Indonesian Journal of Pharmaceutical and Clinical Research
Journal homepage: https://idjpcr.usu.ac.id

Isolation and identification of diosgenin and flavonoids compound from ethanol extract of *Dioscorea hispida* Dennst. tuber

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ARTICLE INFO

Article history:
Received : 24 March 2024
Revised : 05 September 2024
Accepted : 05 November 2024
Available online 16 November 2024

E-ISSN: 2620-3731
P-ISSN: 2615-6199

How to cite:
Syahputra, H., Masfria, M., Amana, M. R., & Rivai, M. (2024). Isolation and identification of diosgenin and flavonoids compound from ethanol extract of *Dioscorea hispida* Dennst. tuber. *Indonesian Journal of Pharmaceutical and Clinical Research*, 7(1), 7-15.

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http://doi.org/10.32734/idjpcr.v7i1.15994

ABSTRACT

The tuber of Dioscorea hispida Dennst. has potential for development as a synthetic pharmaceutical ingredient due to the presence of secondary metabolites such as diosgenin and flavonoids, which exhibit biological activity within the body. Further exploration of this potential requires the isolation and identification of these compounds. Diosgenin was isolated from the ethanol extract using thin-layer chromatography, while flavonoids were isolated from the ethyl acetate fraction using paper chromatography. The pure isolates of both compounds were identified using UV-Vis spectrophotometry and FT-IR spectroscopy. The UV-Vis spectrophotometric analysis of diosgenin revealed maximum absorption wavelengths at 205 and 453 nm. Flavonoid isolates exhibited maximum absorption wavelengths of 264 nm for the first isolate, 265 nm for the second, 276 nm for the third, and 272 nm for the fourth. FT-IR spectroscopic analysis of the diosgenin isolate indicated the presence of functional groups such as -OH, aromatic C=C, and aromatic C-O, while the flavonoid isolate showed the presence of O-H, aromatic C-H, aliphatic C-H, C=O, C=C, and C-O. These identification results confirm that the isolates obtained indeed contain diosgenin and flavonoids.
Keyword: Isolation, Identification, Diosgenin, Flavonoids, *Dioscorea hispida* Dennst

ABSTRAK

Umbi Dioscorea hispida Dennst. mempunyai potensi untuk dikembangkan sebagai bahan obat sintetik di bidang farmasi karena mengandung senyawa metabolit sekunder seperti diosgenin dan flavonoid yang memiliki aktivitas biologis di dalam tubuh. Penggalian lebih jauh potensi tersebut memerlukan isolasi dan identifikasi senyawa-senyawa tersebut. Isolasi senyawa diosgenin dilakukan dari ekstrak etanol menggunakan kromatografi lapis tipis, sedangkan flavonoid diisolasi dari fraksi etil asetat menggunakan kromatografi kertas. Isolat murni kedua senyawa tersebut diidentifikasi menggunakan spektrofotometri UV-Vis dan FT-IR. Identifikasi diosgenin secara spektrofotometri UV-Vis menunjukkan nilai panjang gelombang maksimum pada 205 dan 453 nm. Isolat flavonoid menunjukkan nilai panjang gelombang maksimum 264 nm untuk isolat pertama, 265 nm untuk isolat kedua, 276 nm untuk isolat ketiga, dan 272 nm untuk isolat keempat. Analisis spektroskopi FT-IR terhadap isolat diosgenin menunjukkan adanya gugus fungsi -OH, C=C aromatik, dan C-O aromatik, sementara isolat flavonoid menunjukkan adanya O-H, C-H aromatik, C-H alifatik, C=O, C=C, dan C-O. Hasil identifikasi ini menegaskan bahwa isolat yang diperoleh memang mengandung diosgenin dan flavonoid.
Keyword: Isolasi, Identifikasi, Diosgenin, Flavonoid, *Dioscorea hispida* Dennst

1. Introduction

Indonesia has enormous food resources, including tubers. Despite low production costs and easy cultivation, some Indonesian tubers are rarely used. The tuber of *Dioscorea hispida*, a member of the tuber

group (*Dioscorea*), is one example where the tuber has a forest habitat. It is known that *Dioscorea hispida* has cyanogenic glycosides, which play a role in cyanide production [1]. When these cyanogenic glycosides break down completely, they can produce cyanide, harming your health. However, apart from harmful molecules, some are beneficial to health, such as diosgenin and flavonoids. Several studies have found that this root has qualities that make it effective against hypertension, obesity, cancer, and oxidative stress. The presence of secondary metabolites in the tuber of Dioscorea is responsible for its therapeutic effect. Phytochemical screening has found secondary metabolites in the tuber of Dioscorea, including alkaloids, saponins, tannins, glycosides, phenolics, and flavonoids [2,3].

