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RESEARCH ARTICLE

DETERMINATION OF TANNIN CONTENT AND ANTIBACTERIAL ACTIVITY OF *CHROMOLAENA ODORATA* L.

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Abstract

Aims and objectives: To ascertain the antibacterial capability using TLC-bioautography and the tannin content using the UV-Vis spectrophotometric method in the ethanol extract of kopasanda (*Chromolaena odorata* L.) leaves.
Methods: Tannins quantitatively utilizing the UV-Vis spectrophotometric technique using the folin ciocalteu reagent at a wavelength of up to 687 nm. Antibacterial potential against gastrointestinal infection-causing bacteria (*Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, and *Shigella dysentriae*) is assessed using TLC-bioautography technique.
Results: The tannin content of the ethanol extract of kopasanda leaves (*Chromolaena odorata* L.) is 41.9064±0.26 mgTAE/g extract. *Escherichia coli*, *Shigella dysentriae*, *Salmonella typhi*, and *Vibrio cholerae*, respectively, create inhibitory zones in the antibacterial potential test that yields 7, 8, 8, and 7 stains. Tannins, flavonoids, alkaloids, and saponins are believed to be among the chemicals found in the TLC results obtained with stain spraying reagents 1, 3, 5, and 4, respectively.
Conclusions: The average tannin concentration of the ethanol extract of kopasanda leaves (*Chromolaena odorata* L.) is 41.9064±0.26 mgTAE/g extract, and it has antibacterial properties against germs that cause gastrointestinal illnesses.
Keywords: *C. odorata* L., Folin-ciocalteu, tannins, TLC bioautography.

INTRODUCTION

The weed known as kopasanda (*Chromolaena odorata* L) grows in the wild. In many parts of Indonesia, kopasanda has long been used to cure wounds, reduce blood sugar, enhance blood flow, and treat skin infections, swelling, and itching. According to studies on the findings of phytochemical screening tests, ethanol extract of kopasanda leaves contains flavonoids[1], polyisoprenoids[2], terpenoids, alkaloids, quinines[3,4], and steroids. It also has the ability to prevent the body from forming cholesterol and may also act as an antioxidant and anti-inflammatory[5,7] and antidiabetic[8]. The presence of flavonoids and phenolics, which might prevent the oxidation process, causes this situation[2,3].

One of the reasons for the high rates of morbidity and mortality worldwide is infectious diseases. Microorganisms might be viruses, fungus, or bacteria that cause infections. The usage of antibiotics is an attempt to combat infectious infections. Additionally, endophytic fungus found in Kopasanda may be able to create antibacterials against bacteria that cause skin infections[9]. Kopasanda leaves include saponins, tannins, phenols, alkaloids, and flavonoids, all of which have antibacterial properties[10]. Additionally, it has antimicrobial properties against microorganisms that cause gastrointestinal problems[11] and skin illnesses[10].

Diarrhea is one instance of a digestive system illness that is highly prevalent. Stool that is mushy to liquid in consistency and occurs three or more times in a single day is referred to as diarrhea. Fever, stomach pain, decreased appetite, exhaustion, and weight loss are all symptoms of diarrhea. Sudden fluid and electrolyte deficiencies brought on by diarrhea can result in a number of problems, including organ damage, hypovolemic shock, coma, and fluid loss. In addition to contaminated food, allergies, and malnourishment, the primary causes of diarrhea are typically microorganisms including Shigella, Rotavirus, *Entamoeba histolytica*, *Salmonella* sp, *Yersinia* sp, *Vibrio cholerae*, and *Vibrio para* hemolyticus[12].

MATERIALS AND METHODS

The sampels used in this research are ethanol extract from leaves of *C. odorata* L. were collected in east Seram distric, Maluku-Indonesia. Voucher specimens

were prepared and deposited at the Botanical Division, Laboratory of Pharmacognosy-Phytochemistry Faculty of Pharmacy, Universitas Muslim Indonesia for future reference.

