Indonesian Journal of Global Health Research
Volume 6 Number S5, October 2024
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

PHYTOCHEMICAL ANALYSIS, FTIR SPECTROSCOPIC OF FLAVONOID AND ANTIBACTERIAL ACTIVITY OF *Crassocephlum crepidiodes* (Benth.) *S. Moore* LEAF AND HERBS EXTRACT

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ABSTRACT
Staphylococcus aureus is a bacteria that causes various skin diseases. C. crepidiodes contains flavonoid compounds which are thought to be responsible for antibacterial activity. This research aims to identify flavonoid compounds and antibacterial activity in the leaves (LC) and herbs (HC) of C. crepidiodes. LC and HC were extracted with 70% ethanol using the maceration method. The secondary metabolites were evaluated by phytochemical analysis and flavonoid compound identification using the Spectrophotometer Fourier Transform Infra-Red (FTIR) test and the antibacterial activity was conducted by the Well method against S. Aureus. The results showed that the secondary metabolite compounds of LC were different from HC and the differences FTIR spectra in transmittance peaks in the identified functional group hydroxyl stretching vibrations correlate with the antibacterial activity of S. Aureus. The antibacterial activity test of C. crepidiodes leaf extract had an average inhibitory zone concentration of 8.35mm to 15.09mm. In comparison, herbal extracts had an average inhibitory zone of 13.61mm to 18.11mm. HC (average inhibitory zone of 13.61mm to 18.11mm) has greater antibacterial activity than LC (average inhibitory zone concentration of 8.35mm to 15.09mm). The herbs parts of C. crepidiodes can be recommended as active antibacterial natural ingredients. This understanding is important to improve the quality of traditional medicine raw materials.

Keywords: C. crepidiodes; flavonoid; staphylococcus aureus

How to cite (in APA style)
Sari, R. K., Susilowati, S., & Wahyudi, D. (2024). Phytochemical Analysis, Ftir Spectroscopic of Flavonoid and Antibacterial Activity of Crassocephlum Crepidiodes (Benth.) S. Moore Leaf and Herbs Extract. Indonesian Journal of Global Health Research, 6(S5), 419-426. https://doi.org/10.37287/ijghr.v6iS5.4540.

INTRODUCTION

Infectious diseases are one of the diseases that disrupt health and occur not only in Indonesia but throughout the world. The cause of infectious diseases is not only viruses but also bacteria. There are more than 45% of deaths caused by infectious diseases (Nurfitasari, 2018). According to WHO (2014), infectious diseases kill 3.5 million people every year. The high incidence of contagious diseases is triggered by the ability of pathogenic microorganisms to transmit and cause infections. One of the bacteria that causes infections is *Staphylococcus aureus*. *Staphylococcus aureus* is a gram-positive bacteria on the skin, mouth, and digestive tract. Treatment of bacterial infections can be done with antibiotics. Antibiotics are drugs given to treat various infections caused by bacteria. Examples of antibiotics used to treat infections include tetracycline, clindamycin, and chloramphenicol (Phadmacanty *et al*., 2016).

Indonesia has biodiversity and many types of natural plants that are used for medicinal purposes. The use of natural ingredients has natural and safe properties, and fewer side effects, and its benefits have been proven empirically (Niah & Baharsyah, 2018). The use of natural ingredients as antibacterial drugs is *C. crepidiodes*. *C. crepidiodes* is a weed plant that belongs to the *Asteraseae* family and grows naturally in damp places. C. crepidiodes has

health benefits in treating headaches, stomach ulcers, stomach disorders, and treating wounds (Yunus & Malik, 2022). According to a study (Rose *et al*., 2020). *C. crepidiodes* leaf extract has activity against *Staphylococcus aureus* which contains alkaloids and flavonoids and has antibacterial effects. Meanwhile, according to research (Lestari, 2015). Based on the information, *C. crepidiodes* leaf extract with a percentage of 10% is able to inhibit the growth of *Staphylococcus aureus* in the inhibition zone of 6.5mm in the moderate category. According to (Husna *et al*., 2022) flavonoid-producing plant parts are not only found in leaves and flowers but also in all parts of the plant: roots, stems, leaves, flowers, skin and seeds. So far, no research has been conducted on *C. crepidiodes* herbs against *Staphylococcus aureus*, so research is needed to develop knowledge about *C. crepidiodes* plants. This study aims to further analyze the identification of flavonoid compounds and the antibacterial activity of *C. crepidiodes* leaves and herbs to *Staphylococcus aureus* bacteria.

