Analysis of Function Groups in Manihot esculenta and Hedyotis corymbosa Extracts Using Fourier Transform Infrared (FTIR) as Potential Immunomodulators

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Abstract: Immunomodulators are substances that can help improve immune system function. Immunomodulators are clinically used for the treatment of immune disorders, such as cancer, HIV/AIDS, malnutrition, allergies and others. The use of synthetic drugs is effective in restoring immune system imbalances, but is at risk of causing side effects, so the search for safer natural alternatives is important. Cassava leaves (Manihot esculenta) and pearl grass (Hedyotis corymbosa) are plants rich in bioactive compounds, such as flavonoids, saponins, and triterpenoids, which have potential as immunomodulators. The mechanism of immunomodulation is thought to be through suppression of proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-17) and an increase in anti-inflammatory cytokines (IL-4, IL-10), while the effect on IL-2 is variable and IFN-γ tends to be lowered, thus supporting the achievement of a balanced immune response. This study aims to analyze the functional groups in Manihot esculenta and Hedyotis corymbosa extracts using Fourier Transform Infrared (FTIR) as a potential immunomodulator. The extracts were obtained through maceration method with 96% ethanol. The analysis showed that both extracts have main functional groups OH (alcohol/phenol), C=O (carbonyl), and CH (alkane) that support immunomodulatory activity. Hedyotis corymbosa extract showed a higher concentration of bioactive compounds, which could potentially provide a stronger immunomodulatory effect compared to Hedyotis corymbosa extract.

1 INTRODUCTION

The human body is composed of various organs that coordinate to form both organ systems and functional systems. These two systems work together to form a unified whole called the body system. The immune system is part of the body's system that plays a role in defending the body against the entry of foreign objects, ensuring that bodily functions are not disrupted (Perdana, 2021). Immunity is the body's response to the presence of foreign substances, both through molecular and cellular mechanisms. The immune system involves T cells produced by the thymus and B cells formed in the spinal cord. T cell activity and development can be enhanced by administering immunomodulatory compounds (Sukmayadi et al., 2014).

Immunomodulators are compounds that play a role in optimizing immune system function. Immunomodulatory mechanisms generally occur through suppression of pro inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-17) and an increase in anti inflammatory cytokines (IL-4, IL-10), while IL-2 levels vary and IFN-γ tends to be decreased, thus achieving a balanced immune response (Leyva López et al., 2016). Individuals with a good immune system tend to be more protected from disease attacks, while in sick people, increasing immunity is important so that immunostimulant drugs are often given. Substances that are able to stimulate the immune systembiological response modifiers (BRM) are divided into two types: biological and synthetic. Examples of biological materials include interferon and monoclonal antibodies, while synthetic materials include muramyl dipeptide (MDP) and levamisole (Rosida and Handojo, 2019).

The use of synthetic immunomodulators can cause side effects, such as microscopic bleeding in the gastrointestinal tract, decreased respiratory function, and decreased platelet count. Possible side effects of immunostimulants include increased uric acid levels, urticaria, and agranulocytosis. Immunosuppressants can cause liver toxicity and gastrointestinal disturbances (Alkandahri et al., 2018). Further research is needed to assess the potential of immunomodulators derived from natural ingredients. Extracts and isolations from natural ingredients can function as immunostimulants with fewer side effects and play a role in suppressing or reducing viral infections.Manihot esculenta And Hedyotis corymbosais a traditional medicinal plant known to have activity as an immunomodulator.

Traditional medicinal plants have long been used as a source of bioactive compounds that play a role in maintaining health and boosting the immune system. One such plant with potential is cassava leaves (Manihot esculenta), which is known to contain various compounds with immunomodulatory activity. These compounds include flavonoid glycosides such as rutin, kaempferol-3-O-rutinoside, and quercetin derivatives, which function to suppress pro inflammatory cytokines while stimulating the production of immunoprotective cytokines. Furthermore, the content of trigonelline, oleamide, and condensed tannins also supports the immunomodulatory effect through antioxidant and anti-inflammatory mechanisms (Fioroni et al., 2023). This is also in line with research conducted by Boukhers et al., (2022) that the leaf extract Manihot esculenta has immunomodulatory activity through concentration-dependent inhibition of proinflammatory mediators such as IL-6, TNF-α, MCP-1, NO, and PGE_2in vitro. Extract Hedyotis corymbosa it is also known to have immunomodulatory potential supported by the content of bioactive compounds in the form of flavonoids (apigenin, kaempferol, luteolin, catechin), phenolic acids (gallic acid, ascorbic acid), and triterpene betulinic acid which play a role in antioxidant and anti-inflammatory mechanisms (Wijayanti, 2017). Characterization of bioactive compounds in Manihot esculenta and Hedyotis corymbosa can be done using various analysis methods, one of which is FTIR (Fourier Transform Infrared). This method is capable of detecting the main functional groups in an extract, thus providing an initial picture of the types of secondary metabolites contained. FTIR analysis is widely used because it is fast, sensitive, and accurate, and can support the identification of compounds with potential immunomodulatory properties. Several previous studies only reported that Manihot esculenta and Hedyotis corymbosa has immunomodulatory activity, but the study focused more on biological tests and did not link this activity to the characteristics of functional groups using FTIR. Based on this description, this study was conducted to analyze the functional groups in the extract Manihot esculenta and Hedyotis corymbosa using the FTIR method as a scientific basis in exploring the potential of both as sources of natural immunomodulators

