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Article

Isolation and Characterization of Flavonoid from *Mimosa pudica*

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Muhamad Afham[1*], Rudi Hendra[2], Hilwan Yuda Teruna[2]

[1]Department of Chemistry, Faculty of Engineering and Maritime Technology, Universitas Maritim Raja Ali Haji, Tanjungpinang, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Riau, Pekanbaru, Indonesia

Abstract. Indonesia has a rich biodiversity, including the putri malu plant (*Mimosa pudica* L.) which is known to have secondary metabolites with medicinal potential but has not been fully utilized. The purpose of this work is to isolate and analyze secondary metabolites from *M. pudica*. The aerial portions were dried and macerated in methanol before being partitioned using n-hexane, dichloromethane, and ethyl acetate solvents. The ethyl acetate extract was separated using liquid vacuum chromatography, then recrystallized and purity tested (KLT, melting point, and HPLC). The chemical structure was characterized using UV-Vis, FTIR, and NMR (^{1}H and ^{13}C) spectroscopy. One of the 17 fractions generated pure yellow crystals with a melting point of 242-244°C and single prominent peak on HPLC. The study revealed that the chemical was quercetin, a flavonoid with a variety of biological functions. This study demonstrates that *M. pudica* include active flavonoid compounds that can supportaid in the development of herbal medications based on natural ingredients. These findings lay the groundwork for additional research into its bioactivity and pharmacological potential, particularly as an antidiabetic drug and antioxidant.

Corresponding Author :
Muhamad Afham
Department of Chemistry, Faculty of Engineering and Maritime Technology, Universitas Maritim Raja Ali Haji, Tanjungpinang, Indonesia
Email : muhamadafham@umrah.ac.id

1. Introduction

Indonesia possesses significant biodiversity and is the greatest distributor of tropical flora, which has been extensively utilized by the community as therapeutic resources based on anecdotal evidence, sometimes without knowledge of the precise constituents of these medicinal plants [1]. Natural resources have long served as a foundation for medicinal discoveries. The presence of bioactive compounds and the therapeutic potential of various plants continue to encourage scientific investigation into natural products for drug development. According to the World Health Organization (WHO), approximately 80% of the global population continues to depend on traditional medicine as their main source of healthcare [2]. Approximately 20% of identified plant species are utilized in pharmacological research, contributing positively to human health, including cancer therapies and other ailments [3].

The Mimosa pudica plant, commonly known as the "sensitive plant," is characterized by its unique ability to fold its leaves in response to physical touch and reopen them after a short period [4]. This plant is known as a ground-covering weed with invasive characteristics, as it grows relatively faster than other plants and is resistant to various abiotic stresses [5]. *M. pudica* plants are recognized as a substantial source of bioactive compounds and possess significant pharmacological potential [6]. Secondary metabolites are biologically active chemicals that can safeguard plants against pests and diseases, as well as their surrounding environment [7-8]. Phytochemical investigations of this plant have identified the presence of metabolite chemicals including flavonoids, saponins, steroids, phenolics, glycosides, alkaloids [9], essential oils [10], terpenoids, and carbohydrates [11].

Currently, *Mimosa pudica* plays a significant role in Greco-Arab, Ayurvedic, and traditional Chinese medicine a due to its wide range of therapeutic benefits. In Taiwan, this plant was used as an ornamental plant and utilized for green manure, animal fodder, and honey production. In Traditional Chinese Medicine (TCM), it is now employed to manage conditions such as wounds, insomnia, anxiety, traumatic injuries, neurasthenia, rheumatoid arthritis, diabetes, depression, and tuberculosis [12]. Moreover, studies have documented its traditional use in Nepal for treating fever and digestive disorders like dyspepsia [13]. This plant has been reported to possess many bioactivities, including anthelmintic, wound healing, anti-inflammatory, antidiabetic, antibacterial, antioxidant, anticytotoxic and antidiarrheal properties [4],[13-15].

Numerous studies have reported the biological activities of crude extracts from *Mimosa pudica*; however, the majority have been confined to preliminary phytochemical screening and bioactivity assays. Comprehensive identification and isolation of pure bioactive constituents, particularly those supported by detailed spectroscopic characterization, remain limited and underexplored. Consequently, researchers aim to isolate and characterize pure chemicals from *M. pudica* plants. This research commences with the extraction and isolation of compounds, succeeded by their purification and spectroscopic elucidation to ascertain the chemical structure of the compound.

