第 49 卷 第 2 期
2022 年 2 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 2
February 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.2.15

Chemical Compounds Profile by LC-MS/MS and Assessment of Antioxidant and Antibacterial properties of *Caesalpinia pubescens* Fruits Extracts

Praptiwi[1*], Ridwan[2], Ahmad Fathoni[1]

[1] Research Center for Chemistry, National Research and Innovation Agency (BRIN), Gd. 452, Puspitek Serpong Gate, Muncul, Kec. Serpong, Kota Tangerang Selatan, Banten, Indonesia

[2] Research Center for Biology, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor KM. 46 Cibinong, Bogor, West Java, Indonesia

Abstract: This study presents the profile of chemical compounds by LC-MS/MS and evaluates antioxidant and antibacterial properties of *Caesalpinia pubescens* fruits extracts. The study aimed to evaluate the antioxidant activity and antibacterial activity of *Caesalpinia pubescens* fruits extract and determine the active extract's chemical compounds by LCMS/MS. Total phenolic content (TPC) and total flavonoids content (TFC) were determined by the colorimetric assay, respectively. The antioxidant activity was performed by TLC-bioautography, determination of the IC_{50}, antioxidant activity index (AAI), and the inhibition of β-carotene bleaching. This study also determined the correlation between TPC, TFC, and antioxidant activities. Evaluation of antibacterial activity was performed against *S. aureus* and *E. coli*. The results showed that the highest TPC was obtained in methanol extract (108.54 ± 1.92 mg GAE/g extract), and the highest TFC was obtained in dichloromethane extract (33.38 ± 0.86 mg QE/g extract). Methanol and ethyl extracts have powerful DPPH free radical scavenging with the IC_{50} value range of 8.27 - 11.24 µg/ml and AAI values of 2.74 - 3.72. The DPPH radical free scavenging capacity has a high positive correlation with the TPC values with an r-value of 0.950. The increased concentration of extracts correlated with the increased inhibition of β-carotene bleaching, with Pearson's coefficient (r) in a range of 0.624 - 0.962. The 1000 ppm of methanol extract had the best antioxidant activity, significantly different from positive control and other extracts ($P<0.05$). The dichloromethane and methanol extracts possess moderate antibacterials against *S. aureus* with a MIC value of 256 µg/mL. This study is the new on the antibacterial and antioxidant activity of *C. pusbescens* fruit extracts, and the first study regarding flavonoid and phenolic contents and the other chemical composition in the *C. pusbescens* fruit extracts.

Keywords: *Caesalpinia pubescens*, fruits, antioxidant activity index, antibacterial, LC-MS/MS.

液相色谱-串联质谱法的化学成分谱及毛毛竹果提取物抗氧化和抗菌特性的评估

摘要：本研究通过液相色谱和串联质谱法展示了化合物的概况，并评估了毛毛竹果提取物的抗氧化和抗菌特性。本研究旨在通过液相色谱-串联质谱法评价毛毛苢果实提取物的抗氧化活性和抗菌活性，并确定活性提取物的化学成分。分别通过比色法测定总酚含量和总黄酮含量。抗氧化活性通过薄层色谱-生物自显影、测定抑制剂浓度、抗氧化活性指数和抑制β-胡萝卜素漂白来进行。该研究还确定了总酚含量、总黄酮含量和抗氧化活性之间的相关性。对金黄色葡萄球菌和大肠杆菌进行抗菌活性评估。结果表明，甲醇提取物中总酚含量最高（108.54±1.92 毫克没食子酸当量/克提取物），二氯甲烷提取物中总黄酮含量最高（33.38±0.86 毫克 量化宽松/克提取物）。甲醇和乙基提取物具有强大的 2,2-二苯基-1-苦基肼 自由基清除能力，抑制剂浓度范围为 8.27 - 11.24 微克/毫升，问责制、敏捷性和内部创业值为 2.74 - 3.72。 2,2-二苯基-1-苦基肼自由基清除能力与总酚含量值呈高度正相关，r 值为 0. 950。提取物浓度的增加与对 β-胡萝卜素漂白的抑制作用增加相关，皮尔逊系数（r）在 0.624 - 0.962 范围内。 1000 百万分之几甲醇提取物的抗氧化活性最好，与阳性对照及其他提取物有显着差异（P<0.05）。二氯甲烷和甲醇提取物对金黄色葡萄球菌具有中等抗菌作用，最低抑菌浓度 值为 256 微克/毫升。本研究是对白毛苢果实提取物抗菌和抗氧化活性的新研究，也是对毛毛苢果实提取物中黄酮类和酚类含量及其他化学成分的首次研究。

Received: November 12, 2021 / Revised: December 31, 2021 / Accepted: January 09, 2022 / Published: February 28, 2022

About the authors: Praptiwi, Ahmad Fathoni, Research Center for Chemistry, National Research and Innovation Agency (BRIN), Kota Tangerang Selatan, Indonesia; Ridwan, Research Center for Biology, National Research and Innovation Agency (BRIN), Bogor, Indonesia

Corresponding author Praptiwi, praptiwip@yahoo.com

关键词：毛竹，果实，抗氧化活性指数，抗菌，液相色谱-串联质谱。

1. Introduction

Plants have been used in traditional medicine to cure various diseases for thousands of years. 35,000-70,000 plant species have been tested for medicinal use [1]. Furthermore, over 80% of the world's population depends on traditional medicines [2]. The importance of plants in medicine is related to their bioactive compounds. The search for new bioactive compounds has been extensively performed. However, the search for new bioactive compounds is still important to overcome antibacterial resistance and other diseases. On the other hand, plants are the richest sources of organic compounds that have contributed significantly to the supply of new chemical entities that have been applied in medicine, cosmetics, food, and agrochemicals [3].

The genus *Caesalpinia* belongs to the family of Caesalpiniaceae and consists of more than 500 species distributed in the tropics and subtropics [3]. Several species of the genus *Caesalpinia* have been known to produce bioactive compounds with various biological activities. A previous study reported that *C. pulcherrima* has various medicinal properties for treating skin diseases, fever, and tumors and also has antimicrobial activity against several bacteria species and antioxidant activity [4]. Chemical compounds dihydrobonducellin and 2-methoxydihydrobonducellin, isolated from *C. pulcherrima,* are more potent antioxidants compared to vitamin C [5]. Chemical compounds in *C. pulcherrima* were diterpenoids, peltoginoids, flavonoids, chalcones, and homoisoflavonoides [4]. The methanolic seed extract of *C. bonducella* exhibited antibacterial activity similar to kanamycin [6]. It also displayed high DPPH-free radical scavenging activity [7].

One species of *Caesalpinia* that has not been studied is *Caesalpinia pubescens* (Desf.) Hattink (Fig. 1). It has four synonyms, i.e., *Caesalpinia ignota* Blanco, *Mezoneuron glabrum* Desf., *Mezoneuron pubescens* Desf., *Mezoneurum pubescens* Desf [8]. This species is distributed in Malesia: Sumatra (Bangka), Java (all parts, also Madura and Kangean Is.), Philippines (C Luzon), Celebes (SW and Salajar), Lesser Sunda Islands (Bali, Lombok, Sumbawa, Flores, Timor, Wetar) [9]. It is scandent shrubs with a height reaching 10-15 m. Lombok Island has the local name kentok mayung, and the fruits have been used as a vegetable due to their delicious taste. They are believed to have health benefits such as maintaining youthfulness and strengthening stamina [10]. Based on the previous study, several species of C*aesalpinia* have potent antioxidant activity, so the authors are interested in evaluating the antioxidant extract of *C. pubescent* related to their flavonoids and phenolic content and the antibacterial property. The antioxidant potential, flavonoids, and phenolic content of *C. pubescens* fruits were not studied yet.

Fig. 1 *Caesalpinia pubescens* (Desf.), Hattink

2. Materials and Methods

2.1. Plant Material Preparation

The fruits of *Caesalpinia pubescens* were collected from Sukadana Village, Pujut Sub-District, Central Lombok, West Nusa Tenggara, Indonesia (-8.8966766,116.3198675). The plant specimen was identified and authenticated at the Herbarium Bogoriense, Botany Division- Research Center for Biology. The fruits collected from the field were washed, air-dried, and grounded to powder.

