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Identification of Chemical Constituents in Ethanolic Extract of *Hibiscus sabdariffa L.* Calyces (Roselle) by FTIR and GC-MS: Preliminary Phytochemical Screening

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Abstract

Indonesia is widely recognized for its rich biodiversity, which has long served as a valuable resource for traditional herbal medicine. In recent years, interest in plant-based therapies has grown significantly. One such plant, Hibiscus sabdariffa L., commonly known as Rosella, is valued for its therapeutic properties. These benefits arise from the combined effects of its bioactive compounds, allowing it to address a range of health issues. Research has shown that Rosella contains notable levels of flavonoids, saponins, tannins, and alkaloids—all of which contribute to its antibacterial activity. Techniques such as Fourier-transform infrared (FTIR) spectroscopy and gas chromatography–mass spectrometry (GC-MS) are employed to identify these chemical constituents. FTIR analysis has detected functional groups like amines, fatty acids, aldehydes, carboxylic acids, and aromatic rings. Additionally, GC-MS results have indicated the presence of major compounds belonging to the methyl ester group. Noteworthy identified substances include Hexadecanoic Acid Methyl Ester, Hexadecanoic Acid Ethyl Ester, and Benzenepropanoic Acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, Methyl Ester. A review of existing prior research suggests that these compound groups exhibit significant pharmacological potential, including the usage for antioxidant, anticancer, antibacterial, antidiabetic, and anti-inflammatory effects.

Keywords: Herbal Medicine, Roselle, GC-MS, FTIR

1. Introduction

Indonesia possesses a significant component of global biodiversity, qualifying it as a megadiverse nation. The ethnopharmacological application of these vast biological resources as primary materials for medicinal formulations is a long-standing and well-documented practice. The utilization of various botanicals such as ginger (*Zingiber officinale*), lime (*Citrus aurantiifolia*), cloves (*Syzygium aromaticum*), and andrographis (Andrographis paniculata) as sources for phytomedicinal compounds is extensively reported in peer-reviewed scientific literature. Following this tradition, a prominent species within Indonesia's flora, Roselle (*Hibiscus sabdariffa*), is now emerging as a subject of intensive scientific investigation.[1]

The most widely utilized botanical part of the Roselle plant is the calyx, distinguished by its characteristic deep red pigmentation. Consumption of Roselle calyces is reputed to confer therapeutic benefits, including antioxidant, antidiabetic, and antibacterial activities. The chemical constituents responsible for the red coloration are considered pivotal in contributing to the overall pharmacological efficacy of Roselle as a phytomedicinal agent. Consequently, it is imperative to conduct phytochemical analysis to identify and isolate the specific

bioactive compounds responsible for validating the efficacy of *Hibiscus sabdariffa L* as a source material for herbal-based pharmaceuticals.[2,3] This research involved the laboratory analysis of an alcoholic extract derived from Roselle (*Hibiscus sabdariffa L*) calyces. Spectroscopic characterization was employed to elucidate the molecular structure of the bioactive compounds within the extract by identifying their constituent functional groups. Specifically, this molecular identification was performed using Fourier Transform Infrared (FT-IR) spectroscopy to ascertain the chemical profile of the extract.[4]

The measurement results include the molecular vibration spectrum found in the ethanol extract. Subsequently, compound characterization was carried out using gas chromatography instrumentation coupled with mass spectrometry, known as gas chromatography tandem mass spectrometer (GC-MS), to qualitatively identify the chemical compound components. The resulting chromatogram and mass spectrum were used to interpret the presence of chemical compounds identified in the Roselle flower extract using ethanol as the solvent.[5] Roselle calyx has potential as a herbal medicine due to their content of active compounds. Experimental identification of these compounds is necessary to provide scientific justification for the use of Roselle as a herbal drug candidate.[6]

This study aims to explore the chemical compounds that can be used as candidates in the medicinal activity from Roselle calyx. The identified chemical compounds will be correlated with the observed therapeutic activities and effects experienced from the recent research reported. The expected outcome of this research is to serve as a source of information to initiate the development of traditional medicine from a pharmacological perspective. Furthermore, the downstream application of this research is anticipated to support the use of Roselle calyx as a herbal medicine within the community and inform the potential of Roselle calyx.