2. Experimental Section

2.1. Tools and Materials

This research employs a mortar and pestle, analytical balance, substance bottle, ultrasonic apparatus, distillation apparatus, rotary evaporator, Liquid Vacuum Chromatography apparatus, UV lamp (Cole Permer 254 and 366 nm), Fisher Johns melting point determination apparatus, IR Spectrophotometer (Shimadzu, IR Prestige-21), UV-Vis Spectrophotometer (Shimadzu UV-1800), HPLC (Shimadzu), and NMR Spectroscopy (Agilent).

The specimen utilized in this research is the *Mimosa pudica* plant. The ingredients include n-hexane, dichloromethane (DCM), ethyl acetate, methanol, dimethyl sulfoxide (DMSO), $FeCl_3$ staining reagent, aluminum foil, silica gel GF254 KLT thin-layer chromatography plate, silica gel 60 GF254 (230-400 mesh) and additional chemicals as per the established protocols.

2.2. Research Procedures

The research approach commenced with sample preparation, followed by isolation and extraction, separation by VLC method, recrystallization, and structural characterisation. Biological activity testing of the pure compound was not conducted in this study. It is intended to be included in subsequent investigations to validate the bioactive potential of the isolated constituent.

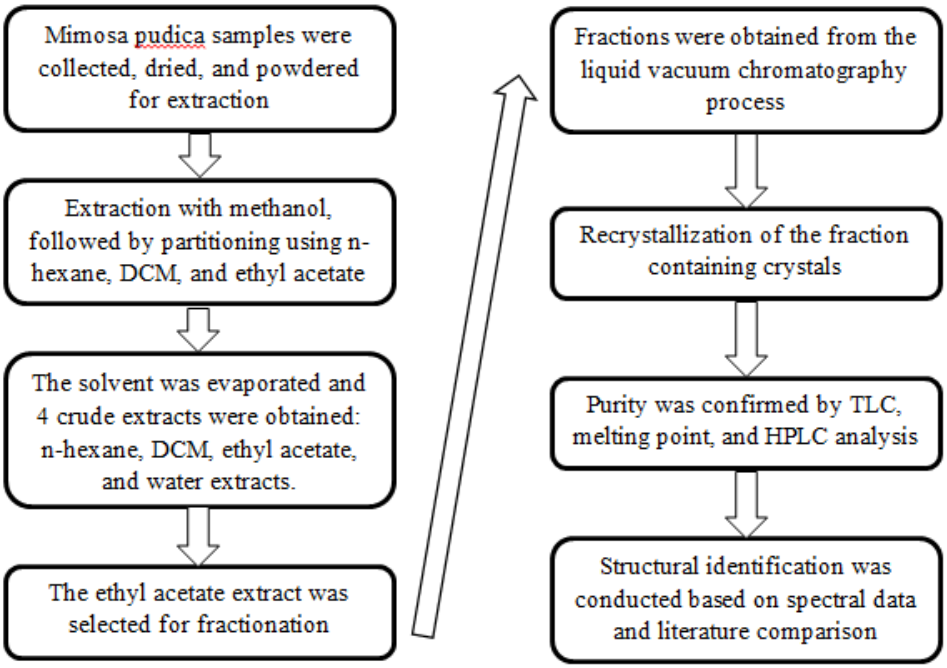

Figure 1. Schematic/flowchart of research

2.2.1. Sample Preparation

Specimen of *M. pudica* (putri malu) were collected at the Riau Main Stadium grounds located on Jalan Naga Sakti, Tampan District, Pekanbaru, Riau. *M. pudica* specimens were desiccated for three weeks at ambient temperature. The airborne components were ground into powder in preparation for maceration.

2.2.2 Extraction and Isolation of Secondary Metabolite Compounds

Four thousand five hundred grams of *M. pudica* plant powder were macerated with methanol solvent many times until the resulting macerate was devoid of color. The resulting maserat was evaporated using a rotary evaporator until a concentrated methanol extract was achieved. The dense methanol extract was subsequently partitioned utilizing n-hexane, dichloromethane, and ethyl acetate solvents in specific ratios for each partition. The partitioned extract was evaporated using a rotary evaporator, and upon drying, it was weighed with an analytical balance, resulting in four extracts: n-hexane, DCM, ethyl acetate, and water extracts.