2.2. Extraction of *Caesapinia pubescens* Fruit

The powdered fruits were macerated with hexane, dichloromethane, ethyl acetate, and methanol. The sample was extracted thrice in every solvent. The filtrate was collected and concentrated to dryness by using a rotary evaporator. The crude extracts were placed in a glass bottle and stored at 4°C for further use.

2.3. TLC-Bioautography for DPPH-Free Radical Scavenging Activity

Evaluating the DPPH-free radical activity of *C.pubescens* extracts was carried out by the bioautography-DPPH method on the TLC plate (Silica gel 60 F_{254}, Merck). Ten µl of extract (10 mg/ml) was transferred onto the TLC plate. After drying the plate, the hexane extract was developed with n-hexane: ethyl acetate (3: 2), the Dichloromethane and Ethyl acetate extract were developed with dichloromethane: methanol (10:1), and the methanol extract was developed with chloroform: methanol: distilled water (6:4:1). The developed plates were dried and sprayed with 0.2% DPPH solution in methanol. Yellow spots on purple backgrounds after 30 minutes indicated the active compound with antioxidant activity [11].

2.4. Determination of Total Phenolic Content

The total phenolic content was determined using the Folin Ciocalteu test. Gallic acid solutions in ethanol at the 6.25-200 µg /mL concentration were used as standard solutions. In comparison, the test sample was prepared at a 1000 µg/mL concentration in ethanol. Ethanol is used as a blank. Standard solutions of gallic acid (0.2 mL) and extract (0.2 mL) were added with 0.2 mL 50% Folin-Ciocalteu phenol reagent then mixed using vortex for 1 minute. The mixture solution was added with 4 mL of 2% sodium carbonate solution (Na_2CO_3) and stored under the dark at room temperature for 30 minutes. The absorbance of the mixed solution was read at a 750 nm wavelength with a UV-Vis spectrophotometer. The total phenolic content is expressed as mg Gallic Acid Equivalent (GAE) per g extract.

2.5. Determination of Flavonoid Content

The aluminum chloride colorimetric assay measured total flavonoid content. Quercetin was used as a standard solution and made at 3.125-100µg/ml concentration range. Ethanol was used as a blank solution, and the sample was prepared at the concentration of 1000 µg/mL in ethanol. An aliquot (0.5 ml) of standard solutions was added into the vial containing 1.5 ml ethanol. The standard solutions and extract were added 0.1 ml of 10% $AlCl_3$, 0.1 ml of $CH_3COONa.3H_2O$ 1M, and 2.8 ml aqua dest. The solution was mixed, and the absorbance was measured at 417 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE) per g extract.

2.6. Determination of DPPH-Free Radical Scavenging Activity

The DPPH-free radical activity of the extracts was performed by microdilution on 96-well microplates. Each well in the first to the eight rows was filled with 100 µl of methanol. Five µl of extract (10,240 µg/ml) were filled well in the first row. Then, 95 µl of methanol was added and homogenized. Every extract was done in triplicate. Serial dilution was done by transferring 100 µl from the first row into the second row and homogenized. Again, one hundred µl from the second row was transferred into the third row. On the 8^{th}, 100 µl was discarded. After the dilution process was complete, each well was added with 100 µl of methanolic DPPH solution (61.50 µg/ml). The microwell plates were incubated at room temperature for 90 minutes in dark conditions. Catechin was used as a positive control. The absorbance was observed at 517 nm using Varioskan Flash (Thermo Scientific). Percent inhibition concentration (IC) was calculated as follows:

$$\mathrm{IC}\ (\%) = \left(\frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}}\right) x 100\% \tag{1}$$

where A control is the absorbance of DPPH without samples, A sample is the absorbance of DPPH at different concentrations of extracts. The IC_{50} values of DPPH were obtained from linear calibration between the percentage of inhibition and sample concentration. The antioxidant activity index (AAI) is acquired by the formula below:

$$\mathrm{AAI} = \frac{\text{The final concentration of DPPH}}{\mathrm{IC_{50}}} \tag{2}$$

2.7. Correlations between TPC, TFC, and DDPH free radical scavenging activity

Correlation assessment between antioxidant activity and total phenolic content (TPC) or between antioxidant activity and total flavonoid content (TFC) was carried out by Pearson's correlation ($P<0.01$) using SPSS 16 for Windows.

2.8. *β*-Carotene Bleaching Assay

The β-carotene solution was prepared by dissolving 10 mg of β-carotene (Sigma-Aldrich) in 10 ml chloroform. Pipet 0.2 ml of the solution into a flask and add 20 mg of linoleic acid and 200 mg of Tween-20. Chloroform was evaporated completely using a rotary vacuum evaporator at 40ºC. After evaporating, 50 ml of distilled water was added slowly to the residue, followed by vigorous shaking to form an emulsion. Aliquots of 4.8 ml of the emulsion were added to a series of test tubes containing 0.2 ml of the sample or BHT (butylated hydroxytoluene) (Merck). The ranging concentrations were from 1 to 1000 ppm. The absorbance was read immediately using UV/Vis Spectrophotometer at the wavelength of 470 nm against a blank (an emulsion without β-carotene). The tubes were placed in a water bath at 50ºC; the absorbance was measured again at 470 nm after 60 min incubation. A control treatment was 0.2 ml ethanol, while BHT was used as the reference. Antioxidant activity was calculated with the following equation:

$$\mathrm{AA} = \frac{\mathrm{DR_c} - \mathrm{DR_s}}{\mathrm{DR_c}} x\ 100 \tag{3}$$

where AA is the antioxidative activity of β-carotene or bleaching inhibition; DR_C is the degradation rate of the control {DR_C = [ln(a/b)/60]}; DR_S is the degradation rate in the presence of the sample {DR_S = [ln(a/b)/60]}; where a is the absorbance at 0 min; and b is the absorbance after 60 min.

2.9. Antibacterial Activity: Determination of Minimum Inhibitory Concentration (MIC) Value

Two-fold broth microdilution in a 96-well microplate was carried out to determine the extracts' Minimum Inhibitory Concentration (MIC) value against *Staphylococcus aureus* Ina CC B4 and *Escherichia coli* InaCC B5. Wells in the first row were loaded with 100 μL of double strength of Mueller Hinton Broth, 10 μL of extract (10.24 mg/mL Dimethyl sulfoxide (DMSO), and 90 sterile aqua dest and homogenized. Wells of the 2nd to 4th row was filled with 100 μL Mueller Hinton Broth. One hundred μL of the 1st well was taken out and transferred into the 2nd well of a vertical row and homogenized. Serial dilution was carried out until the 4th row, and at the 4th row, 100 μL of the mixture was discarded. After that, 100 μL of suspension of bacteria (10^6 CFU/mL) was added to each well, followed by incubation of the microwell plate for 18 hours at 37^{o}C. After incubation, each well was added with 10 μL INT. The lowest concentration before the color changes is the MIC value.

2.10. Identification of Compounds of the Extracts using LC-MS-MS Analysis

The extracts of *C. pubescens* fruit were diluted with methanol. The soluble extract was filtered using a 0.45-micron Millipore filter. Five μL filtrate of the sample was injected into the LC-ESI-QTOF system (XEVO G2-XS QToF). LC-MS analysis was performed using a binary pump and UPLC-MS (Waters Acquity UPLC I-Class). The LC is connected to QTOF mass spectrometer coupled to ESI. The MS was used with positive ionization mode. The ESI parameters are source temperature 120°C, gas atomizer 50 L/h, and source voltage +2.0 kV. Full scan mode was from 100 m/z to 1200 m/z. UPLC column was Acquity UPLC® BEH C8 1. 7 μm 2.1 x100 mm, column temperature 40.0 °C. Solvent A was 0,1 % Formic Acid (FA) in H_2O; solvent B was 0,1% FA in acetonitrile. Solvents were set at a total flow rate of 0.300 mL/min. Isocratic elution system was run at 0–1.0 min with ratio 95:5; linear gradient of solvent A was from 95% to 60% at 1.0-11.0 min; isocratic elution system was run at 11.0–13.0 min with ratio 0:100; linear gradient of solvent A was from 0% to 95 % at 16 min.