2. Method

This study is a research-based investigation involving laboratory experiments in the field of chemistry. The chemical composition of the Roselle flower sample will be analyzed using spectroscopy-based instruments, namely Fourier Transform Infrared (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS), both of which are supported by reference libraries or databases for compound identification.

The research samples were obtained from plants cultivated by residents of Pulau Panggung Village, Semende Darat Laut District, Muara Enim Regency, South Sumatra Province. The samples were confirmed to be Roselle calyx (*Hibiscus sabdariffa L.*) through a determination test conducted at the Biology Laboratory, Faculty of Teacher Training and Education, Universitas Muhammadiyah Palembang. Sampling was carried out using a simple random sampling technique by randomly selecting Roselle calyx from local plantations in Pulau Panggung Village. To minimize variability during the sampling process, a homogenization procedure was performed prior to further experimental analysis.

The collected Roselle samples were prepared by separating the calyces from the rest of the flower. The samples were then dried in an open-air dryer for approximately 48 hours. Once dried, the Roselle was homogenized using a blender, producing particles with a defined diameter of 1 mm. The resulting Roselle

powder was subjected to an extraction process using ≥99.9% ethanol (GC), gradient grade, suitable for HPLC, LiChrosolv® (Sigma Aldrich: 1.11727), over a 24-hour maceration period.

The extract obtained from the maceration process was filtered using Whatman No. 40 filter paper with an 8 µm pore size. Filtration was conducted manually based on gravity flow, resulting in two components: the filtrate and the residue. The next step involved purification of the extract using a rotary evaporator. This separation process was carried out under low-pressure evaporation with the aid of a vacuum pump, aiming to completely remove the ethanol solvent and obtain a pure sample concentrate for further qualitative analysis using laboratory instruments. The extraction was conducted in the three cycles and the extract was combined to obtain the homogenized extraction results.

The Roselle extract concentrate was then transferred into a sealed storage bottle, wrapped in aluminum foil, and stored in a chiller at 4°C. This storage method was used to prevent degradation of the chemical compounds present in the Roselle extract prior to analysis.[7]

The presence of flavonoid compounds in the Roselle ethanol extract was qualitatively identified using the magnesium reduction test. In this procedure, approximately 0.1 grams of metallic magnesium was added to the extract solution, followed by the gradual addition of 10 drops of concentrated hydrochloric acid (HCl). This reaction promotes the reduction of flavonoids, especially flavones and flavonols, leading to the formation of colored complexes. A positive reaction is indicated by the appearance of a deep red coloration in the solution, which signifies the presence of flavonoid structures. In contrast, the absence of color change or indistinct coloration suggests a negative result, indicating that flavonoids are not present in detectable concentrations.[8,9]

The detection of alkaloid compounds was carried out using Mayer's reagent, a classical qualitative test for alkaloids. This reagent consists of a solution of mercuric chloride ($HgCl_2$) and potassium iodide (KI) in distilled water, which reacts with nitrogen-containing alkaloid compounds to form insoluble complexes. A positive test is demonstrated by the formation of a white or cream-colored precipitate, indicating the presence of alkaloids in the extract.[10] This result suggests that Roselle may contain nitrogenous bases with potential pharmacological properties.