Ethanol 96% (Merck), distilled water (Brataco Chemical), ammonia (Merck), gallic acid (Sigma), $FeCl_3$ (Merck), Folin iocalteau (Merck), potassium ferricyanide (Merck), Na_2CO_3 (Merck), test microbes *Escherichia coli* (ATCC 25923), *Salmonella typhi* (NCTC 786), *Shigella dysentriae* (ATCC 13313), *Vibrio cholera* (ATCC 14035), N-hexanes (Merck), methanol (Merck), ethyl acetate (Merck), chloroform (Merck), DMSO (Merck), Nutrient Agar medium (Merck), Dragendorf (Merck), aluminum chloride (Merck), Lieberman Burchard (Merck), 0.9% physiological NaCl solution (Otsuka). All reagent compounds were mixture properly.

The study was carried out through experimental procedures conducted in a laboratory setting, employing the spectrophotometric UV-Vis and TLC-bioautography method.

Sample extraction

The 500 g of powdered *C. odorata* L. leaves were macerated at room temperature and extracted three times using 2 L of 96% ethanol each. To get 60 g of a dark, gooey extract, the filtrates were mixed together and evaporated at a lower pressure.

Gallic acid standard curve

Add 1 ml of the Folin ciocalteau reagent (1:10) to 1 ml of gallic acid standard solution concentration series 1, 2, 3, 4, 5, and 6 ppm, and incubate for 5 minutes. Incubate for five minutes after adding 1 ml of a 7.5% Na_2CO_3 solution. Incubate for an additional 80 minutes in the absence of light. A UV-Vis spectrophotometer was used to measure the absorption at the highest wavelength, which is 687 nm, following this incubation period.

Analysis of tannin of kopasanda leaves (*C. odorata* L.)

Dissolve 10 milliliters of distilled water with 10 milligrams of ethanol extract of kopasanda leaves (*Chromolaena odorata* L.). Add 1 ml of Folin ciocalteau (1:10) to 1 ml of extract solution in a pipette, and then let it sit for 5 minutes. Add 1 milliliter of a 7.5% Na_2CO_3 solution, and let it sit for five minutes. Keep incubating in the dark for another 80 minutes. The greatest wavelength of absorption, 687 nm, was measured with a UV-Vis spectrophotometer.

KLT-bioautography analysis

Fill a petri dish with 20 µL of the test bacterial solution and 9 ml of Nutrien Agar (NA) medium (10 g:120 ml). For 60 minutes, the eluted TLC plate (eluent ethyl acetate:chloroform=4:6) is adhered to the NA medium's surface. Incubate once for twenty-four hours at 37°C.

Statistical analysis

The following formula was used to calculate the tannin concentration: tannin concentration = (C x V x FP)/W, where C stands for the regression equation's X value, V for the volume of solution (ml), FP for the amount of dilution, and W for the extract's weight (gram).

RESULTS AND DISCUSSION

In terms of tannin content, kopasanda is one specimen. As active secondary metabolite chemicals, tannins can stop bleeding, repair burns, and create a protective layer around wounds and kidneys. For many years, tannins have been used as a quick fix for diarrhea, dysentery, and bleeding. Tannins can serve as biological antioxidants in addition to their numerous biological roles as protein precipitators and metal chelators[13]. As active secondary metabolite molecules, tannins are recognized to provide a number of benefits, including antibacterial, anti-diarrhea, astringent, and antioxidant effects. The complex components of organic substances known as tannins include phenolic chemicals that precipitate proteins from their solutions, combine with these proteins, and are hard to separate and crystallize[14].

As insecticides, growth regulators, and defenses against predators, tannins and related substances are found in a variety of plant species. If a protein contains at least 12 hydroxyl groups and 5 phenyl groups, tannins can bind to it. In other plants, tannins and polyphenolic components have unique qualities, maintain pathogen pathways operate as pro- and pre-biotics, and help people stay energetic, healthy, and immune[11].

Table 1: Tannin content of ethanol extract of kopasanda leaves (*C. odorata* L.).