3. Result and Discussion

3.1. TLC-Direct Bioautography for DPPH-Free Radical Scavenging

DPPH is a stable free radical commonly used to evaluate antioxidant activity because this assay is easy, fast, reliable, and simple. The DPPH is more stable, accessible than other chromogenic [12], and sensitive enough to detect antioxidant activity at low concentrations. The antioxidant components change the purple color of DPPH to yellow because antioxidants quench radicals. TLC-Bioautography for evaluating antioxidant activity by DPPH-free radicals was presented in Fig. 2. Chemical compounds with antioxidative properties showed yellow spots or yellow bands on a purple background. The yellow spots or bands indicated the antioxidant compounds of the extract. TLC-bioautography of the different extracts showed different profiles. Hexane extract only had one yellow spot on the TLC plate, while dichloromethane, ethyl acetate, and methanol extract have a yellow band (Fig. 2). It may indicate that extract dichloromethane, ethyl acetate, and methanol have several antioxidant compounds.

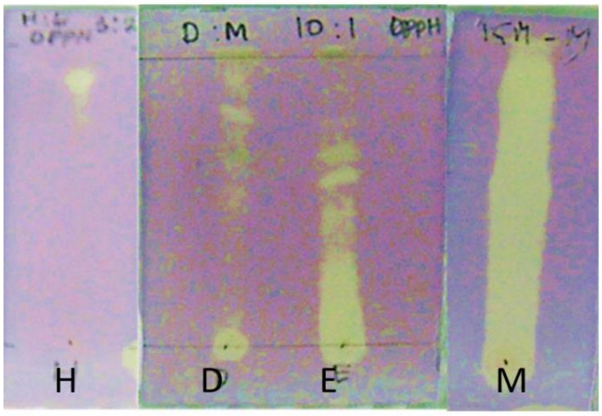


Fig. 2 TLC-bioautography profile of Hexane (H), Dichloromethane (D), Ethyl Acetate (E), and Methanol (M) Extracts of *C.pubescens* fruits after sprayed with DPPH in methanolic solution. H extract was developed in H: E (3:2), D and E extracts were developed in D: M (10:1), while M extract was developed in Chloroform:Methanol: Water (6:4:1)

3.2. Total Phenolic Content (TPC)

Phenolic is the secondary metabolite produced by plants with redox properties [13] and contributes to the antioxidant activity by scavenging free radicals or preventing the free radical's formation by hydroperoxides. Table 1 shows that increasing the polarity also increases the TPC. TPC of *C. pubescens* fruits was in the following order: n-Hexane> Dichloromethane> Ethyl acetate> Methanol fraction. The TPC of *C. pubescens* was significantly varied. It may be due to the solvent's polarity that dissolves the plant's endogenous compounds with the same polarity. The increase in polarity of the solvent increases the concentration of the phenolic, which indicates that phenolic is more soluble in the more polar solvent.

3.3. Total Flavonoid Content (TFC)

Flavonoids are a group of plant phenolic compounds characterized by the structure of benzo-r-pyrone, commonly found in fruits and vegetables [14]. Flavonoids have scavenging effects by scavenging free radicals or chelating processes in-vivo or in-vitro [15]. Table 1 showed that the lowest TFC was in the methanol fraction, while the highest was in the dichloromethane.

3.4. Determination of Antioxidant Capacity by DPPH-Free Radicals

All extracts with different solvent polarities have antioxidant activity (Fig. 2 and Table 1). The existence of antioxidant components in *C. pubescens* is likely to strengthen stamina and maintain youthfulness as local people in Lombok. Another study showed that supplementation of L-arginine and antioxidants increased exercise performance in the elderly [16]. Ethyl acetate and methanol extracts displayed very strong antioxidant activity; however, methanol extract displayed the strongest capability to scavenge free radicals DPPH. It could be attributed to the phenolic content in those extracts. It indicated that ethyl acetate and methanol extract more antioxidant compounds than n-Hexane and dichloromethane.

Table 1 TPC, TFC, IC_{50}, and AAI of *C. pubescens* fruits extract (*: Antioxidant category based on AAI value: weak < 0.5 < moderate < 1 < strong < 2 < very strong)

Extract	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	IC_{50} (ug/mL)	AAI	Antioxidant Category Based on AAI Value*
n-H	$18.81^{d} \pm 0.03$	$30.80^{b} \pm 0.02$	$24.27^{d} \pm 0.01$	$1.27^{d} \pm 0.00$	Moderate
D	$33.84^{c} \pm 0.01$	*$33.40^{a} \pm 0.02$*	$48.51^{e} \pm 0.01$	$0.63^{e} \pm 00$	Moderate
E	$82.56^{b} \pm 0.01$	$23.86^{c} \pm 0.01$	*$11.22^{c} \pm 0.02$*	*$2.74^{c} \pm 0.01$*	*Very strong*
M	*108.56^{a}* ± 0.01	$9.58^{d} \pm 0.01$	*$8.26^{b} \pm 0.02$*	*$3.72^{b} \pm 0.01$*	*Very strong*
C+	NA	NA	*$2.42^{a} \pm 0.02$*	*$12.71^{a} \pm 0.11$*	*Very strong*

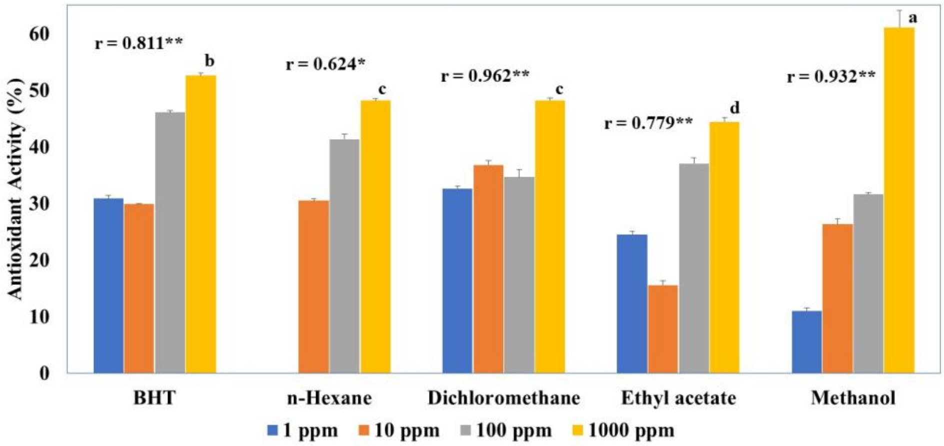
Where n-H: n-hexane extract, D: dichloromethane extract, E: ethyl acetate extract, M: methanol extract, C+: catechin as a positive control. NA: not assessed. GAE: gallic acid equivalent. QE: quercetin equivalent. IC50: inhibition concentration of 50% or the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. AAI: antioxidant activity index. Duncan's Multiple Range-Tests (DMRT) Statistical analysis of variance was performed using SPSS 16.0. The experiment was performed in triplicate and expressed as mean ± SD. Values in each column with the different letters are significantly different ($P<0.05$).

3.5. Correlations between Phenolic Content, Flavonoid Content, and Antioxidant Activity

Pearson's correlation carried out correlation assessment between antioxidant activity and total phenolic content (TPC) or between antioxidant activity and total flavonoid content (TFC). The result showed that increasing the value of TPC (gallic acid equivalents) contributes to the AAI value; however, the increasing TFC value (quercetin equivalents) does not contribute to the AAI value. There was a positive and significantly very strong Pearson's correlation between TPC and AAI value ($r = 0.950$), while a negative and significantly very strong Pearson's correlation between TFC and AAI value ($r = -0.921$) ($P<0.01$). A negative and high correlation between TPC and IC_{50} ($r = -0.743$), while TFC and IC_{50} ($r = 0.727$) had a positive and high correlation. The decrease in IC_{50} value would increase antioxidant activity. The increase in TPC is positively correlated with the antioxidant activity index due to the redox properties of the phenolic compounds in plants. Phenolic compounds can neutralize free radical lipid and prevent the decomposition of hydroperoxides into free radicals. Phenolic compounds can donate hydrogen atoms or electrons and free electrons.

