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FOURIER-TRANSFORM INFRARED SPECTROSCOPY ANALYSIS OF MAULI BANANA (MUSA ACUMINATA) EXTRACT AS A POTENTIAL PULP WOUND HEALING

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ABSTRACT
Background: *Pulp wound healing is a critical aspect of endodontics, aiming to preserve the vitality and function of dental pulp tissues. Natural compounds have gained attention for their potential therapeutic applications in dentistry. Mauli banana (Musa acuminata) is a tropical fruit in South Kalimantan as one of the natural plants that have the ability as antibacterial, antifungal, anti-inflammatory, antioxidant, and immunomodulatory effects contains various bioactive molecules with potential pulp wound healing properties.* ***Purpose:*** *to identify and evaluate the functional groups present in mauli banana stem extracts.* ***Methods:*** *This study is true experimental research, the samples were mauli banana stem that were extracted with maceration technique and were subjected to Fourier-Transform Infrared Spectroscopy test.* ***Results:*** *Identification of functional groups of organic compounds from mauli banana are as follows: a) Peak 3638.95 cm^{-1} show O-H (nonbonded hydroxy group, OH stretch primary) component alcohol, b) Peak 3265.82 $cm^{-1}$is C-H (alkane), c) Peak 2928.09 cm^{-1} refer to OH (carboxylic acid hydrogen bonds) and aldehydes groups, d) peak 1571.35 cm^{-1} refers to amide, C=O - asymetric stretching, e) peak 1400.82 cm^{-1}, refers to CO_2- symetric stretching and carboxylate (carboxylic acid salt) CH_2 scissoring, f) peak 1030.17 cm^{-1} refers to C=C (alkanes), aliphatic phosphate, g) Peak 520.53 cm^{-1} refers to inorganic phosphates.* ***Conclusion:*** *FTIR spectra analysis of mauli banana stem extract show the presence of O-H hydroxy groups, aldehydes groups, carbonyl groups, aliphatic groups, carbonyl groups, amides groups and C-O stretching vibrations suggests that the extract contains various bioactive molecules with potential pulp wound healing properties.*

Keywords: *Fourier-Transform Infrared Spectroscop, Mauli Banana Stem Extracts, Pulp Wound Healing*
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INTRODUCTION

The dental pulp plays a crucial part in maintaining tooth vitality by providing nutrients and defense mechanisms against microbial invasion. However, pulpal wounds may develop when dental pulp tissues are exposed to external

factors mainly caries or trauma, potentially result in infection and necrosis. [1]. External factors such as caries is destruction to tooth hard tissue that occurs progressively. If the caries are not treated, they will spread to the pulp area and result in inflammation of the pulp. Inflamed pulp referred to as pulpitis. Pulpitis is caused by bacteria, trauma, heat due to cavity preparations and the toxic effects of restorative materials. Pulpitis is divided into two types: reversible and irreversible pulpitis. Pulpitis is determined by the treatment prognosis. Pulpitis is stated to be reversible when it is anticipated that the pulp condition will improve once the irritating stimulus has been eliminated. In teeth with irreversible pulpitis, it is highly unlikely that removal of the irritant alone can restore the pulp to its natural state.[2] In order to prevent these issues and maintain the function and vitality of the tooth pulp, effective pulp wound healing agents are essential.

Natural products have received a lot of interest in dentistry research due to their ability to enhance wound healing while minimizing side effects.[3] Bananas are among the most popular fruits in the world and provide many employment opportunities. Mauli banana (*Musa acuminata*) is a tropical fruit abundant in South Kalimantan as one of the natural plants that have the ability as antibacterial, antifungal, anti-inflammatory, antioxidant, and immunomodulatory effects.[4] These characteristics make it a potential candidate for pulp wound healing. Its potential use in dental applications has been explored due to its bioactive compounds, including flavonoids, polyphenols, and alkaloids, which are known for their antioxidant and anti-inflammatory properties.[4,5] Basic Health Research (RISKESDAS) results in 2018 indicate that 48,0% of Indonesians and 58,4% of Southern Kalimantan people use traditional medicine to treat health.[6] Mauli banana stem contain the bioactive compound tannins 67.59%; saponins 14.49%; alkaloids 0.34% and flavonoids 0.25%.[7] A preliminary study of the *Liquid Chromatography High Resolution Mass Spectrometry* (LC-HRMS) test that was not published yet by Puspitasari in 2021 found that Mauli bananas also contain compounds such as choline, eucalyptol, cinnamic acid, caffeic acid, citral, linolenic acid, ascorbic acids, carboxylic acids, flavonols, apigenin, isoleucine, choline and many more.