Evidence-based usage of *Dioscorea hispida* as a traditional medicine already exists; however, it is not widely practiced. This utilization takes advantage of the biological activity present in the secondary metabolites of *Dioscorea hispida*, such as diosgenin and flavonoids. Diosgenin is saponin that occurs naturally in Dioscorea sp. legumes and tubers [4,5]. Diosgenin is a precursor to many pharmaceutically used synthetic steroids. Studies have shown that the intestine may absorb diosgenin, that it plays a significant role in regulating cholesterol metabolism, that it has the potential as a therapeutic agent for many disorders such as cancer and hypercholesterolemia, and that it has both esetogenic and anti-tumor actions [6–8]. in comparison, flavonoids are antioxidant-rich phenolic compounds. Because flavonoids can bind to a free radical and change its properties, they help mitigate the harm of free radicals, including cell or tissue destruction and cancer development [9,10].

The optimal development of the two metabolites found in *Dioscorea hispida* holds great potential in the pharmaceutical industry. By conducting continuous research, these compounds can be effectively produced using modern methods, either as pharmaceutical drugs or as a foundation for synthesizing novel drugs. Such advancements would significantly contribute to the improvement of public health and overall welfare. The scientific process holds significant importance in ensuring that traditional medicine is not solely reliant on anecdotal evidence but instead supported by scientific research. This validation is crucial for integrating traditional medicine into contemporary formal healthcare systems. Therefore, it becomes imperative to first isolate and identify diosgenin and flavonoids from *Dioscorea hispida* tubers

2. Materials and Methods

2.1 Materials

The tools used in this study were quality glassware, a set of paper chromatography tools (PC) (Merck), hot plates (Thermo Scientific), analytical balances (mettler toledo), oven (Memmert), TLC plates (Merck), rotary evaporator (Stuart RE300), FT-IR spectrophotometer (Shimadzu IR-Prestige-21) and ultraviolet spectrophotometer (Shimadzu UV-1800), TLC scanner (CAMAG), and vortex (Boeco).

The *Dioscorea hispida* tubers were obtained from Deli Serdang sub-district, Indonesia. Plant identification was conducted at the Medanese Herbarium (MEDA), located within the Faculty of Mathematics and Natural Sciences at the University of North Sumatra. The materials and reagents used are pro-analysis from Merck and Smart-Lab, namely aluminum chloride, acetic acid, hydrochloric acid, sulfuric acid, butanol, ethanol, ethyl acetate, ferric chloride, methanol, n-hexane, lead acetate, chloroform, isopropanol, diosgenin, KBr powder, and toluene.

2.2 Extraction and ethyl acetate fraction of *Dioscorea hispida* tuber

Dioscorea hispida tuber simplicia powder, as much as 1000 g, is placed in a container with 10 l of 96% ethanol solvent. Soak for 6 h, stirring occasionally, then let rest for 18 h. Use a filter to separate the maceration. Repeat the extraction process at least once using the same solvent, half of the initial volume. The maceration results were then concentrated with a rotary evaporator to become a thick extract. Diosgenin will be extracted from this thick extract, while flavonoids will be extracted from the ethyl acetate part using liquid-liquid extraction. As much as 10 g of the condensed extract was added with 40 ml of ethanol and 100 ml of hot water, homogenized, then put into a separatory funnel and partitioned with 100 ml of n-hexane until the Liebermann-Burchard reagent did not give a positive result, leaving n-hexane and water fractions. The water fraction was separated with 100 ml of ethyl acetate until the ethyl acetate fraction became colorless, and $FeCl_3$ did not affect the ethyl acetate fraction. The ethyl acetate and water fractions were isolated, and the ethyl acetate fraction's flavonoid concentration was studied [11,12].

2.3 Phytochemical screening

Standard protocols were employed to conduct phytochemical screening to detect the presence of different phytoconstituents, including flavonoids, glycosides, alkaloids, tannins, saponins, and steroids/triterpenoids [13].