For the identification of tannins, the ethanol extract of Roselle was reacted with lead (II) acetate solution, which is commonly used in phytochemical screening due to its high specificity toward polyphenolic structures such as tannins. Upon reaction, the presence of tannins is confirmed by the formation of a brown precipitate, which results from the complexation between tannin molecules and lead ions. This visual indication serves as strong evidence for the presence of hydrolyzable or condensed tannins in the extract.[8,9] The presence of saponin compounds was determined through a foam test, which involves the reaction of the extract with a solution of sodium carbonate (Na_2CO_3). Saponins are known for their surfactant properties and ability to lower surface tension, resulting in stable frothing upon agitation. In this test, the development of stable, persistent white foam on the surface of the solution is considered a positive indication of saponin content.[8,9] The presence of saponins in Roselle is noteworthy, given their known roles in modulating cholesterol levels and contributing to antimicrobial activity.

The identification of chemical compounds using FTIR spectrophotometry was conducted at the Integrated Testing Laboratory, Sriwijaya University. The processed Roselle flower extract was characterized using Fourier Transform Infrared (FTIR) vibrational spectroscopy to determine the functional groups present in the target analyte compounds. The extracted sample was analyzed using a Thermo Scientific Nicolet iS10 FTIR instrument, operated in Attenuated Total Reflection (ATR) mode. The spectral scan range was set from 4000 to 450 cm^{-1}, with a spectral resolution of 0.1 cm^{-1}.[4]

The comprehensive chemical characterization, the ethanolic extract of rosella calyx was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) at the Integrated Testing Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University. The analysis was performed in a single run, employing a constant carrier gas flow and a programmed oven temperature ramp to systematically separate the chemical constituents based on their volatility. Prior to injection, the sample was meticulously prepared to ensure optimal results and protect the instrument; the concentrated extract was diluted 100 times using denatured ethanol (Merck, Catalog No. 1009741011) in a volumetric flask. This diluted solution was subsequently filtered through a PVDF syringe filter to remove any particulate matter before the clear filtrate was transferred into a 2 mL vial for automated analysis.

The GC-MS instrument used was a Thermo Scientific GC-MS system, model TSQ™ 9000 Triple Quadrupole. The analytical column employed featured a mid-polar stationary phase. Sample injection was carried out using a splitless injection system, with an injection volume set at 1 microliter. The inlet temperature was maintained at 250°C, and the column operation was set to constant flow mode with a column flow rate of 1 mL/min. The oven temperature program started at 70°C, then increased steadily to 290°C at a rate of 10°C per minute. The final temperature of 290°C was held for 15 minutes, followed by a post-run phase where the column temperature was overridden for an additional 1 minute. Mass spectrometry (MS) spectra were recorded over a mass-to-charge ratio (m/z) range of 30–800 amu. The MS system parameters included a transfer line temperature of 290°C, an ion source temperature of 270°C, and ionization was performed in Electron Ionization (EI) mode with positive polarity.

Data acquisition used centroid mode.The acquired mass spectra were compared to the NIST Library database using Thermo Scientific's Xcalibur™ software integrated with the TSQ™ 9000 system. Compound identification was evaluated based on the percentage match score relative to the reference spectra in the database.

3. Result

Following the completion of all experimental procedures, the obtained extract samples were thoroughly characterized to identify potential chemical compounds with promising herbal medicinal properties. This detailed characterization aimed to uncover bioactive constituents that could contribute to the therapeutic efficacy of the extract. After successfully identifying the chemical profile of the Roselle *(Hibiscus sabdariffa L.*) extract, the next phase involved exploring its practical applications. This includes evaluating the potential uses of *Hibiscus sabdariffa L.* in herbal medicine formulations, pharmacological studies, or as a natural

remedy, thereby bridging the gap between laboratory findings and real-world medicinal applications.

3.1. Phytochemical Screening Result

Phytochemical screening methodology was employed to identify the presence of four major groups of compounds in the test material of flavonoids, alkaloids, tannins, and saponins. These compound groups were detected using qualitative identification methods by reacting the test samples with specific reagents described in section 2.3. The results confirmed the presence of all four groups of compounds in Roselle (*Hibiscus sabdariffa L.*). These findings are illustrated in Figure 1 below.

