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Qualitative and quantitative phytochemicals of leaves, bark and roots of *Antiaris toxicaria* Lesch., a promising natural medicinal plant and source of pesticides

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Abstract

Antiaris toxicaria Lesch. of the Moraceae family is a tree that grows endemic to Indonesia and has a height of about 20-30 m. This study aimed to screen the phytochemical constituents of leaf, bark and its root. The plant materials were collected from the Samarinda Botanical Garden. Latex of this species in Indonesia is known as a source of blowpipe poison. In other countries *Antiaris* sp. plant parts (leaves, bark and seeds) are used in ethnobotany practice as raw material for traditional medicine. The leaves, bark and seeds of this plant are used in the treatment of syphilis, leprosy, cancer and used as laxatives for sore throats. Screening of the phytochemical constituents of the samples began by tracing the macromolecules of alkaloids, steroids, tannins, phenolic compounds, flavonoids and saponins with various tests. Alkaloids, saponins, tannins, phlobatnins, flavonoids and terpenoids were detected in the chemical analysis. High performance liquid chromatography diode array detection (HPLC-DAD) was conducted. HPLC screening of *A. toxicaria* extracts revealed the presence of galic acid, catechins, chlorogenic acid, caffeic acid, ellagic acid, epigallocatechin, routine, isoquercitrin, quercitrin, quercetin and kaempferol. The study revealed the array of secondary metabolites present in the plant that can be used in medicinal preparations and will be candidate species for developing a natural insecticide.

Keywords

Toxicology, natural insecticide, HPLC, phytochemicals, *Antiaris toxicaria*

Introduction

Antiaris toxicaria Leach. (Moraceae) is a tree that grows endemic to Indonesian and has hight of about 20-30 m, to sometimes it can grow up to 40 m. This plant has white latex and the leaves grow alternately asymmetrically (1). It has a thick flat crown and gray bark with white patches. The flowers are small, greenish-white in color and produce red velvety fruit. The diameter of the stem can be as large as 70-80 cm, allowing it to make wide board sheets for use in the home furnishings manufacturing business (2, 3). In Kenya, Sudan and Nigeria, the *Antiaris* sp. is a shade plant in the "shelter belt" at the side of the road. The leaves, bark and seeds are used as traditional medicine or ethnobotany. Extracts of bark, leaves and roots are used as traditional medicine for the treatment of chest pain (4). The leaves are used for the treatment of syphilis (1). The sap is used as a laxative agent for sore throats and leprosy. In Cameroon, the sap is used in

the treatment of cancer. On the island of Borneo, the sap of this plant is taken to poison chopsticks, arrows and spearheads. The effects of toxic *A. toxicaria* sap on the Rattus norvegicus have been studied in detail (5), The sap of this plant has a high toxicity to vertebrate pests so that in the future the sap extract and its active ingredients are candidates for natural rodenticide materials.

Management of Plant Pest Organisms (OPT) is currently managed in a conventional way by utilizing pesticides. The active ingredients of pesticides are toxic materials that can poison humans, animals and the environment. Pesticide residue contamination in vegetables in Samarinda City is approaching the consumption threshold (6). The search for pest control from natural ingredients sourced from plants and entomopathogens as pest control is a prospective step forward. Isolation of plant active ingredients directly, identifying any compound ingredients contained in plant parts that are indicated to be toxic is generally carried out (7). Natural compounds are mostly classified as alkaloids, steroids, tannins, phenolic compounds, flavonoids and saponins (8).

In the process of harvesting the wood of *A. toxicaria*, leaves, twigs, bark and roots only become waste that is scattered on the forest floor. In other countries where it is exported, this plant litter is still very valuable as a raw material for various herbal medicines. This phenomenon raises the question whether there are still useful constituents of *A. toxicaria* plant wastes. Research on phytochemical constituents in plant parts and the use of *A. toxicaria* has not been widely carried out in Indonesia, even in East Kalimantan Province, which has an abundance of biodiversity. This study aimed to screen the phytochemical constituents of the leaf, bark and roots of *A. toxicaria*.

Materials and Methods

This research was carried out in October-December 2019. The plant materials of *Antiaris toxicaraia* were collected from the Samarinda Botanical Gardens and Ritan Baru village, Kec. Tabang Kab. Kutai Kertanegara. Extracts and phytochemical screening of the samples were Analyst at the Biochemistry Laboratory of Brawijaya University, Malang.