Replication	Weight of extract (mg)	Absorbance	Tannin content (mgTAE/g extract)	Average tannin content (mgTAE/g extract)
1	0.0101	0.681	42.1785	
2	0.0100	0.666	41.5629	41.9064±0.26
3	0.0100	0.672	41.9779	

A linear equation, Y=0.144X + 0.065 was derived from tannin concentration research utilizing the UV Vis spectrophotometry method with Folin-ciocalteu reagent. This equation has a correlation coefficient (r) of 0.9952 and a coefficient of variation based on function (Vx0) of 0.5976 ≤2.0 is the acceptance parameter.

One of the secondary metabolites that the kopasanda plant produces is tannin, which has a variety of pharmacological properties, including antibacterial ones. According to the results of the TLC bioautography method used to study the antibacterial activity of ethanol extract of kopasanda leaves (*C. odorata* L.) against bacteria that cause gastrointestinal infections, seven stains inhibit the growth of *Escherichia coli* (EC), eight stains inhibit *Shigella dysentriae* (SG), eight stains inhibit *Salmonella typhi* (ST), and seven stains inhibit *Vibrio cholerae* (VC).

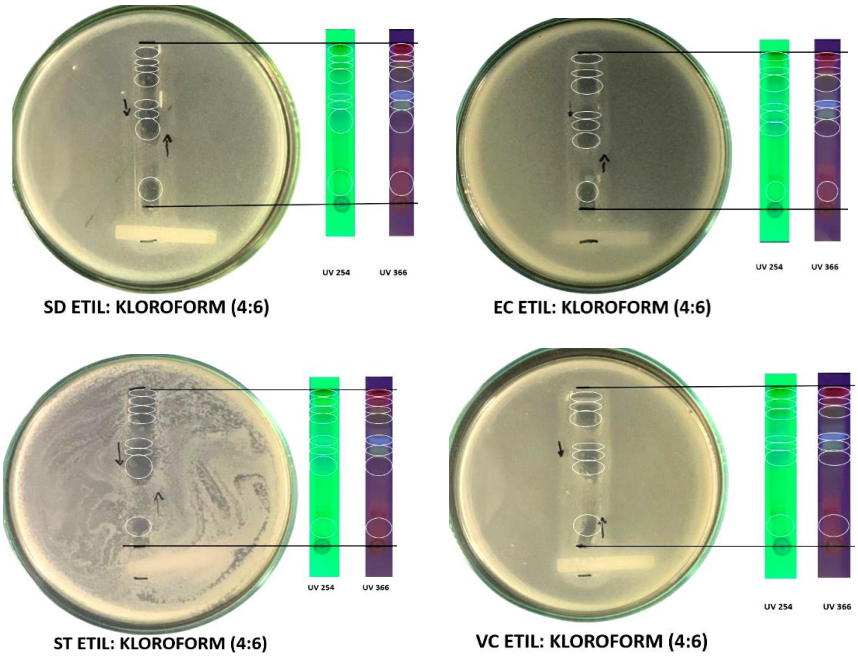

Figure 1: Antibacterial activity of ethanol extract of kopasanda leaves (*C. odorata* L.) against bacteria causing gastrointestinal infections using TLC bioautography method.

If any of the secondary metabolites' chemical components (tannins, flavonoids, sterols, saponins, and alkaloids) act as an antibacterial agent against the relevant test microorganisms, an inhibitory zone is created. This clarifies how these chemical components can prevent test bacteria from growing through a variety of processes, including preventing the production of bacterial cell walls by reacting with the lipoprotein layer, which prevents the formation of bacterial cell walls and causes bacterial cell lysis.

Through the precipitation of proteins, tannin chemical compounds have antibacterial properties. By reacting with cell membranes, inactivating enzymes, and deactivating the functions of genetic material, tannins have an antimicrobial impact.

Table 2: Rf value of TLC stains of ethanol extract of kopasanda leaves (*C. odorata* L.) that produce inhibition zones against bacteria that cause gastrointestinal infections.