3.2. *Fourier Transformation Infrared* (FTIR) Spectroscopy Test Result

To identify the chemical constituents within the test sample, spectroscopic characterization was performed utilizing Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. This analytical technique provides detailed information on the molecular vibrations of various functional groups, which is essential for predicting the chemical structures of the compounds present in the sample. The comprehensive results of this analysis, including the specific molecular vibration data and the full IR spectrum, are summarized for reference in Table 1 and visually depicted in Figure 2.

The interpretation of the FTIR spectral data is presented in Table 1. The translated data indicate the molecular vibrations associated with each functional group identified in the Roselle flower extract.

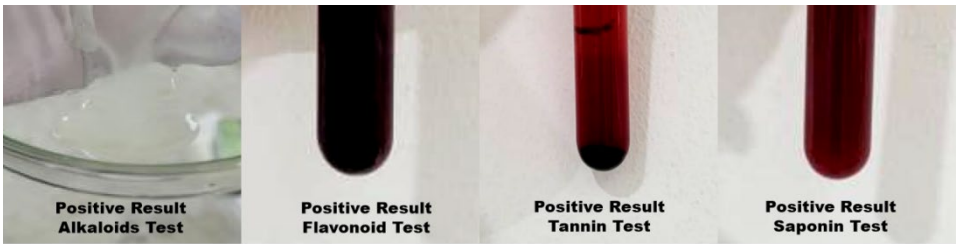

Figure 1. Phytochemical Test Results Showing: (a) Positive Alkaloid Test; (b) Positive Flavonoid Test; (c) Positive Tannin Test; and (d) Positive Saponin Test

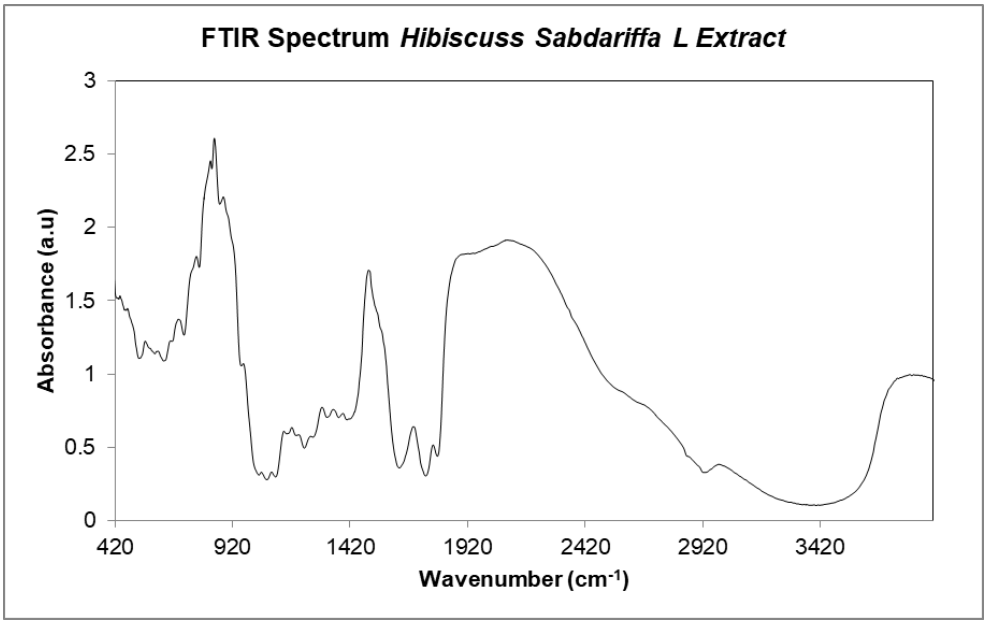

Figure 2. FTIR Spectrum of the Roselle (Hibiscus sabdariffa L.) Extract Sample

Table 1. FTIR Measurement Results of Roselle Flower Extract Sample (*Hibiscus sabdariffa L.*)

