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Original Research Article

Phytochemical and Antioxidant Activity of the Ethanol Extracts of Songgolangit Plant (*Tridax procumbens* L.)

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ABSRTACT

The songgolangit plant is used by some Indonesians as a traditional wound medicine. Therefore, this study was conducted to determine the content of phytochemical compounds and antioxidant activity in ethanol extracts of songgolangit plants (Tridax procumbens L.) so that the use of this plant is more widespread and scientifically proven to contain compounds that can function as drugs. This research uses the GC-MS (Gas Chromatography-Mass Spectrometry) method because this method can separate compounds that are mixed together and can identify various compounds even in low concentrations. Antioxidant activity testing uses the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) method, this method is often used because it is fast, simple, and does not require high costs for screening free radical capture activity. Extract testing via GC-MS showed that the leaf sample of the songgolangit plant had 15 compounds, the stem sample had 11 compounds, and the flower sample had 11 compounds. Only three compounds were found equally in both leaf, stem, and flower. These compounds were 1,2,5-oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide with a function as an antifungal activity. The 4,6-dichloro-5,7-dinitro-2,1,3-benzothiadiazole compound has been no specific research on this compound. Meanwhile 5,8-epoxy-15-nor-labdane was a compound that had antidiabetic function. Songgolangit plants contain alkaloids, tannins, terpenoids, flavonoids, steroids, organic compounds, fatty acids, amines, naphthalene acids and catecholamines. Antioxidant activity analysis showed that songgolangit plants had an IC_{50} value of 81 µg/mL in leaf, 80 µg/mL in stem, and 81µg/mL in flower, and all had strong antioxidant activity category.

Keywords: *Tridax procumbens*, phytochemistry, antioxidant, Gas Chromatography-Mass Spectrometry

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Introduction

The songgolangit plant is a plant that belongs to the Asteraceae family. This plant is a medicinal plant that contains secondary metabolite compounds such as flavonoids, alkaloids, steroids and terpenoids. In some areas In Indonesia, the songgolangit plant is widely used as traditional medicine, one of which is used to heal wounds. In India, the songgolangit plant has long been used in Ayurveda[1]. One study in Tamil Nadu India also mentioned that native Indians use all parts of the songgolangit plant as juice to heal wounds. In Guatemala, the leaves of the songgolangit plant are used as an antibacterial, antifungal, and are used as an antiviral treatment as well as treating mucosal inflammation, pharyngitis, diarrhea and skin infections. Songgolangit leaves can be used as fruit juice to treat wounds and stop bleeding.

Secondary metabolites in several parts of plants are very complex and diverse, this is because some metabolites are synthesized through special regulatory pathways and special transport pathways in certain organs, tissues and cells [2]. The leaves, stems and flowers of the songgolangit plant can contain several different compounds, influenced by environmental factors and the vegetative and generative growth phases of the plant.

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Due to differences in the life cycles of different plants, large amounts of compounds often appear at certain stages of plant growth. The large number of secondary metabolite compounds produced by plants is caused by various physiological changes [3].

Several genetic studies show that the production of secondary metabolites in a plant is influenced by genetic control, which is estimated to be around 15-25% of the genes in the plant genome contributing to the formation of secondary metabolites [4].

Leaf age[5], harvest season[6], and growth stage[7] all influence the compound content in leaves. In flowers, this may be directly related to the developmental characteristics of floral organs, the spatio-temporal expression characteristics of genes regulating the biosynthesis of the chemical composition of volatile substances and the proteins they encode [8,9]. Derived from its anatomical and physiological functions, it can contain compounds such as alkaloids which play a role in defense against herbivores and pathogens. The compound content in the stem is influenced by the growth period, planting season and growing year. Therefore, species and tissue specificity determined by genetic factors all influence the synthesis and accumulation of compounds in a plant. Environmental factors such as sunlight, temperature, humidity and soil type can also influence the production of secondary metabolite compounds. Plants can produce certain compounds in response to environmental stress. Apart from that, gene expression can also influence differences in compound content. Gene expression refers to the process by which genetic information in DNA is translated into protein or RNA products that can regulate cell activity.