2 METHOD

Tools and Materials

The tools used are scissors, blender, analytical scales, glass jars, stirring rods, filter paper, Erlenmeyer flasks, rotary evaporators, FTIR instruments (Fourier Transform Infrared), and a dropper. The materials used are leavesManihot esculenta,Hedyotis corymbosa, 70% ethanol, aluminum foil and plastic wrap.

Sample Preparation

The leaf of Manihot esculenta and Hedyotis corymbosa are dried in the hot sun for 24 hours until there is no water content. The next step is to grind the leaves using a blender, then sieve them with a sieve and weigh out 250 grams of the sample to be used in the extraction process.

Leaf Extraction Manihot esculenta and Hedyotis corymbosa

A sample of 250 grams was dissolved with 125 ml of 70% ethanol, the ratio of ingredients to solvents was 1:10, then placed in a storage cabinet for 2x24 hours at room temperature and stirred occasionally. The next stage after 2x24 hours, filtered using filter paper, the filtrate obtained is concentrated with a rotary evaporator at a temperature of 50 C. The results of Manihot esculenta and Hedyotis corymbosa extracts were obtained.

Testing Using FTIR (Fourier Transform Infrared)

The first step is to turn on the FTIR testing tool (Fourier Transform Infrared) and the computer connecting the software used for analysis, then dripped The sample is placed in the sample holder. The FTIR instrument is operated to produce an FTIR spectrum from the sample. The spectrum is read by comparing it to the FTIR table

Data Analysis

The data obtained is presented in the form of variables that reflect the results of the analysis of the two extracts that have been tested using the FTIR (Fourier Transform Infrared) instrument. The methodology used involves a literature review to strengthen understanding of the characteristics of detected compounds. The results of the analysis are then presented in tables and graphs, which aim to provide a clear and informative visual representation of the data obtained, thus facilitating further interpretation and analysis.

Literature Review

This research also includes a literature review to strengthen the analysis of FTIR results. The reviewed literature focuses on research discussing bioactive compounds from Manihot esculenta and Hedyotis corymbosa and its relationship to diseases involving the immune system, such as inflammation, infection, and autoimmune disorders. A literature search was conducted using Google Scholar and ScienceDirect with the keywords "Manihot seculenta immunomodulator”, “Hedyotis corymbosa immunomodulator” and “bioactive compounds of medicinal plants as potential immune system”. The selected articles were published in English and Indonesian between 2015 and 2024, both in the form of original research and reviews. The collected data were then analyzed narratively to connect the results of the FTIR functional group identification with the potential immunomodulatory activity

3 RESULT

FTIR analysis is used to characterize the properties of organic compounds. The properties of a substance typically depend on the molecular structure, which contains specific functional groups. Functional groups are groups of atoms that provide unique identities to a class of carbon compounds and influence their chemical properties (Made & Wayan, 2021).

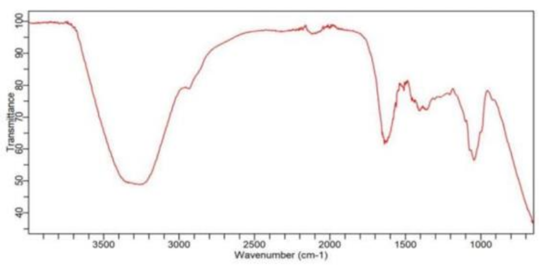

Figure 1. 1 Spectrum FTIR Manihot esculenta

Table 1. 1 Result Analysis FTIR Manihot esculenta

No.	Number of Waves	Functional	Group Intensity
1.	1045,51826	Alcohol, Ether, Carboxylic Acid, Ester (C-O)	Strong (0.31393)
2.	1364,20565	Nitro (NO2) Alkane (C-H) Compound	Strong (0.56082)
3.	1509,57182	Cincin Aromatik (C=C)	Changing (0.65631)
4.	1638,16498	Alkena (C=C)	Changed (0,39097)
5.	3263,28428	Phenols, Alcohol Monomers, Hydrogen Bonding Alcohols (O-H)	Medium (0.19140)

The results of measurements using FTIR spectroscopy on Manihot esculenta extract show several functional groups seen in table 1.1. C-O group (alcohol, ether, carboxylic acid, esters) at a wave frequency of 1045.5 cm^{-1}. The C-H group (aromatic ring) and NO2 compound content were at a peak of 1509.5 cm^{-1} and a hydroxyl bond (O-H) was seen at a peak of 3263, 28428 cm^{-1}. There is an aromatic compound with a carbon bond of C=C at a peak of 1509.5 cm^{-1}, and an alkene functional group (C=C) with a peak of 1638.1 cm^{-1} with variable intensity.