2.2.3. Fractionation by Liquid Vacuum Chromatography

The ethyl acetate extract of *M. pudica* was isolated using liquid vacuum chromatography column. Twenty grams of ethyl acetate extract were pre-adsorbed with silica gel and pulverized to achieve homogeneity, thereafter placed at the top of the column. The column was eluted with solvents sequentially from 100% n-hexane, to a mixture of n-hexane and ethyl acetate, then to 100% ethyl acetate, followed by a mixture of ethyl acetate and methanol, and finally 100% methanol. The elution

findings were collected in an Erlenmeyer flask and the solvent was evaporated. Subsequently, the solution is transferred into numbered vials, and the solvent is let to evaporate at ambient temperature.

2.2.4. Recrystallization

The separation products, manifested as solids or crystals, subsequently advance to the recrystallization phase. Recrystallization involves dissolving the precipitate in a suitable solvent and allowing the undissolved solids or crystals to settle. The produced crystals are subsequently analyzed for purity.

2.2.5. Purity Analysis

Recrystallized crystals are assessed for purity by various methods, including the KLT test, melting point determination, and HPLC analysis. In the KLT test, a crystal is deemed pure if it yields a single stain.A melting point test was performed with the Fisher-Johns melting point apparatus. A melting point difference of 2°C or less signifies that the crystal is pure. Purity assessment can also be conducted with HPLC. The crystal is deemed pure if the chromatographic findings exhibit a singular dominating peak.

2.2.6. Characterization

The pure crystals were analyzed utilizing UV-Vis Spectrophotometry, FT-IR, and NMR Spectroscopy.

3. Results and Discussion

3.1. Extraction and Isolation

The main step in obtaining bioactive compounds from natural materials is extraction. Extraction is a method that involves the separation of active secondary metabolites from plants using a suitable solvent. This process is frequently employed to quantify or isolate compounds [16]. The extraction of *M. pudica* was conducted using the solvent solvent partitioning method (Kupchan partitioning technique) with some modifications in accordance with the findings of [17]. This method was selected due to its simplicity and effectiveness in the separation of compounds from multiple extracts based on the polarity of the solvent [18]. Table 1 illustrates the extraction outcomes.

Table 1. Extraction results of *M. Pudica* plant

Sample Weight (g)	Extract	Weight (g)	Color
4500	n-hexane	35.1	Dark green
	dichlorometane (DCM)	36.8	Dark green
	ethyl acetate	43.2	Dark green
	water	52.5	Brown

3.2. Fractionation

According to the findings of various studies, the ethyl extract is thought to contain many bioactive compounds such as flavonoids, saponins, phenolics, terpenoids and tannins [19], so the extract is separated using Liquid Vacuum Chromatography to separate secondary metabolites into several fractions, making it easier to isolate active compounds from this plant. Vacuum liquid chromatography was employed in this study owing to its effectiveness in facilitating the efficient separation of large quantities of compounds, aided by the application of reduced pressure via a vacuum pump [20].The extract chromatography findings can be found in Table 2.

Table 2. Chromatography obtained from ethyl acetate extract of *M. Pudica* plant

No. Fractions	No. Vial	Eluent (200 mL)	Weight (g)	Color
1	F_1	H = 100 %	0.0094	Yellow
2	F_2	H:E = 80:20	0.2013	Bright green
3	F_3	H:E = 60:40	1.4473	Green
4	F_4	H:E = 40:60	3.5022	Green
5	F_5	H:E = 20:80	1.3450	Greenish yellow
6	F_6	E = 100 %	0.8789	Greenish yellow
7	F_7	E:M = 90:10	1.2198	Greenish yellow
8	F_{8A}	E:M = 80:20	0.5424	Dark green
9	F_{8B}	E:M = 80:20	0.9486	Dark green
10	F_9	E:M = 70:30	0.8064	Brown
11	F_{10}	E:M = 60:40	1.1009	Brown
12	F_{11}	E:M = 50:50	0.5079	Brown
13	F_{12}	E:M = 40:60	0.8020	Dark red
14	F_{13}	E:M = 30:70	0.4507	Dark red
15	F_{14}	E:M = 20:80	0.8789	Dark red
16	F_{15}	E:M = 10:90	0.3348	Dark red
17	F_{16}	M = 100 %	0.1865	Dark red

3.3. Recrystallization and Characterization of Secondary Metabolite Compounds

From the 17 fractions recovered from VLC, only fraction 5 generated crystals when recrystallized. The crystal was assigned the designation 20-06-C-FV. The crystal was then submitted to purity and characterisation testing. The melting point, KLT, and HPLC tests were used in this work to confirm purity, whereas UV-Vis, FT-IR, and NMR spectroscopy were used to characterize pure substances.