3.6. *β*-Carotene Bleaching Assay

β-carotene bleaching assay is often used to determine antioxidant activity. In the *β*-carotene bleaching method, peroxyl free radicals are formed from linoleic acid oxidation at the incubation process at 50°C. Free radicals will attack the *β*-carotene molecule to absorbance decreases when observed at a wavelength of 470 nm. *β*-carotene oxidation will be minimized in the presence of antioxidants. The amount of decomposed β-carotene is related to the antioxidant activity of the extract. Antioxidants will neutralize free radicals derived from linoleic acid. The results of *β*-carotene bleaching rates of *C. pubescens* extracts were presented in Fig. 3. It showed that the antioxidant activity depended on the concentration of extracts. The increase in the concentration of the extracts was positively correlated to the increase in antioxidant activity or the increase in inhibition of *β*-carotene bleaching, with the Pearson's coefficient (r) range of 0.624-0.962 (the correlations are significant at the range of 95 – 99 % confidence levels). Antioxidant activity of the methanol extract at a concentration of 1000 ppm had the best antioxidant activity (61.08 ± 2.99 %) compared to positive control (BHT; 52.57 ± 0.40 %) and all other extracts (n-hexane, dichloromethane and ethyl acetate extracts with antioxidant activity of 48.15 ± 0.39; 48.22 ± 0.38; and 44.39 ± 0.83 %, respectively). The methanol extract's antioxidant activity was significantly higher than all extracts and positive control ($P<0.05$). The increasing concentration of all extracts increases antioxidant activity due to their higher phenolic and flavonoid content. The phenolic and flavonoid groups are highly responsible for the antioxidant activity of the plant extracts.



Statistical analysis of variance was performed by Duncan's multiple-range tests using SPSS 16.0 for 1000 ppm. Experiment was performed in triplicate and expressed as mean ± SD. Values of antioxidant activity in 1000 ppm of the extract concentration with different letter are significantly different (P<0.05). The Pearson's coefficient of correlation (r) between concentration (ppm) and antioxidant activity (%) was performed by Pearson correlation. (**): Correlation is significant at the 0.01 level. (*): Correlation is significant at the 0.05 level.

.

Fig. 3 The β-carotene bleaching inhibition of *C. pubescens* extracts

3.7. Antibacterial Activity: Minimum Inhibitory Concentration (MIC) Value

Antibacterial activities of the extracts were determined through their minimum inhibitory concentration (MIC) values against S.*aureus* InaCC B4 and E.*coli* InaCC B5. The MIC of extracts against *S.aureus* ranged from 256 - >256 µg/mL, while the MIC value of extracts against *E.coli* were >256 µg/mL (Table 2). There were 2 extracts (dichloromethane and methanol extracts) showed moderate antibacterial activity against *S.aureus* with the MIC value of 256 µg/mL. However, all extracts showed weak to moderate antibacterial activity against *E. coli* (MIC>256 µg/mL).

Table 2 Antibacterial activity of C. pubescens fruit extracts (*: Antibacterial category based on MIC values, strong < 100 µg/mL < moderate < 500 µg/mL < weak < 1000 µg/mL < inactive)

Samples	MIC value against *S. aureus* (µg/mL)	Antibacterial Category	MIC value agains *E. coli* (µg/mL)	Antibacterial Category
n-Hexane extract	>256	Weak-Moderate	>256	Weak-Moderate
Dichloromethane Extract	*256*	*Moderate*	>256	Weak-Moderate
Ethyl acetate Extract	>256	Weak-Moderate	>256	Weak-Moderate
Methanol Extract	*256*	*Moderate*	>256	Weak-Moderate
Chloramphenicol (Positive Control)	<2	*Strong*	4	*Strong*

3.8. Identification of Compounds of the Extracts using LC-MS/MS analysis

The chromatogram and mass fragmentation of the LC-MS/MS analysis showed the five major identified compounds of the extracts (Table 3 and Fig. 4). The chromatogram of n-hexane extract showed five main peaks at retention times of 9.13, 9.25, 10.09, 10.26, and 10.34 minutes (Table 3). Each peak was then fragmented and resulted in the fragmentation spectra with mass observed (*m/z*) 445.2124, 507.2287, 431.3526, 429.3726, and 413.3777 (*m/z* observed is $[M+H]^+$) with the error mass range of -0.1 to 0.9 mDa. Based on the internal spectra database, the compounds were identified as aurantiamide acetate, asperphenamate, cerevisterol, stigmastan-3,6-dione, and spinasterol at retention times of 9.13, 9.25,10.09, 10.26, and 10.34 minutes, respectively. Based on the peak area and detector count, the dominant compound in the n-hexane extract is aurantiamide acetate. This amino acid derivative compound isolated from the stem bark of *Albizia adianthifolia* has antioxidant and antimicrobial activities [17].

The LC-MS/MS analysis of dichloromethane extract showed five main peaks at retention times of 93.64, 9.12, 9.44, 9.47, and 9.51 minutes. Table 3 shows each peak was then fragmented and resulted in the fragmentation spectra with mass observed (*m/z*) for $[M+H]^+$ or $[M+Na]^+$, such as 195.0879, 445.2126, 609.2717, 593.2765, 535.2710 with the error mass range of -0.1 to 0.9 mDa. Based on the internal spectra database, the compounds were identified as (-)-bornesitol, aurantiamide acetate, azedarachin C, unknown (candidate mass $C_{34}H_{40}O_9$), and pyrophaeophorbide A at retention times of 93.64, 9.12, 9.44, 9.47, and 9.51 minutes, respectively. Based on the peak area and detector count, the dominant compounds in the dichloromethane extract are unknown compounds and azedarachin C. This limonoid derivative compound can be found in the *Melia azedarach* plant, and it has the capacity as an insect antifeedant [18].

The LC-MS/MS analysis of ethyl acetate extract showed five main peaks at retention times of 3.65, 5.10, 9.46, 9.47, and 9.52 minutes. Each peak was then fragmented and resulted in five fragmentation spectra with mass observed (*m/z*) 195.0877, 303.0505, 609.2712, 593.2764, and 535.2708 (*m/z* observed is $[M+H]^+$ or $[M+Na]^+$). Based on the internal spectra database, the compounds were identified as (-)-bornesitol, quercetin, azedarachin C, unknown (candidate mass of $C_{34}H_{40}O_9$, pyrophaeophorbide A at retention times of 3.65, 5.10, 9.46, 9.47, and 9.52 minutes, respectively (Table 3). Based on the peak area and detector count, the dominant compound in the ethyl acetate extract is similar to dichloromethane extract, namely unknown compound and azedarachin C.

The LC-MS/MS analysis of methanol extract showed five main peaks at retention times of 1.10, 3.63, 4.36, 5.12, and 9.36 minutes. Each peak was then fragmented and resulted in five fragmentation spectra with mass observed (*m/z*) 195.0877, 303.0505, 609.2712, 593.2764, and 535.2708 (*m/z* observed is $[M+H]^+$ or $[M+Na]^+$). Based on the internal spectra database, the compounds were identified as valine, (-)-bornesitol, kaempferol-3-O-rutinoside, quercetin, unknown (candidate mass $C_{29}H_{47}NO_4$ at retention times of 1.10, 3.63, 4.36, 5.12, and 9.36 minutes, respectively. Based on the peak area and detector count, the dominant compounds in the methanol extract are unknown compounds and quercetin (Table 3). Quercetin is a potent antioxidant flavonoid and, more specifically, a flavonol found in onions, grapes, berries, cherries, broccoli, and citrus fruits. It is a versatile antioxidant known to possess protective abilities against tissue injury induced by various drug toxicities and also has the capacity as α-glucosidase inhibitory activity, therapeutic effects for inflammation, obesity, and type 2 diabetes [16, 19-20].

