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QUALITATIVE SECONDARY METABOLITE AND *FT-IR* PROFILES OF THE METHANOLIC EXTRACT FROM *Muntingia calabura* L. LEAVES

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ABSTRACT

The kersen plant (*Muntingia calabura* L.), which grows wildly in Aceh Province, particularly in the city of Banda Aceh, receives little attention from the public because most people are unaware of the benefits of the kersen leaf. In general, kersen leaf extract can be used to treat a variety of diseases, as a health maintenance supplement, and most notably as an anti-diabetic medication. The objective of this study was to determine the chemical composition of kersen leaf extract as an anti-diabetic bioactive substance using thin-layer chromatography and a Fourier transform infrared spectrum. The stages and research techniques were carried out by maceration method using an ethanol solvent, followed by phytochemical testing of organic components in kersen leaf ethanol extract using a TLC method utilizing n-hexane: ethyl acetate (5:5) eluent, and finally, the identification of the kersen ethanol extract was conducted using FT-IR. Based on the phytochemical screening result using thin layer chromatography, the sample with the addition of NaOH and $FeCl_3$ reagents gave distinct colors, namely reddish orange yellow and blackish blue, which revealed that the chemical compounds were dominated in kersen leaf are flavonoid and phenolic. This is also supported by the presence of broad peak spectra for the O-H group, as well as sharp peak spectra for the C-H group of flavonoid and phenolic compounds, at wave numbers 3408 cm^{-1} and 2372 cm^{-1}, respectively. Flavones, flavanol, Auron, and phenol compounds are the specific flavonoid and phenolic chemical compounds found in kersen leaves.

Keywords: Kersen Leaf, Ethanol Extract, Isolation, Characterization, Flavonoids.

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INTRODUCTION

Indonesia is a country that is rich in biodiversity, sourced from natural ingredients, that can be used as a source of traditional medicinal plants. One of them is the kersen leaf, also known as a cherry leaf, or in Latin, it is called as *Muntingia calabura* L. According to previous research, there are 40,000 plant species in Indonesia, but only about 1300 plant species are used as traditional medicine.[1] This is because most people are unaware that plants that grow wild in their environment have the potential to be used as traditional medicine for certain diseases and health supplements. There are bioactive substances in these plants that can be used as medicinal ingredients for a variety of diseases.[2] Bioactive compounds have been defined as plant-derived compounds that can be used in human life.[3,4] Their uses as alternative medicines hold a significant contribution to improving the medical capacity, especially during the ongoing COVID-19 pandemic.[5-7] Bioactive compounds can be used as anti-inflammatory, antioxidant, anti-cancer, and antibacterial agents. The kersen plant is one of the plants that have the potential to be used in traditional medicine. Kersen plants have enormous potential because all parts of them, including the bark, fruit, and leaves, contain bioactive compounds. Water extract from kersen leaves has been reported for its anti-diabetic properties.[8] The richness of secondary metabolites such as flavonoids, alkaloids, and saponins in

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kersen leaves has also been witnessed.[9,10] These three compounds have the potential to be used as antibacterial, antioxidant, and anti-cancer additives.[11-13] Herein, we investigated the methanolic extract from kersen leaves collected from the Banda Aceh area and revealed its qualitative phytochemical and Fourier transform infrared (FT-IR) spectral profiles.

EXPERIMENTAL

Materials

The plant specimen used in this research was *M. calabura* L. collected from the Banda Aceh area with the following coordinate: 5032'23" N 95020'06" E, 9.75 km. Analytical grade chemicals used in this research included methanol 96%, HCl 2 N, Dragendroff's reagent, Mayer's reagent, NaOH, Liberman Buchard's reagent, Pb acetate, $AlCl_3$, KOH 10%, $FeCl_3$ 5%, ethyl acetate, chloroform, n-Hexane, butanol, glacial acetic acid, and H_2SO_4 10%, where all of them were purchased from Merck (Selangor, Malaysia).

Kersen Leaf Extractions (*Muntingia calabura* L.)