In previous studies mentioned extract of mauli bananas stem has antimicrobial properties against Streptococcus mutans and Candida albicans, non-toxic, antioxidant, anti-inflammatory and immunomodulator by increasing the number of macrophages.[8,9] Carabelly *et al* mentioned that Mauli banana stem extract gel concentrations of 6.25%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, 98% has antimicrobial properties were able to reduce the viability of dual-species biofilms of *Streptococcus mutans* and *Lactobacillus acidophilus* which play a role in the development of caries.[7] Another study reported that 25% concentration of mauli banana stem extract has also been reported to have antibacterial activity against *Staphylococcus aureus w*hen combined with basil leaf extract.[10] As an antifungal, 25% concentration of mauli banana stem extract has antifungal activity against Candida albicans.[11] Mauli banana stem extract gel also has anti-inflammatory activities. A 37.5% concentration of mauli banana stem extract gel showed increased number of macrophage cells in the pulp of wistar rats on the 3rd day compared to $Ca(OH)_2$. The results of this study indicate that mauli banana has the potential to be a healing agent in vital pulp therapy such as mechanical pulp perforations tested on rat teeth.[4] A 37.5% mauli banana stem extract concentration indicates high TGF-β expression. [4,12] In addition, 50% EBPM concentration has a strong anti-inflammatory effect by reducing nuclear factor kappa B (NF-κB) expression.[12,13]

It has been widely employed in dentistry to characterize the chemical composition of dental materials and determine their interaction with biological tissues. In the context of pulp wound healing agents, that can provide insights into the chemical composition and structural characteristics of materials.[14] Knowing the material's composition and structural and chemical features is desirable, as they are placed directly or closely to vital tissues. *Fourier-Transform Infrared Spectroscopy* (FTIR) is a powerful analytical technique to characterize the chemical composition and structural characteristics of natural extracts.[15]

In this study, FTIR is used to evaluate the functional groups and chemical compatibility present in mauli banana stem extract. These analytical methods use infrared radiation absorption measurements to identify functional groups within a sample. This technique is moderately sensitive, very precise, and rapid. For the FTIR process, samples come into contact with infrared (IR) radiation. The IR radiation has an effect on the molecules in the samples' atomic vibrations, resulting in a particular energy absorption and/or transmission. The FTIR is useful for detecting and localizing certain chemical vibrations in the samples using IR radiations.[16] The purpose of this study was to identified and explained functional groups of mauli banana stem extract as potential pulp wound healing.

MATERIALS AND METHODS

The study is true experimental research that was conducted in the Laboratory of Faculty of Mathematics and Natural Sciences (FMIPA) Lambung Mangkurat University, Laboratory of the Faculty of Medicine, Lambung Mangkurat University Banjarbaru, also Laboratory of the Department of Materials and Metallurgical Engineering Sepuluh Nopember Institute of Technology Surabaya in June to July 2023. The main ingredient used was mauli banana stem extract. The FTIR characterization was carried out with FTIR spectrometer (Nicolet™ iS™ 10 FTIR Spectrometer). This tool specification including spectral Range 7800 to 350 cm^{-1} optimized, mid-infrared KBr beamsplitter and 11,000 to 375 cm^{-1} XT KBr extended range mid-infrared optics. This study used spectra at a wavelength range of 400-4,000 cm^{-1}.

Plant determination test

Mauli banana stems are supplied from the SMK PP Banjarbaru. Mauli banana stems were determined at the Laboratory of FMIPA Lambung Mangkurat University. Plant determination tests are carried out to prevent errors in the collection of materials. Test result certificate show that the plant is classified in termes of species of *Musa acuminata Linn.*

Mauli banana stem extraction

The extraction process was carried out at FMIPA Laboratorium, Banjarbaru. The chosen Mauli banana stems are those that are 15 to 21 weeks old, with blossomed flowers, fruit that appears to be fully developed, and picked stalks that have fallen. The portion of the Mauli banana stem that was harvested had a total of four stems and was 10 cm from the root hump. Mauli banana stem were extracted with maceration technique. Fresh mauli banana stems were collected and thoroughly washed, cut into little pieces, and dried for 3 days in an oven that was set with a temperature of 40-60˚C.

The dried Mauli banana stems were grinded with a blender to obtain simplicia powder. After being ground into simplicia powder, dried mauli banana stems were immersed for 3 days, the extract was produced by stirring and filtering a 70% ethanol solution up to 1 cm above the surface of the sample. At temperatures below 40-50˚C a rotary vacuum evaporator is employed to create thick extracts. The ethanol concentration of Mauli banana stem extract is determined using potassium dichromate compound (K_2Cr_2O7). The ethanol-free extract does not change color when exposed to the reagent.