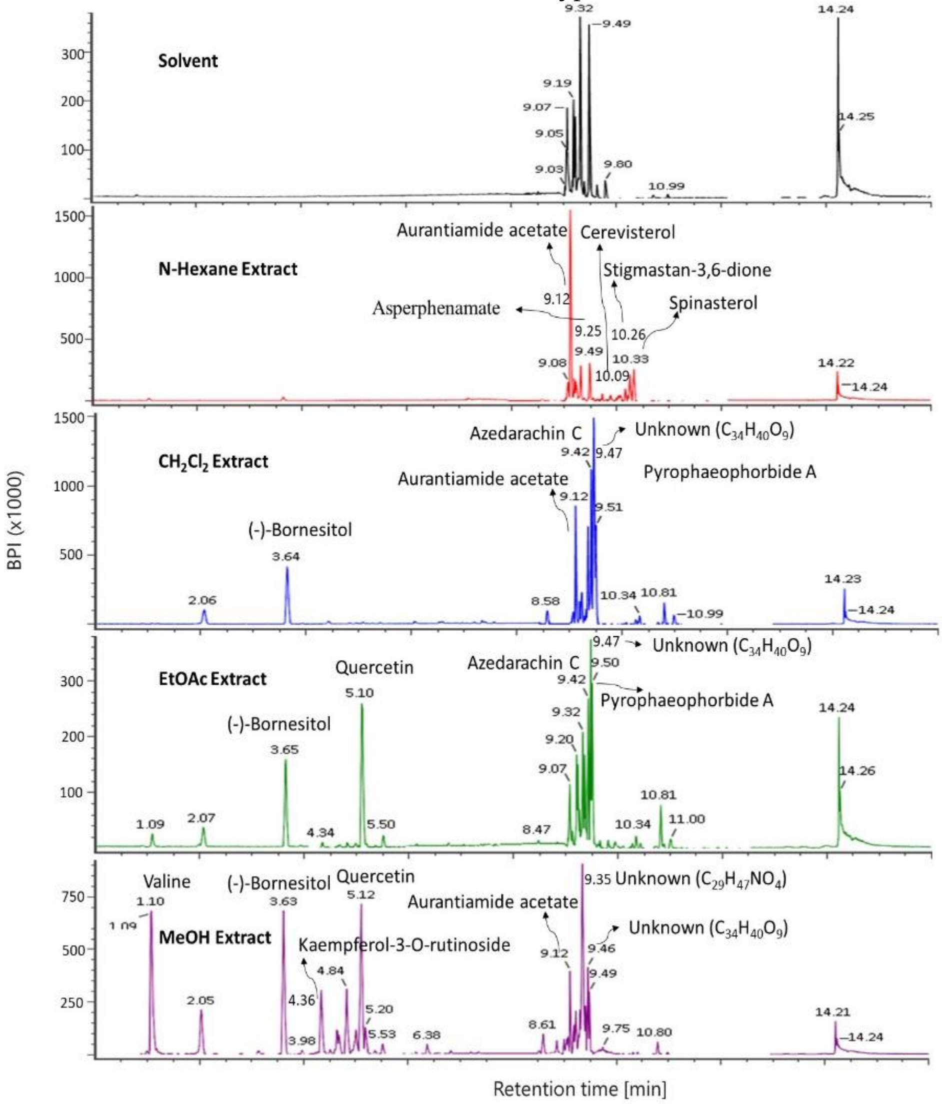


Fig. 4 Several chromatograms of *C. pubescens* fruit extracts

Table 3 Five identified compounds in *C. pubescens* fruit extracts

RT	Compounds name	Observed *m/z*	Δ Mass (mDa)	Adducts	Formula	Detector count
	n-Hexane Extract					
9.13	*Aurantiamide acetate*	*4.452.124*	*0.2*	*+H*	$C_{27}H_{28}N_2O_4$	*1,316,391*
9.25	Asperphenamate	5.072.287	0.9	+H	$C_{32}H_{30}N_2O_4$	121,419
10.09	Cerevisterol	4.313.526	0.6	+H	$C_{28}H_{46}O_3$	156,838
10.26	Stigmastan-3,6-dione	4.293.726	-0.1	+H	$C_{29}H_{48}O_2$	304,367
10.34	Spinasterol	4.133.777	-0.1	+H	$C_{29}H_{48}O$	308,355
	Dichloromethane Extract					
3.64	(-)-Bornesitol	1.950.879	1.6	+H	$C_7H_{14}O_6$	355,121
9.12	Aurantiamide acetate	4.452.126	0.4	+H	$C_{27}H_{28}N_2O_4$	380,4
9.44	*Azedarachin C*	*6.092.717*	*4.7*	*+Na*	$C_{32}H_{42}O_{10}$	*1,759,209*
9.47	Candidate Mass $C_{34}H_{40}O_9$	5.932.765	1.9	+H	$C_{34}H_{40}O_9$	1,064,641
9.51	Pyrophaeophorbide A	5.352.710	0.6	+H	$C_{33}H_{34}N_4O_3$	552,221
	Ethyl Acetate Extract					
3.65	(-)-Bornesitol	1.950.877	1.4	+H	$C_7H_{14}O_6$	143,038
5.10	Quercetin	3.030.505	0.6	+H	$C_{15}H_{10}O_7$	300,234
9.46	*Azedarachin C*	*6.092.712*	*4.2*	*+Na*	$C_{32}H_{42}O_{10}$	*336,374*
9.47	Candidate Mass $C_{34}H_{40}O_9$	5.932.764	1.9	+H	$C_{34}H_{40}O_9$	426,889
9.52	Pyrophaeophorbide A	5.352.708	0.4	+H	$C_{33}H_{34}N_4O_3$	148,96
	Methanol Extract					
1.10	Valine	1.180.862	0.0	+H	$C_5H_{11}NO_2$	396,393
3.63	*(-)-Bornesitol*	*1.950.879*	*1.6*	*+H*	$C_7H_{14}O_6$	*424,729*
4.36	*Kaempferol-3-O-rutinoside*	*5.951.670*	*1.3*	*+H*	$C_{27}H_{30}O_{15}$	*587,014*
5.12	*Quercetin*	*3.030.498*	*-0.2*	*+H*	$C_{15}H_{10}O_7$	*772,732*
9.36	Candidate Mass $C_{29}H_{47}NO_4$	4.963.402	0.5	+Na	$C_{29}H_{47}NO_4$	982,821