Based on the description above, phytochemical tests using the GC-MS (Gas Chromatography-Mass Spectrometry) method and antioxidant tests using the DPPH (2,2 Diphenyl-1-Picrylhydrazyl) method were carried out to support the utilization of songgolangit plants by the community according to the compounds they contain. Antioxidant testing is expected to determine the ability of antioxidant activity in songgolangit plants. According to Iheanacho et al. (2023)[10] free radicals appear to cause cell damage which is a major factor in diabetes,

inflammation, kidney failure, brain dysfunction, and stress and others. So that the presence of antioxidant compounds in a plant affects the ability of a plant as an herbal plant. In the previous year's research[11] , analgesic activity of the songgolangit plant was tested, while in this study phytochemical testing was carried out on three samples of songgolangit plant parts, namely leaves, stems and flowers to compare useful compounds and differences in content in each part of the plant and antioxidant activity. Differences in the geographical location of plants can affect their content so that the songgolangit plant in this study is focused on plants located in Bali. It is hoped that the results obtained from this study can provide good education to the community in the future. Some of these herbal medicines may have components that are important for human metabolism, disease prevention, and healing, but on the other hand, they may also contain non-beneficial components that can pose health hazards to the body system because their safety and efficacy have not been confirmed.[12] Therefore, it is important to understand the compound content in herbal plants used for various medical purposes in order to provide good benefits to the people who utilize herbal plants as treatment.

Materials and Methods

This research was conducted from March to November 2023. This research was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Udayana University and the Denpasar Police Criminal Investigation Laboratory. Plant sample was carried out on 21 March 2023 and the location of the growth of songgolangit plants (T. procumbens) at the Experimental Garden, Faculty of Agriculture, Udayana University, at Pulau Moyo street number 16X, Pedungan, Denpasar, Bali (-8.706695137625747, 115.21564416510238), with the characteristics of healthy plants and not attacked by pests. Voucher number songgolangit plants is PTBG0000010470 herbarium voucher specimen from Ta'u Island (Manu'a Islands/American Samoa/Pacific region).

The materials used in this study were leaves, stems, and flowers of songgolangit plants (Tridax procumbens L.), 96% ethanol, and simplisia of leaves, stems, and flowers of songgolangit plants. While the tools used in this study are cutters, glass jars, 3-layer tissue, ovens, blenders, evaporators, eppendorf tubes, measuring flasks, spectrophotometer UV and other tools in the laboratory.

Samples were taken and sorted into leaves, stems and flowers and weighed as the initial wet weight of the sample. After that, the samples were washed using running water and air-dried for several hours at room temperature ± 25°C. The air-dried samples were then put into a 30 L Drying Oven / Lab Oven / Universal Oven Model 9035A 300', made in China at 45°C for 3x24 hours until a constant dry weight was obtained.

Process of Making Simplisia, Maceration, and Evaporation Process of Songgolangit Plant Leaves, Stems, and Flowers

The sample maceration process refers to research by Rudiana et al. (2023)[13], with modifications to the solvent used. Dried leaf, stem, and flower samples from the Songgolangit plant were then sorted again. Simplisia is obtained by blending or grinding the sample to make it easier for further extraction. The smaller the surface area of the sample, the faster the extraction process. The results obtained were 500g each of leaf, stem and flower simplisia of songgolangit plants from 1kg of plant wet weight. Samples of songgolangit leaves, stems, and flowers were macerated using 96% ethanol solvent in a ratio of 1:10. Simplisia that has been macerated is left for 3x24 hours with 1x24 hour stirring and adding solution. The filtrate obtained from the maceration results was then taken, the remaining pulp was macerated again. The filtrate obtained from the leaves, stems and flowers amounted to 1 liter. The filtrate obtained was then evaporated using a vacuum rotary evaporator at 40°C with a speed of 95. The results obtained will be a thick extract as much as 4ml of leaf extract, 3ml of flower extract and 4ml of stem, which is then continued with antioxidant and antimicrobial tests and taken to the Forensic Laboratory of Bareskrim Polri Denpasar Branch to carry out phytochemical tests using the GC-MS (Gas Chromatography-Mass Spectrometry) method.

Phytochemical Analysis using the GC-MS (Gas Chromatography-Mass Spectrometry) Method

Phytochemical Analysis using the GC-MS to research by Manurung et al. (2022)[14], with modifications to the solvent used. The first step that must be taken at this stage is to dilute the thick sample using ethanol. Then the next sample was injected into the injector in the amount of 1 micro liter, with an initial temperature of 70°C to a final temperature of 290°C, the temperature increase was 5°C/minute (Denpasar Forensic Lab, 2023). Next, the sample will have its compounds identified by a machine. In this study, the column used was an Agilent HP 5MS UI which had a length of 30 m with a diameter of 0.250 mm and a column thickness of 0.25 µm. The observation variables observed and analyzed at this stage are the benefits and molecular formulas of each compound found in the leaf, stem and flower samples of the songgolangit plant.

