

Physicochemical and phytochemical standardization, and antibacterial evaluation of *Cassia alata* leaves from different locations in Indonesia

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Abstract

Physicochemical analysis for simplicia and extract, respectively: water content 2.12–4% for simplicia; drying losses 3.93–5.47% and 8.51–19.76%; total ash 5.14–9.41% and 6.22–17.07%; total ash acid-insoluble content 0.29–5.41% and 0.52–3.82%; total ash water-soluble content 1.26–7.14% and 1.43–8.54%; water-soluble content 19.60–39.43% and 58.45–77.51%; ethanol-soluble content 13.99–33.76% and 59.79–75.39%. The phytochemical analysis showed that the extracts contain alkaloids, flavonoids, saponins, tannins, and terpenoids. Total flavonoids and total phenolics content were 9.86–15.74% QE/g and 6.67–7.65% GAE/g, respectively. Based on LC-MS results, the extract contained emodin, kaempferol, kaempferol-3,7-diglucoside, and kaempferol-3-O- β -D-glucopyranoside. The extracts possessed antibacterial activity against bacteria tested.

Keywords

antibacterial activity, *Cassia alata*, herbal medicine, physicochemical, phytochemical, standardization

Introduction

Cassia alata (synonym: *Senna alata* (L.) Roxb.), is a herb plant from the Fabaceae family, found in intertropical areas. It is commonly known as 'Ath thora', 'Eth thora' (Sri Lanka), candle bush/tree (Malaysia), candle stick, Carrion Crow Bush, Winged Senna, Empress Candle plant, dadmardan (India), Roman Candle trees (Fiji), and ring-worm shrub (Habtemariam 2019). In Indonesia, *Cassia alata* commonly known as ketepeng cina, but has several local names, such as ketepeng kebo (Java), ketepeng badak (Sunda), acon-aconan (Madura), sajamera (Halma-

hera), kupang-kupang (Ternate), tabankun (Tidore), daun kupang, daun kurapan, and gelinggang (Sumatra) (Asmah et al. 2020). As a traditional medicine, in many countries in the world *C. alata* leaves are used to treat digestive, dermatologic, anti-infectious, antidiabetic, and miscellaneous diseases (Hennebelle et al. 2009), including Indonesia where it is used for treating fungus on the skin which can cause hives by grinding or rubbing directly on the affected skin (Fatmawati et al. 2020).

Several studies have been reported the pharmacological activities of *Cassia alata* leaf extract, such as antibacterial (Ibrahim and Osman 1995), antifungal (Palanichamy

and Nagarajan 1990; Timothy 2012), antiviral (Angelina et al. 2017, 2020), antioxidant (Sagnia et al. 2014), cytotoxicity (Sugumar et al. 2019), antitumor (Pieme et al. 2008), anti-inflammatory (Moriyama et al. 2003), antidiabetic (Palanichamy et al. 1988), antihepatotoxic and hepatoprotective effects (Mohammed et al. 2017). Some of the reported chemical constituents are phenolics (rhein, chrysophanol, kaempferol, aloe emodin, and glycosides), anthraquinones (alatinone and alatonal), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol) (Oladeji et al. 2020).

The chemical composition of medicinal plant not only varies because of varieties or species difference and genetic factors, but also depends on external variables, such as environmental conditions, i.e. type of soil, levels of precipitation, light intensity, humidity, etc., agricultural practices, and post-harvest handling, i.e. drying, storage, processing. Inconsistency and varying its biological effects encourage standardization of phytopharmaceuticals in order to obtain reproducible results in pharmacological, toxicological, and clinical studies (Nafiu et al. 2017).

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility, which is used as a tool for quality control process (Kunle 2012). World Health Organization (1998) emphasized the need to ensure the quality of medicinal plant products, hence described a series of tests for assessing the quality of medicinal plant materials, i.e. macro and microscopic examination, determination of foreign organic matter, ash, extractable matter, moisture content, contaminants, chromatographic examination, qualitative and quantitative chemical evaluation, etc.

Several publications, United States Pharmacopoeia, British Herbal Compendium, British Herbal Pharmacopoeia, Chinese Pharmacopoeia, Physician's Desk Reference (PDR), Ayurvedic Pharmacopoeia of India carry monographs for herbal raw material. In Indonesia, based on the General Standard Parameter of Medicinal Plant Extract Handbook, released by the Ministry of Health of Indonesia (Depkes RI 2000), parameters which need to be standardized for herbal medicines are specific and non-specific parameters. Specific parameters are related to compounds found in plants, including organoleptic, water-soluble content, ethanol-soluble content, components and levels of chemical compounds. Meanwhile, non-specific parameters are not directly related to pharmacological activity but can affect the safety and stability aspects of the resulting extract or preparation, include ash content, acid-insoluble ash content, water-soluble ash content, water content, bacterial contaminants, mold and yeast contaminants and heavy metal contaminants. However, the information about *C. alata* standardization is inadequate. Previous studies reporting standardization of *C. alata* were limited to groups of compounds instead of marker compounds (Gritsanapan and Mangmeesri

2009). In the view of this background, the aims of our studies were to investigate and determine the physicochemical and phytochemical contents supplemented with data on marker compound and the antibacterial activity *C. alata* leaf obtained in four locations in Indonesia for further standardization of its usage as herbal medicine raw materials.

Materials and methods

Plant materials

The leaves of *Cassia alata* were collected from four different locations in Indonesia: Bogor Botanical Garden, West Java (6°35'51.0"S, 106°47'58.4"E), Bogor, West Java (6°23'03.3"S, 106°40'46.5"E), South Tangerang, Banten (6°21'13.1"S, 106°39'31.6"E) and Banjarmasin, South Kalimantan (3°19'38.7"S, 114°35'26.8"E). The plants were identified at Herbarium Bogoriense, Department of Botany, Indonesian Institute of Sciences, Research Center of Biology.

C. alata leaves were dried and ground using a blender to get a simplicia powder. The powder was macerated with 70% ethanol, with the ratio of 1:5 (ratio of simplicia powder and solvent) and stirred by a shaker at 110 rpm for 18 h. The extraction was repeated until the macerate color resembled the color of the solvent. Then, the extract was evaporated with a vacuum evaporator at 50 °C, yielding of the thick extract (Angelina et al. 2020).



Figure 1. *Cassia alata* leaves collected from South Tangerang, Indonesia.