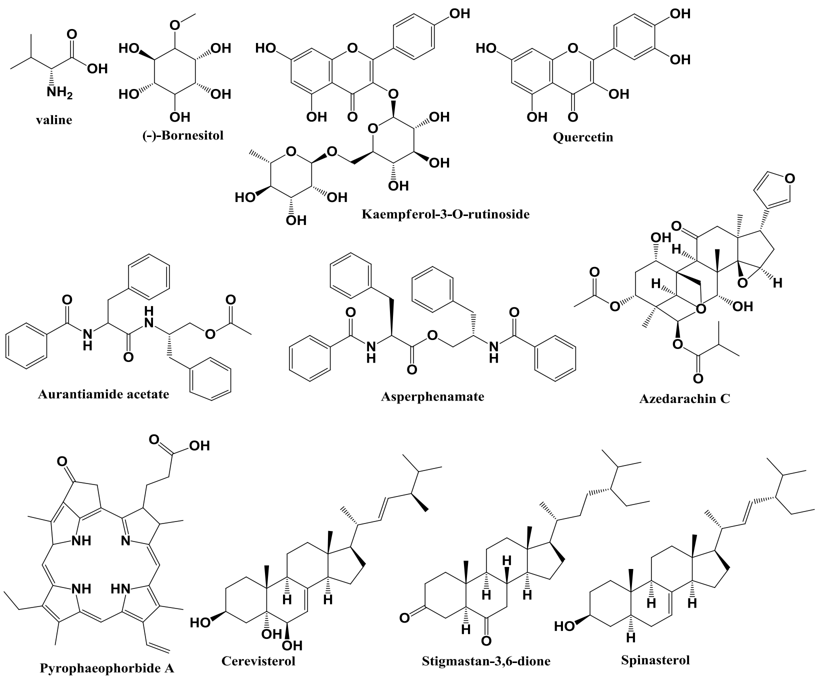


Fig. 5 Chemical structure of *C. pubescens* compounds

The chemical structure of the identified compounds of the extracts indicated that several classes of compounds such as flavonoid, phenolic, steroid, limonoid, and amino acid derivatives have various benefits for health (Table 4 and Fig. 5). The main compounds of the extracts may play a role in their capacities as antioxidant and antibacterial activity. The dichloromethane extract (with predominantly azedarachin C and unknown compound, $C_{34}H_{40}O_9$) and methanol extract (dominated by quercetin and unknown compound, $C_{29}H_{47}NO_4$) were moderate antibacterials against *S. aureus* InaCC-B4. From TLC-DB, it can be seen that all extracts can reduce free radicals or all extracts have the capacity as antioxidant activity due to the bioactive metabolite such as flavonoid, phenolic, and other compounds. The LC-MS/MS data revealed that flavonoids in the methanol extract such as quercetin and kaempferol-3-O-rutinoside might contribute to antioxidant activity (DPPH radical free scavenging and *β*-carotene bleaching inhibition) as well as antibacterial activity against as *S. aureus*, a positive-Gram bacteria.

Table 4. Some Compounds of *C. pubescens* and Their Benefits for Health.

	Component name	**Class Compound**	**Benefits**	**Literature**
1	Valine	Amino acid	Improves net photosynthetic rate and fruit quality	[21]
2	(-)-Bornesitol	cyclohexanol	Antihypertensive activity, potential use against chronic diseases such as diabetes	[22-23]
3	Kaempferol-3-O-rutinoside	Flavonoid glycoside	α-glucosidase inhibitory activity, potential use as *antifungal, antioxidant,* and anticholinesterase activities	[20, 24]
4	*Quercetin*	Flavonoid	α-glucosidase inhibitory activity, therapeutic effects for inflammation, obesity, and type 2 diabetes	[17, 20]
5	Aurantiamide acetate	Amino acid derivative	*Antioxidant and antimicrobial* activities	[19]
6	Asperphenamate	Amino acid derivative	Antitumor activity	[25]
7	*Azedarachin C*	Limonoid	Antidiabetic and Antioxidant agents	[26]
8	Pyrophaeophorbide A	Amino acid derivative	Tumor photodynamic therapy	[27]
9	Cerevisterol	Steroid	Antimicrobial activity	[28]
10	Stigmastan-3,6-dione	Steroid	Antibacterial activity	[29]
11	Spinasterol	Steroid	Antitumor activity	[30]

Evidence of the antioxidant and antibacterial properties of the fruits of *C. pubescent* can explain the use of *C. pubescent* fruit to strengthen stamina and maintain the health of the local people in Lombok.

4. Conclusion

The study results indicated that the fruit extracts of *C. pubescent* possess antioxidant activity and antibacterial activities. The DPPH radical free scavenging capacity positively correlates with the TPC values (r, 0.950). The *β*-carotene bleaching inhibition of the methanol extract in the concentration of 1000 ppm is the highest antioxidant activity and significantly different from all other extracts and positive control ($P<0.05$). The dichloromethane extract and methanol extract have moderate antibacterial against *S. aureus*. The LC-MS/MS data revealed that flavonoids in the extracts such as quercetin, kaempferol-3-*O*-rutinoside, and azedarachin C might contribute to DPPH radical free scavenging and *β*-carotene bleaching inhibition as well as antibacterial activity against Gram-positive bacteria, i.e., *S. aureus*. This study is the new report on antibacterial and antioxidant activities, the flavonoid and phenolic contents, and the other chemical composition in the *C. pusbescens* fruit extracts. This study still has limitations with only fruit samples, and the test is still *in vitro* testing. However, this study demonstrated the potential antibacterial and antioxidant activity of the *C. pusbescens* fruit extracts. Further research is needed using other parts of this plant and in vivo activity tests, including using experimental animals to prove antibacterial and antioxidant activity. In addition, the next research is needed to obtain pure compounds and perform the antioxidant and antibacterial test.

5. Acknowledgment

The authors acknowledge the facilities scientific and technical support from Advanced Characterization Laboratories Serpong, National Research and Innovation Agency through e-Layanan Sains (Elsa), Badan Riset dan Inovasi Nasional (BRIN). The authors also would like to thank Ersaliany Nurul P. Qodrie, and Andi Saptaji Kamal for their help and support assistance on the laboratory works.

6. Author Contributions

All authors contribute to obtaining/collecting data and writing the manuscript, and all authors are the main contributors.

References

[1] BOY H. I. A., RUTILLA A. J. H., SANTOS K. A., TY A. M. T., YU A. I., MAHBOOB T., TANGPOONG J., and NISSAPATORN V. Recommended Medicinal Plants as Source of Natural Products: A Review. *Digital Chinese Medicine*, 2018, 1(2): 131-142. https://doi.org/10.1016/S2589-3777(19)30018-7

[2] MSOMI N. Z., & IMELANE M. B. C. Herbal Medicine. In: BUILDERS P. F (Ed.) *Herbal Medicine*. IntechOpen, Lomdon, 2018. https://doi.org/10.5772/intechopen.72816

[3] ZANIN J. L. B., DE CARVALHO B. A., MARTINELI P. S., DOS SANTOS M. H., LAGO J. H. G., SARTORELLI P., VIEGAS C. J., and SOARES M. G. The genus Caesalpinia L. (Caesalpiniaceae): phytochemical and pharmacological characteristics. *Molecules*, 2012, 17(7): 7887–902. https://doi.org/10.3390/molecules17077887

[4] DAS B., THIRUPATHI P., RAVIKANTH B., KUMAR R. A., SARMA A. V. S., and BASHA S. J. Isolation, synthesis, and bioactivity of homoisoflavonoids from Caesalpinia pulcherrima. C*hemical and Pharmaceutical Bulletin*, 2009, 57(10): 1139–1141. https://doi.org/10.1248/cpb.57.1139

[5] RAO V. M., DAMU G. L. V., SUDHAKAR D., SIDDAIAH V., and RAO C. V. New efficient synthesis and bioactivity of homoisoflavonoids. *Arkivoc*, 2008, 11: 285-294. http://dx.doi.org/10.3998/ark.5550190.0009.b28

[6] SAEED M. A., & SABIR A. W. Antibacterial activity of Caesalpinia bonducella seeds. *Fitoterapia*, 2001, 72(7): 807–809. https://doi.org/10.1016/s0367-326x(01)00292-1

[7] SHUKLA S., MEHTA A., JOHN J., SINGH S., MEHTA P., and VYAS S. P. Antioxidant activity and total phenolic content of ethanolic extract of Caesalpinia bonducella seeds. *Food and Chemical Toxicology*, 2009, 47(8): 1848–1851. https://doi.org/10.1016/j.fct.2009.04.040

[8] THE PLANT LIST. *A Working List of All Plant Species*. 2013. www.theplantlist.org

[9] FLORA OF GREECE. *Vascular Plants Checklist of Greece*. 2020. www.portal.cybertaxonomy.org

[10] DADUS A. Private Communication, 2017.

