta Adenina



Pharmacognosy Journal, 14(3):584-590

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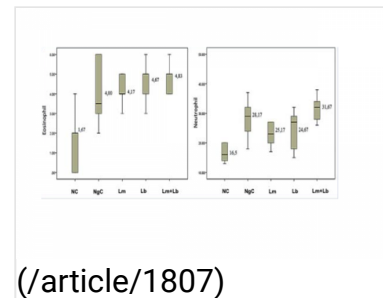
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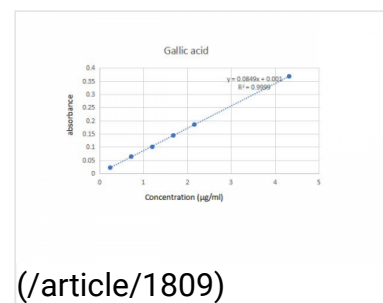
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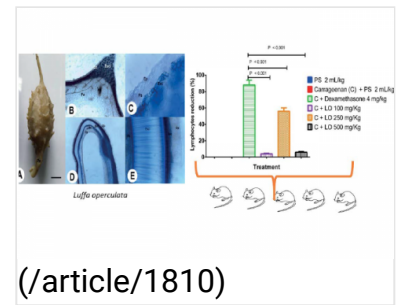
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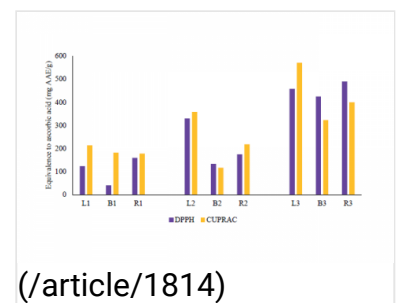
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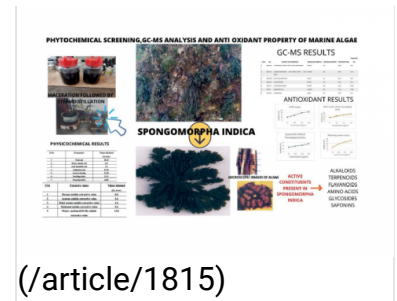
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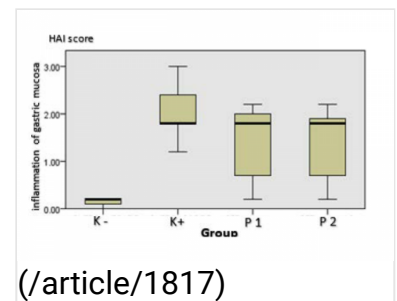
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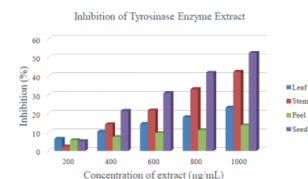
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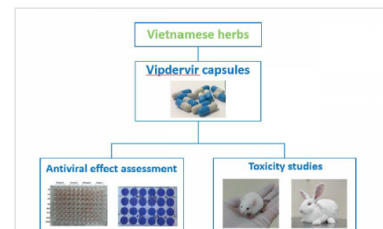
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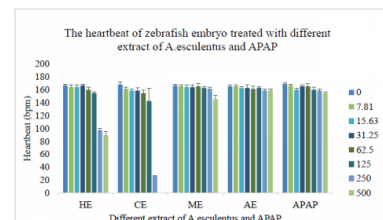
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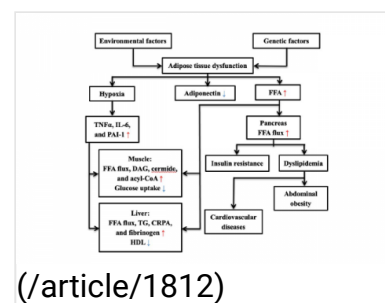
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Phytochemical Evaluation and Antioxidant Activity of Virginia tobacco Leaves (*Nicotiana tabacum* L. var *virginia*) Fractions with DPPH and FTC Methods