Solvents, reagents, and standards

The chemical used in the study were 70% ethanol, ether, methanol, HCl, CHCl₃, H₂SO₄, HNO₃, FeCl₃, NaNO₂, AlCl₃, NaOH, H₂O₂, Na₂CO₃, NaCl, Mayer reagent, Dragendorff reagent, Bouchardat reagent, Folin-Ciocalteu, Quercetin, Gallic acid, purchased from Merck.

Microbial culture media used were Peptone Water, Potato Dextrose Agar (PDA), Selenite Cystine Broth (SCB), Luria Broth (LB), Mueller-Hinton Agar (MHA), and Brain Heart Infusion (BHI), purchased from Himedia.

Organoleptic tests

The organoleptic test was carried out to observe the shape and color of simplicia and extract according to (Depkes RI 2000).

Physicochemical analysis

Physicochemical parameters include loss on drying, water content, total ash content, acid-insoluble ash content, water-soluble ash content, water-soluble extract content, and ethanol-soluble content.

Determination of loss on drying (LOD)

LOD was determined by gravimetric determination. 2–5 g of sample was placed in crucible porcelain, dried at 105 °C for 60 min, then moved into a desiccator. This process was repeated until the constant weight was achieved. LOD was expressed as gram per gram of air-dried (World Health Organization 1998).

Determination of water content

Water content was determined by gravimetric method. 1 g of sample was heated in the oven at 105°C for 5 h, and then weighed. The process was continued with 1 h intervals until the difference between 2 consecutive weighings is not more than 0.25% (Depkes RI 2000).

Determination of total ash content

1 g of sample was placed in a silicate crucible and weighed. Sample was spread in an even layer in the crucible, and the material ignited by gradually increasing the heat to 500–600 °C until free from carbon, cooled in a desiccator, and weighed. Repeatedly until a fixed weight is obtained (World Health Organization 1998).

Determination of acid-insoluble ash content

Ash obtained from ash content testing was boiled with 25 mL HCl (~70 g/l) TS for 5 min. The ash is filtered with non-ash filter paper and washed with 5 mL hot water. The insoluble matter was transferred to the crucible, dried on a hot-plate and ignited to constant weight, and placed in a desiccator for 30 min, then weighed without delay. Content of acid-insoluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

Determination of water-soluble ash content

Containers containing total ash were added with 25 mL of water and boiled for 5 min. Material that does not soluble is collected into a glass cup or ashless filter paper. Then, it was washed with hot water and ignited in a cup for 15 min

at a temperature of 450 °C until the weight remained. The reduction of the residue weight in mg is total ash weight. The water-soluble ash is calculated in mg per g of air-dried material (World Health Organization 1998).

Determination of water- and ethanol-soluble extract

5 g of extract was macerated with 100 mL of water for 6 h for water-soluble extract determination, and then saturated with CHCl_3 . For ethanol-soluble determination, it was macerated with ethanol. They were shaken frequently, and allowed to stand for 18 h. The extract produced was filtered and poured into a volumetric flask. 20 mL of extract was transferred to a porcelain cup, evaporated until dry. The residue was heated using an oven at 105 °C to receive constant weight. The soluble extract was calculated in g per g of air-dried material (Depkes RI 2000).

Qualitative phytochemical analysis

Alkaloid screening

10 mg of extract was dissolved with 2 mL of 5% HCl and filtered. The filtrate was divided into 4 tubes. Drops of Mayer, Dragendorff, and Bouchardat reagents were added to each tube. The fourth filtrate serves as a positive control. The formation of a yellowish-white precipitate (Mayer), red–orange precipitate (Dragendorff), and brown precipitate (Bouchardat) indicates the presence of alkaloids (María et al. 2018).

Flavonoid screening

A few drops of concentrated HCl were added to a small amount of the extracts of the plant material. Immediate development of a red color was taken as an indication of the presence of flavonoids (Abdel-motaal et al. 2016).

Tannin screening

0.5 g of extract was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% FeCl_3 was added and observed for brownish green or a blue-black coloration (Edeoga et al. 2005).

Phenolic screening

0.2 g of thick extract was added with 2 mL of 5% FeCl_3 solution. A positive result is indicated by the formation of a bluish color (Adusei et al. 2019).

Saponin screening

0.5 g of extract was shaken with 10 mL of hot water. If foam produced persists for 5 min, it indicates the presence of saponins (Iqbal et al. 2015).

Terpenoid screening

Terpenoids screening was determined by Salkowski test 2 mL of sample was mixed in 2 mL of CHCl_3 , and 2 mL concentrated H_2SO_4 was carefully added to form

a layer. A reddish brown color produced in the lower chloroform layer indicates the presence of terpenoid (Bargah 2015).

Identification major compound using LC-MS

Mass spectrometry was performed on a Xevo, G2-XS QToF (Waters MS Technologies) according to the method from (Angelina et al. 2020). Ionisation type is ESI. The scan range was from 100 to 1200 m/z. The capillary and cone voltage was set at 0.8 kV and 30 kV, respectively and was used positive electro spray mode. The desolvation gas was set to 1000 L/h at 500 °C and the cone gas was set to 50 L/h and the source temperature was 120 °C. The UPLC analysis was performed using a Waters Acquity Ultra Performance LC system. Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 C18 column (100 mm × 2.1 mm, 1.7 µm) at a column temperature of 40 °C. The mobile phase consisted of solvent A (0.1% formic acid in water, v/v) and solvent B (0.1% formic acid in acetonitrile), with gradient polarity from 95:0.5 (A:B) to 0.5:95 (A:B). The flow rate was 0.3 mL/min. The column and auto sampler were maintained at 40 °C and 20 °C, respectively and the injection volume was 1 µL. The data acquisition and processing were performed using UNIFI. The parameter used was retention time (RT) in the range of 1–15 min. Determination of the dominant compound was carried out with semi quantitative determination based on the high intensity of the compound on the LC-MS/MS graph.

Quantitative phytochemical analysis

Determination of total flavonoids content

Total Flavonoid Content (TFC) was assessed by spectrophotometric method with minor modification (Dewi and Maryani 2015). For each sample, 500 µL of the samples (1000 µg/mL) were mixed with 2.2 mL of aquadest and 150 µL of 5% NaNO₂. After 5 min, 150 µL of 10% AlCl₃ was added. Then, 6 min later, 2 mL of 1M NaOH was added. The absorbance was read at 510 nm. Quercetin was taken as standard for the calibration curve. The total flavonoid content was calibrated using the calibration curve based linear equation. The total flavonoid content was expressed as mg quercetin equivalent/g dry extract.