Plant material

The bark, leaves and root each 500 g were air dried and then oven dried at 60-800 ^{0}C for 3 days. The dried materials were ground into powder, the bark, leave and root (± 1500 g) its was macerated in 80% methanol volume 5 l for 72 hrs and then filtered. The filtrate was concentrated with vacuum rotary evapotor (Heidolph) to 72.8 g or (1.12 %) and then stored in a sample bottle for qualitative and quantitative analyses.

Phytochemical screening test

Flavonoid test

The plant powder equivalent 500 g was heated with 10 ml of ethyl acetate over a steam heater for 3 min, then the mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia 20 %, Dark yellow color indicates the presence of flavonoids (9).

Alkaloid test

The plant powder consist 0.5 g was added with 5% ethyl ether and fat will setle if for 15 minutes. Then extracted with 5 ml of dilute HCl 20 % left for 20 min in a cup of boiling water. The solution was removed and cooled to room temperature ± 30 degree celcius and was centrifuged for 10 min at 3000 rpm. 1 ml of Maeyer's reagent was added to filtrate than 1 ml of the mixture solotion was added with dropped with Dragendorff's reagent, after which it was observed whether the solution became cloudy or a precipitate formed (10).

Saponin test

Weighing 2.0 g of plant powder was boiled with distilled water in a test tube and filtered. As much volume 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously and observed for the formation of a stable persistent froth. The foam is mixed with 3 drops of olive oil and shaken vigorously to form an emulsion (9).

Anthraquinone test

The plant powder approximately 0.5 g was shaken with 10 ml of benzene and filtered, the filtrate was added with 10% ammonia and the mixture was shaken well. Changes in the color of the solution to pink/violet or red color indicate the presence of anthraquinones (10).

Terpenoid test

Plant powder of 0.5 g was dissolved in 5 ml of methanol; 2 ml of the extract was treated with 1 ml of 2,3-dinitrophenyl hydrazine dissolved in 100 ml of 2 M HCl. Yellow-orange staining is indicative of terpenoids (10).

Tannin test

The coarse powder as much 0.5 g the was stirred in 10 ml of distilled water and filtered, after filtering the filtrate was added with ferric chloride ($FeCl_3$) 1.1 g reagent. The appearance of a dark green, purple, blue or black color indicates the presence of tannins in the samples (11).

Anthraquinone test

The raw powder equivalent 0.5 g was shaken with 10 ml of benzene then filtered, added 10% ammonia solution to the mixture, shaken well. Formation of pink/violet or red color indicates the presence of anthraquinone (10).

Quantitative analysis

Quantitative analysis of phytochemical constituents of leaf, bark and root samples of *A. toxicaria* was carried out using high performance liquid chromatography diode array detection (HPLC-DAD) (Shimadzu, Kyoto, Japan) having Prominence Auto Sampler (SIL-20A) equipped with Shimadzu LC-20AT reciprocating pump connected to DGU 20A5 degasser with CBM 20A integrator, series diode SPD-M20A detector and LC solution 1.22 software SP1.

Results and Discussion

Phytochemical macromolecular

Test work of leaf powder, bark and roots in Table 1, show that there are a number of macromolecules including alkaloids, saponins, tanins, flavonoids and terpenoid present in the plant parts. Alkaloids in plants function as toxins that can protect them from insects and herbivores, growth regulating factors and metabolite product compounds that are able to supply nitrogen and other elements needed by plants (12).

Table 1. Macromolecule constituents of *A. toxicaria.*

Molecule	Existence
Alkaloid	+
Saponin	+
Tannin	+
Phlobatannins	+
Anthraquinone	-
Flavonoid	+
Terpenoid	+

Alkaloids are basic compounds that have polar properties. The presence of compounds such as alkaloids from the bark of the plant can interfere with the nervous system (neuromuscular toxic) inhibiting the feeding power of larvae of the insects (13). The way the alkaloids work is by inhibiting the action of the acetylcholinesterase enzyme which has the function of hydrolyzing acetylcholine. Acetylcholine, when stable, functions to transmit nerve impulses, then undergoes hydrolysis with the help of the acetylcholinesterase enzyme, resulting in the accumulation of acetylcholine which damages the nervous system. Then, the larva's body will also experience a more transparent color change and its body movement slows down (14). Saponins are used because they have broad activities such as antibacterial, antifungal, ability to lower cholesterol in the blood and inhibit the growth of tumor cells. Saponins are compounds that have glycoside properties that are widely distributed in higher plants (15). It can form a colloidal solution when shaken to form foam and do not disappear when acid is added. Saponin molecules taste bitter, foamy when mixed with water, have anti-exudative properties, have inflammatory properties, and have hemolytic properties (damage red blood cells). Some saponins work as antimicrobials. Steroidal saponins consist of a steroid core (C27) which has a carbohydrate molecule. This compound is hydrolyzed to form an aglycone called sapogenin (16). This type of saponin has an antifungal effect. In animals, it shows inhibition of smooth muscle activity. Steroids are excreted after the coagulation of glucotonic acid, and are the main ingredients in the biosynthesis of corticosteroid drugs. This type of saponin has an aglycone in the form of a steroid obtained from the secondary metabolism of plants (17). Some of the properties of saponins include: a strong poison component of fish and amphibians; has a bitter taste; the liquid forms a foam; hemolyze erythrocytes; difficult to purify (18).