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ABSTRACT

Introduction: Virginia tobacco (*Nicotiana tabacum* var. *virginia*) is one of the most extensive varieties of tobacco plants. *Nicotiana tabacum* var. *virginia* leaves known to contain alkaloids, saponins, tannins, phenol, flavonoids, triterpenoids and glycosides. In addition to cigarette raw materials, tobacco is also able to be efficacious as an antibacterial, antifungal and bioinsecticide. **Objective:** The aim of this study to determine the total phenol and total flavonoids and also antioxidant activity of Virginia tobacco leaves fractions. The fraction with the largest compound content was evaluated further for its antioxidant activity. **Methods:** Virginia tobacco leaves was fractioned into dichloromethane (DCM), ethyl acetate, butanol and water fractions. Phenol levels were determined with Follin-Ciocalteu reagent using the UV-Vis spectrophotometer method measured at 743.50 nm and gallic acid as a reference compound. Total flavonoid levels were determined with AlCl₃ reagent using the UV-Vis spectrophotometer method measured at 434.50 nm and quercetin as a reference compound. Antioxidant activity was evaluated with DPPH and Ferric Thiocyanate (FTC) method and the standard used was quercetin. **Results:** Total phenol levels in Virginia tobacco leaves of DCM, ethyl acetate, butanol and water fractions respectively were 191.2386 mgGAE/g, 201.2913 mgGAE/g, 180.5714 mgGAE/g, 212.8692 mgGAE/g. Total Flavonoid levels respectively were 6.0927 mgQE/g, 6.9659 mgQE/g, 5.1112 mgQE/g, 8.3346 mgQE/g. Antioxidant of water fraction was evaluated further using DPPH and FTC method with IC50 respectively were 75.9148µg/ml and 67.8972 µg/ml. **Conclusion:** Overview of total phenol and flavonoid levels fractions and antioxidant can be used as an additional initial reference for Virginia tobacco leaves development as source of medicinal substances.

Key words: Antioxidant, DPPH, Phenol, Flavonoid, FTC, Virginia tobacco.

INTRODUCTION

Indonesia is one of the countries in Asia with abundant of biodiversity. It has the largest tropical rainforest in the world with high humidity that allows the growth of various types of plants. The plant has been used for various purposes, especially for traditional remedies. The utilization of traditional remedies in Indonesia is part of the national culture and has been widely used for centuries ago. However, the effectiveness and safety have not been supported by adequate research.¹

In improving the quality, safety and usefulness of traditional medicines, it is necessary to standardize raw materials in the production of traditional medicines including the standardization of plant extracts and fractions. The extraction quality standardization parameters consist of non-specific parameters (drying losses and specific gravity, the total ash content, moisture content, residual solvent and also pesticide, heavy metal contamination and the microbial contamination) and specific parameters (identity, organoleptic, dissolved compounds in certain solvents, phytochemical screening, total class of chemical content, specific chemical content.²

Virginia tobacco (*Nicotiana tabacum* var. *virginia*) is one of the world's widest varieties of tobacco plants. This variant is planted around 1.8-2.0

million hectares. Recently, 1.5-2 million hectares of tobacco growing areas worldwide. Many researches has reported that *Nicotiana tabacum* var. *virginia* contains more than 4000 chemical compounds.³ Previous research reported that Virginia tobacco leaves contained triterpenoids, alkaloids, glycosides, saponins, flavonoids, tannins and phenols. In addition as raw materials for cigarettes, tobacco is also efficacious as an antibacterial,⁴ antifungal and bioinsecticide.⁵ Andjani *et al.* (2019). proved that, *Nicotiana tabacum* L. bio-oil at dosage 5000 mg/kg body weight was not toxic against female winstar rats. There is no mortality and also no significant change in behavior and the body weight.⁶

The chemical compound of *Nicotiana tabacum* are phenol and flavonoids, which are responsible for their health benefits.⁷ According to multiple reports in the literature, phenolic and its derivate exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden.⁸ Considering the important role and function of phenolic and flavonoid compounds, it is necessary to conduct research on analysis of phenolic and flavonoid levels of Virginia tobacco leaves (*Nicotiana tabacum* var. *virginia*) and assesses the antioxidant using two different methods with a different mechanisms. So that the data in the chemical content of the group will be preliminary information about the characteristics of the extract associated with pharmacological effects.