Wave-number (cm^{-1})	Vibration Mode	Prediction Chemical Compound Group
522	C–H bending	Aromatic groups
778.29	N–H out-of-plane bending	Primary or secondary amines
832.22	$=CH_2$ wagging	Alkenes (vinyl group)
1064.79	C–O stretching	Alcohols, carboxylic acids
1103.11	C–O–C bending	Ethers, esters, or nucleic acids
1225.3	$–PO_2^-$ asymmetric stretching	Phospholipids or phosphate esters
1321.7	$–CH_3$ symmetric bending	Lipids or methyl groups
1408.05	=CH (cis) bending/rocking	Unsaturated fatty acids, amino acids
1629.38	C=C (cis) stretching	Alkenes (cis isomer)
1741.31	C=O (ester) stretching	Esters
1791.15	C=O stretching (anhydride or strained ring)	Anhydrides, strained ketones (e.g., lactones)
2929.9	$–CH_2–$ asymmetric stretching	Alkanes (saturated hydrocarbons, lipids)
3401.9	O–H stretching (broad)	Alcohols, phenols (hydrogen-bonded)

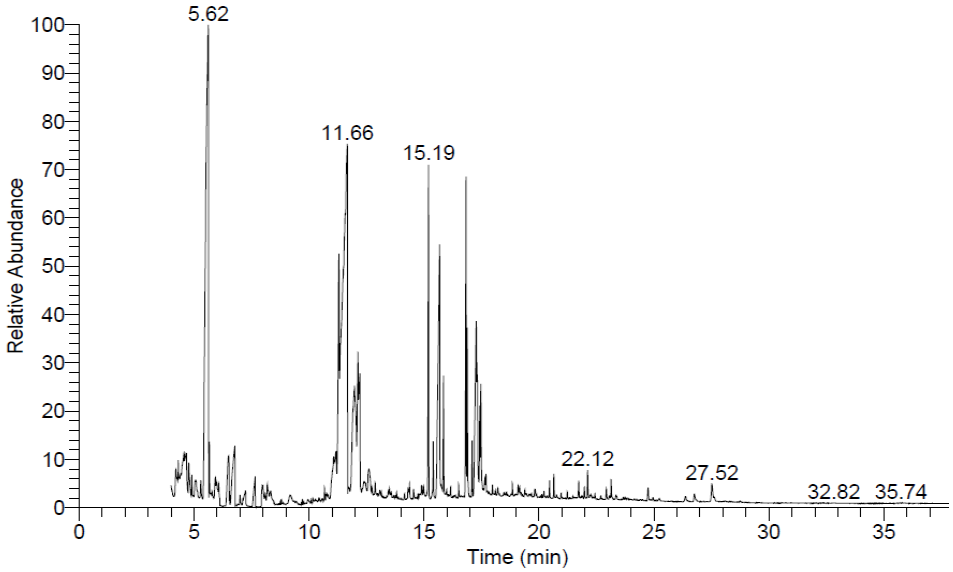

Figure 3. Chromatogram of Roselle Flower Extract Sample from GC-MS Analysis

Table 3. Chemical Compound Identification Result by GC-MS Screening

Retention Time (min)	Identified Chemical Compound	Abundance-Based Area Percentages
5.62	3-Pentenoic Acid, 3- Ethyl-, Methyl Ester	21.91%
11.66	Butanedioic Acid, 3-Hydroxy-2,2-Dimethyl-, Dimethyl Ester	24.44%
15.19	Hexadecanoic Acid, Methyl Ester	3.34%
15.40	Benzenepropanoic Acid, 3,5-Bis (1,1-Dimethylethyl)-4-Hydroxy-, Methyl Ester	0.60%
15.85	Hexadecanoic Acid, Ethyl Ester	1.00%
17.47	Octadecanoic Acid	1.17
27.52	Gamma-Sitosterol	0.53%

3.3. Gas Chromatography–Mass Spectrometry (GC-MS) Test Results

The GC-MS analysis revealed several candidate compounds with potential antibacterial activity, as indicated by the peaks observed in the chromatogram. Data interpretation was carried out by assessing the match quality between the mass fragmentation spectra of the sample and reference spectra from the built-in library database available in the ThermoScientific TSQ™ 9000 Triple Quadrupole GC-MS system, using the Xcalibur™ software.