Tannins are generally soluble in water, except for those tannins which have a very high molecular weight. Tannins are phenolic compounds that have a high molecular weight, which is 50 to 20000. Tannin compounds are compounds that have a bitter taste and react with proteins, amino acids and alkaloids containing many hydroxyl and carboxyl groups to form strong complex bonds with proteins and other macromolecules so that the very bitter taste is not liked by insects. The leather industry uses it for tanning leather to make it durable and easy to process and use (19). Tannins are also used for tanning (waving) nets, ropes and sails to make them more resistant to seawater. In addition, tannins are used as dyes, adhesives. phlobatannins and cardenolids, as antibacterial, antiamoebic and antifungal compounds that can cure syphilis.

Flavonoids are chemical compounds that have insecticidal properties. Flavonoids work as respiratory inhibitors. Most of the flavonoids act as inhibitors or reduce the rate of chemical reactions. Several groups of flavonoids also act as antioxidants. treat allergies, viral infections, arthritis and certain inflammatory conditions. repair cells damaged by free radicals (20) Terpenoids are one of the chemical compounds of natural ingredients that are widely used as drugs. The role of terpenoids as allelopathy to inhibit the growth of competing plants and as insecticides against vertebrate animals (18).

HPLC Analysis

HPLC screening of extracts of *A. toxicaria* revealed the presence of gallic acid (retention time (tR) = 12.40 min; peak 1), catechins (tR = 16.35 min; peak 2), chlorogenic acid (tR = 23.08 min; peak 3) , caffeic acid (tR = 25.39 min; peak 4), ellagic acid (tR = 30.79 min; peak 5), epigallocatechin (tR = 33.56 min; peak 6), rutin (tR = 39.18 min ; peak 7), isoquercitrin (tR = 43.97 min; peak 8), quercitrin (tR = 47.73 min; peak 9), quercetin (tR = 52.03 min; peak 10) and kaempferol (tR = 64, 15 min; peak 11) (Fig. 1 and Table 2). Phytochemical screening detects the

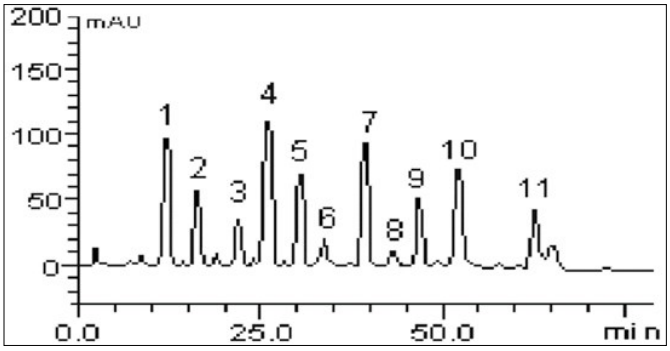
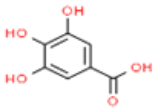
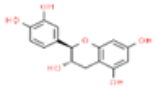
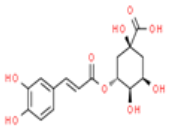
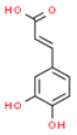
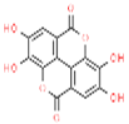
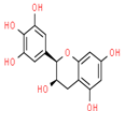
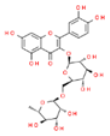
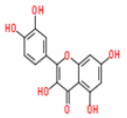
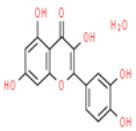


Fig. 1. High-performance liquid chromatographic profiles of *Antiaris toxicaria* Lesch root ash extract: gallic acid (**1**), catechins (**2**), chlorogenic acid (**3**), caffeic acid (**4**), ellagic acid (**5**), epigallocatechin (**6**), rutin (**7**), isoquercitrin (**8**), quercitrin (**9**), quercetin (**10**) and kaempferol (**11**).

presence of saponins, tannins, phlobatannins, flavonoids and terpenoids in the powdered extracts of leaves, bark and roots of *A. toxicaria*.