Cite this article: Yati K, Gozan M, Mardiasuti, Anggia V, Prastiwi R, Jufri M. Phytochemical Evaluation and Antioxidant Activity of Virginia tobacco Leaves (*Nicotiana tabacum* L. var *virginia*) Fractions with DPPH and FTC Methods. *Pharmacogn J.* 2022;14(3): 544-548.

MATERIAL AND METHODS

Plant extract

Ethanol extract of Virginia tobacco leaves was obtained from the Agency for the Assessment and Application of Technology (BPPT), Indonesia extracted with reflux method.³

Chemicals and standards

Quercetin, gallic acid, Folin Ciocalteu and AlCl_3 were purchased from Merck and other chemical reagent for analytical analysis.

Physicochemical parameter evaluation of extract

Physicochemical parameters i.e. moisture content, total ash, water soluble ash content, acid insoluble ash content were evaluated according to the official methods described in Indonesian Pharmacopeia.²

Moisture content

Determination of drying loss according to Indonesian Pharmacopeia.²

Total Ash

The extract was put into the crucible silicate that has been equalized and tamed, incinerate slowly until the charcoal runs out, cool and weigh. If the charcoal cannot be removed, add hot water, stir, filter through ash-free filter paper. Filter paper along with the rest of the filtering in the same crucible is incinerated to a fixed weight. Total ash content is calculated against the weight of the test material, expressed in% w/w.

$$\text{Total ash value} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

W_0 = empty crucible weight

W_1 = weight of crucible and extract before flattened

W_2 = weight of crucible and dried extracts

Water-soluble ash content

Ash obtained from the determination of ash content, boil with 25 ml of water for 5 minutes. Collect insoluble parts then filter with ash-free filter paper, wash with hot water, and incinerate for 15 minutes at a temperature of no more than 450 °C, until the weight remains then weigh.

$$\text{Water soluble ash value} = \frac{\text{total weight} - \text{Empty crucible weight}}{\text{Extract weight}} \times 100$$

Acid insoluble ash content

Ash obtained from the determination of ash content, boiled with 25 ml dilute hydrochloric acid for 5 minutes, collect acid-insoluble parts, filtered through ash-free filter paper, then wash with hot water, incinerate to a fixed weight. Acid insoluble ash content was calculated against the weight of the test material, expressed in% w/w.

$$\text{Acid insoluble ash value} = \frac{\text{total weight} - \text{Empty crucible weight}}{\text{Extract weight}} \times 100$$

Determination of phenolic level

The analysis was carried out according to the procedure performed by Lim & Murtijaya (2007) with a few modifications and gallic acid was used as a reference compound. 50.0 mg gallic acid was dissolved in 0.5 ml ethanol, then diluted with distilled water to a volume of 100.0 ml. 300 μL of gallic acid solution added 1.5 ml of the Folin-Ciocalteu reagent (1:10), then shaken and allowed to stand for 3 minutes. 1.2 ml of 7.5% Na_2CO_3 solution was added and shaken until homogeneous, and allowed to stand at room temperature in the operating time range. The absorbance of gallic acid was read at maximum wavelength. The

series concentration of gallic acid was made and recorded at maximum wavelength. The standard calibration curve was determined from the linear regression equation between series concentrations of gallic acid (x) and the absorbance of the gallic acid reaction with the Folin-Ciocalteu reagent (y). 10.0 mg of Virginia tobacco leaves fractions were dissolved to 10.0 ml with a mixture of methanol: distilled water (1: 1). 300 μL sample solution was allowed the procedure previously described, read at maximum wavelength and the procedure was carried out triplicate. Phenol levels of the samples obtained were expressed as equivalence to gallic acid (GAE).⁹