Preparation of FTIR test

The samples were made by mixing the extract with KBr and crushing the pellet. This sample preparation method had a few drawbacks and required professional knowledge to obtain a high level of consistency in daily operations. When employing the potassium bromide KBr technique to analyze an invariable sample, special considerations such as pellet thickness, particle dispersion, ensuring vacuum condition during the pressing and pressure influence must be taken into account. The sample's spectra were recorded after being scanned between 500 and 4000 cm^{-1}. A produced KBr pellet should have a material content of 2-10% of its overall weight. A sample of 1 to 5mg and a pellet size of 13 mm were needed to generate a 300g KBr pellet. After being fully blended with the KBr powder to form a pellet, the examined samples were crushed into a powder, which was then evaluated by FTIR.

The FTIR characterization aimed to identify the existing molecular bonds and analyze them on each identified functional group. The analysis conducted refers to bond bending and stretching. The FTIR produced graphs or curves of relative transmittance (%) to wave number (cm^{-1}) as its end product. Infrared radiation comprised a number of invisible frequency bands. At a wave number of 4,000–200 cm^{-1}, measurements were made in the midinfrared area of the infrared spectrum. The radiation's generated energy would cause the molecules in the materials to vibrate. Each kind of chemical bond or functional group had a separate and tailored infrared absorption band. This technique proved very useful for identifying organic and organometallic compounds.

The measurements of functional group in each sample are placed inside the FTIR cell with a hole leading to the radiation source. The sample is allowed to be exposed to infrared radiation on the FTIR device. The specimen then absorbs some incoming IR, while the unabsorbed infrared is transmitted through the sample surface so that the infrared beam escapes to the detector and the measured signal is then sent to the computer and recorded in the form of peaks according to the functional set that is typical for each molecule.[17]

Spectral data were collected and analyzed to identify characteristic peaks corresponding to functional groups within the extract. The resulting spectrum, displayed as a graph, revealed the percentage of transmittance that varied for each infrared radiation frequency. Infrared dispersion spectrophotometer used a monochromator to select wavelengths. In the situation that a specific infrared radiation frequency passed on an organic

compound sample, the compound will absorb the frequency of the radiation. The wavelength was expressed as the frequency measurement on the axis' horizontal line. Infrared radiation is one type of light frequency that has an unique energy. The energy will be transmitted to the compound if the frequency of light that the compound absorbs is discovered throughout the experiment. The state of the complex's molecules would depend on how much energy was absorbed by the chemical.

RESULTS

The infrared spectra of mauli banana stem extract are presented in figure 1. The typical functional group are listed in table 1.

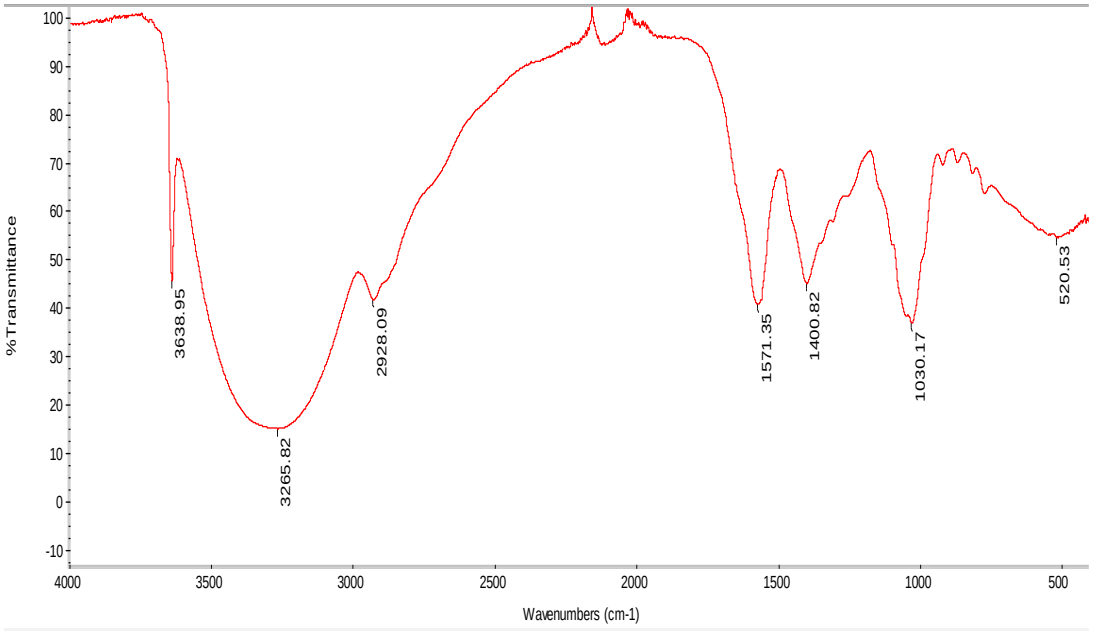

Figure 1. FTIR graph of mauli banana stem extract

Table 1. Identification of functional groups of organic compounds from mauli banana stem extract.