M. calabura L. samples were cleaned and air-dried. A total of 1.5 kg samples were then subsequently weighed, mashed, and extracted through maceration using 2.5 L of ethanol for 3 days. After that, the extract was filtered to separate the filtrate from the residue. The filtrate was then evaporated using a low-pressure rotary evaporator, yielding 150 g of a concentrated extract.

Thin-Layer Chromatography (TLC)

Phytochemical tests with TLC were performed on groups of compounds that tested positive with reagents in phytochemical tests. TLC identification was performed on a 60F254 silica plate. Each plate measures 1x10 cm^2 in size. The ethanol extract of kersen leaves was spotted with a capillary tube 1 cm from the plate's bottom edge, then dried and eluted with each mobile phase of the compound group.[14]

FT-IR Spectroscopy

The spectrum of FT-IR analysis was used to characterize the organic compounds in kersen leaf extract based on the functional groups following the protocol from a previously published report.[15-17]

RESULTS AND DISCUSSION

Extraction of Active Compounds

Kersen leaf (*Muntingia calabura* L.) is a wild plant that can be used in traditional medicine. According to published literature, standardization of secondary metabolite content is one of the scarcity parameters for traditional medicinal raw materials.[18] In this study, the maceration method was used to extract secondary metabolites in kersen leaves. Maceration was chosen because it can be done at room temperature for three days of immersion.[19] The filtrate obtained from the extraction is then concentrated using a rotary evaporator to obtain a thick extract of 150 g, with a product yield of about 10%.

Phytochemical Profile

Phytochemical screening tests were performed to determine the type of secondary metabolites present in a sample/simplicia. The results of the phytochemical screening test for secondary metabolite compounds are shown in Table-1. The active phase observations of the kersen leaf extract were performed using the thin layer chromatography (TLC) method.

Separation and Purification Method Using TLC

The secondary metabolite content of the resulting extract was determined using thin-layer chromatography with an n-hexane: ethyl acetate eluent ratio of 5:5. The active phase results of compounds found in kersen leaves were depicted in Fig.-1. The TLC test on steroid metabolites produced positive results, as evidenced by the appearance of a green color after spraying the sample with the Liberman-Burchard reagent. According to a published report[19], steroid testing with Lieberman Burchard's reagent followed by 5 minutes of heating at 105°C can produce a positive reaction characterized by the appearance of a bluish-green stain. Furthermore, the identification of terpenoid compounds by TLC test yielded positive results; as seen in the test results after the sample was sprayed with the Liberman-Burchard reagent, an orange-yellow color appeared on the plate.

Table-1: Phytochemical Screening Test Result of Kersen Leaf Ethanol Extracts

Secondary metabolites	Reagent	Appearance	Screening result
Steroid	Liebermann Burchard	Green/dark green/blue/dark Blue	+
Terpenoid	LiebermannBurchard	Amber-yellow/orange/red-orange/purple	+
Flavanoid	NaOH	Yellow/orange/red-orange	++
Fenol	$FeCl_3$	Dark blue, dark green, black	++
Alkaloid	Dragendorff (DD)	Orange/brick red/brown	+
Saponin	Distilled water	Stable foam	+
Saponin Steroid	HCl + Liebermann Burchard	Green/blue	+
Saponin Terpenoid	HCl + Liebermann Burchard	Amber yellow/orange/ orange-red/purple	+

(+) indicate the presence of secondary metabolites.

Flavonoid testing of secondary metabolites yielded positive results as well. This is demonstrated by the results of the reaction of NaOH with flavonoid compounds, which produce a yellow color, and the identification of phenolic compounds using $FeCl_3$ reagent, which produces a blue-black color, indicating the presence of phenolic compounds in kersen leaf extract. Moreover, testing for alkaloid compounds with Dragendorff's reagent produced a brownish-red color, indicating a positive alkaloid. Furthermore, the saponin compound content was determined by adding distilled water to the sample and shaking it to produce foam. The resulting foam indicates that saponin compounds are present in the kersen leaf extract. Finally, steroid and terpenoid saponins were tested with HCL reagent in combination with Liebermann Burchard, yielding positive results with the appearance of green and orange-yellow colors. The findings are consistent with previously published studies,[9,10,20-23] in which the test results show the content of secondary metabolites in the form of steroids, terpenoids, flavonoids, and phenolics. Slightly different, in this study, it was discovered that terpenoid saponins and steroidal saponins were also found in kersen leaf extract grown in Banda Aceh.