Determination of total phenolics content

The total phenol content was determined by using the Folin-Ciocalteu method (Dewi and Maryani 2015) with few modifications. For each sample, 500 µL of the samples (1000 µg/mL) was added to 3.5 mL distilled water and 250 µL of 2N FC reagent. The mixture was incubated at room temperature for 8 min, and then 250 µL of 20% Na₂CO₃ was added to the mixture, and incubated again for 2 h. The absorbance was read at 765 nm. Gallic acid was taken as standard for the calibration curve. The total phenol content was expressed as mg gallic acid equivalent/g dry extract.

Metal contamination evaluation

The concentration of lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). 1 g samples were digested with 9 mL HNO₃ and 3 mL H₂O₂. The mixture was heated at 180 °C for 25 min. After this destruction process, the sample was diluted with 0.5M HNO₃ until the volume reaches 50 mL and analyzed with Agilent 5110 ICP-OES.

Microbial contamination evaluation

Microbial contamination was investigated on *Escherichia coli* (LB), *Salmonella* sp & *Shigella* sp (SCB), *Pseudomonas aeruginosa* (Peptone Water), Total Plate Count (NA), Yeast & Mould Count (PDA). All mediums were dissolved with aquadest and sterilize 1 mL of sample was placed at the petri dish (NA and PDA) and tubes (LB, SCB and Peptone Water), and mixed with 9 mL of each media tested. The plates and tubes were incubated at 27 °C for 24 h for bacteria and 3 days for molds/yeast.

Antibacterial activity

Antibacterial activity was assessed against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, and *Bacillus subtilis* ATCC 6633. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the broth microdilution assay according to (Jorgensen and Ferraro 2009; Septama and Panichayupakaranant 2017) with few modifications. The bacteria were incubated with MHA at 37 °C for 24 h, and then transferred in the sterile 0.9% NaCl. The turbidity of the suspension was adjusted to the 0.5 McFarland standard solution, which was equivalent to 10⁸ CFU/mL. The MIC was performed using a 96-well microplate, consisted of 100 µL BHI for each well. For starting, 100 µL of 1000 µg/mL extract was added and diluted until final concentration used was 500, 250, 125, 62.5 and 31.25 µg/mL. Afterwards, 100 µL of bacteria suspension was added. MIC was done at 37 °C after 24 h of incubation. MIC was defined as the lowest concentration of extracts at which the microorganism tested did not demonstrate visible growth. The MBC tests were performed by scratching suspension from the microplate at MIC, below and above MIC value. MBC was defined as the lowest concentration yielding negative subcultures or only one colony.

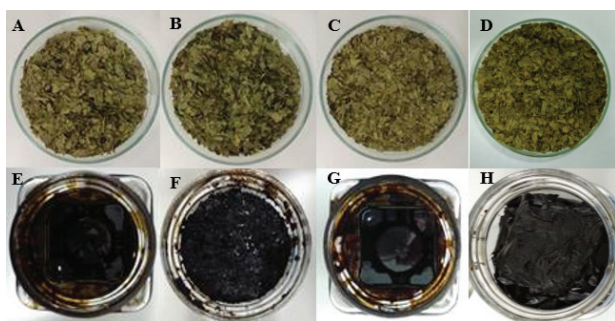
Results and discussion

Organoleptic test

The results of organoleptic observations from shape and color of simplicia and *C. alata* leaf can be seen in Fig. 2 and Table 1. The data obtained are used as a standard for good simplicia raw materials and extracts before being used for further testing. The simplicia of *C. alata* leaf from Bogor,

Table 1. Result of organoleptic test of simplicia and extracts *C. alata* leaf.

Parameters	Bogor	Bogor Botanical Garden	South Tangerang	Kalimantan
Simplicia				
Shape	Coarse powder	Coarse powder	Coarse powder	Coarse powder
Color	Brownish green	Brownish green	Brownish green	Brownish green
Extracts				
Shape	Thick	Thick	Thick	Thick
Color	Blackish brown	Blackish brown	Blackish brown	Blackish brown

**Figure 2.** (A–D) The organoleptic profiles of *C. alata* simplicia. A: Bogor; B: Bogor Botanical Garden; C: South Tangerang; D: Kalimantan. It had rough shape with a brownish green color. (E, F) The organoleptic profiles of *C. alata* ethanol extract. E: Bogor; F: Bogor Botanical Garden; G: South Tangerang; H: Kalimantan. It was thick and brownish black in color.

Bogor Botanical Garden, South Tangerang and Kalimantan had a rough shape with a brownish green color. While the 70% ethanol extract was thick and brownish black in color.

Physicochemical analysis

Table 2 shows the result of physicochemical analysis of *C. alata* simplicia and extracts. The loss of drying (LOD) determines the maximum limit range of the compound lost during the drying process. During the drying process, the

Table 2. Results of physicochemical analysis from *C. alata* leaf simplicia and extracts.

Parameters	Bogor	Bogor Botanical Garden	South Tangerang	Kalimantan
Simplicia				
Loss on drying (%)	5.09	5.15	5.47	3.93
Water content (%)	-	3.44	2.12	4
Total ash content (%)	6.94	9.41	7.10	5.14
Acid-insoluble ash content (%)	0.65	0.39	5.41	0.29
Water soluble ash content (%)	1.93	7.14	1.26	1.95
Water soluble content (%)	19.60	39.43	27.23	29.72
Ethanol soluble content (%)	22.91	13.99	24.18	33.76
Extracts				
Loss on drying (%)	9.35	19.76	17.38	8.51
Water content (%)	-	-	-	-
Total ash content (%)	8.91	17.07	6.22	6.94
Acid-insoluble ash content (%)	3.05	3.82	2.44	0.52
Water soluble ash content (%)	8.54	7.97	1.43	2.79
ater soluble content (%)	77.51	66.38	73.71	58.45
Ethanol soluble content (%)	59.79	69.53	75.39	75.20

air and compounds with a lower boiling point than air will evaporate (Depkes RI 2000). According to the results, losses on drying from simplicia from the lowest were *C. alata* from Kalimantan 3.93%, Bogor 5.09%, Bogor Botanical Garden 5.15%, and South Tangerang 5.47%. While losses on drying of extract from the lowest were Kalimantan 8.51%, Bogor 9.35%, South Tangerang 17.38%, and Bogor Botanical Garden 19.76%, respectively. The LOD value of the extract we examined was higher than the previous study (Gritsanapan and Mangmeesri 2009) whose LOD value was no more than 6%. One of the causes is due to the difference in solvents in the extraction process. In this study we used ethanol 70% while in the previous study used ethanol 80%. The high water content in the solvents we use is one of the things that cause the extract's LOD value to be higher.