Determination of flavonoid level

The analysis was carried out according to the procedure performed by Chang *et al.* (2002) with a few modifications used AlCl_3 and quercetin was used as a reference compound. The standard solution was made at a concentration of 5 μg / ml, then added 3 ml of p.a ethanol, 0.2 ml of 10% AlCl_3 , 0.2 ml of 1M sodium acetate, and aquadest to a volume of 10 ml. Absorbance was read at maximum wavelength. 100 mg Virginia tobacco leaves fractions were dissolved in a volumetric flask with 10 ml ethanol. 1 ml solution added with 3 ml ethanol, then added with 0.2 ml AlCl_3 10% and 0.2 ml natrium asetat 1M reagent. The solution was added with distilled water up to 10 ml. The mixture was incubated at room temperature according to the operating time range. Absorbances were measured at the maximum wavelength and analysis was carried out in triplicate. Flavonoid content was obtained as equivalent to quercetin. The equation of the standard calibration curve was obtained from linear regression between the concentration of quercetin (x) and absorbance (y). Flavonoid levels of the samples obtained were expressed as equivalence to quercetin (QE).¹⁰

Determination antioxidant activity

The fraction with a high level of phenolic and flavonoid content was analyzed further for its antioxidant activity with DPPH and FTC methods.

1,1-Diphenyl-2-Picrylhidrazil (DPPH) method

Virginia tobacco leaves fraction antioxidant capacity was determined according to a method performed by Molyneux (2004). 10 mg DPPH dissolved in 10 ml methanol. 2.0 ml of the standard solution was pipetted then dissolved in a 100 ml volumetric flask. 0.2 ml of methanol mixed with 3.8 ml of DPPH then the maximum wavelength of DPPH solution was determined in a range of 400–800 nm. 10 mg of tobacco leaves fraction dissolved in 100 ml volumetric flask with methanol then prepared with various concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. The absorbance of the sample was measured at λ_{max} for 30 minutes. The data obtained were processed to get the percentage inhibition value. Tests were carried out in triplicate.¹¹

Ferric Thiocyanate (FTC) method

The capacity of Virginia tobacco leaves fraction to inhibit the peroxides formation from linoleic acid was determined with the FTC methods described by Zahin *et al.* (2009). 1 ml of 50 mM linoleic acid in 100% ethanol was added with 1 ml of phosphate buffer pH 7 0.1 M, then incubated for 24 hours at 40 °C in a dark room. The incubated solution was taken as much as 100 μL then added with 2.35 ml of 75% ethanol and 50 μL of ammonium thiocyanate 30%. Then add 50 μL FeCl_3 0.02 M in 3.5% HCl. The maximum wavelength was determined the range of 380–780 nm. The fraction was prepared with various concentrations (20, 40, 60, 80 and 100 ppm). The mixture was incubated according to the operating time range that has been determined before in a dark room temperature. Absorbances were measured at the maximum wavelength.¹²

RESULTS

Extraction and fractionation

Ethanol extract of Virginia tobacco leaves were obtained from the Agency for the Assessment and Application of Technology (BPPT) then fractionated with DCM, ethyl acetate, butanol and water solvents. All fractions then evaporated *in vacuo*. DCM, ethyl acetate, butanol and water fraction were analyzed further to determine phenolic, and flavonoid content.

Physicochemical evaluation

The nonspecific parameters of the extract were determined including water content, total ash content, water-soluble ash content and acid insoluble ash content in the extract. Results are shown in Table 1.

Determination of phenolic content

Quantitative analysis of Phenolic levels was determined by the UV-Vis spectrophotometry method whose absorbance was measured at 743.50 nm. The phenolic level of Virginia tobacco leaves fractions is shown in table 2.

Determination of flavonoid content

Quantitative analysis of flavonoid levels was performed using UV-Vis spectrophotometry method. The maximum wavelength is obtained at 443.50 nm. The flavonoid level of Virginia tobacco leaves fractions is shown in table 3.

Determination of antioxidant activity

DPPH method

Based on the analysis of phenolic and flavonoid content, the water fraction gave the highest value than the other fraction on both compounds analyzed. Therefore, the ability of water fractions to counteract free radicals was further evaluated by DPPH method. In this study, the maximum wavelength was obtained at 515 nm. The antioxidant activity of water fraction compared to the standard is shown in Table 4. Based on the research, antioxidant activity was increased according to water fraction concentration with the IC₅₀ of 75.9148 µg/ml compared to quercetin was 6.7918 µg/ml. In this study,

Table 1: Physicochemical Evaluation of ethanol extract of Virginia tobacco.