Wavenumber (cm^{-1})	**Functional group**
3638.95	O-H (Nonbonded hydroxyl group, OH stretch Primary).[18–20]
3265.82	O-H bound, N-H stretching. [18–20]
2928.09	H-C=O. [21,22]
1571.35	C=O - asymetric stretching. [23]
1400.82	bend CH_2, CO2- symetric stretching.[20]
1030.17	inorganic phosphates, C – C, aliphatic phosphate (P-O-C stretch). [24]
520.53	inorganic phosphates. [24]

Identification of functional groups of organic compounds from mauli banana stem based on the FTIR spectra as shown in Table 1 and figure 1 are as follows: a) Peak 3638.95 cm^{-1}, identification of the organic compound functional group is O-H (nonbonded hydroxy group, OH stretch primary) component alcohol, hydrogen bond with a percent intensity of 44%, b) Peak 3265.82 cm^{-1}, identification of the functional group of organic compounds is C-H (alkane) with a percent intensity of 16 %, c) Peak 2928.09 cm^{-1} , identification of the functional group of organic compounds is OH (carboxylic acid hydrogen bonds) and aldehydes groups, with a percent intensity of 40 %, d) the peak 1571.35 cm^{-1} refers to functional group of amide, C=O - asymetric stretching, with a percent intensity of 40%, e) peak 1400.82 cm^{-1}, refers to functional group CO_2- symetric stretching and carboxylate (carboxylic acid salt) CH_2 scissoring, with a percent intensity of 44%; f) peak 1030.17 cm^{-1} refers to functional group C = C (alkanes), aliphatic phosphate (P-O-C stretch) with percent intensity of 34 %; g) Peak 520.53 cm^{-1}, identification of the functional group

of inorganic phosphates with a percent intensity of 60% .

DISCUSSION

This study was conducted to determine the functional group of mauli banana stem extract as potential pulp wound healing using FTIR spectrophotometer. The resulting spectrum in a graph showed the percentage of transmittance that varied at each frequency of infrared radiation, range to 16 until 60%.[17] The energy needed for a bond to vibrate was related to the energy of infrared radiation. The number of frequencies passed through the compound (not absorbed) would be computed as a percentage of transmittance (%).[20,25] The absorbance value indicates the number of frequencies absorbed by the compound. The absorbance value is inversely proportional to the frequency transmittance value of the compound that is not adsorbed. The higher the absorbance value, the lower the transmittance value, which means that the compounds released or absorbed are higher. This reason causes the percentage difference in the transmittance value. If there is 100% transmittance, none of the IR frequencies will be absorbed by the compound. However, this condition has never happened. There was always a small amount of this frequency absorbed and giving a transmittance of as much as 95%. This very high absorption could provide important information about the bonds in the compound contained in mauli banana extract. [17]

The study of Mothilal et al., stated that *M. acuminata* extract contains phenols, amines, aldehydes, carbonyl group, and amide compounds.[24] Preliminary study showed that mauli bananas also contain compounds such as choline, eucalyptol, cinnamic acid, caffeic acid, citral, linolenic acid, ascorbic acids, carboxylic acid, flavonols, apigenin. The studies were linked to the peaks that present in the mauli banana stem spectra in the present study.