METHOD

Tools and materials: The tools needed are stirring rods, test tubes, droppers, Bunsen, Petri dishes, micropipettes, autoclaves, cork borers, incubators, syringes, and FTIR devices. The materials used are HCl reagents, magnesium powder, FeCl3, distilled water, Mayer, Dragendroff, *Staphylococcus aureus* bacterial cultures, MHA media, 10% DMSO, leaves and herb of *C. crepidiodes* , 0.9% NaCl. **Sample Preparation:** The location of the *C. crepidiodes* plant sampling was taken at Ceto Temple, Karanganyar, Central Java, Indonesia. The plants taken were fresh plants with green color, pointed tips, and no strange objects attached to the plants. Determination of C. crepidiodes plants was carried out with the aim of knowing to avoid mixing with other plants and identifying whether the plants used were really *C. crepidiodes* plants. Determination of *C. crepidiodes* plants was carried out at the UPF Tawangmangu Traditional Health Service, Dr. Sardjito Hospital Indonesia. 4kg each of fresh leaves and herbs with green color and free from foreign objects were collected, then washed, and dried using a 50°C oven until they became dry Simplicia, then ground and sieved using a 40 mesh sieve. **Extraction method:** 400 grams each of leaves and herbs *Simplicia* powder was extracted with maceration methid for 3 days using 70% ethanol by stirring once a day. After 3 days, it was filtered and the macerate was evaporated on a Rotary evaporator temperature of 50 ° C became a thick extract and was weighed to calculate the yield.

Phytochemical Screening: The Flavonoid test was carried out with using the Wilstater Cyanidin method by 50mg extract added with Magnesium powder and HCl. Positive results are indicated by the formation of a reddish-orange color. The Alkaloids test was carried out with 50mg of extract divided into 2 tubes, tube 1 is inserted with 1 ml of Mayer's reagent. A positive result is a white precipitation. Then, 1 ml of Dragendroff reagent is inserted into tube 2. Positive alkaloid if a brick red precipitate appears. The phenolic test was carried out with 50mg of extract added with $FeCl_3$ drop by drop. Positive results if there is a blue-black color change. The Saponin test was carried out with 50mg of extract plus warm distilled water and shaken for 30 seconds. Positive saponin results will produce foam with a height of 1-10cm (Maimunah *et al*., 2020).

Flavonoid Identification with FTIR Spektrophotometer: The sample is prepared by using a small amount taken and placed directly on the FTIR surface and recorded in the wavenumber region 4000-500 cm-1. **Antibacterial Activity testing:** Pure bacteria are taken in 1-2 oses, transferred to BAP media by scratching in a zig-zag pattern, and incubated at 37°C for 18-24 hours. The rejuvenated test bacteria were sampled as much as 1-2 ose and poured into a test tube containing 0.9% NaCl and homogenized. The turbidity of the suspension was compared with McFarland's 0.5%. 1 ml of *Staphylococcus aureus* suspension was put into a petri dish, and MHA was added, homogenized, and allowed to harden. Use a cork borer to make 5 wells in solid media. Each well was filled with 50µl of test solution. And incubation was carried out at 37°C for 24 hours. The antibacterial activity of the sample is indicated by the diameter of the inhibition zone in the well area. Each sample was tested at concentrations of 20%, 40% and 60% in 10% DMSO. Tests were carried out with Chloramphenicol (50µg dissolved in 5 ml of 10% DMSO) as a positive control (Maimunah *et al*., 2020).

RESULT

The yield of LC was 21.8% and HC was 25.6%. The results of the phytochemical screening of the sample are shown in Table 1.

Table 1.
Phytochemical Screening of C. crepidiodes Leaf and Herbs Extract

Compound	Reagent	Test Results	
		LC	HC
Flavonoid	HCL + Mg Powder	+	+
Alkaloid	Mayer	-	+
	Dragendorff	-	+
Phenolic	FeCl3	+	+
Saponins	Aquades	-	-