Antioxidant Analysis using the DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Method

Antioxidant Analysis using the DPPH to research by Riskianto et al. (2022)[15], with modifications to the solvent used. The first stage carried out is the process of preparing the DPPH solution. Second, extract samples of leaves, stems and flowers of the songgolangit plant were diluted using 96% ethanol and then vortexed. After that the sample was centrifuged at 3000 rpm for 15 minutes and filtered. The resulting filtrate was pipetted at 0.5 ml + 3.5 ml DPPH, then vortexed and the sample left for 30 minutes. Measurements used a spectrophotometer UV VIS Visible Spektrofotometer model 752AP made in China with a wavelength of 517 nm.

Results and discussion

Results of GC-MS Analysis of Songgolangit Plant Leaf, Stem and Flower Samples

GC-MS analysis data is in the form of a chromatogram with peak compound results read by a mass spectrometer according to the retention time (RT). Chromatogram results that have been tested show the highest peak compound in songgolangit leaf samples, namely RT 31,940 on Morpholine compounds with uses as anti-cancer with an area of 40.49%. In the chromatogram results of the stem sample, it is known that the highest peak compound is RT 29,823 with the compound 4-Iodo-2,5-Dimethoxyamphetamine which functions as Treating symptoms of traumatic brain injury and symptoms of daytime sleepiness in cases of narcolepsy and chronic fatigue syndrome. Chromatogram results on flower samples the highest peak compound is located at RT 29.821 with Palmitic Acid compound as used in the cosmetics field to treat dry and scaly skin. Compounds that have a low boiling point will come out earlier than compounds that have a higher boiling point, this is due to the difference in evaporation temperature. Therefore, the retention time of each compound is determined by the boiling point of the compound. The following are the compounds contained in songgolangit ethanol extract with an equal value of >90. The compound content of songgolangit plant ethanol extract from GC-MS can be seen in Table 1.

Extract testing via GC-MS showed that the leaf sample of the songgolangit plant had 15 compounds, the stem sample had 11 compounds, and the flower sample had 11 compounds. Compounds that have area under curve (AUC) highest owned by Neophytadiene compounds function as Antidepressants, anticonvulsants and sedatives. While the lowest is the compound 2-(4-Chlorophenyl)-1,3-Oxazine-4,6-Dione as Anti-bacterial and Agathenic Acid as It is located in human blood, and its function is unknown. Some parts of the Songgolangit plant act as antidiabetic, antimicrobial, anti-inflammatory, and antioxidant drugs16. This is in accordance with the results obtained in the table that the songgolangit plant has compounds as antimicrobials, anti-inflammatory and antioxidants found in its leaves, stems and flowers.

Comparison of Compound Content of Extracts of Songgolangit Plant leaf, stem, and flower

Based on the results obtained from the GC-MS test, the three samples were found to contain different compounds. In Table 1, it is known that

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there are only 3 compounds that are the same between the leaf, stem, and flower samples of Songgolangit. These compounds, namely 1, 2,5-Oxadiazol-3 carboxamide, 4,4'-azobis-, 2,2'- dioxide with 3.01%, are most abundant in the leaves and function as antifungal compounds. The 4,6-dichloro-5,7-dinitro-2,1,3-benzothiadiazole compound has the largest area percentage in the stem and there has been no specific research on this compound. Meanwhile, 5,8-Epoxy-15-Nor-Labdane is a compound that has antidiabetic function and is mostly found in stems with a percentage of 7.07%. Thirty compounds derived from the songgolangit plant can be classified with the structure of the compounds. It can be seen in Table 2.

Test of Antioxidant Activity of Songgolangit Plant Leaf, Stem and Flower Extracts

The results of the antioxidant activity test are expressed in IC50 values. Inhibitory concentration (IC50) is the concentration of extract that can inhibit radical oxidation activity by 50% 37. The lower the IC50 value obtained, the stronger the antioxidant activity. The results of the antioxidant activity test for extracts of songgolangit leaves, stems and flowers can be seen in Table 3.