[11] PRAPTIWI, FATHONI A., and ILYAS M. Diversity of endophytic fungi from Vernonia amygdalina, their phenolic and flavonoid contents and bioactivities. *Biodiversitas*, 2020, 21(2): 436–441. https://doi.org/10.13057/biodiv/d210202

[12] MUNTEANU IG, and APETREI C. Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, 2021, 22(7), 3380. https://doi.org/10.3390/ijms22073380

[13] ARYAL S., BANIYA M. K., DANEKHU K., KUNWAR P., GURUNG R., and KOIRALA N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, 2019, 8(4): 96. https://doi.org/10.3390/plants8040096

[14] BAKAR M. F. A., MOHAMED M., RAHMAT A. B., and FRY J. *R. Phytochemicals and antioxidant activity of different parts of bambangan (Mangifera pajang) and tarap (Artocarpus odoratissimus). Food Chemistry*, 2009, 113: 479–483. https://doi.org/10.1016/ j.foodchem.2008.07.081

[15] PAI T., SAWANT S. Y., GHATAK A., CHATURVEDI P., GUPTE A., and DESAI N. S. Characterization of Indian beers: chemical composition and antioxidant potential. *Journal Food Science and Technology*, 2013, 52: 1414–1423. https://doi.org/10.1007/s13197-013-1152-2

[16] CHEN S., JIANG H., WU X., and FANG J. Therapeutic Effects of Quercetin on Inflammation, Obesity, and Type 2 Diabetes. *Mediators of Inflammation*, 2016, 2016: 9340637. https://doi.org/10.1155/2016/9340637

[17] TAMOKOU J. D., SIMO MPETGA D. J., KEILAH LUNGA P., TENE M., TANE P., and KUIATE J. R. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of Albizia adianthifolia (Mimosoideae). *BioMedCentral Complementary Medicine and Therapies*, 2012, 12(1): 99. https://doi.org/10.1186/1472-6882-12-99

[18] HUANG R. C., OKAMURA H., IWAGAWA T., TADERA K., and NAKATANI N. Azedarachin C. a limonoid antifeedant from Melia azedarach. *Phytochemistry*, 1995, 38(3): 593-594. http://dx.doi.org/10.1016/0031-9422(94)00707-Z

[19] DAVID A. V. A., ARULMOLI R., and PARASURAMAN S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacognosy Reviews*, 2016, 10(20): 84–89. https://doi.org/10.4103/0973-7847.194044

[20] HABTEMARIAM S. A-glucosidase inhibitory activity of kaempferol-3-O-rutinoside. *Natural Product Communications*, 2011, 6(2): 201–203. https://doi.org/10.1177%2F1934578X1100600211

[21] LI S., PENG F., XIAO Y., GONG Q., BAO Z., LI Y., and WU X. Mechanisms of High Concentration Valine-Mediated Inhibition of Peach Tree Shoot Growth. *Frontiers in Plant Science*, 2020, 11. https://doi.org/10.3389/fpls.2020.603067

[22] MOREIRA L., SILVA G., CÂMARA D., PÁDUA R., LEMOS V., BRAGA F., and CORTES S. The Cyclitol L-(+)-Bornesitol as an Active Marker for the Cardiovascular Activity of the Brazilian Medicinal Plant Hancornia specios. *Biological and Pharmaceutical Bulletin*, 2019, 42(12): 2076–2082. https://doi.org/10.1248/bpb.b19-00601

[23] TOMAZI R., FIGUEIRA Â. C., FERREIRA A. M., FERREIRA D. Q., DE SOUZA G. C., DE SOUZA PINHEIRO W. B., PINHEIRO NETO J. R., DA SILVA G. A., DE LIMA H. B., and DA SILVA HAGE-MELIM L. I. Hypoglycemic Activity of Aqueous Extract of Latex from *Hancornia speciosa* Gomes: A Study in Zebrafish and In Silico. *Pharm*, 2021, 14(9): 856. https://doi.org/10.3390/ph14090856

[24] DO NASCIMENTO J. E. T., RODRIGUES A. L. M., DE LISBOA D. S., LIBERATO H. R., FALCÃO M. J. C., DA SILVA C. R., NOBRE JÚNIOR H. V., BRAZ FILHO R., DE PAULA JUNIOR V. F., ALVES D. R., and DE MORAIS S. M. Chemical Composition and Antifungal In Vitro and In Silico, Antioxidant, and Anticholinesterase Activities of Extracts and Constituents of Ouratea fieldingiana (DC.) Baill. *Evidence-Based Complementary and Alternative Medicine*, 2018, 2018: 1748487. https://doi.org/10.1155/2018/1748487

[25] SUBKO K., WANG X., NIELSEN F. H., ISBRANDT T., GOTFRESDSEN C. H., RAMOS M. C., MACKENZIE T., VICENTE F., GENILLOUD O., FRISVAD J. C., and LARSEN T. O. Mass Spectrometry Guided Discovery and Design of Novel Asperphenamate Analogs From Penicillium astrolabium Reveals an Extraordinary NRPS Flexibility. *Frontiers in Microbiology*, 2021, 11; 618730. https://doi.org/10.3389/fmicb.2020.618730

[26] YAZID F., HASANAH N. B., ROSMALENA, HANAFI M., and PRASASTY V. D. Antidiabetic and antioxidant potential of Vernonia amygdalina leaf extract in alloxan-induced Sprague-Dawley rats. *Online Journal of Biological Scien*ces, 2020, 20(4): 190-200. https://doi.org/10.3844/ojbsci.2020.190.200

[27] ZHANG X., WU Y., LI Z., WANG W., WU Y., PAN D., GU Z., SHENG R., TOMÁS T., ZHANG H., RODRIGUES J., GONG Q., and LUO K.

Glycodendron/pyropheophorbide-a (Ppa)-functionalized hyaluronic acid as a nanosystem for tumor photodynamic therapy. *Carbohydrate Polymers*, 2020, 247: 116749. https://doi.org/10.1016/j.carbpol.2020.116749

[28] APPIAH T., AGYARE C., LUO Y., BOAMAH V. E., and BOKAYE Y. D. Antimicrobial and Resistance Modifying Activities of Cerevisterol Isolated from Trametes Species. *Current Bioactive Compounds*, 2020, 16(2): 115-123. https://doi.org/10.2174/1573407214666180813101146

[29] SINAGA N. I., HANAFI M., and YANTIH N. Identification of chemical compounds and antibacterial activity of 96% ethanol extract from moringa oleifera lam. Leaves against MRSA (methicillin resistant staphylococcus aureus). *International Journal Applied Pharmaceutics*, 2021, 13(SI2): 111-114. https://doi.org/10.22159/ijap.2021.v13s2.21

[30] SEDKY N. K., EL GAMMAL Z. H., WAHBA A. E., MOSAD E., WALY Z. Y., EL-FALLAL A. A., ARAFA R. K., and EL-BADRI N. The molecular basis of cytotoxicity of α-spinasterol from Ganoderma resinaceum: Induction of apoptosis and overexpression of p53 in breast and ovarian cancer cell lines. *Journal of Cellular Biochemistry*, 2018, 119(5): 3892–3902. https://doi.org/10.1002/jcb.26515

参考文:

[1] BOY H. I. A., RUTILLA A. J. H., SANTOS K. A., TY A. M. T., YU A. I., MAHBOOB T., TANGPOONG J., 和 NISSAPATORN V. **推荐**药用植物作为天然产品的来源：综述。数字中医, 2018, 1(2): 131-142. https://doi.org/10.1016/S2589-3777(19)30018-7

[2] MSOMI N. Z., 和 IMELANE M. B. C. 草药。在：BUILDERS P. F（编辑）草药。 **英泰公开**赛，伦敦, 2018. https://doi.org/10.5772/intechopen.72816

[3] ZANIN J. L. B., DE CARVALHO B. A., MARTINELI P. S., DOS SANTOS M. H., LAGO J. H. G., SARTORELLI P., VIEGAS C. J., 和 SOARES M. G. **仙人掌 (槐科) 属**：**植物化学和**药理特性。分子, 2012, 17(7): 7887–902. https://doi.org/10.3390/molecules17077887

[4] DAS B., THIRUPATHI P., RAVIKANTH B., KUMAR R. A., SARMA A. V. S., 和 BASHA S. J. **仙人掌 中高异黄**酮的分离、合成和生物活性。化学和制药公报, 2009, 57(10): 1139–1141. https://doi.org/10.1248/cpb.57.1139

[5] RAO V. M., DAMU G. L. V., SUDHAKAR D., SIDDAIAH V., 和 RAO C. V. **同异黄**酮的新高效合成及生物活性。阿尔基沃克, 2008, 11: 285-294. http://dx.doi.org/10.3998/ark.5550190.0009.b28

[6] SAEED M. A., 和 SABIR A. W. **石竹 种子的抗菌活性**。鱼翅目, 2001, 72(7): 807–809. https://doi.org/10.1016/s0367-326x(01)00292-1

[7] SHUKLA S., MEHTA A., JOHN J., SINGH S., MEHTA P., 和 VYAS S. P. **石竹 种子乙醇提取物的抗氧化活性和**总酚含量。食品和化学毒理学, 2009, 47(8): 1848–1851. https://doi.org/10.1016/j.fct.2009.04.040

[8] THE PLANT LIST. **所有植物物种的工作清**单. 2013. www.theplantlist.org

[9] FLORA OF GREECE. **希腊**维管植物清单. 2020. www.portal.cybertaxonomy.org

[10] DADUS A. **私人通**讯, 2017.