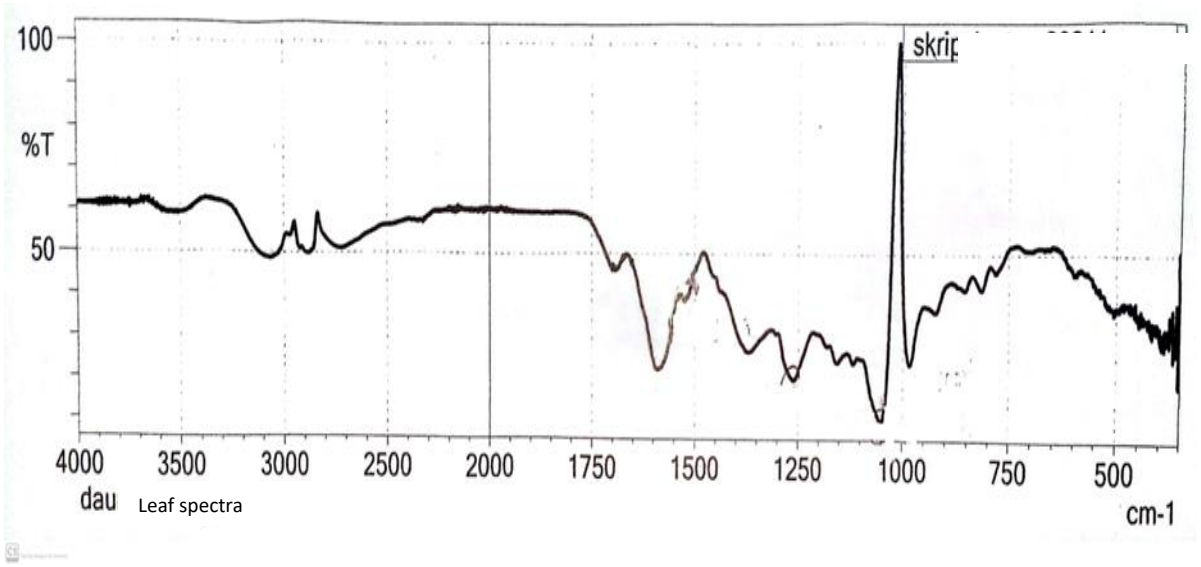
Information :
(+) positive contains compounds
(-) negative contains compounds

The results of the infrared spectra of LC and HC are shown in Figures 1 and 2. The identification results of the functional groups of flavonoid compounds in each sample can be seen in Table 2.

Table 2.
Functional groups of flavonoid compounds in LC and HC based on infrared spectra

		Wave Number (cm^{-1})	
Flavonoid group	Reference (Heneczkowski et al., 2001)	LC	HC
-C=O carbonyl	1820-1660 s	1653 s	1654 s
-OH phenolic	3400 – 3200 m	3100 w	3100 w
-C-OH deformation vibration	1377 – 1188 s	1260 m	1260 m
-C-OH streching vibration	1168 – 1112 m-s	1156 s 1051 s	1010 s

Information. s: strong, m: medium, w: weak.



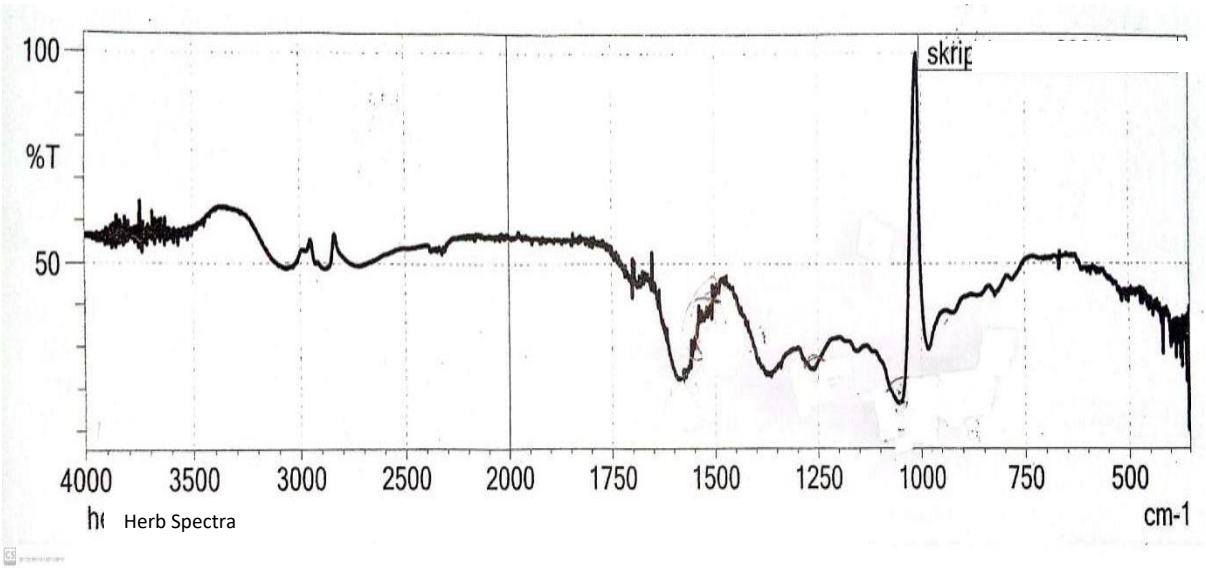
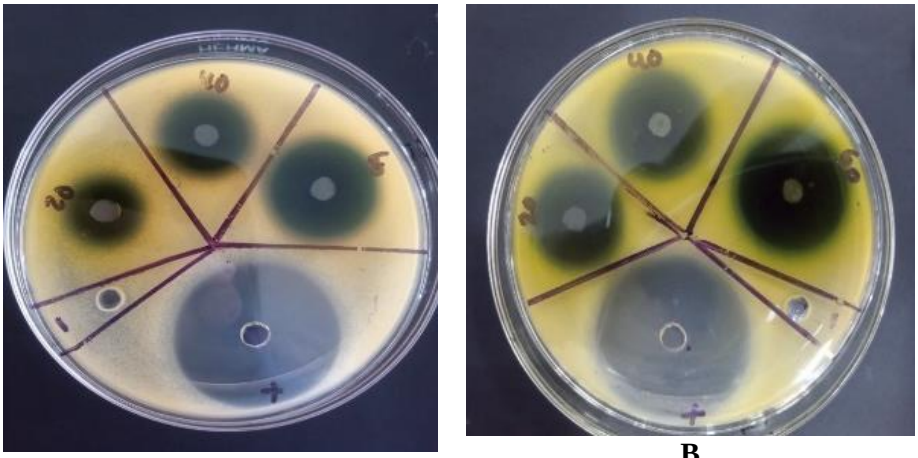