Compound 20-06-C-FV has a yellow powder and a melting point of 242-244°C. The KLT test yielded an Rf value of 0.70 with an eluent ratio of n-hexane:ethyl acetate of 1:1, while the HPLC test yielded a single dominant peak with a retention time of 15.46 minutes. These results indicated that the chemical obtained was pure.

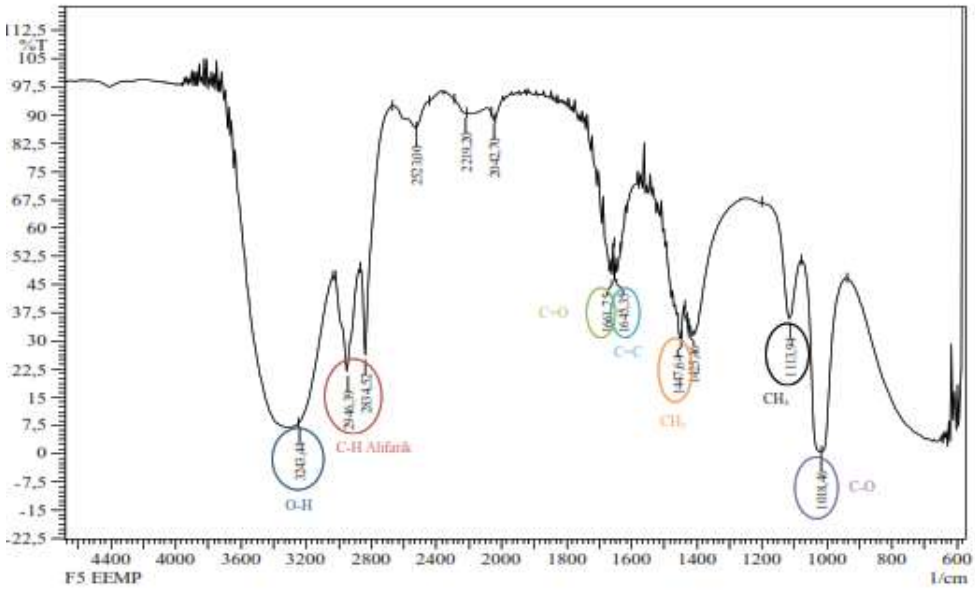

Figure 2. FTIR spectrum of 20-06-C-FV compound

The UV-Vis spectra revealed maximal absorbance at 288 nm, confirming the existence of benzoyl bands in the flavonol skeleton. The IR spectrum (Figure 2) revealed that this chemical has an OH group that absorbs in a broad band at 3243 cm^{-1}. The molecule is considered to feature an aliphatic C-H group based on high absorption at wave numbers 2946 and 2835 cm^{-1}. This molecule contains a C=O group at wave number 1662 cm^{-1} as well as an alkene stretch band (C=C) at 1645 cm^{-1}. C-O bonds can be detected at wave number 1018 cm^{-1} with modest absorption intensity.