Parameters	Result (%w/w)
Water content	16.43%
Total ash content	7.10 %
Acid insoluble ash content	0.36 %
Water-soluble ash content	3.47 %

Table 2: Phenolic content analysis from ethanol extract of Virginia tobacco.

Fractions	Absorbance	Phenolic Content (mgGAE/g)	Average (mgGAE/g)
DCM	0.661	194.3708	191.2386
	0.656	188.9604	
	0.666	190.3846	
Ethyl acetate	0.695	201.7234	201.2913
	0.697	200.4320	
	0.689	201.7185	
Buthanol	0.626	180.8988	180.5714
	0.632	184.6921	
	0.622	176.1234	
Water	0.729	210.0786	212.8692
	0.734	216.5896	
	0.724	211.2141	

Table 3: Flavonoid content analysis from ethanol extract of Virginia tobacco.

Fractions	Absorbance	Flavonoid content (mgQE/g)	Average (mgQE/g)
DCM	0.490	6.0897	6.0927 ± 0,089
	0.485	6.0043	
	0.487	6.1841	
Ethyl acetat	0.552	6.9187	6.9659 ±0,060
	0.554	6.9456	
	0.560	7.0335	
Buthanol	0.412	5.0281	5.1112 ±0,086
	0.417	5.1056	
	0.424	5.1999	
Water	0.653	8.2707	8.3346 ±0,088
	0.655	8.2976	
	0.664	8.4355	

Table 4: Antioxidant activity of water fraction with DPPH method.

Sample	Concentration (ppm)	Absorbance	%Inhibition	IC ₅₀ (µg/mL)
Quercetin	2	0.5248	22.6643%	6.7918µ/mL
	4	0.4483	33.9357%	
	6	0.3658	46.0949%	
	8	0.2946	56.4397%	
	10	0.2149	68.3318%	
Water fraction	20	0.5046	26.5822%	75.9148µ/mL
	40	0.4304	37.3781%	
	60	0.3707	46.0643%	
	80	0.3304	51.9278%	
	100	0.2915	67.5876%	

Table 5: Antioxidant activity of water fraction with FTC method.

Sample	Concentration (ppm)	Absorbance	%Inhibition	IC ₅₀ (µ/mL)
Quercetin	2	0.4331	35.5697%	6.0528µg/mL
	4	0.3833	42.9782%	
	6	0.3296	50.9669%	
	8	0.2866	57.3638%	
	10	0.2539	62.2285%	
Water fraction	20	0.4614	31.3597%	67.8972 µg/mL
	40	0.3925	41.6096%	
	60	0.3373	49.8215%	
	80	0.3060	54.4779%	
	100	0.2743	59.1936%	

the results of quercetin gave a value that was close to the research of Rini *et al.* of 5.631 µg/ml.

FTC method

Furthermore, the capacity of water fraction in reducing the formation of peroxide in fat peroxidation was measured and the Ferric thiocyanate complex formed was read at 484 nm. The antioxidant activity of water fraction compared to the standard shown in Table 5. This study showed that water fraction Virginia tobacco leaves can prevent the formation of fat peroxidation from linoleic acid with IC₅₀ was 67.8972 µg/ml compared to standard was 6.0528 µg/ml.

DISCUSSION

Virginia tobacco (*Nicotiana tabacum* var. *virginia*) is one of a variety of tobacco that is mostly used as the main raw material for cigarettes

proven to be efficacious for many important pharmacological activities. The development of this variety for other purposes besides cigarettes is expected to increase its economic value. The present study, ethanol extract of Virginia tobacco leaves was obtained from the Agency for the Assessment and Application of Technology (BPPT), Indonesia which is extracted with the reflux method. the use of the extraction method is the main point that must be considered. The use of the reflux method provides several benefits to the compound extraction process. Hidayat *et al.* (2019) proved that the antioxidant activity possessed by black Sumatran incense resin which is extracted using the reflux method is greater than *Jatropha curcas* L. and smaller than *Sargassum serratifolium* extract.¹³