Figure 1. FTIR Spectra of LC and HC on 4000 – 500 cm^{-1}



A **B**

Figure 2**.** Inhibition zone of C. crepidiodes extract against *S. Aureus*. A. LC extract at 3 concentrations (20%, 40% 60%) and positive control; B. HC extract at 3 concentrations (20%, 40% 60%) and positive control.

Table 2.
Size of the inhibition zone of *C. crepidiodes* extract against *S. Aureus*

Sample	Size of the inhibition zone (mm)			
	Replication 1	Replication 2	Replication 3	Average
LC 20%	6.91	8.30	9.85	8.35
LC 40%	9.1	9.62	13.52	10.74
LC 60%	13.08	15.63	16.58	15.09
Chloramphenicol	22.60	24.43	26.69	24.67
Media	0	0	0	0
HC 20%	12.2	13.51	15.12	13.61
HC 40%	14.28	14.48	16.50	15.09
HC 60%	18.45	17.80	18.07	18.11
Chloramphenicol	25.72	27.00	27.43	26.72
Media	0	0	0	0

DISCUSSION

The choice of extraction solvent is very important to extract active plant ingredients based on solubility and intensity of interaction with the matrix. Some studies showed that flavonoid polarity is compatible and more efficient with organic solvents, such as methanol, but non-toxic and biodegradable alternatives, such as ethanol are more recommended because they reduce the impact of organic solvents on the environment with better extractability (Chaves *et al*., 2020). Ethanol concentration affects the solubility of flavonoid and phenolic compounds (Wilorianza *et al*., 2023). 70% ethanol can attract higher flavonoids than 96% ethanol (Khairunnisa *et al*., 2022). The maceration method was chosen because it is simple and does not require heating which can affect the stability of compounds in plants. It is possible that changes that occur in the solvent during the extraction process can affect the stability of flavonoids. It is known that temperature affects the stability of flavonoids and is more sensitive to heating (Susilowati & Desi, 2022). Heating extraction shows degradation of flavonoid compounds, so the maceration extraction method as a cold extraction method is more recommended than the heat extraction method, even though the types of flavonoids have different responses (Chaaban *et al*., 2017). the yield of *C. crepidiodes* herb extracts is higher compared to leaves. The extract yield can be influenced by the part of the plant used, the greater the extract yield indicates the more compounds it contains (Senduk *et al*., 2020).