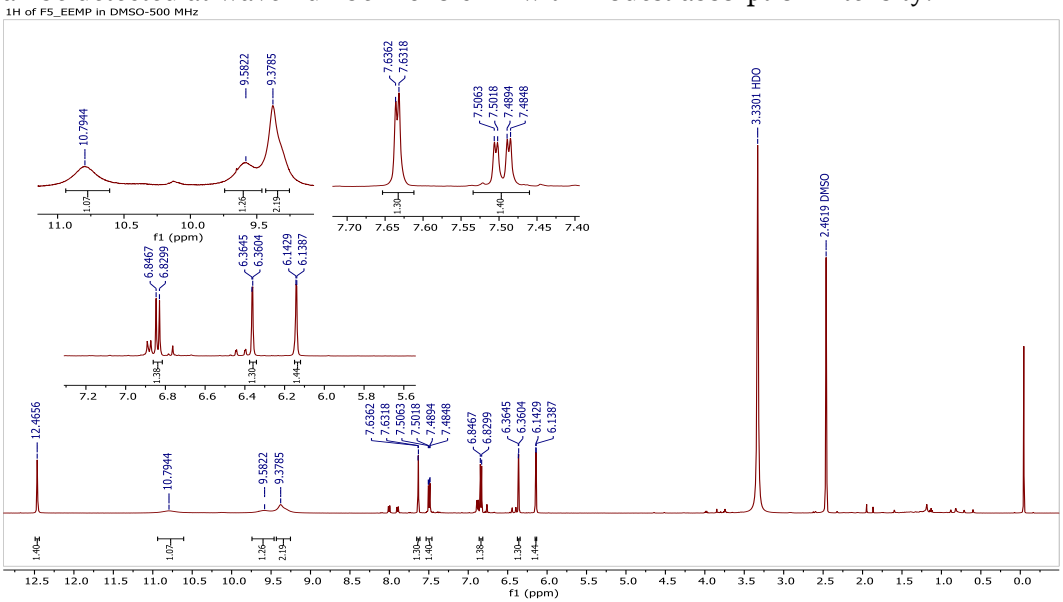

Figure 3. [1]H-NMR spectrum of 20-06-C-FV compound

In the [1]H-NMR spectrum (Figure 3), showed a singlet signal at δ 12.47, which represented the hydroxyl group at C-5. Signals at δ 6.14 and δ 6.36 showed as doublets, with J_m values of 2.1 Hz and 2.0 Hz, respectively. For H-6 and H-8 protons on ring A, the signals suggested that they were meta Meanwhile, the other three aromatic signals at δ 7.63 (d, J_m = 2.2 Hz), 6.84 (d, J_o = 8.4 Hz) and 7.50 (dd, J_m = 2.2 Hz, J_o = 8.5 Hz) were designated as H-2', H-5' and H-6' on ring B. The H-6' proton signal is a doublet of doublets as a result of meta (J_m = 2.2 Hz) and ortho (J_o = 8.5 Hz) interactions with H-2' and H-5' protons.

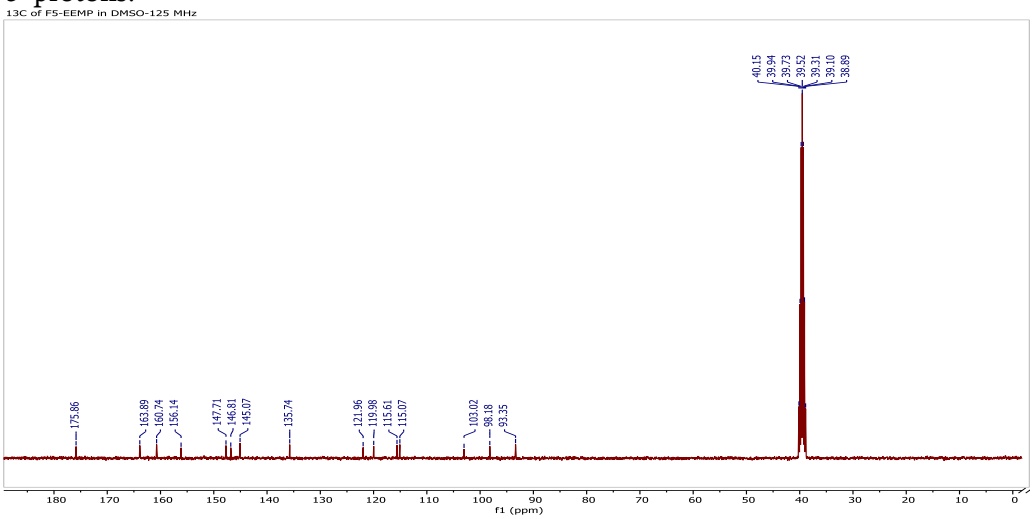

Figure 4. [13]C-NMR spectrum of 20-06-C-FV compound

The ^{13}C-NMR spectrum (Figure 4) revealed the existence of 15 aromatic carbon signals. The carbonyl signal was detected at δ 175.86 (C-4). Additionally, chemical shifts at δ 135.74, 160.74, 163.89, 145.07, and 147.71 reveal oxygenated flavone core carbons at C-3, C-5, C-7, C-3', and C-4', respectively. Based on the characterization results and comparison with literature data, 20-06-C-FV substance was recognized as a 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, also known as quercetin compound [21]. Table 3 shows the comparison results for 20-06-C-FV compound.

