Volume 4, Nomor 2, Juni 2023 **ISSN : 2774-5848 (Online)**
ISSN : 2774-0524 (Cetak)

PHYTOCHEMICAL SCREENING, TOTAL ANTIOXIDANT ACTIVITY AND TOXICITY TEST ON METHANOL EXTRACT OF ANDROGRAPHIS PANICULATA LEAF

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ABSTRACT
Antioxidants are compounds that can inhibit the oxidation of a molecule and neutralize radicals that have a negative impact on cells. In addition, antioxidants can also inhibit the activity of reactive oxygen species (ROS) by regulating the superoxide dismutase enzyme. When the level of ROS is greater than that of antioxidants, an imbalance state called oxidative stress can occur. In this case, antioxidants play an important role in preventing ROS activity. Sambiloto or, "King of Bitter," is one of the herbal plants that contain antioxidants and often used as traditional medicine. Phytochemicals are chemical compounds produced by herbal plants, which can act as anti-inflammatory, antidiabetic, antimicrobial, antiparasitic, antidepressant, anti-cancer, antioxidant, and wound healing. This study aims to increase knowledge about the antioxidant ability and toxicity of Sambiloto leaf. This study used an in-vitro experimental and bioassay study consisting of qualitative phytochemical tests (Harborne), total antioxidant capacity with DPPH (Blois), total phenolic content (Singleton and Rossi), total alkaloid content (Trivedi et al) and toxicity test with BSLT. The results showed that the phytochemical test of Sambiloto leaf extract contained alkaloids, cardio glycosides, flavonoids, glycosides, phenolics, saponins, quinones, steroids, terpenoids, tannins, coumarins, and betacyanins; total antioxidant capacity (IC50=104.22 µg/mL); mean total phenolic content (392.19 µg/mL); mean total alkaloid content (9.47 µg/mL); toxicity test (LC50=107.54 g/mL). It can be concluded that Sambiloto leaf extract has the potential to be antioxidant and cytotoxic.

Keywords *: Andrographis paniculate; phytochemical; antioxidant; DPPH; BSLT*

ABSTRAK
Antioksidan adalah senyawa yang dapat menghambat oksidasi molekul dan menetralisir radikal yang berdampak negatif pada sel. Selain itu, antioksidan juga dapat menghambat aktivitas spesies oksigen reaktif (ROS) dengan mengatur enzim superoksida dismutase. Ketika tingkat ROS lebih besar dari antioksidan, keadaan ketidakseimbangan yang disebut stres oksidatif dapat terjadi. Dalam hal ini, antioksidan berperan penting dalam mencegah aktivitas ROS. Sambiloto atau, "Raja Pahit," adalah salah satu tanaman herbal yang mengandung antioksidan dan sering digunakan sebagai obat tradisional. Fitokimia adalah senyawa kimia yang diproduksi oleh tanaman herbal, yang dapat bertindak sebagai antiinflamasi, antidiabetes, antimikroba, antiparasit, antidepresan, anti kanker, antioksidan, dan penyembuhan luka. Penelitian ini bertujuan untuk meningkatkan pengetahuan tentang kemampuan antioksidan dan toksisitas daun Sambiloto. Penelitian ini menggunakan penelitian eksperimental dan bioassay in-vitro yang terdiri dari uji fitokimia kualitatif (Harborne), kapasitas antioksidan total dengan DPPH (Blois), kandungan fenolik total (Singleton dan Rossi), kadar alkaloid total (Trivedi et al) dan uji toksisitas dengan BSLT. Hasil penelitian menunjukkan bahwa uji fitokimia ekstrak daun Sambiloto mengandung alkaloid, kardio glikosida, flavonoid, glikosida, fenolik, saponin, kuinon, steroid, terpenoid, tanin, kumarin, dan betasianin; kapasitas antioksidan total (IC50 = 104,22 µg / mL); rata-rata kandungan fenolik total (392,19 µg / mL); rata-rata kandungan alkaloid total (9,47 µg / mL); uji toksisitas (LC50 = 107,54 g / mL). Dapat disimpulkan bahwa ekstrak daun Sambiloto berpotensi menjadi antioksidan dan sitotoksik.