GC-MS interpretation was performed by comparing the measured mass spectra with reference data from the spectral library. The data were processed, and the identified chemical compounds are presented in Table 3.

4. Discussion

4.1. Phytochemical Testing

In the qualitative phytochemical tests conducted in this study, a color change was observed, indicating a positive result for the presence of flavonoid compounds in the concentrated Roselle flower extract obtained using ethanol as the solvent. This color change from red to deep red occurred after the reaction between the sample, magnesium metal, and hydrochloric acid (HCl). In this reaction,

the target flavonoid groups interact with magnesium, leading to the formation of a carbonyl bond. The subsequent addition of 10 drops of concentrated HCl facilitates the formation of a flavylium salt, which exhibits a deep red color. This change serves as a qualitative indicator of the presence of flavonoids in the sample.[11]

In the qualitative test for alkaloids, the ethanol extract of Roselle also produced positive results when tested using Mayer's reagent. A white precipitate formed, indicating the presence of alkaloid compounds. The reaction is based on the interaction between potassium tetraiodomercurate(II) (the active component in Mayer's reagent) and the nitrogen atoms in alkaloid structures. This results in the formation of a potassium-alkaloid complex that precipitates due to its low solubility in water.[12]

The presence of tannins was tested by reacting the ethanol extract of Roselle with lead (II) acetate. This is a standard test for detecting tannin compounds in plant-based extracts. In this study, the reaction resulted in the formation of a brown precipitate, confirming a positive result for tannins. The underlying reaction involves the dissociation of lead acetate in water to form Pb^{2+} ions, which then react with tannin molecules in the sample to form lead-tannate complexes. These complexes are poorly soluble in water and therefore precipitate, serving as a visual indicator of tannin presence.[13]

For saponin testing, the ethanol extract of Roselle showed a positive result, as evidenced by the formation of stable white foam when the extract, dissolved in water, was reacted with a sodium carbonate solution.[13] Based on this series of rapid phytochemical screening tests, it was concluded that the Roselle flower extract sample contains several bioactive compound groups and is thus suitable for further analysis using advanced laboratory instrumentation. This includes Fourier Transform Infrared (FTIR) spectrophotometry to identify functional groups in the concentrated extract, followed by Gas Chromatography–Mass Spectrometry (GC-MS) for chemical compound profiling.

4.2. FTIR Testing Evaluation

The FTIR spectral analysis of the ethanol extract of Hibiscus sabdariffa L. (Roselle) revealed the presence of various functional groups indicative of bioactive phytochemicals. A broad and strong absorption band at 3401.90 cm^{-1} corresponds to the O–H stretching vibration, characteristic of hydroxyl groups typically found in alcohols and phenolic compounds, which are common constituents in plant extracts and are associated with antioxidant activity.

A peak at 2929.90 cm^{-1} is attributed to the asymmetric C–H stretching vibration of methylene ($–CH_2–$) groups, indicative of aliphatic hydrocarbon chains. This suggests the presence of alkane-type structures, possibly within lipids or long-chain fatty acids. The absorption band at 1629.38 cm^{-1} is more accurately assigned to C=O stretching of conjugated ketones or carbonyl groups (often seen in flavonoids or quinones), or C=C stretching in aromatic or olefinic compounds. If the compound contains aromatic rings, this band may also arise from C=C aromatic ring vibrations rather than cis-alkenes. A sharp peak observed around 1741 cm^{-1} indicates the C=O stretching vibration of ester functional groups, which could suggest the presence of lipid esters or flavonoid glycosides. Additionally, the band at 1791.15 cm^{-1}, if present, may be attributed to anhydride C=O stretching, though this wavenumber is relatively high and uncommon in natural products unless strained cyclic anhydrides

or similar structures are present—this requires cautious interpretation.