[11] PRAPTIWI, FATHONI A., 和 ILYAS M. **杏仁斑**鸠菊内生真菌的多样性、酚类和类黄酮含量及生物活性。生物多样性, 2020, 21(2): 436–441. https://doi.org/10.13057/biodiv/d210202

[12] MUNTEANU IG, 和 APETREI C. **用于确定抗氧化活性的分析方法**：综述。国际分子科学杂志, 2021, 22(7), 3380. https://doi.org/10.3390/ijms22073380

[13] ARYAL S., BANIYA M. K., DANEKHU K., KUNWAR P., GURUNG R., 和 KOIRALA N. **尼泊**尔西部野菜的总酚含量、类黄酮含量和抗氧化潜力。植物, 2019, 8(4): 96. https://doi.org/10.3390/plants8040096

[14] BAKAR M. F. A., MOHAMED M., RAHMAT A. B., 和 FRY J. R. **班班甘** (长芒果) **和 塔拉普** (香椴木) **不同部位的植物化学物**质和抗氧化活性。食品化学, 2009, 113: 479–483. https://doi.org/10.1016/ j.foodchem.2008.07.081

[15] PAI T., SAWANT S. Y., GHATAK A., CHATURVEDI P., GUPTE A., 和 DESAI N. S. **印度啤酒的特征**：**化学成分和抗氧化潜力。食品科学与技**术杂志, 2013, 52: 1414–1423. https://doi.org/10.1007/s13197-013-1152-2

[16] CHEN S., JIANG H., WU X., 和 FANG J. **槲皮素**对炎症、肥胖和 2 **型糖尿病的治**疗作用。炎症介质, 2016, 2016: 9340637. https://doi.org/10.1155/2016/9340637

[17] TAMOKOU J. D., SIMO MPETGA D. J., KEILAH LUNGA P., TENE M., TANE P., 和 KUIATE J. R. **合**欢 (**含羞草科**) **茎皮中乙酸乙**酯提取物、组分和化合物的抗氧化和抗菌活性。 **生物医学中心** 补充医学和疗法, 2012, 12(1): 99. https://doi.org/10.1186/1472-6882-12-99

[18] HUANG R. C., OKAMURA H., IWAGAWA T., TADERA K., 和 NAKATANI N. 苦参碱 C 一种来自 苦楝 的柠檬苦素拒食剂。植物化学, 1995, 38(3): 593-594. http://dx.doi.org/10.1016/0031-9422(94)00707-Z

[19] DAVID A. V. A., ARULMOLI R., 和 PARASURAMAN S. **槲皮素的生物学重要性概述**：**一种生物活性**类黄酮。生药学评论, 2016, 10(20): 84–89. https://doi.org/10.4103/0973-7847.194044

[20] HABTEMARIAM S. **山奈酚-3-O-芸香苷的 A-葡萄糖苷酶**抑制活性。天然产物通讯, 2011, 6(2): 201–203. https://doi.org/10.1177%2F1934578X1100600211

[21] LI S., PENG F., XIAO Y., GONG Q., BAO Z., LI Y., 和 WU X. **高**浓度缬氨酸介导的桃树苗生长抑制机制。植物科学前沿, 2020, 11. https://doi.org/10.3389/fpls.2020.603067

[22] MOREIRA L., SILVA G., CÂMARA D., PÁDUA R., LEMOS V., BRAGA F., 和 CORTES S. 环醇 L-(+)-**冰片醇作**为巴西药用植物 汉考草 **心血管活**动的活性标志物。生物医药公报, 2019, 42(12): 2076–2082. https://doi.org/10.1248/bpb.b19-00601

[23] TOMAZI R., FIGUEIRA Â. C., FERREIRA A. M., FERREIRA D. Q., DE SOUZA G. C., DE SOUZA PINHEIRO W. B., PINHEIRO NETO J. R., DA SILVA G. A., DE LIMA H. B., 和 DA SILVA HAGE-MELIM L. I. 茇茇草 戈麦斯 乳胶水提取物的降血糖活性：斑马鱼和计算机研究。医药, 2021, 14(9): 856. https://doi.org/10.3390/ph14090856

[24] DO NASCIMENTO J. E. T., RODRIGUES A. L. M., DE LISBOA D. S., LIBERATO H. R., FALCÃO M. J. C., DA SILVA C. R., NOBRE JÚNIOR H. V., BRAZ FILHO R., DE PAULA JUNIOR V. F., ALVES D. R., 和 DE MORAIS S. M. 莨菪菜 的提取物和成分的化学成分和抗真菌体外和计算机模拟、抗氧化和抗胆碱酯酶活性。循证补充和替代医学, 2018, 2018: 1748487. https://doi.org/10.1155/2018/1748487

[25] SUBKO K., WANG X., NIELSEN F. H., ISBRANDT T., GOTFRESDSEN C. H., RAMOS M. C., MACKENZIE T., VICENTE F., GENILLOUD O., FRISVAD J. C., 和 LARSEN T. O. 质谱引导从星形青霉中发现和设计新型氨基苯甲酸酯 类似物揭示了一种非凡的非核糖体肽合成酶灵活性。微生物学前沿, 2021, 11; 618730. https://doi.org/10.3389/fmicb.2020.618730

[26] YAZID F., HASANAH N. B., ROSMALENA, HANAFI M., 和 PRASASTY V. D. 斑鸠菊叶提取物在四氧嘧啶诱导的 斯普拉格-道利 大鼠中的抗糖尿病和抗氧化潜力。在线生物科学杂志, 2020, 20(4): 190-200. https://doi.org/10.3844/ojbsci.2020.190.200

[27] ZHANG X., WU Y., LI Z., WANG W., WU Y., PAN D., GU Z., SHENG R., TOMÁS T., ZHANG H., RODRIGUES J., GONG Q., 和 LUO K. 羽衣甘蓝/焦脱镁叶绿酸-a-功能化透明质酸作为用于肿瘤光动力治疗的纳米系统。碳水化合物聚合物, 2020, 247: 116749. https://doi.org/10.1016/j.carbpol.2020.116749

[28] APPIAH T., AGYARE C., LUO Y., BOAMAH V. E., 和 BOKAYE Y. D. 从栓菌属中分离的 脑甾醇 的抗菌和耐药性修饰活性。当前的生物活性化合物, 2020, 16(2): 115-123. https://doi.org/10.2174/1573407214666180813101146

[29] SINAGA N. I., HANAFI M., 和 YANTIH N. 辣木 96% 乙醇提取物的化合物鉴定和抗菌活性。叶对抗耐甲氧西林金黄色葡萄球菌。国际应用药剂学杂志, 2021, 13(SI2): 111-114. https://doi.org/10.22159/ijap.2021.v13s2.21

[30] SEDKY N. K., EL GAMMAL Z. H., WAHBA A. E., MOSAD E., WALY Z. Y., EL-FALLAL A. A., ARAFA R. K., 和 EL-BADRI N. 来自灵芝的 α-菠菜甾醇的细胞毒性的分子基础：在乳腺癌和卵巢癌细胞系中诱导凋亡和 p53 过表达。细胞生物化学杂志, 2018, 119(5): 3892–3902. https://doi.org/10.1002/jcb.26515