The study result of mauli banana stem extract spectra show that hydroxy groups (O-H Stretching) at wavenumber 3638.95 cm^{-1} and amine groups at 3265.82 cm^{-1}. Peaks at 3638.95 cm^{-1} show that hydroxy groups (O-H Stretching) were inline with the references that refers to around 3400 cm^{-1} indicated the presence of hydroxyl groups, typically found in phenolic compounds, which are known for their antioxidant and anti-inflammatory properties.[19,20] Those properties are needed in the pupal wound healing. The process of pulp wound healing consists of the following stages; haemostasis, inflammatory response, cell proliferation and tissue remodeling. The phase of inflammation is crucial to the healing of wounds. The prolonged inflammatory phase, however, might impede wound healing. [30]

In the present study, the presence of OH stretching vibrations were atrributed to peaks 3638.95 cm^{-1} were suggested to caffeic acid, compound contained in mauli banana stem.[26.] Caffeic acid, in pulp wound healing may promote collagen deposition, re-epithelialization and has anti-oxidative, and anti-inflammatory properties and effects of immunomodulatory actions.[27,28, 30]

Peak at 2928.09 cm^{-1} shows aldehyde functional groups that referes to the bands between 2800-3000 cm^{-1}, it's suggested the presence of aromatic or aliphatic compounds CH stretching that were potentially contributing to the extract's wound healing properties. Aldehydes are organic compounds which incorporate a carbonyl functional group and divided into aromatic aldehydes and aliphatic aldehydes.[21,22] Aldehydes is suggested to contribute to antimicrobial prevent wound infection development and anti-inflammatory activity resulting in pulp wound healing acceleration. Peak at 2928.09 cm^{-1} in the present study is also estimated to show the presence of functional groups characteristic for cinnamamide derivative which is the content of Mauli banana stem, namely aliphatic CH groups and also in the presence of C=O amide at peaks 1571.35 cm^{-1}. Cinnamic acid and its derivatives have anti-inflammatory and free radical scavenging capabilities. They may also increase neovascularization, which is essential for typical wound healing. The release of growth factors is one of the primary mechanisms of neovascularization. [30]

Peaks 1571.35 cm^{-1} showed CO_2-asymetric stretching is refers to amide functional group that is indicated the presence of the carbonyl group of aromatic acids found in lignin and hemicellulose in raw banana stem, which could be associated with compounds having antioxidant and antimicrobial properties.[23] In this peak refers to the ring's C=O group was probably connected to the formation of intramolecular hydrogen bands with the valence vibration of the (O-H) hydroxy groups in the structure form of apigenin. Apigenin has antioxidant, anti-inflammatory and antimicrobial properties.[29]

The peak around 1030.17 cm^{-1} was refers to the bands between 1000-1300 cm^{-1} indicated the presence of C-O stretching vibrations of polyols, possibly indicating the presence of polyphenols or flavonoids such as hydroxyflavonoids.[19] The spectra of mauli banana stem extract showed the presence of functional group that refers to the bioactive compound tannins and flavonoids. Although FTIR analysis was not conclusive, it

showed strong evidences of the presence of tannins. Tannins show antimicrobial activity, which plays an important role in wound healing applications. Flavonoids are a very important class of polyphenols, as they are plant compounds that have antimicrobial, antioxidant and anti-inflammatory properties. Their anti-inflammatory property stimulates phagocytic activity and cellular immunity.[19] Peaks 1030.17 cm^{-1} is also refer to inorganic phosphates, bands in the region of 1000-1200 cm^{-1}, indicating the presence of cellulose and hemicellulose from the banana stem extract.[24] Meanwhile, at peak 520.53 $cm^{-1,}$ Inorganic phosphates have very characteristic spectra. There are two strong bands at around 1000 cm^{-1} and 550 cm^{-1}.

Peaks at 1400.82 cm^{-1} refers to carboxylic acid salts typically show characteristic asymmetric stretching absorption from the CO_2- group in the 1650-1550 cm^{-1} region. The corresponding symmetric stretching absorption occurs at around 1440-1335 cm^{-1}.[20] Carboxylic acid has an anti-inflammatory property. Anti-inflammtory compound acts via inhibition of key inflammatory mediators, signaling pathways, and molecules.

Fourier-Transform Infrared Spectroscopy analysis of Mauli Banana (*Musa acuminata*) extract has provided valuable insights into its chemical composition. Further research is needed to isolate and characterize specific compounds within the Mauli Banana extract responsible for its pulp wound healing effects. Clinical studies and in vitro experiments are warranted to evaluate the extract's efficacy and safety in the context of dental pulp wound healing. If successful, Mauli Banana extract could emerge as a promising natural agent for promoting pulp tissue repair and preserving dental pulp vitality. Based on the study, it can be concluded that FTIR spectra analysis of mauli banana stem extract show the presence of O-H hydroxy groups, aldehydes groups, carbonyl groups, aliphatic groups, carbonyl groups, amides groups and C-O stretching vibrations suggests that the extract contains various bioactive molecules with potential pulp wound healing properties. We gratefully acknowledge the support of University of Lambung Mangkurat and LPPM for research funding sources of Lecturer’s Obligatory Program for Research 2023 (615/UN8/PG/2023).

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