The band at 1064.79 cm^{-1} can be assigned to C–O stretching vibrations, which are typical of alcohols, esters, or ether linkages. Similarly, 1103.11 cm^{-1} may correspond to C–O–C asymmetric stretching, common in polysaccharides or glycosidic bonds. The band at 1408.05 cm^{-1} is better attributed to O–H bending (from phenolic –OH) or possibly CH_3 symmetric bending rather than "cis =C–H bending," which typically appears in a different region. The absorption at 1321 cm^{-1} is consistent with CH_3 symmetric bending vibrations, indicative of lipid-related compounds or methyl-substituted aromatics. The band at 1225 cm^{-1} could be due to P=O stretching vibrations from phosphate esters, suggesting the presence of phospholipids or nucleotides. The absorption at 778.29 cm^{-1}, assigned to N–H bending, may be more accurately interpreted as C–H out-of-plane bending in aromatic compounds, since N–H bending typically appears at higher wavenumbers (~1600–1650 cm^{-1} for primary amines). Collectively, these spectral features indicate the presence of various functional groups linked to bioactive compound classes such as phenolics, flavonoids, carboxylic acids, amino acids, lipids, and alkanes in the ethanol extract of Roselle. These findings support the preliminary phytochemical results and provide the basis for further compound identification through Gas Chromatography–Mass Spectrometry (GC-MS), as shown in Figure 2.[14]

4.3. Evaluation of GC-MS Testing

The GC-MS chromatogram of the ethanol extract of Hibiscus sabdariffa L. revealed several prominent peaks, indicating the presence of key chemical constituents. The most abundant compound was detected at a retention time of 5.62 minutes, with a relative abundance of 21.91%. Mass spectral fragmentation at m/z 82, 55, 142, and 110 matched the reference profile for 3-Pentenoic Acid, 3-Ethyl-, Methyl Ester, confirming its identification. Another major compound was observed at a retention time of 11.66 minutes, showing the highest relative abundance of 24.44%. The corresponding mass spectrum exhibited fragment ions consistent with Butanedioic Acid, 3-Hydroxy-2,2-Dimethyl-, Dimethyl Ester, based on spectral library matching.

A third significant peak appeared at a retention time of 15.19 minutes with a relative abundance of 3.34%. The fragment ions observed at m/z 74, 87, and 143 were identified as characteristic of Hexadecanoic Acid, Methyl Ester (commonly known as methyl palmitate), a saturated fatty acid ester commonly found in plant-derived oils. An additional ester compound, Hexadecanoic Acid, Ethyl Ester, was detected at a retention time of 15.85 minutes, with a relative abundance of 1.00%. Within the class of carboxylic acids, Octadecanoic Acid (stearic acid) was also identified, along with a cyclic hydrocarbon derivative, γ-Sitosterol, which was present at 0.53% abundance. A compound structurally related to antioxidant phenolic esters, Benzenepropanoic Acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, Methyl Ester, was identified at a retention time of 15.40 minutes with a relative abundance of 0.60% and a library match score of 94.67%. This compound's identification aligns with previous findings reported by Sehim et al. (2023), who detected the same compound in Roselle flower extracts using methanol as a solvent.[15,16]

4.4. Potential Chemical Compound from *Hibiscus sabdariffa L.* as the Natural Medicine

A series of qualitative phytochemical screenings was conducted on the Roselle calyx extract samples in the laboratory. The results confirmed the positive presence of four major phytochemical groups: Flavonoids, Alkaloids, Tannins, and Saponins. These findings highlight the diverse range of bioactive compounds present in the tested samples. To further characterize the chemical constituents, Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups associated with the bioactive compounds detected in the qualitative tests. FTIR analysis revealed the presence of key functional groups including aldehydes, alcohols, aromatics, and esters, which are typically found within the molecular structures of alkaloids, tannins, flavonoids, and saponins. This molecular fingerprinting corroborated the qualitative phytochemical screening, confirming the chemical diversity of the extract.