Kata kunci : Andrographis paniculate; fitokimia; Antioksidan; DPPH; BSLT

INTRODUCTION

Geographically, Indonesia is a tropical country known for its biodiversity, such as herbal plants. Herbal plants contain phytochemical compounds that are efficacious in preventing and treating certain diseases. The World Health Organization noted that around 65% of people in developed countries and 80% of developing countries use herbal plants as traditional medicines. Since ancient times, the Indonesian people have believed that herbal plants have many benefits. To increase these benefits, BPOM, together with several universities in Indonesia, is currently researching nine superior plants up to the clinical trial stage (Jayakumar et al., 2013; Nugroho, 2017; Sukmawati, 2018).

Phytochemicals are chemical compounds produced by herbal plants. Phytochemical compounds with secondary metabolites play an important role in medicine, where phytochemicals themselves can act as anti-inflammatory, antidiabetic, antimicrobial, antiparasitic, antidepressant, anti-cancer, antioxidant, and wound healing (Asaduzzaman & Asao, 2018). Some of the compounds contained in herbal plants include alkaloids, tannins, saponins, flavonoids, phenolics, steroids, terpenoids, and others.

Antioxidants are compounds that are able to inhibit the oxidation of a molecule and neutralize radicals that negatively impact on cells. In addition, antioxidants can also inhibit the activity of reactive oxygen species (ROS) by regulating the superoxide dismutase enzyme (Aziz et al., 2019; Sing, 2016).

Some cell macromolecules, such as proteins, lipids, carbohydrates, and nucleic acids, can be damaged by reactive oxygen species (ROS). Oxidative stress is a condition of free radical imbalance that occurs when the level of ROS is greater than that of antioxidants. This is where antioxidants play an essential role as the primary defense against cell damage caused by free radicals. Antioxidants are produced endogenously by body cells to exogenous agents that can be obtained from *sambiloto* leaf (Azwanida, 2015; Jayakumar et al., 2013).

Sambiloto *(Andrographis Paniculata)*, one of the herbal plants of the Acanthaceae family, is widely used to overcome health problems, where the main bioactive compounds in this plant are andrographolide. In China, India, Thailand, and Malaysia, *sambiloto* leaf treat sore throat, flu, and upper respiratory tract infections (Jayakumar et al., 2013).

The lack of knowledge about antioxidants and secondary metabolites in *sambiloto* leaf encourages the researcher to conduct this research. Therefore, this study aims to add insight into the antioxidants and metabolite compounds contained in *sambiloto* leaf.

METHOD

This study used an in-vitro experimental study consisting of phytochemicals, antioxidants capacity, total phenolics, total alkaloids, and bioassays consisting of toxicity tests. This research was carried out at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tarumanagara, Jakarta, from November 2021 to May 2022.

RESULT

Table 1 shows the result of phytochemical tests carried out on *sambiloto* leaf in the form of alkaloids, cardio glycosides, flavonoids, glycosides, phenolics, saponins, quinones, steroids, terpenoids, tannins, coumarins, anthocyanin, betacyanin.

Table 1. Phytochemical results of *sambiloto* leaf extract

Phytochemical Compounds	Method Name	Results
Alkaloids	Mayer	+
Cardio glycosides	Keller Kiliani	+
Flavonoids	NaOH	+

Glycosides	Modified Borntrager	+
Phenolics	Folin Ciocalteau	+
Saponins	Foam	+
Quinones	H_2SO4	+
Steroids	Liebermann Burchard	+
Terpenoids	Liebermann Burchard	+
Tannins	Ferric Chloride	+
Coumarins	NaOH	+
Anthocyanin	NaOH	-
Betacyanin	NaOH	+

Phytochemical test results on *sambiloto* leaf extract showed phytochemical compounds such as alkaloids, cardio glycosides, flavonoids, glycosides, phenolics, saponins, quinones, steroids, terpenoids, tannins, coumarins, and betacyanin..