Total alkaloid content was 243.71 mg/g extract and total flavonoid was 155.85 mg (Table 2). The DAD HPLC

Table 2. Composition of phytochemical constituents of ash extract leaf, bark and root samples of *Antiaris toxicaria* Lesch.

Constituents			**Composition**			
					LOD (µg/mL)	**LOQ (g/µmL)**
Index/ACD/IUPAC			**%**	**mg g^{-1}**		
Gallic acid 3,4,5-Trihydroxybenzoic acid	$C_7H_6O_5$		3.07	33.64	0.037	0.123
Catechin(2S,3R)-2-(3,4-Dihydroxyphenyl)-3,5,7-chromanetriol	$C_{15}H_{14}$		1.61	12.11	0.028	0.092
Clorogenic acid (1S,3R,4R,5R)-3-{[(2E)-3-(3,4-Dihydroxyphenyl)-2-propenoyl]oxy}-1,4,5-trihydroxycyclohexane-carboxylic	$C_{16}H_{18}$		0.83	8.36	0.016	0.054
Caffeine acid	$C_9H_8O_4$		3.59	38.91	0.012	0.039
Ellagin acid 2,3,7,8-Tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione	$C_{14}H_6O$		2.20	21.18	0.035	0.115
Epigallocatechin (2R,3R)-2-(3,4,5-Trihydroxyphenyl)-3,5,7-chromanetriol)	$C_{15}H_{14}$		0.45	5.82	0.007	0.023
Rutin 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside	$C_{15}H_{14}$		3.03	32.78	0.021	0.070
Isoquercitrin*			0.21	3.76	-	-
Quercitrin*			1.54	18.67	-	-
Quercetin 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one hydrate (1:1)	$C_{15}H_{12}$		2.67	28.23	0.009	0.029
Kaempfero	$C_{15}H_{10}$		1.30	18.75	0.014	0.047

detected the presence of gallic acid, catechins, chlorogenic acid, caffeic acid, ellagic acid, epigallocatechin, rutin, isoquercitrin, quercetin and kaempferol and some ingredients because the titers were small so they were not detected.

Caffeic acid (caffeic) was the most detected phenolic compound (33.64 mg/g extract) and rutin was the detected flavonoid (32.78 mg g^{-1} extract) (Table 2 and Fig. 1) This secondary metabolite is a plant defense material against

other organisms. Flavonoids as secondary metabolites in plants in the phenolic structure of more than 8000 from simple molecules such as phenolic acids to highly polymerized substances such as tannins, phlobatannins, flavonoids and terpenoids are known polyphenolic compounds reported to be responsible for most of the biological activities in plants and plant defense against interference with other organisms (21). The main classes of polyphenols, based on their structure, are phenolic acids, flavonoids, stilbenes and lignans.

Flavonoids account for about two-thirds of the phenolics in our diet (20) and are one of the most abundant known polyphenols classified into 6 major groups; flavonols, flavones, flavanones, flavan-3-ols, anthocyanins and isoflavones. The results of the search for flavonoid active ingredients, which are polyphenols, were detected quite a lot in *A. toxicaria*. Polyphenols as precursors of phenylalanine (shikimic acid). Flavonoids are the most studied polyphenol group of more than 4000 have been identified and categorized into six subclasses: flavonols, flavones, flavanones, flavan-3-ols, anthocyanins and isoflavones. Flavonoids are also macromolecules that are widely contained in the food consumed by humans and animals and invertebrates. Several subclasses of flavonoids protect against chronic diseases such as liver damage, kidney injury. A wide variety of phenolic compounds, found in fruits, vegetables and many plants. Phenolic compounds (such as caffeic, ellagic and ferulic acids, sesamol and vanillin) have been studied for the inhibition of atherosclerosis (hardening of the arteries caused by cholesterol plaque buildup) (22).

Conclusion

Phytochemical screening of a series of assays detected macromolecules such as alkaloids, saponins, tannins, phlobatannins, flavonoids and terpenoids from *Antiaris toxicaraia*. The HPLC traced the presence of quercetin, rutin, isoquercetirin, caffeic acid and gallic acid. The presence of chemical constituents in simplesia is an indication that the leaf, bark and root of *A. toxicaria* can be used to produce constituents of medicinal compounds and candidate constituents of important natural pesticides.