For precise compound identification, Gas Chromatography coupled with Tandem Mass Spectrometry (GC-MS/MS) was utilized as the definitive analytical tool to profile the metabolite composition in the ethanol extract of Roselle calyx. The GC-MS results revealed the presence of multiple chemical compounds, predominantly from the ester class. Esters are known for their significant pharmacological properties, including all the potential applications as the antioxidants, antibacterials, and hepatoprotective effects, making them promising candidates for medicinal applications. In particular, Hexadecanoic Acid, Methyl Ester (commonly known as methyl palmitate), identified in the Roselle extract, has been previously reported by Gupta et al. (2023) to possess hepatoprotective properties when isolated from Pistia stratiotes L. leaves, suggesting its role in liver function protection.[17] Moreover, Daben et al. (2017) demonstrated that this compound exhibits anti-inflammatory and antibacterial activities, making it valuable in therapeutic formulations.[18]

Another notable compound detected was γ-Sitosterol, which exhibits unique bioactive characteristics. Naikwadi et al. (2023) reported the presence of γ-Sitosterol in Woodfordia floribunda Salisb extracts and demonstrated its potent anti-inflammatory effects through computational modeling studies.[19] Furthermore, Octadecanoic Acid (stearic acid), also identified in this study, holds considerable significance in pharmaceutical manufacturing. This compound serves as an excipient or protective agent in drug formulations.[20] Recent studies have also shown that Octadecanoic Acid can induce apoptosis—programmed cell death—in malignant breast cancer cells, while sparing non-cancerous cells, by targeting critical cell cycle checkpoints, thus inhibiting cancer cell proliferation.[21]

Overall, these findings substantiate the potential of Roselle flower extracts as a valuable source of bioactive compounds with diverse therapeutic applications, warranting further pharmacological and clinical investigations. Further studies are required to evaluate the effect of chemical component presence with the activity of antibacterial, anticancer, antidiabetic, and hepatoprotective activity. This study was limited in terms of chemical component characterization; therefore, further in vitro investigations are recommended to evaluate the biological activities of the Roselle extract.

5. Conclusion

Based on the results of the FTIR analysis, the presence of key functional groups including esters, aldehydes, and aromatic compounds was identified in the Roselle flower extract. These functional groups indicate the presence of diverse organic compounds that contribute to the bioactivity of the extract. Further chemical profiling using Gas Chromatography-Mass Spectrometry (GC-MS) revealed a variety of ester compounds with different carbon chain lengths. These esters were successfully identified and characterized through mass spectral matching against reference libraries. The chemical classes identified in the Roselle flower extract have been extensively reported in the literature for their diverse pharmacological potentials. Ester-containing compounds present in the extract may contribute to antibacterial activity by inhibiting the growth of pathogenic bacteria, indicating their potential as natural antimicrobial agents. Certain detected constituents also exhibit cytotoxic properties against cancer cells, suggesting a promising role in anticancer drug development. Moreover, some bioactive compounds may possess antidiabetic effects through modulation of glucose metabolism and improvement of insulin sensitivity. Additionally, several chemical constituents demonstrate hepatoprotective properties by reducing oxidative stress and inflammation, thereby protecting against liver damage. Overall, these findings indicate that the Roselle flower extract comprises a rich profile of bioactive molecules with broad therapeutic potential, warranting further biochemical and pharmacological investigations to elucidate their mechanisms of action and confirm their clinical relevance.

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