Identification of Extract Compound using FT-IR

The structure of the compounds in kersen leaf extract, which is dominated by flavonoid compounds, was then tested using an infrared spectrum (FT-IR). To determine the composition of the dominant compounds found in kersen leaves, infrared spectrum testing was performed. The spectrum results are depicted in Fig.-2.

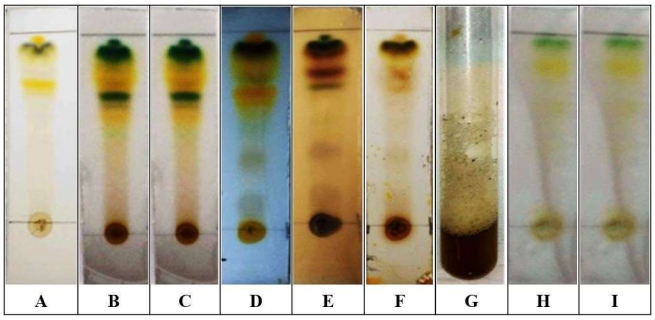

Fig.-1: TLC Identification Test Results of the Methanolic Extract: (A) Before Treatment (Control), (B) Steroid Metabolites, (C) Terpenoid Metabolites, (D) Flavonoid Metabolites, (E) Phenol Metabolites, (F) Alkaloid Metabolites, (G) Saponin Metabolites, (H) Steroid Saponin Metabolites, And (I) Terpenoid Saponin Metabolites

As previously shown, kersen leaf extract contains flavonoid compounds such as flavones, flavanols, and aurones. This is evidenced by the appearance of peaks at wavenumber 1047, 1166, 1222, 1302, and 1379 cm^{-1}, indicating the presence of a C=O group, and a peak at 3408 cm^{-1}, indicating the presence of an O-H group. The test results show the absorption of the C=O group at a wavenumber of 1705 cm^{-1} and the OH

group at a wavenumber of 3394 cm^{-1}. Furthermore, the absorption of aromatic C-H groups at a wavelength of 692 cm^{-1} demonstrated that kersen leaf extract contains terpenoid and steroid compounds. According to published literature,[24,25] terpenoid compounds are derived from isoprene molecules CH_2=C(CH_3)-CH=CH_2, the carbon skeleton of which is formed by joining two or more C5 units. This is consistent with the findings in this study, which show that kersen leaf extract contains alkaloid compounds at wavelengths of 2372, 2852, and 2926 cm^{-1} (aliphatic C-H group). According to the literature, alkaloid compounds contain C-H and C-N, N-H, and N-C=O groups.[26]

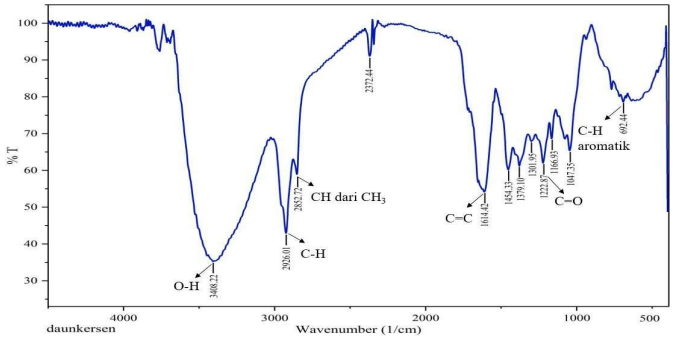

Fig.-2: FT-IR Spectrum of Methanolic Extract from Kersen Leaves

CONCLUSION

Phytochemical screening tests on an ethanol extract of kersen leaves yielded positive results for several organic compounds. The compositions of flavonoids and phenols were the most dominant. The FT-IR spectrum characterization evidenced the presence of O-H and C-H functional groups confirming the presence of flavonoids such as flavones, flovanols, aurons, and phenols.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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