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Authors contributions

All authors contributed equally.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Berhaut J. Flore Illustree du Senegal: Dicotyledones, Tome VI: Linacées à Nymphéacées. Dakar: Ministère du Développement Rural et de l'Hydraulique. 1979:402-05.
2. Berg CC, Hijman ME, Weerdenburg JC. Flore du Cameroun: Moracées. Paris: Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie. 1985:104-10.
3. Ahmad M, Lim CP, Akowuah GA, Ismail NN, Hashim MA, Hor SY et al. Safety assessment of standardised methanol extract of *Cinnamomum burmannii*. Phytomedicine. 2013; Sep 15;20 (12):1124-30. https://doi.org/10.1016/j.phymed.2013.05.005
4. Okogun JI, Spiff AI, Ekong DE. Triterpenoids and betaines from the latex and bark of *Antiaris africana*. Phytochemistry. 1976. https://doi.org/10.1016/S0031-9422(00)94464-9
5. Subiono T. Ligan Activity of *Antiaris toxicaria* Lesch. and the role of toxicarioside in crude extract: *In Silico*, *In Vitro* and *In Vivo* Approaches.
6. Rahmadi A, Hanafi MH, Setianingrum D, Widyastuti AD, Susilawati R. Physical, microbial and pesticide contaminations on fresh vegetable and fruit marketed in Samarinda-Indonesia. InIOP Conference Series: Earth and Environmental Science 2020 Feb 1 (Vol. 443, No. 1, p. 012062). IOP Publishing. https://doi.org/10.1088/1755-1315/443/1/012062
7. Huang S, Wang L, Liu L, Fu Q, Zhu D. Nonchemical pest control in China rice: a review. Agronomy for Sustainable Development. 2014 Apr;34(2):275-91. https://doi.org/10.1007/s13593-013-0199-9
8. Moronkola DO, Oladosu IA. Chemical Compositions of *Lonchocarpus cyanescens* Benth.,(Fabaceae)—Case study of its volatile oils and two triterpenoids. American Journal of Plant Sciences. 2013; Jul 30;2013. https://doi.org/10.4236/ajps.2013.48199
9. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; Aug 19;4(7):685-88. https://doi.org/10.5897/AJB2005.000-3127
10. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.
11. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000; Nov 1;55(6):481-504. https://doi.org/10.1016/S0031-9422(00)00235-1
12. Wink M. Ecological roles of alkaloids. Modern alkaloids. 2008; Jan 8:3-52.
13. Xu T, Li D, Jiang D. Targeting cell signaling and apoptotic pathways by luteolin: cardioprotective role in rat cardiomyocytes following ischemia/reperfusion. Nutrients. 2012; Dec;4(12):2008-19. https://doi.org/10.3390/nu4122008
14. Wizen G, Gasith A. Color variability and body size of larvae of two *Epomis* species (Coleoptera, Carabidae) in Israel, with a key to the larval stages. ZooKeys. 2011; (119):37. https://doi.org/10.3897/zookeys.119.1451
15. Faithfull NT. Methods In Agricultural Chemical Analysis A Practical Handbook. Cabi; 2002. https://doi.org/10.1079/9780851996080.0000
16. Desai SD, Desai DG, Kaur H. Saponins and their biological activities. Pharma Times. 2009; Mar;41(3):13-16.
17. Pagare S, Bhatia M, Tripathi N, Pagare S, Bansal YK. Secondary metabolites of plants and their role: Overview. Current Trends in Biotechnology and Pharmacy. 2015;9(3):293-304. https://doi.org/10.5958/2249-0035.2015.00016.9

18. Polya G. Biochemical targets of plant bioactive compounds: a pharmacological reference guide to sites of action and biological effects. CRC press; 2003 May 15. https://doi.org/10.1201/9780203013717

19. Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn. against acetaminophen-induced liver damage in rats. Food and Chemical Toxicology. 2010 Aug 1;48(8-9):2200-05. https://doi.org/10.1016/j.fct.2010.05.047

20. Sathishkumar T, Baskar R, Shanmugam S. Optimization of flavonoids extraction from the leaves of *Tabernaemontana heyneana* wall using L16 orthogonal design. Nature and Science. 2008; 6(3):10-21.

21. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; Oct;15 (10):7313-52. https://doi.org/10.3390/molecules15107313

22. Decker EA. The role of phenolics, conjugated linoleic acid, carnosine and pyrroloquinoline quinone as nonessential dietary antioxidants. Nutrition Reviews. 1995; Mar 1;53(3):49-58. https://doi.org/10.1111/j.1753-4887.1995.tb01502.x

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