Determination of water content in the sample was set to maintain the quality of the extract. The less water content in crude and extracts, the less possibility of contamination by mold growth. According to the literature (Depkes RI 2008), the water content should not be more than 10%. The water content of simplicia is between 2.12%–4%. Hence, it can be concluded that the water content of all samples of simplicia *C. alata* leaf met the standard.

Determination of total ash content was carried out to measure the total amount of external and internal mineral in simplicia or extract from the initial process to the end of manufacture. The high value of the total ash content indicates that the sample contains minerals. Total ash content in simplicia was 5.4%–9.41% and in extract was 6.94%–17.07%. In this test there are two parameters to determine type of nutrient absorbed by plants, acid-insoluble ash content and water-soluble ash content. Water-soluble ash content was tested by dissolving the ash into water, and determined the amount of mineral soluble in water, while the acid-insoluble ash content indicates the amount of mineral insoluble in acid. The high value of acid-insoluble ash content indicates that the sample has a silicate component associated with soil or sand, silver, lead or mercury (Salim 2016). Acid-insoluble ash content in simplicia was 0.29%–5.41% and in extract was 0.52%–3.82%. Water-soluble ash content in simplicia was 1.26%–7.14% and in extract was 1.43%–8.54%. Based on the results, the value of water-soluble ash content is higher in simplicia and extract indicates that the sample does not contain much silica component or other impurities.

The soluble content parameter determines the amount of chemical components in simplicia and extracts in certain solvents. The chemical compounds in the sample which can be dissolved are related to reproducibility in the pharmacodynamic activity of the crude drugs. The ability of each plant to dissolve in water or other organic solvents is different. Water solvent used to dissolve polar compounds and ethanol to dissolve the less polar compounds (Wirnawati et al. 2020). Water-soluble content in simplicia was between 19.60%–39.43%, and in extract was 58.45%–77.51%. The ethanol-soluble content in simplicia was 13.99%–33.76% and in extract was 59.79%–75.39%. Based on the results (Table 2) water-soluble content value

in simplicia and extract is higher than the levels of ethanol-soluble content, which indicates that the sample of simplicia and extract contain more polar substances which make it easier to dissolve in water than ethanol.

Phytochemical analysis

Phytochemical screening was carried out to identify the phytoconstituents present in the *C. alata* leaf extract. Based on the screening (Table 3), the compounds contained in the ethanol extract of *C. alata* leaves from Bogor, Bogor Botanical Garden, South Tangerang, and Kalimantan are alkaloid, flavonoid, terpenoid, saponin and tannin. The results obtained are in accordance with the research conducted by (Kumavat et al. 2011) which showed that the phytochemical components contained in the ethanol extract of *C. alata* leaves were alkaloids, tannins, steroids, glycosides, sterols, terpenoids, flavonoids. As well as research conducted by (Raji et al. 2015; Nagarajan 2018) which showed that the content of phytochemical compounds in the ethanol extract of *C. alata* leaves were phenols, steroids, flavonoids, terpenoids and alkaloids.

Table 3. Qualitative and quantitative phytochemical determination of extract *C. alata* leaf extract.

Parameters	Bogor			
	Bogor	Bogor Botanical Garden	South Tangerang	Kalimantan
Alkaloid				
Meyer	-	-	-	-
Bouchardat	+	+	+	+
Dragendorf	+	+	+	+
Flavonoid	+	+	+	+
Terpenoid	+	+	+	+
Saponin	++	++	++	++
Tanin	+	+	+	+
Total flavonoids content (%QE)	14.13	10.15	9.86	15.74
Total phenolics content (%GAE)	6.75	6.67	7.65	-

Alkaloids are semi polar, have a nitrogen atom that has a lone pair of electrons that will bond covalently to the iodine ion in the reagent and have substituents such as phenol, amine, amide and methoxy (Kurmukov 2013). Alkaloids qualitative tests were carried out using Dragendorf, Meyer and Bouchardat reagents. The results showed the presence of alkaloids with the formation of orange sediment in Dragendorf's reagent and brown sediment in Bouchardat's reagents, while in Meyer's reagents there was no sediment. Terpenoids consist of several compounds including volatile monoterpenes, sesquiterpenes, volatile diterpenes, non-volatile triterpenes and sterols. It

is characterized by the formation of a red, pink or violet color. The results showed that the extract of *C. alata* leaf contained terpenoids. Saponin is a secondary metabolite belonging to surface compounds and found in many plants. Saponins are characterized by the presence of foam after shaking in aqueous solution. The results showed that the extract of *C. alata* leaf contains saponins. Tannins are compounds that are commonly found in vascular plants, have a phenol group and astringent taste (Kurmukov 2013). Based on the results, the extract of *C. alata* leaf contained tannin compounds. This was indicated by a change in the color of the solution to blackish green.

The measurement data using LC-MS is seen at Table 4 and the chromatograms are shown in Fig. 3. The chemical constituents detected were emodin, kaempferol, kaempferol-3,7-diglucoside, and kaempferol-3-O-β-D-glucopyranoside (Fig. 3). It can be seen that the % area detected from each compound from four sources in Indonesia was different, hence it can be concluded that the phytochemicals of extracts from different locations were different.

From our previous study (Angelina et al. 2020), the ethanolic extract of *C. alata* leaf contained 5,7,2',5'-tetrahydroxy-flavone, daturametelin H, and kaempferol-3,7-diglucoside. Metabolic profiles of medicinal plants are mainly controlled by genetic factors, but their production is also influenced by environmental factors (Muraina et al. 2008; Mohammadi Bazargani et al. 2021). In another studies, it has been reported that chemical constituents of *C. alata* leaf ethanol extract were aloe emodin, emodin, ω-hydroxyemodin, lunatin, physcion, ziganein, apigenin, 7,4'-dihydroxy-5-methoxyflavone, diosmetin, kaempferol, luteolin, trans-dihydrokaempferol, trans-resveratrol (Promgool et al. 2014). From numerous studies, flavonol compounds which have been identified were kaempferol, kaempferol-3-O-β-D-glucopyranoside, 3,5,7,4'-tetrahydroxy flavone, 2,5,7,4'-tetrahydroxy isoflavones, anthraquinone and kaempferol 3-O-gentiobioside (Fatmawati et al. 2020). Alkaloid compounds from *C. alata* leaves have also been identified, such as adenine, chrysoeriol, quercetin, 5,7,40-trihydroflavanone, kaempferol-3-O-beta-D-glucopyranosyl-(1→6)-beta-D-glucopyranoside, n-dotriacontanol, n-triacontanol, stearic acid, palmitic acid, diomestin, luteolin (Joel et al. 2017), and 1,3,5-trihydroxy-7-methylanthracene-9,10-dione (Promgool et al. 2014; Joel et al. 2017; Fatmawati et al. 2020).