No.	Rf value of the TLC stain that produces the inhibition zone			
	E. coli	*S. dysentriae*	*S. typhi*	*V. cholerae*
1	0.09	0.09	0.09	0.11
2	0.51	0.49	0.49	0.55
3	0.60	0.60	0.60	0.62
4	0.67	0.67	0.67	0.69
5	0.80	0.80	0.82	-
6	0.87	0.87	0.87	0.85
7	-	0.91	0.93	0.91
8	0.96	0.96	0.96	0.96

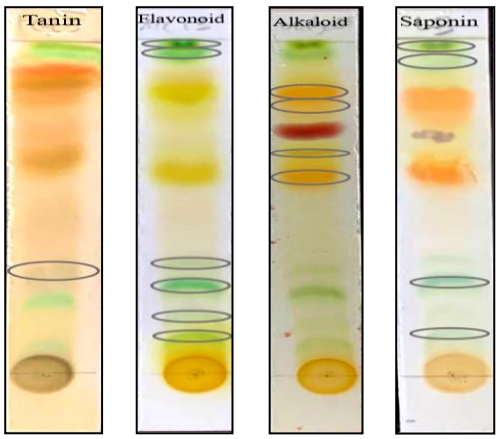

Figure 2: The stain of the TLC results of ethanol extract of kopasanda leaves (*C. odorata* L.) with stain reagents.

Table 3: Stains of compound groups from TLC results of ethanol extract of kopasanda leaves (*C. odorata* L.) with stain reagents.

No.	Group of compounds	Spotting reagent	Positive results	Rf value
1	Tanin	$FeCl_3$	Blue to blue blackish	0.309
2	Flavonoid	$AlCl_3$	Yellow to green yellowish	0.05 0.6 0.84
3	Alkaloid	Dragendorf	Orange to brown	0.6 0.67 0.73 0.8 0.85
4	Saponin	Liebermann-Burchard	Green	0.11 0.27 0.93 0.98

Inhibiting reverse transcriptase and DNA topoisomerase prevents the formation of bacterial cells, which is how tannins work as antibacterials. By activating microbial cell adhesins, activating enzymes, and interfering with protein transport in the inner cell layer, tannins exhibit antibacterial activity. Additionally, tannins target polypeptides in the cell wall, causing the development of the cell wall to become less complete. As a result, bacterial cells are lysed by physical and osmotic pressure, which ultimately leads to their death. Tannin toxicity may be explained by the complexation of iron ions with tannins. For a number of reasons, including the reduction of DNA ribonucleotide precursors, aerobic microorganisms need iron. Because tannins have a significant ability to bind iron, they cannot generate the reverse transcriptase and DNA topoisomerase enzymes of bacterial cells[15].

Based on the spraying reagents employed, the TLC results' stains are thought to include one stain of tannin-containing compounds, three flavonoid-containing compounds, five alkaloids-containing compounds, and four saponins containing compounds.

Limitation of study

This study's primary focus is on secondary metabolites found in plants, as well as the general characteristics and mechanisms of tannins' antibacterial action. Specific information or experimental findings about tannins' antibacterial activity or how they compare to other known antibacterials are lacking from this study. Although the theoretical underpinning of tannins is significant, the effectiveness of tannins as antibacterials is not empirically supported by this investigation. Additional experimental research (in vitro or in vivo assays) is required to ascertain the true antibacterial effect ability and compare it with currently available antibacterials in order to draw significant conclusions regarding the efficacy of tannins as antibacterials for bacteria that cause gastrointestinal infections. Furthermore, the possible adverse effects, bioavailability, and useful applications of tannins—all crucial factors for their usage in medicine and health-were not covered in this study.

CONCLUSIONS

The average tannin concentration of the ethanol extract of kopasanda leaves (*C. odorata* L.) is 41.9064±0.26 mgTAE/g extract, and it has antibacterial properties against germs that cause gastrointestinal illnesses.

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AUTHOR'S CONTRIBUTIONS

Rahmawati: Writing the original draft, method, and investigation. **Baits M:** formal analysis, data organization**. Naid T:** data curation, data organization, visualization. **Azmi N:** visualization, review. **Rumata RW:** literature survey, conceptualization. All authors reviewed and approved the final version of the article.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

There is no conflict of interest around this work.