Table 3 shows that the antioxidant activity of extracts in leaf, stem, and flower samples based on classification according38 is classified as strong with an IC50 value of 81µg/mL in leaf and flower, while the antioxidant activity found in stem samples has an IC50 value of 80 µg/mL. The antioxidant compounds found in the leaf are 3',7-Dimethoxy-4',5,8-trihydroxyflavone 8-glucoside with an AUC (Area Under Curve) value of 4.04%, in the stems there is the compound 2-[2-(2- Phenyl-1,3-dioxolan-2-yl)phenoxy]ethane-1-amine with an AUC percentage of 3.00%, while the compound in the flower is 2,3-Dihydro-5,5',7,7'-Tetrahydroxy-2- (4-Hydroxyphenyl)[3,8'-Bi-4H-1-Benzopyran]-4,4'-Dione with an AUC of 0.61%.

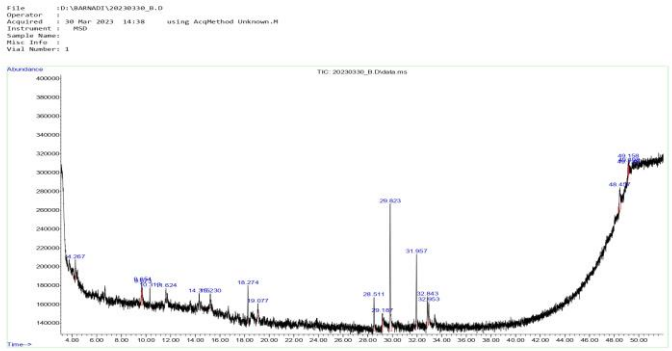

Figure 2: GC-MS chromatogram of songgolangit plant stem extract

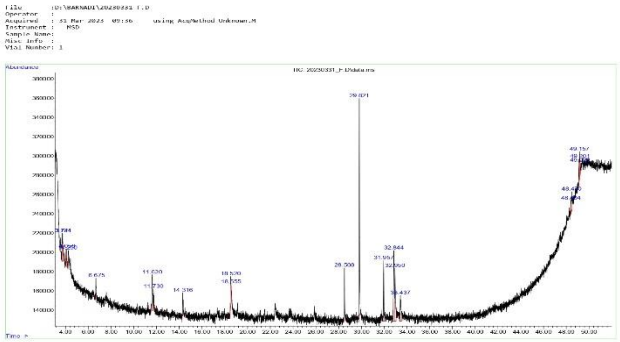

Figure 3: Chromatogram of GC-MS results of songgolangit plant flower extracts

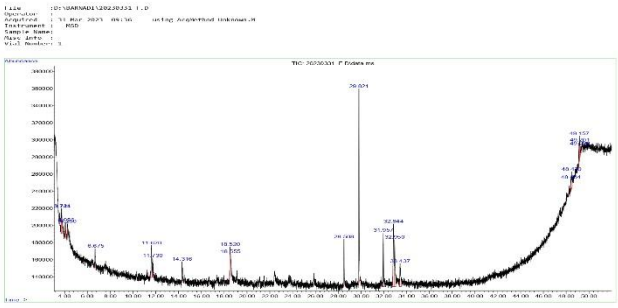

Figure 3: Chromatogram of GC-MS results of songgolangit plant flower extracts

Table 3: IC50 values of songgolangit plant leaf, stem, and flower extracts

Sample Extract	Value IC_{50} (µg/mL)	Antioxidant power
Leaf	81	Strong
Stem	80	Strong
Flower	81	Strong

Description: antioxidant power according[38];
Very strong: < 50 µg/mL, strong: 50-100 µg/mL, medium: 100-250 µg/mL,
weak: 250-500 µg/mL, not active: > 500 µg/mL.

Conclusion

Songgolangit plant leaf contained 15 compounds, the stem and flower samples each contained 11 compounds. Among these compounds there were 3 compounds which found in leaf also found in stem and flower part of the plant. The 3 compounds namely 1,2,5-oxadiazol-3 carboxamide, 4,4'-azobis-, 2,2'-dioxide, 4,6-dichloro-5,7-dinitro-2,1,3-benzothiadiazole, and 5,8-epoxy-15-nor-labdane. Songgolangit plants contained alkaloid, tannin, terpenoid, flavonoid, steroid, organic compound, fatty acid, amine, naphthalene acids, and catecholamine. The leaf, stem, and flower had IC_{50} antioxidant activity of 81 µg/mL, 80 µg/mL and 81 µg/mL respectively which all categorized to strong antioxidant activity. The results of this study can be used as a guideline for making drugs according to the compounds contained in the songgolangit plant

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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