Determination of physicochemical parameters of the extract is useful to determine the quality of extract as one of raw materials of traditional medicine. Water content is essential to provide a minimum limit or range of the amount of water content in the extract. Based on the result, the water content of the ethanol extract of Virginia tobacco meets the requirements of Indonesian Pharmacopeia. Determination of total ash content gives an overview of extract mineral content, starting from the initial process to extract so that the total ash content parameters are related to the purity and contamination of an extract. The results showed that the level of minerals in an extract is still low.²

The chemical compound was one of the important parameters that related to the activity. In previous research ethanol extract of Virginia tobacco showed a positive result to the alkaloid, phenolic, flavonoid and saponin.⁴ Phenolic and its derivatives are compounds that have been widely studied for various pharmacological activities that are important and beneficial. Many studies have reported the advantages of phenolic compounds, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative agents.¹⁴ Phenolic compounds have received increasing interest in human health due to their beneficial effects against several diseases like cancers attributed in particular to their antioxidant activity.¹⁵ *Nicotiana tabacum* L. var. Virginia leaf extract obtained from Ethanolic Heat Reflux Extraction (EHRE) had shown insecticidal activity against *Gryllus bimaculatus imago* and *Galleria mellonella* larvae. The values of LC₅₀ were 38.5 mg/ml for *Gryllus bimaculatus* and 36.6 mg/ml for *Galleria mellonella*.¹⁶

Flavonoids are the largest group of phenolic compounds containing two aromatic rings linked by three atoms of carbon bridge (C₆-C₃-C₆). They include mainly flavones, isoflavones, flavonols, flavans, flavanones and anthocyanidins.¹⁷ This compound is one of the polyphenol compounds which has the various property that is important for human health in inhibiting alpha-amylase enzyme which is a key enzyme that is responsible to digest carbohydrates in the digestive tract.¹⁴ Besides that, the flavonoid is phenolic compound compounds that are highlighted for their antioxidant activity and the most widely distributed phenolic compounds in plant foods and also the most studied ones.¹⁸

Analysis of phenolic and flavonoid levels in the present study showed that ethanol extract of Virginia tobacco contained 153.7414 ± 0.281 mg GAE/g of phenolic compound and 3.5337 ± 0.1029 mg QE/g of flavonoid compound. It was an initial report that can be useful in the development of Virginia tobacco as a source of phenolic compound. Virginia tobacco (*Nicotiana tabacum* var Virginia) with high production capacity in various regions are more widely used to produce the white cigarette with high production capacity in various regions which may harm health. But this research indicated that Virginia tobacco still has pharmacological activities that are beneficial to our health. Interestingly, this plant can inhibit the oxidation process which can be caused by oxidants that can come from cigarette smoke.

CONCLUSION

Tobacco leaf is a plant that is widely found in Indonesia and is used as a commodity for export to various countries due to Indonesia has tobacco