REFERENCES

1. Nurwahidah. Analysis of absorbance values in determining flavonoid content of kopasanda leaves extraction results (*Chromoleana odorata* L.). [Makassar]: Universitas Islam Negeri Alaudin; 2021. http://repositori.uin-alauddin.ac.id/id/eprint/19021
2. Basyuni M, Yulia V, Sumardi S. Polyisoprenoids profile in kopasanda (*Chromolaena odorata* L.) leaves senescence. In: AIP Conference Proceedings. AIP Publishing; 2021; 2342(1):1-4. *https://doi.org/10.1063/5.0045331*
3. Nurhajanah M, Agussalim L, Iman SZ, Hajiriah TL. Analysis of the antiseptic content of kopasanda leaves (*Choromolaena odorata*) as a basis for making wound gel. Bioscientist : Jurnal Ilmiah Biologi 2020;8(2):284.

4. Sirithiapaporn A, Jiraungkoorskul W. Wound healing property review of siam weed *Chromolaena odorata* L. Pharmacogn Rev 2017;12(21):35–8. *https://doi.org/10.4103/phrev.phrev_53_16*
5. Siregar AJ, Mambang DEP. Anticholesterol activity test of ethanol extract of tekelan leaves (*Chromolaena odorata* L.) R. King & H. Rob) in white male rats (Rattus Norvegicus). Farmasainkes: Pharmacy Sci Cases J 2021; 1(1). *https://doi.org/10.32696/fjfsk.v1i1.818*
6. Rahmawati, Baits M, Putri H, Nurhikmah. *In vitro*, antidiabetic and anticholesterol effects of kopasanda (*Chromolaena odorata* L.). JFFI; 2024.
7. Rahmawati, Mamat Pratama, Zainal Abidin, Yuli wahyuni, Esa yuliasundarsani. *In vitro* antiinflamation and antioxidant activity of etanol extract of *Chromolaena odorata* L. Makassar; 2023.
8. Rahmawati, Widiastuti H, Puspita D. Antidiabetic potential of ethanol extract of kopasanda leaves (*Chromolaena odorata* L.) by UV Vis spectrophotometry method. Makassar; As-Syifaa J Farmasi 2024; 16(2):117-125. *https://doi.org/10.56711/jifa.v16i2.1095*
9. Rusli, Kosman R, Melinda P. Search for endophytic fungi in kopasanda (*Chromoleana odorata* L.) leaves that have potential as antibacterial producers against bacteria that cause skin infections. As-Syifaa J Farmasi 2020; 12(1):64-69. *https://doi.org/10.33096/ja.v12i1.622*
10. Nurhanifah, Ratnah St, Pakadang SR. Antibacterial potential of kopasanda (*Chromolaena odorata* L.) leaves extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Farmasi J Akfarindo 2022;7(2):94-99. *https://doi.org/10.37089/jofar.vi0.132*
11. Patangrao VK. Tannins-antimicrobial chemical components. Int J Technol Sci 2016; 9:5-9.
12. Hutasoit DP. Effect of food sanitation and *Escherichia coli* bacteria contamination on diarrhea. JIKSH 2020;9(2): 779-786. *https://doi.org/10.35816/jiskh.v10i2.399*
13. Khasanah SN, Sutaryono, Addin Q. Analysis of tannin content of methanol extract of telang flower (*Clitoria ternatea* L.) by UV-Vis spectrophotometric method. Ilmu Farmasi J 2021; 10(2):31-35. *https://doi.org/10.61902/cerata.v12i2.307*
14. Pratama M, Razak R, Rosalina VS. Analysis of total tannin content of ethanol extract of clove flower (*Syzygium aromaticum* L.) using UV-Vis spectrophotometric method. Fitofarmaka Indonesia J 2019; 6(2): 368-373. *https://doi.org/10.33096/jffi.v6i2.510*
15. Maliana Y, Khotimah S, Diba F. Antibacterial activity of *Garcinia mangostana* Linn bark against the growth of Flavo bacterium and Enterobacter from *Coptotermes curvignathus* Holmgren. Protobiont 2013; 2(1): 7-11.