A total antioxidant capacity test on *sambiloto* leaf extract was carried out using the DPPH method with vitamin C (IC_{50} 5,4 µg/mL) as a standard for comparison. Table 2 shows the test results of the total antioxidant capacity of *sambiloto* leaf extract.

Table 2. Calculate the percentage of inhibition and IC_{50} based on the concentration of the sample

Concentration (µg/mL)	Percentage of Inhibition (%)	IC_{50} (µg/mL)
100	47,62	104,22
200	65,75	
300	78,02	
400	90,11	

From this study, it is obtained that IC_{50} is as big as 104,22 µg/mL. If IC_{50} gets lower, it indicates higher antioxidant levels in the sample. Thus, it can be said that the results of the total antioxidant capacity test in the *sambiloto* leaf extract showed that the levels of ascorbic acid in vitamin C is higher than the antioxidants levels in the *sambiloto* leaf. However, vitamin C levels can cause side effects on the stomach.

The phenolic level of the *sambiloto* leaf extract was tested using the method of Singleton and Rossi with tannins as a standard of comparison. Table 3 shows the results of the phenolic level test in the *sambiloto* leaf extract.

Table 3. Absorbance value and level of phenolic extract

Tube	Absorbance	Phenolic Level (µg/mL)	Average of Phenolic Level (µg/mL)
I	0,146	414,79	392,19
II	0,113	369,59	

In this study, the average phenolic level in the *sambiloto* leaf was 392,19 µg/mL. The alkaloid level test in the *sambiloto* leaf extract used berberine chloride as a standard of comparison. Table 4 shows the results of the alkaloid level test in the *sambiloto* leaf extract.

Table 4. Absorbance value, alkaloid level, and total level average of sambiloto leaf

Tube	Absorbance	Alkaloid level (µg/mL)	Average of Alkaloid Level (µg/mL)
I	0,354	4,94	9,47
II	0,316	4,53	

In this study, the average alkaloid level in the *sambiloto* leaf was 9,47 µg/mL. A toxicity test on the *sambiloto* leaf extract was carried out using the BSLT method. Table 5 shows the toxicity test results on the *sambiloto* leaf extract.

Table 5. Concentration, concentration log, % of mortality, and LC_{50} *sambiloto* leaf extract

Concentration (µg/mL)	Concentration log	% Of mortality	LC_{50} (µg/mL)
50	1,70	17,021	107,54
100	2,00	41,304	
150	2,18	64,000	
200	2,30	80,702	
250	2,40	92,424	

In this study, it is obtained that LC_{50} amounts to 107,54 µg/mL. The value of LC_{50} obtained is the concentration of *sambiloto* leaf extract required to kill 50% of Artemia Salina shrimp larvae. If LC_{50} is getting lower, then the toxicity will be higher.

DISCUSSION

This finding is in line with Prabowo, Bangsawan, and Andriani's research (2014), where flavonoids, tannins, terpenoids, and saponins compounds were found in *sambiloto* leaf extract. Pandey, Saini, dan Raja (2019) also found the compound of glycosides in *sambiloto* leaf extract. The results of research conducted by (Bhargavi & Kaloori, 2018) stated that *sambiloto* leaf also contain cardio glycosides with ethanol extract and phenolics with methanol and ethanol extract. Nurhafiza (2015) also stated that alkaloids and steroids were also found in *sambiloto* leaf extract. The research conducted by Murugan, Elangovan, and Singh (2017) found the content of quinones in other Andrographis genera. The study by Gurupriya and Cathrine (2021) found coumarin compounds in one of the plants of the Andrographis. Thus, it can be said that *sambiloto* leaf can act as anti-inflammatory, anti-cancer, anti-parasitic, anti-microbial, antidiabetic, antioxidant, and wound healing (Asaduzzaman & Asao, 2018).