leaves which was one of the best qualities in the world. Virginia tobacco is one of the primary commodities in the production of cigarettes that provides a large income for the government but unfortunately, it harms human health. Determining the levels of phenols and flavonoids can be an opportunity in the search for sources of natural antioxidants. Besides that, analysis of phenol and flavonoid content from plant extracts can expand the utilization of Virginia tobacco plants for other uses as a source of traditional medicinal plants.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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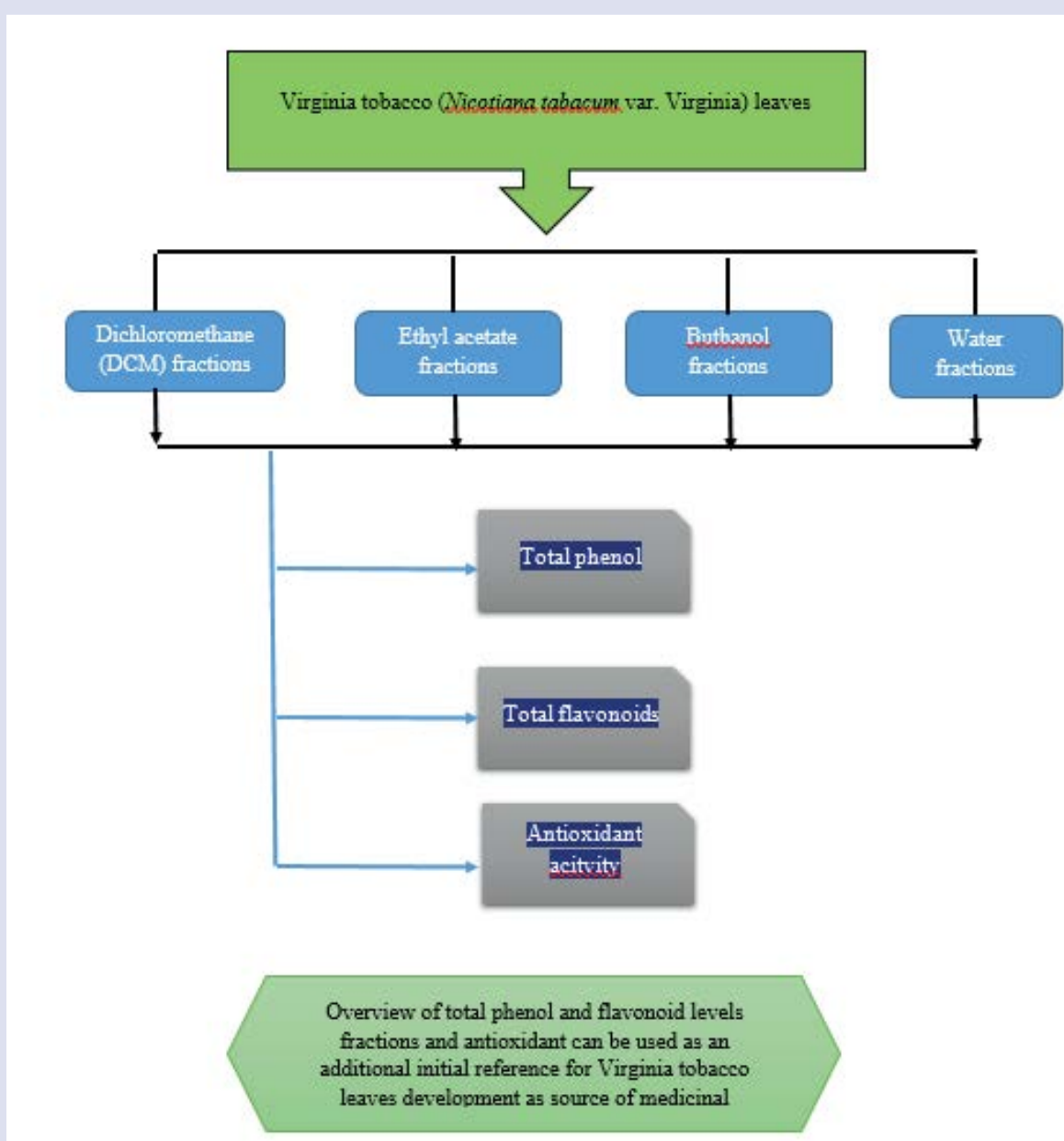
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GRAPHICAL ABSTRACT