Based on Table 1, the phytochemical screening test on LC and HC showed differences in the composition of secondary metabolite content. LC contains flavonoids and phenolics while HC contains flavonoids, phenolics, and alkaloids. Flavonoid testing using the Wilstater Cyanidin method was carried out with reagents Concentrated HCl and Mg powder will produce an orange, yellow, or even color if positive for flavonoids (Hanani, 2015). Mg powder and HCl were added aims to reduce the benzopyrone nucleus contained in the flavonoid structure so that flavilium salts are formed (Rumagit *et al*., 2015). The addition of concentrated HCl causes an oxidation reaction to occur reduction between Mg powder as a reducer and flavonoid compounds (Feronica & Muhammad, 2024). In the alkaloid test, the results were negative for the leaf extract, while the herbal extract showed positive results with the presence of a white precipitate in the Mayer test, and a brick red precipitate with Dragendorff's reagent. This difference in results is due to the alkaloid content in plant tissue being less than 1% of the alkaloid compounds found in various parts of the plant (Azizah *et al*., 2018). In the phenol test, the extract of *C. crepidiodes* leaves and C. crepidiodes herbs showed positive results. It contains phenolic compounds that change color to black-green after the sample reacts with $FeCl_3$, color changes and hybridization appear (Putri *et al*., 2019). In the saponin test, the extract of C. crepidiodes leaves and *C. crepidiodes* herbs showed negative results on the sample after being shaken and the foam produced did not last long, only a few seconds. The saponin foam is in the form of micelles with glycosyl groups on the outside and non-polar humus on the inside (Habibi *et al*., 2018).

Flavonoid compound identification of FTIR spectra showed that the LC and LC contain all of the core functional groups in flavonoid compounds including -C=O carbonyl, -OH phenolic, -C-OH deformation, and stretching vibrations (Table 2). The FTIR method is very suitable for the use of qualitative and quantitative identification of flavonoid compounds from plants (Wulandari *et al*., 2016). The advantage of FTIR is that the sample preparation process becomes simpler and only requires a small amount of sample. The mechanism of FTIR technology starts from a light source that hits a prism at a critical angle then is reflected through the sample and hits the detector, causing the wavelength to appear in the IR spectrum and be visible on the monitor screen (Arsista *et al*., 2021). However, the flavonoid functional groups of LC and HC were different in the C-OH stretching or hydroxyl group. Based on

Figure 1, LC has two absorptions and HC has one absorption. The results showed that each part of the plant has different plant characteristics even though it contains the same secondary metabolite group

Based on Figure 2 and table 3, the higher sample concentration will increase the diameter of the inhibition zone for *S. Aureus*. HC showed larger inhibition zone diameters at all test concentrations. The difference was declared significant with a p-value test result of 0.007 (less than 0.05). This showed that differences in plant parts will influence the activity of inhibiting bacterial growth. It has also been proven that each part of the Plumeria rubra L. plant has different antibacterial activity against different *S. Aureus* (Nuryanti & Haryoto, 2023). The difference in antibacterial activity of C. crepidiodes leaf extract shows a significant difference as seen in table 3. *C. crepidiodes* herb extract has a larger inhibition zone than *C. crepidiodes* leaf extract. This is because the antibacterial activity of *C. crepidiodes* herb extract against *Staphylococcus aureus* is better than *C. crepidiodes* leaf extract. The antibacterial activity of plants is influenced by the composition of secondary metabolites in each part of the plant used (Akinduti *et al*., 2022). Herbs are parts of plants that consist of all parts of the plant, so they contain a more complex composition of secondary metabolites compared to the leaves alone. Herbal medicine preparations are generally composed of various parts such as roots, stems, leaves, flowers, seeds and skin containing various secondary metabolite compounds which can increase bioactivity and minimize side effects (Badaring *et al*., 2020).

Flavonoids are secondary metabolite compounds that responsible for the antibacterial activity of *C. Crepidiodes* (Omotayo *et al*., 2015). The mechanism of the antibacterial effect of flavonoids is the inhibition of cell membrane function and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids can form complex compounds with extracellular proteins and damage bacterial cell membranes. The mechanism of alkaloids in antibacterial agents is to affect the peptidoglycan component of bacterial cells, preventing the formation of cell wall layers and causing bacterial lysis and death (Saptowo *et al*., 2022). The antibacterial mechanism of phenolics inhibits the activity of bacterial enzymes and changes the properties of proteins, causing the metabolic activity of bacterial cells to cause death (Sadiah *et al*., 2022).

CONCLUSION

The leaf and herb extracts of *C. crepidiodes* have different metabolite secondary profiles and flavonoid spectra. The antibacterial activity of the herb extract has greater antibacterial activity than the leaf extract. Further analysis is needed to identify flavonoid compounds in leaves and herbs using UPLC or other more appropriate methods to interpret the amount and type of flavonoid content in each sample to confirm the relationship with its antibacterial activity.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Research and Community Service Sekolah Tinggi Ilmu Kesehatan Nasional for their kind support during and all other lab studies.

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