The total flavonoids content (Table 3) ranged from 9.86% to 15.74% QE. The highest concentration was found in *C. alata* from Kalimantan (15.74%) followed by Bogor (14.13%), Bogor Botanical Garden (10.5%) and South Tangerang (9.86%). Quantitative analysis of total

Table 4. LC-MS analysis of *C. alata* leaf extract.

Compounds	Bogor				Bogor Botanical Garden		South Tangerang		Kalimantan		
	Name of Compound	Molecular weight	Molecular formula	RT	% Area	RT	% Area	RT	% Area	RT	% Area
Emodin		270.05282	C ₁₅ H ₁₀ O ₅	6.66 & 7.58	2.64	6.58 & 7.5	3.99	6.58 & 7.5	3.03	6.60 & 7.53	2.24
Kaempferol		286.04774	C ₁₅ H ₁₀ O ₆	6.73	8.04	6.65	12.86	6.66	9.63	6.67	12.06
Kaempferol-3,7-diglucoside		587.15338	C ₂₇ H ₃₀ O ₁₆	4.48	9.77	4.48	14.59	4.48	18.41	4.48	37.70
Kaempferol-3-O-β-D-glucopyranoside		425.10056	C ₂₁ H ₂₀ O ₁₁	4.99	3.04	4.98	4.17	4.98	4.18	4.98	3.31

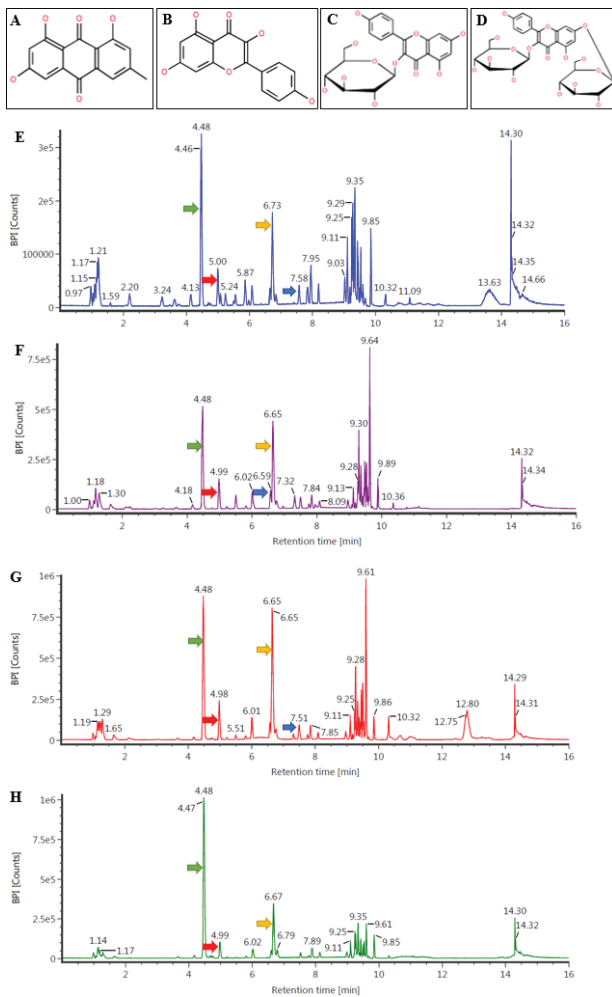


Figure 3. (A–D) The chemical constituents detected in *C. alata* leaf ethanol extract. **A:** Emodin. **B:** Kaempferol. **C:** Kaempferol-3-O- β -D-glucopyranoside. **D:** Kaempferol-3,7-diglucoside). (E–H) The chromatograms from LC-MS of *C. alata* leaf ethanol extract. **E:** Bogor. **F:** Bogor Botanical Garden. **G:** South Tangerang. **H:** Kalimantan. The chemical constituents are marked by green arrow: kaempferol-3,7-diglucoside; red arrow: kaempferol-3-O- β -D-glucopyranoside; yellow arrow: kaempferol, blue arrow: emodin.

flavonoid compounds was carried out with measurements based on the formation of complexes between $AlCl_3$ with ketone groups on C-4 atoms and hydroxyl groups on C-3 or C-5 atoms adjacent to flavones and flavonols (Nurlinda et al. 2021). The standard solution in this study was quercetin, a flavonol that is widespread in plants, and made up to 60–75% of flavonoids (Nurlinda et al. 2021).

The concentration of total phenolic content is given in terms of GAE/g of the extract (Table 3) and ranges from 6.67 to 7.65% GAE. The highest amount of phenolic content was observed in *C. alata* extract from South Tangerang (7.65%), followed by Bogor (6.75%), and botanical garden (6.67%). Phenolic compounds are widely distributed in a variety of plants. Natural phenolic can range from simple molecules, such as phenolic acid, to highly polymerised compounds, such as tannins (Debebe et al. 2016). The total phenolic content of the extract *C. alata* leaf was determined using Folin-Ciocalteu's reagent. Phenols reacted with the Folin-Cio-

calteu reagent in the presence of sodium carbonate to form a blue-coloured complex. The intensity of the blue color is proportional to the amount of reactive phenolic compounds in the sample (Kupina et al. 2019). It is also mentioned in (Debebe et al. 2016), that the presence of compounds in plants is largely influenced by genetic factors and environmental conditions, other factors such as germination, degree of ripeness, variety, processing and storage. Based on the results obtained, it shows that the area where *C. alata* grows affects the total amount of flavonoids and phenolics.

Contamination evaluation

Heavy metal contamination testing aims to determine the levels of metal content, i.e. Pb, Cd, Hg and As contained in extract, which is dangerous and toxic to the body. The results obtained on the levels of Pb, Cd, Hg and As shows the absence of heavy metals in *C. alata* leaf extract (Table 5), hence the samples meet the standards, where the maximum limit for Pb is ≤ 10 mg/kg, Cd ≤ 0.3 mg/kg, Hg ≤ 0.5 mg/kg and As ≤ 5 mg/kg in herbal capsule containing dry extract (Vinet and Zhedanov 2011).