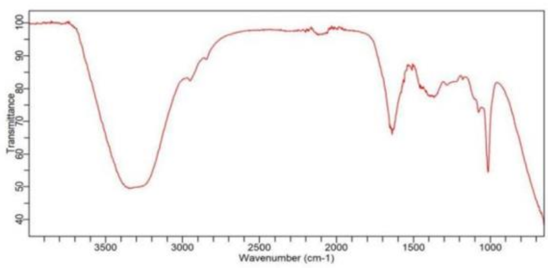

Figure 1. 2 Spectrum FTIR Hedyotis corymbosa

Table 1. 2 Result FTIR Hedyotis corymbosa

No.	Number of Waves	Functional	Group Intensity
1.	1015,69956	Alcohol, Ether, Carboxylic Acid, Ester (C-O)	Strong (0,6276)
2.	1075,33697	Alkohol, Eter, Asam Karboksilat, Ester (C-O)	Strong (0.55603)
3.	1280,34055	Cincin Aromatik (C=C)	Medium (0.68823)
4.	1638,16498	Amina, Amida (N-H)	Changing (0,44743)
5.	1999,71675	Alkena (C=C)	Changing (0.93009)
6.	2120,85523	Alkuna (C=C)	Strong (0,93009)
7.	2202,85666	Alkuna (C=C)	Strong (0,94295)
8.	2845,82244	Alkana (C-H)	Strong (0,81314)
9.	3350,87672	Amina, Amida (N-H)	Medium (0,18067)

Based on the table of FTIR spectroscopy results of pearl grass extract, it was successful to identify the C-O group (Alcohol, Ether, Carboxylic Acid, Ester) at absorption of 1015.6 cm^{-1} to 1075.3 cm^{-1}. C=C (Alkene) group was detected at 1638.1 cm^{-1} to 1999.7 cm^{-1}. At 2202.8 cm^{-1} there is a C≡C (Alkyne) group and at 2845.8 cm^{-1} there is a C=C (Alkanes) group. There is an N-H functional group or amine amide compound with an absorption of 3350.8 cm^{-1} found in pearl grass extract.

4 DISCUSSION

Medicinal plants have long been utilized in various medical traditions because they are rich in secondary metabolites that play an important role in human health. Bioactive compounds such as flavonoids, polyphenols, alkaloids, saponins and terpenoids not only serve as protectors for plants from environmental stress, but also provide pharmacological benefits for humans. These compounds show that secondary metabolites found in plants are able to provide various biological activities, including antioxidants, anti-inflammatory, antimicrobial, and acting as immunomodulators. The role of immunomodulators is very important in maintaining the balance of the body's immune system, namely by regulating the immune response so that it is not excessive but still effective in fighting pathogenic agents (Lin et al., 2018). The existence of these secondary metabolites can be proven through FTIR analysis on cassava leaf extracts (Manihot esculenta) and pearl leaves (Hedyotis corymbosa).

The results of FTIR on cassava leaf extract (Manihot esculenta) showed the presence of a C–O group at 1045.5 cm^{-1}, an aromatic C–H group at 1509.5 cm^{-1}, and an O–H group at 3263.2 cm^{-1}. These O–H and C–O groups indicate the presence of phenolic compounds, especially flavonoids, commonly found in ethanolic extracts. Phenolic compounds act as natural antioxidants that are able to neutralize free radicals and suppress oxidative stress which is generally an inflammatory trigger. This is in line with research conducted by Aprilia et al., (2018) on cassava leaf extract at an absorption of 3452.18 cm^{-1} detected the presence of an O-H hydroxyl group bond. Absorption at 2065.61 cm^{-1} indicates the presence of phenols, absorption of 1635.07 cm^{-1} indicates that C=C (Alkene) bonds and aromatic rings are visible, while absorption of 563.36 cm^{-1} indicates the presence of phosphate groups. Alkene groups, which consist of double bonds (C=C) in the carbon chain, are possible in cassava leaf extracts due to the presence of complex organic compounds in cassava leaves that contain alkene groups. Alkenes are a functional group that is often found in polymers (Imam et al., 2023).