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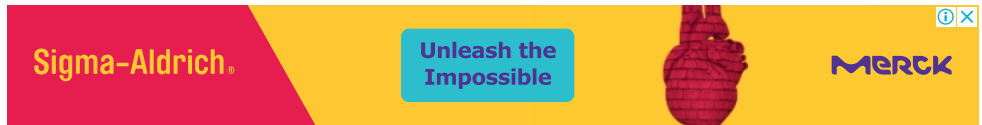
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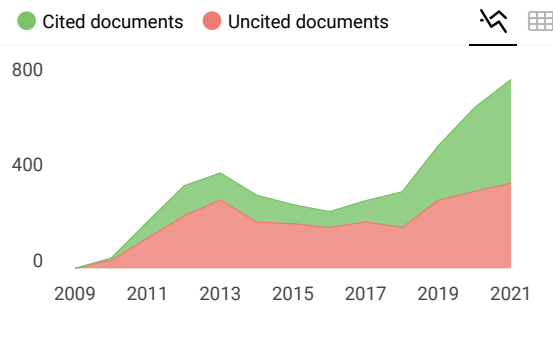
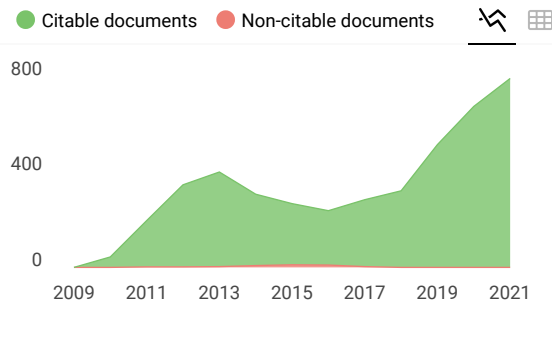
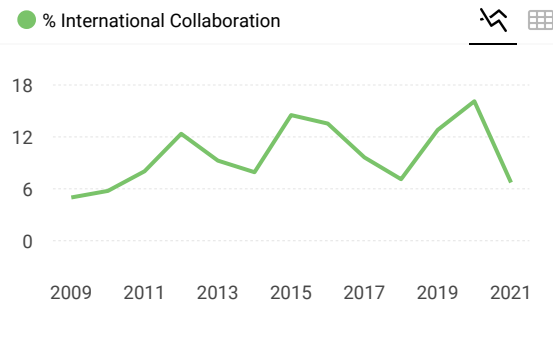
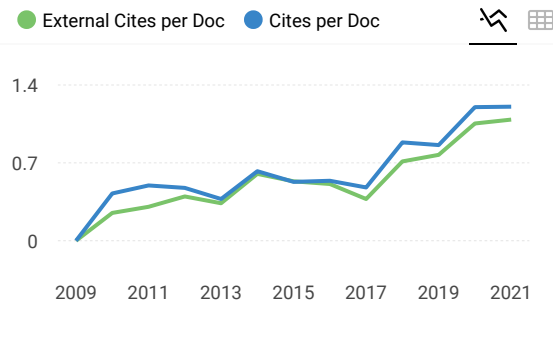
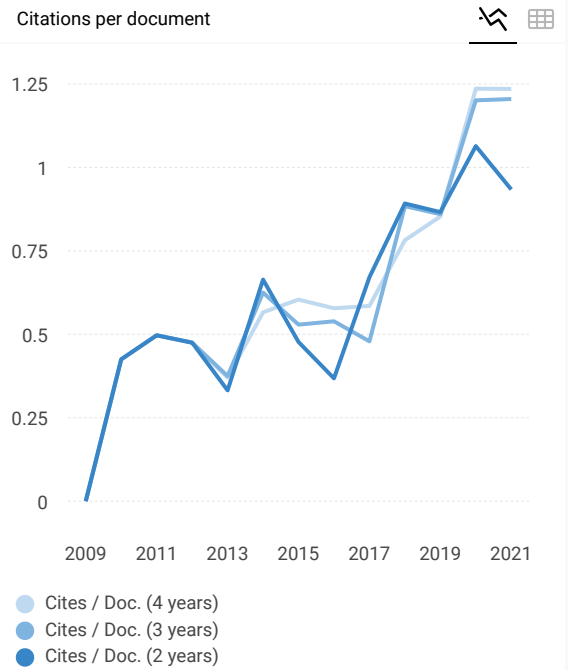
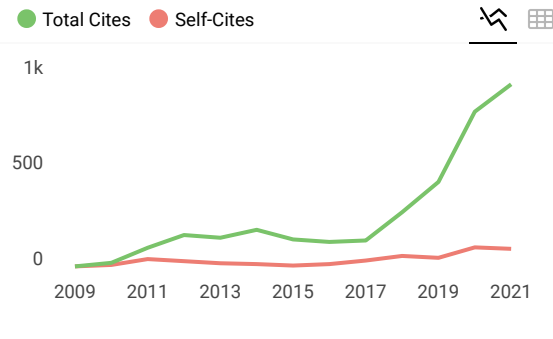
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