Microbial contamination reveals the impurity in me-

Table 5. Metal and microorganism contamination of extract *C. alata* leaf.

Parameters	Bogor	Bogor Botanical Garden	South Tangerang	Kalimantan
Pb	-	-	-	-
Cd	-	-	-	-
Hg	-	-	-	-
As	-	-	-	-
Total plate number	0	0	0	0
Total yeast and mold number	0	1×10^2	0	0
Pathogen bacteria				
<i>Escherichia coli</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-
<i>Salmonella</i> sp	-	-	-	-
<i>Shigella</i> sp	-	-	-	-

dicinal plants, which come from the preparation or final products. Based on microbial contamination test, the total plate number shows the absence of bacteria in the extract of *C. alata* leaf (Table 5), hence the samples meet the standard of (BPOM 2014) which shows that the maximum limit range for the total plate number test is $\leq 10^5$ CFU/g.

The results of total yeast and mold number test extract of *C. alata* leaf from Bogor, South Tangerang and Kalimantan did not contain yeast and mold, while extract of *C. alata* leaf from Bogor Botanical Garden contained yeast and mold of 1×10^2 CFU/g. The result was still below the limit, where the limit of mold and yeast contamination that may be present in capsule preparations containing dry extract is $\leq 10^3$ CFU/g (BPOM 2014).

The identification test of pathogenic bacteria is carried out to detect the presence of pathogenic bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* sp, and *Shigella* sp. Based on the test results, in all samples there were no pathogenic bacteria tested. This met the standard of (BPOM 2014) which shows that the

maximum limit of *E. coli* contamination is ≤ 10 CFU/g, for *Salmonella* and *Shigella* negative/g.

Antibacterial activity

Antibacterial activity of *C. alata* leaf can be seen in Table 6. From the MIC value, the extract from all areas showed similar antibacterial strength against all bacteria tested whether Gram positive bacteria (*S. aureus*, *S. epidermidis*, and *B. subtilis*) or Gram-negative bacteria (*E. coli*). The MIC of all samples against *S. aureus* was 250 $\mu\text{g}/\text{mL}$, meanwhile for the rest of bacteria tested the MIC was 125 $\mu\text{g}/\text{mL}$. From the MBC value, the lowest concentration is from extract from South Tangerang and Kalimantan which was 1000 $\mu\text{g}/\text{mL}$ against *S. aureus* and 250 $\mu\text{g}/\text{mL}$ against other bacteria, whilst the highest MBC was from Bogor which was 1000 $\mu\text{g}/\text{mL}$ against *S. aureus* and 500 $\mu\text{g}/\text{mL}$ against other bacteria. It can be seen that the geographical factor from the plants affects its antibacterial activity.

The antibacterial activity from this study confirms pre-

Table 6. Antibacterial activity of extract *C. alata* leaf ($\mu\text{g}/\text{mL}$).

Bacteria	Bogor		Bogor Botanical Garden		South Tangerang		Kalimantan	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Escherichia coli</i>	125	500	125	250	125	250	125	250
<i>Staphylococcus aureus</i>	250	1000	250	1000	250	1000	250	1000
<i>Staphylococcus epidermidis</i>	125	500	125	500	125	250	125	250
<i>Bacillus subtilis</i>	125	500	125	250	125	250	125	250

vious study (Khan et al. 2001; Somchit et al. 2003) where it reported *C. alata* leaves extract antibacterial activity. The MIC and MBC values against *E. coli* bacteria obtained in this study were similar to the values of MIC and MBC in previous studies (El Mahmood AM 2008). The antibacterial effect of the *C. alata* leaves extracts may be due to presence of flavonoids, phenols, alkaloids, saponins, tannins, and terpenoids detected (Wikaningtyas and Sukandar 2016). Several researches have investigated the isolated compound of *C. alata* which are responsible for antibacterial activity. Bioactive compounds such as kaempferol, luteolin, and aloe emodin isolated from the methanolic leaves extract showed antibacterial activity against MDR bacteria strains, which could be linked to the anthraquinone and flavonoids compounds detected (Joel et al. 2017). Compounds detected in this study

References

- Abdel-motaal FF, Abdel-farid IB, El-sayed MA (2016) Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *Journal of the Saudi Society of Agricultural Sciences* 15: 112–117. <https://doi.org/10.1016/j.jssas.2014.06.002>
- Adusei S, Otchere JK, Oteng P, Mensah RQ, Tei-Mensah E (2019) Phytochemical analysis, antioxidant and metal chelating capacity of

also were reported have antibacterial activity. Emodin isolated from *C. alata* showed strong antibacterial activity against MRSA with MIC value of 4 $\mu\text{g}/\text{mL}$ and moderate antibacterial activity against *B. cereus* and *S. aureus* MIC value of 16 $\mu\text{g}/\text{mL}$, but showed weak antibacterial activity against Gram-negative bacteria (MICs 128 $\mu\text{g}/\text{mL}$) (Promgool et al. 2014). Kaempferol 3-O- β -glucopyranoside exhibited MIC₅₀ values of 13.0 ± 1.5 $\mu\text{g}/\text{mL}$ against MRSA (Hazni et al. 2008). Kaempferol and kaempferol-3,7-diglucoside also have been investigated to have antibacterial activity (Saito et al. 2012; Mahmood 2014). Other studies have also reported that *C. alata* has activity against microorganisms that cause skin diseases in human (Ibrahim and Osman 1995).

Conclusion

In summary, findings from this investigation suggest that the physicochemical and phytochemical properties of *C. alata* from several locations in Indonesia fulfilled the requirements as raw material for herbal drugs and showed similar antibacterial activity. Future experiments aimed at separation and isolation of individual compounds, as well as other *C. alata* pharmacological effects and its mechanisms which are still unexplored sufficiently.

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Conflicts of interest

The authors declare no conflicts of interest.