The research by Selvamathy, Geetha, and Saranya (2010), which used ethanol as a solvent to test antioxidant capacity in the *sambiloto* leaf showed that IC_{50} is as big as 8,5 µg/mL. The same thing was found in the study of Apriliani and Tukiran (2021), which obtained IC_{50} as big as 15,55 µg/mL. The significant difference in IC_{50} can be influenced by the method and solvent used.

Research conducted by Saravanan and US Mahadeva (2015) found that the total levels of phenolic in *sambiloto* leaf were 58,78 GAE/g, while the research conducted by Nurcholis et al. (2012), which used the Folin-Ciocalteau method found that the total phenolic levels in *sambiloto* leaf were 493,37 TAE/g. The presence of phenolic content in *sambiloto* leaf shows that *sambiloto* leaf can be used in pharmacological activities such as anti-inflammatory, antioxidant, anti-aging, and antiproliferative (Lin et al., 2016).

Kurzawa et al. (2015) used the chromatographic method in testing the alkaloid level of the *sambiloto* root and leaf. It was found that the alkaloid levels varied from 50,71 ± 0,36 mg/g to 78,71 ± 0,48 mg/g. The presence of alkaloid compounds in the *sambiloto* leaf indicates that the *sambiloto* leaf has effects of antiproliferative, antibacterial, antivirus, insecticide, and antimetastatic (Shi et al., 2014).

Meyer et al. (1982) stated that the value of LC_{50}, which is less than 1000 ppm, it has cytotoxic and antimitotic properties. Thus, it can be said that after 24 hours of observation, *sambiloto* leaf extract has cytotoxic and antimitotic effects. Research conducted by Nurcholis et al. (2012) that used ethanol as a solvent to test the toxicity of *sambiloto* leaf extract obtained an LC_{50} amounting to 593,2 µg/mL. The difference in LC_{50} obtained can be influenced by the type of solvent, the quality of the *sambiloto* leaf, and the research method used.

CONCLUSION

Based on the study's results, it was found that the phytochemical content of the *sambiloto* leaf extract was in the form of alkaloid, cardio glycosides, flavonoid, glycosides, phenolics, saponins, quinones, steroids, terpenoids, tannins, coumarins, and betacyanin. The results of the antioxidant capacity test of *sambiloto* leaf extract were obtained at 104,22 µg/mL, which indicates that *sambiloto* leaf has a moderate antioxidant capacity (101-250 µg/mL). The results of the phenolic level test showed that the average total phenolic content of the *sambiloto* leaf

extract was 392,19 µg/mL. The results of the alkaloid test showed that the average total alkaloid content of the *sambiloto* leaf extract was 9,47 µg/mL. The results of the toxicity test on the *sambiloto* leaf were 107.54 µg/mL, which indicates that the *sambiloto* leaf has a cytotoxic effect on the larvae of Athermia Salina so that it has the potential as antimitotic. The researcher also suggests that further research on *sambiloto* leaf extract is needed, and further research on experimental animals is needed.

ACKNOWLEDGMENTS

The researcher is very grateful to various parties for their support in preparing this research from beginning to end. Thanks to Dr. dr. Noer Saelan Tadjudin, Sp.KJ as the Dean of the Faculty of Medicine, Faculty of Medicine of Tarumanagara University; dr. Wiyarni Pambudi, Sp.A; IBCLC as the Head of the Research Unit and Scientific Publications of the Faculty of Medicine, Tarumanagara University; Prof. Dr. dr. Frans Ferdinal, MS, as the Advisory Lecturer and Head of the Department of Biochemistry and Molecular Biology Tarumanagara University; dr. David Limanan, M.Biomed as the Academic Advisor and Staff of the Department of Biochemistry and Molecular Biology Tarumanagara University; Mrs. Eny as Staff of the Department of Biochemistry and Molecular Biology Tarumanagara University; and also family and friends who always encourage and provide material and moral support.

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