Research by Meilawaty et al., (2020) states that cassava leaf extract has been shown to be effective in reducing the expression of pro inflammatory cytokines such as TNF-alpha and COX-2, which play an important role in the inflammatory process. This decrease in cytokine levels reduces the activity of the MMP-8 enzyme, which is typically expressed in response to an increase in TNF-α and leads to damage to periodontal tissue fibroblasts. The flavonoids in cassava leaf extract function as anti-inflammatory by inhibiting cyclooxygenase and lyoxyglycenase, thereby reducing the production of prostaglandins. This results in a decrease in vascular permeability and the number of inflammatory cells, including inflammatory mediators. As a result, the inflammatory process is shortened, and the stimulation of fibroblasts to produce MMP-8 is reduced, protecting the integrity of periodontal tissue.

The C=C group at wave numbers 1509.5 1638.1 cm^{-1} strengthens the suspicion of the presence of flavonoid and polyphenol compounds in cassava leaf extract. According to Leyva López et al., (2016), flavonoids can suppress pro inflammatory cytokines such as TNF-α and IL-6 and reduces the activity of pro-inflammatory enzymes, including cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS). This mechanism contributes to the immunomodulatory effect by reducing excessive inflammatory responses while increasing immunoprotective cytokines such as IL-2 and IFN-γ. This suggests that the extract Manihot esculenta has the potential to be used as a natural immunomodulatory agent. This is in line with research conducted by Boukherset al., (2024) cassava leaves (Manihot esculenta) contains secondary metabolites such as flavonoids, polyphenols, and carotenoids that play an important role in maintaining immune system balance. Research conducted on RAW 264.7 macrophage cell cultures showed that cassava leaf extract was able to suppress the production of inflammatory mediators, including TNF-α, IL-6, MCP-1, PGE-2, and NO in a concentration-dependent manner. These results support the potential of cassava leaves as a natural immunomodulatory agent through its ability to reduce excessive inflammatory responses.

Hedyotis corymbosa extract showed the presence of relevant functional groups based on the FTIR results. In this extract, there are C–O groups of wave numbers 1015–1075 cm^{-1}, C=C groups at 1638–1999 cm^{-1}, C≡C groups at 2202.8 cm^{-1}, and N–H groups at 3350.8 cm^{-1}. The presence of O–H and C–O groups indicates that these extracts contain flavonoids and polyphenols, while N–H groups indicate the presence of amine compounds or amides that are often found in alkaloid groups.

The flavonoid compounds found in Hedyotis corymbosa extract are a type of secondary metabolite that functions as an antioxidant and acts as an immunomodulator. According to Bashera et al., (2021) that pearl leaf extract (Hedyotis corymbosa) has significant anti-inflammatory activity, both through in vitro and in vivo tests. This effect is attributed to the content of its secondary metabolites, especially flavonoids and polyphenols, which play a role in suppressing inflammatory mediators. This supports the potential of pearl leaves as a natural immunomodulatory agent. This is in line with research conducted by Firmansyah & Duppa, (2022) showing that flavonoids can increase the production of IL-2, which contributes to lymphocyte proliferation. This lymphocyte proliferation affects CD4+ cells, which then activate Th1 cells. Once activated, Th1 cells affect SMAF (Specific Macrophage Activating Factor), which is made up of various molecules, including IFN-γ. IFN-γ (Interferon-γ) functions to activate macrophages, thereby increasing phagocytosis activity. Thus, flavonoids not only play a role in reducing oxidative stress, but also in modulating the immune system through their influence on immune cells, including macrophages.

Research also conducted by Rahminiwati et al., (2025) showed that administering 70% ethanol extract of pearl leaves (Hedyotis corymbosa) in a mouse model with acute lung inflammation was shown to reduce levels of pro-inflammatory cytokines, particularly TNF-α and IL-6, while IL-10 levels remained unchanged. These results indicate that pearl leaf extract has potential as a natural immunomodulatory agent through a mechanism that suppresses the inflammatory response. This is in line with the research Lin et al., (2018) which shows that the extract Hedyotis corymbosa able to suppress the production of TNF-α, IL-1β, and NO in RAW 264.7 cells.

Manihot esculenta and Hedyotis corymbosa extracts both show the presence of O–H, C–O, and C=C groups which indicate the presence of phenolic compounds, including flavonoids and polyphenols, which play a role in immunomodulatory activity. A more complex diversity of functional groups is found in Hedyotis corymbosa, such as the presence of N–H and C≡C, which indicates the possible presence of alkaloid compounds and other nitrogen metabolites. This comparison confirms that although both plants have the potential to be immunomodulators, the results of the FTIR analysis Hedyotis corymbosa indicates a broader contribution of bioactive compounds to their immunomodulatory activity. However, this conjecture still requires confirmation through advanced biological tests to confirm the association between functional group diversity and the resulting immunomodulatory potential.

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