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Tetrapleura tetraptera. *Heliyon* 5: e02762. <https://doi.org/10.1016/j.heliyon.2019.e02762>

- Angelina M, Hanafi M, Suyatna FD, S Mirawati T, Ratnasari S, Dewi BE (2017) Antiviral Effect of Sub Fraction *Cassia alata* Leaves Extract to Dengue Virus Serotype-2 strain New Guinea C in Human Cell Line Huh-7 it-1. *IOP Conference Series: Environmental Earth Sciences* 101: 012004. <https://doi.org/10.1088/1755-1315/101/1/012004>

- Angelina M, Hanafi M, Suyatna FD, Dewi BE (2020) Drug of action cassia alata leaves extract as antiviral to dengue virus serotype-2 in vitro. *Pharmacognosy Journal* 12: 864–871. <https://doi.org/10.5530/pj.2020.12.124>
- Asmah N, Halimatussakdiah H, Amna U (2020) Analisa Kandungan Senyawa Metabolit Sekunder Ekstrak Daun Ketepeng Cina (*Cassia alata* L.) dari Bireum Bayeun, Aceh Timur. *QUIMICA: Jurnal Kimia Sains dan Terapan* 2: 7–10. <https://doi.org/10.33059/jq.v2i2.2646>
- Bargah R (2015) Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. 4: 7–9. <https://doi.org/10.9790/5736-071016972>
- BPOM (2014) Persyaratan Mutu Obat Tradisional. Badan Pengawas Obat dan Makanan: 1–25.
- Debebe A, Chandravanshi BS, Redi-Abshiro M (2016) Total contents of phenolics, flavonoids, tannins and antioxidant capacity of selected traditional Ethiopian alcoholic beverages. *Bulletin of the Chemical Society of Ethiopia* 30: 27–37. <https://doi.org/10.4314/bcse.v30i1.3>
- Depkes RI (2000) Parameter Standar Umum Ekstrak Tumbuhan Obat.
- Depkes RI (2008) Anonim 2008. *Farmakope Herbal Indonesia*: 1–221.
- Dewi RT, Maryani F (2015) Antioxidant and α -Glucosidase Inhibitory Compounds of *Centella asiatica*. *Procedia Chemistry* 17: 147–152. <https://doi.org/10.1016/j.proche.2015.12.130>
- Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4: 685–688. <https://doi.org/10.5897/AJB2005.000-3127>
- El-Mahmood AM, Doughari JH (2008) Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. *Africa Journal of pharmacy and Pharmacology* 2(7): 124–129. <https://doi.org/10.5897/AJPP.9000226>
- Fatmawati S, Yuliana, Purnomo AS, Abu Bakar MF (2020) Chemical constituents, usage and pharmacological activity of *Cassia alata*. *Heliyon* 6. <https://doi.org/10.1016/j.heliyon.2020.e04396>
- Gritsanapan W, Mangmeesri P (2009) Standardized *Senna alata* Leaf Extract.: 6.
- Habtemariam S (2019) Chapter 26 – Antidiabetic herbal medicines rebranded as dietary supplements. In: Habtemariam S (Ed.) *Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases*. Academic Press, 1049–1134. <https://doi.org/10.1016/B978-0-08-102922-0.00026-2>
- Hazni H, Ahmad N, Hitotsuyanagi Y (2008) Phytochemical Constituents from *Cassia alata* with Inhibition against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Planta Medica* 74(15): 1802–1805. <https://doi.org/10.1055/s-0028-1088340>
- Hennebelle T, Weniger B, Joseph H, Sahpaz S, Bailleul F (2009) *Senna alata*. *Fitoterapia* 80: 385–393. <https://doi.org/10.1016/j.fitote.2009.05.008>
- Ibrahim D, Osman H (1995) Antimicrobial activity of *Cassia alata* from Malaysia. *Journal of Ethnopharmacology* 45(1995): 151–156. [https://doi.org/10.1016/0378-8741\(94\)01200-J](https://doi.org/10.1016/0378-8741(94)01200-J)
- Iqbal E, Abu K, Lim LBL (2015) Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University – Science* 27: 224–232. <https://doi.org/10.1016/j.jksus.2015.02.003>
- Joel S, Tatsimo N, Tamokou J, Tsague VT, Lamshoft M, Sarkar P, Bag PK, Spitteller M (2017) Antibacterial-guided isolation of constituents from *Senna alata* leaves with a particular reference against Multi-Drug-Resistant *Vibrio cholerae* and *Shigella flexneri*. *International Journal of Biological and Chemical Sciences* 11(1): 46–53. <https://doi.org/10.4314/ijbcs.v11i1.4>
- Jorgensen JH, Ferraro MJ (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases* 49: 1749–1755. <https://doi.org/10.1086/647952>
- Khan MR, Kihara M, Omoloso AD (2001) Antimicrobial activity of *Cassia alata*. *Fitoterapia* 72: 561–564. [https://doi.org/10.1016/S0367-326X\(00\)00335-X](https://doi.org/10.1016/S0367-326X(00)00335-X)
- Kumavat U, Shimpi S, Jagdale S, Bindu G (2011) Quality standards of ringworm *Cassia*. *International Journal of Pharmacognosy and Phytochemical Research* 3: 43–46.
- Kunle (2012) Standardization of herbal medicines – A review. *International Journal of Biodiversity and Conservation* 4: 101–112. <https://doi.org/10.5897/IJBC11.163>
- Kupina S, Fields C, Roman MC, Brunelle SL (2019) Determination of total phenolic content using the Folin-C assay: Single-laboratory validation, first action 2017.13. *Journal of AOAC International* 102: 320–321. <https://doi.org/10.1093/jaoac/102.1.320>
- Kurmukov AG (2013) Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan* 1: 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4
- Mahmood T (2014) Luteolin and Kaempferol from *Cassia alata*. Antimicrobial and antioxidant activity of its Methanolic extracts. *Aneela Wahab, Tahira, Sabira Begum, Iffat.*: 3–8.
- María R, Shirley M, Xavier C, Jaime S, David V, Rosa S, Jodie D (2018) Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *Journal of King Saud University – Science* 30: 500–505. <https://doi.org/10.1016/j.jksus.2017.03.009>
- Mohammadi Bazargani M, Falahati-Anbaran M, Rohloff J (2021) Comparative Analyses of Phytochemical Variation Within and Between Congeneric Species of Willow Herb, *Epilobium hirsutum* and *E. parviflorum*: Contribution of Environmental Factors. *Frontiers in Plant Science* 11: e595190. <https://doi.org/10.3389/fpls.2020.595190>
- Mohammed I, Mohamed ASM, Abou elella FM, Mohammed MMD, Hamed AR (2017) Phytochemical, cytotoxicity and antioxidant investigation of *Cassia alata* leaves growing in Egypt. *Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)* 4: 97–105.
- Muraina IA, Adaudi AO, Mamman M, Kazeem HM, Eloff J (2008) Effects of geographical location on the yield and bioactivity of *Anoigeisus leiocarpus*. *Journal of Pharmacy & Bioresources* 5(2). <https://doi.org/10.4314/jpb.v5i2.52995>
- Moriyama H, Iizuka T, Nagai M, Miyataka H, Satoh T (2003) Antiinflammatory Activity of Heat-treated *Cassia alata* Leaf Extract and Its Flavonoid Glycoside. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan* 123: 607–611. <https://doi.org/10.1248/yakushi.123.607>
- Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB (2017) Chapter 7 – Preparation, Standardization, and Quality Control of Medicinal Plants in Africa. In: Kuete V (Ed.) *Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*, Elsevier Inc., 171–204. <https://doi.org/10.1016/B978-0-12-809286-6.00007-8>
- Nagarajan S (2008) Pharmacognostical and phytochemical evaluation of *Cassia alata* Linn. *Journal of Medicinal Plants Studies* 2018, 6(5): 69–77.
- Nurlinda N, Handayani V, Rasyid FA (2021) Spectrophotometric Determination of Total Flavonoid Content in *Biancaea sappan* (*Caesalpinia sappan* L.) Leaves. *Jurnal Fitofarmaka Indonesia* 8: 1–4. <https://doi.org/10.33096/jffi.v8i3.712>
- Oladeji OS, Adelowo FE, Oluoyori AP, Bankole DT (2020) Ethnobotanical Description and Biological Activities of *Senna alata*. Evidence-based