Table 3. Comparison of 20-06-C-FV compound with literature

Carbon	20-06-C-FV		Quersetin [21]	
	^{1}H δ_H (*J*, Hz) 500 MHz	^{13}C δ_C 125 MHz	^{1}H δ_H (*J*, Hz) 500 MHz	^{13}C δ_C 125 MHz
2	-	146.81	-	147.7
3	-	135.74	-	135.7
4	-	175.86	-	176.8
5	-	160.74	-	160.7
6	6.14 (1H, d, J_m = 2.1 Hz)	98.18	6.20 (1H, d, J_m = 2.0 Hz)	98.2
7	-	163.89	-	163.9
8	6.36 (1H, d, J_m = 2.0 Hz)	93.35	6.40 (1H, d, J_m = 2.0 Hz)	94.5
9	-	156.14	-	156.1
10	-	103.02	-	103.0
1’	-	121.96	-	121.9
2’	7.63 (1H, d, J_m = 2.2 Hz)	115.07	7.65 (1H, d, J_m = 2.1 Hz)	115.0
3’	-	145.07	-	145.0
4’	-	147.71	-	145.8
5’	6.84 (1H, d, J_o = 8.4 Hz)	115.61	6.85 (1H, d, J_o = 8.4 Hz)	115.6
6’	7.50 (1H, dd, J_o = 8.5 Hz, J_m = 2.2 Hz)	119.98	7.50 (1H, dd, J_o = 8.4 Hz, J_m = 2.1 Hz)	124.5
5-OH	12.47 (1H, s)	-	-	-
7-OH	10.79 (1H, s)	-	-	-
3’-OH	9.38 (1H, s)	-	-	-
4’-OH	9.58 (1H, s)	-	-	-

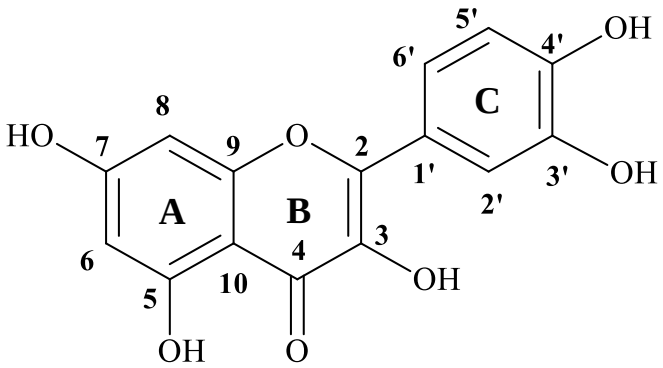

Figure 5. Structure of quercetin

The acquired results are identical to those previously reported by [22-23]. To the best of our knowledge, this is the first report confirming the isolation and full spectroscopic characterization of quercetin from *M. pudica*. Quercetin consists of five hydroxyl groups (Figure 5). The figure shows the chemical characteristics have dehydroxylated B-ring structure. Other important characteristics are the presence of unsaturation in the C-ring and the presence of a 4-oxo in the B-ring [24]. The identification of quercetin in *Mimosa pudica* provides substantial support for the hypothesis that flavonoids constitute the primary bioactive constituents responsible for the plant's pharmacological properties. This observation is in accordance with previous findings reported in related species, wherein quercetin has been recognized as a potent inhibitor of α-glucosidase activity [22].

Quercetin is a chemical molecule found in the flavonoid group. Flavonoids are natural phenolic chemicals present in plant stems, leaves, flowers, and fruits. Flavonoids are often polar chemicals because they include unsubstituted hydroxyl groups. Polar solvents, such as ethanol, methanol, ethyl acetate, or a combination of these solvents, can be employed to extract flavonoids from plant tissues [25]. Flavonoid molecules have been shown to exhibit a wide range of bioactivities, including antioxidant [19], immunomodulator [26], antibacterial [27-28], and so on. Moreover, previous studies have demonstrated the potential applications of this plant as a decomposing agent in biourine production [29], a botanical larvicide [30], and an arsenic hyperaccumulator [31].

The present findings highlight a promising avenue for the development of bioactive compounds derived from readily available wild tropical species. In addition, the methodologies employed in this study offer substantial educational value and may be effectively integrated into laboratory courses in organic and analytical chemistry, particularly in the context of natural product isolation, chromatographic separation techniques, and spectroscopic analysis using UV-Vis, IR, and NMR.

4. Conclusion

This work contributes to the advancement of educational resources in chemistry and supports the broader effort to valorize local medicinal biodiversity for sustainable use. This study effectively isolated and characterized one pure chemical from *Mimosa pudica* extracted with ethyl acetate. The chemical was identified as quercetin, a flavonoid with a high bioactive potential. This finding adds to *M. pudica*'s potential as a source of natural bioactive chemicals for pharmaceutical development, particularly as antidiabetic, antioxidant, and immunomodulatory agents. In the future, more study is needed to examine the biological activity of these chemicals in vitro, in vivo, and in silico.

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