- Complementary and Alternative Medicine 2020: e2580259. <https://doi.org/10.1155/2020/2580259>
- Palanichamy S, Nagarajan S (1990) Antifungal activity of *Cassia alata* leaf extract. *Journal of Ethnopharmacology* 29: 337–340. [https://doi.org/10.1016/0378-8741\(90\)90043-S](https://doi.org/10.1016/0378-8741(90)90043-S)
- Palanichamy S, Nagarajan S, Devasagayam M (1988) Effect of *Cassia alata* leaf extract on hyperglycemic rats. *Journal of Ethnopharmacology* 22: 81–90. [https://doi.org/10.1016/0378-8741\(88\)90233-4](https://doi.org/10.1016/0378-8741(88)90233-4)
- Pieme CA, Penlap VN, Nkegoum B, Ngogang J (2008) *In vivo* Antioxidant and Potential Antitumor Activity of Aqueous Ethanol Extract of Leaves of *Senna alata* (L.) Roxb (Cesalpiniaceae) on Bearing Carcinomatous Cells. *International Journal of Pharmacology* 4(4): 245–251. <https://doi.org/10.3923/ijp.2008.245.251>
- Promgool T, Pancharoen O, Deachathai S (2014) Antibacterial and antioxidative compounds from *Cassia alata* Linn. *Songklanakarin Journal of Science and Technology* 36(4): 459–463.
- Raji P, Sreenidhi J, Sugithra M, Renugadevi K, Samrot AV (2015) Phytochemical screening and bioactivity study of *Cassia alata* leaves. *Biosciences Biotechnology Research Asia* 12: 291–296. <https://doi.org/10.13005/bbra/2202>
- Sagnia B, Fedeli D, Casetti R, Montesano C, Falcioni G, Colizzi V (2014) Antioxidant and Anti-Inflammatory Activities of Extracts from *Cassia alata*, *Eleusine indica*, *Eremomastax speciosa*, *Carica papaya* and *Polyscias fulva* Medicinal Plants Collected in Cameroon. *Cordero MD (Ed.)*. *PLoS ONE* 9: e103999. <https://doi.org/10.1371/journal.pone.0103999>
- Saito ST, Trentin DdS, Marcedo AJ, Pungartnik C, Gosmann G, Silveira JDD, Guecheva TN, Henriques JAP, Brendel M (2012) Bioguided Fractionation Shows *Cassia alata* Extract to Inhibit *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* Growth and Biofilm Formation. *Evidence-Based Complementary and Alternative Medicine* 2012: 6–8. <https://doi.org/10.1155/2012/867103>
- Salim M (2016) Karakterisasi Simplisia dan Ekstrak Kulit Buah Duku (*Lansium domesticum* Corr) dari Provinsi Sumatera Selatan dan Jambi Characterization of Simplisia and The Peel Extract of Duku (*Lansium domesticum* Corr) from South Sumatera and Jambi Province. *Jurnal Kefarmasian Indonesia* 6: 117–128. <https://doi.org/10.22435/jki.v6i2.6226.117-128>
- Septama AW, Panichayupakaranant P (2017) Antibacterial activity of artocarpanone isolated from *Artocarpus heterophyllus* heartwoods against diarrheal pathogens and its mechanism of action on membrane permeability. *Journal of Applied Pharmaceutical Science* 7: 64–68. <https://doi.org/10.7324/JAPS.2017.71109>
- Somchit MN, Reezal I, Elysha Nur I, Mutalib AR (2003) *In vitro* antimicrobial activity of ethanol and water extracts of *Cassia alata*. *Journal of Ethnopharmacology* 84: 1–4. [https://doi.org/10.1016/S0378-8741\(02\)00146-0](https://doi.org/10.1016/S0378-8741(02)00146-0)
- Sugumar M, Doss VA, Maddisetty P, Rangaraju M, Kandhasamy S, Munuswamy S (2019) Pharmacological analysis of hydroethanolic extract of *Senna alata* (L.) for *in vitro* free radical scavenging and cytotoxic activities against HepG2 cancer cell line. *Pakistan Journal of Pharmaceutical Sciences* 32(3): 933–936.
- Timothy SY, Wazis CH, Adati RG, Maspalma ID (2012) Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of *Cassia alata* Linn. *Journal of Applied Pharmaceutical Science* 2(7). <https://doi.org/10.7324/JAPS.2012.2728>
- Vinet L, Zhedanov A (2011) A “missing” family of classical orthogonal polynomials. *Journal of Physics A: Mathematical and Theoretical* 44: 1–37. <https://doi.org/10.1088/1751-8113/44/8/085201>
- Wikaningtyas P, Sukandar EY (2016) Asian Pacific Journal of Tropical Biomedicine. *Asian Pacific Journal of Tropical Biomedicine* 6: 16–19. <https://doi.org/10.1016/j.apjtb.2015.08.003>
- Wirnawati W, Mentari IA, Aryodi Pernando NS (2020) The Characterization The Characterization Of Simplisia And Extract Of Clove Leaves (*Syzigium aromaticum* Merr. Et Perry L) For Herbal Mouthwash Active Ingrediens. *Jurnal Ilmu Kesehatan* 8: 26–33. <https://doi.org/10.30650/jik.v8i1.1377>
- World Health Organization (1998) Quality control methods for medicinal plant materials World Health Organization Geneva, WHO.