2.4 Isolation of diosgenin from ethanol extract

The best determination of the mobile phase was obtained from the results of the orientation ratio of toluene: ethyl acetate (100:0 to 0:100). the orientation results obtained the 5 best mobile phase ratios (5:5, 6:4, 7:3, 8:2, 9:1) and then evaluated preparative TLC as a spotting agent using 50% H_2SO_4 reagent and silica gel stationary phase F_{254}. The ethanol extract was mixed with n-hexane and dissolved. A band was placed 2 cm from the bottom edge of a 20 x 20 cm TLC plate that had been triggered. After the TLC plate was dried, it was placed in a vessel containing a developer solution diluted with the mobile phase. Using the parts already there, the plate is taken out of the tank when it has reached its full potential and then dried. The plate is covered in clear glass in the middle, and 50% H_2SO_4 spot remover is sprayed on the plate's outer edges. The center of the plate parallel to the purple spots was scraped and collected, soaked in ethyl acetate overnight, filtered, and evaporated. The isolates were then TLC-tested for purity [14,15].

2.5 Isolation of Flavonoids from Ethyl Acetate Fraction

Paper chromatography (PC) was used to find the optimal mobile phase for the ethyl acetate fraction of *Dioscorea hispida* tubers using Whatman No. 1 paper stationary phase, 2x20 cm, and several mobile phases, including n-butanol: acetic acid: water (5:1:4). Spots were made on Whatman paper No. 1, and the ethyl acetate fraction was then developed to the limit of the paper before being placed in a chromatography tube saturated with the best mobile phase vapor. Subsequently, the paper is extracted and subjected to a drying process. The value of Rf was determined by observing the outcomes under a UV lamp emitting at 366 nm (UV 366 nm) [16,17].

The ethyl acetate fraction was subjected to preparative PC utilizing the best mobile phase and Whatman No paper stationary phase to separate the flavonoid components. The ethyl acetate fraction is spotted on Whatman paper No. 1 as a ribbon, inserted into a chromatography vessel saturated with the mobile vapor phase, and developed to the boundary line. The document was subsequently extracted, desiccated, and examined under 366 nm ultraviolet illumination. After marking and slicing off the desired portions, the pieces are immersed in methanol for 24 h, shaken every so often, and finally filtered. After soaking up to three times, the extract was collected and concentrated in the open air [16,17].

2.6 Purity Test for Isolates

2.6.1 Diosgenin Isolate Purity Test by One-Way Thin Layer Chromatography

The isolated test was carried out by TLC using toluene: ethyl acetate (8:3) as the mobile phase. The isolate was spotted on a thin layer plate and then put into a chamber saturated with mobile phase vapor. After development is complete, the plate is removed and dried, and the plate is sprayed with 50% H_2SO_4 spot remover and heated in an oven at 110°C [15].

2.6.2 Flavonoid Isolate Purity Test with Two-Way Paper Chromatography

The sample is spotted using a capillary tube on paper and put into a vessel containing a developer. They eluted until developers moved up. After the developer solution reaches the boundary line, the paper is removed from the chromatography vessel and dried in a fume cupboard. The stains obtained were detected using a 365 nm UV lamp. Then the paper's position is rotated 90° from its initial position and dipped into another vessel containing another developer solution. Eluted until the developer moves to a predetermined limit, the paper is removed and dried. The stains obtained were detected using a 365 nm UV lamp [16,17].

2.7 Identification of Isolate Compounds

2.7.1 identification of isolates by UV Spectrophotometry

The isolate was dissolved in methanol, 0.25 mL vanillin reagent was added, and 2.5 mL of 72% H_2SO_4 was added. The mixture was vortexed and heated in a water bath at 60° for 10 min, then cooled for 3-4 min, and the absorbance was seen at a wavelength of 520 nm on a UV-Vis spectrophotometer [18].

2.7.2 Identification with FTIR Spectrophotometer

The identification of flavonoid compounds was achieved through infrared spectrophotometry, wherein the sample powder was mixed with KBr and subjected to testing. Insert the powder into the designated receptacle

of the DRS tool, ensuring that the infrared (IR) beam is directed toward the central region of said receptacle. Subsequently, obtain the infrared spectrum within the 4000-500 cm-1 wave number range.

3. Results and Discussion

3.1 Extraction and Fractionation

A quantity of 28,760 g of *Dioscorea hispida* tubers yielded 4,500 g of *Dioscorea hispida* tuber simplicia powder. Macerate 4500 g *Dioscorea hispida* tuber simplicia powder in 96% ethanol. After being concentrated in a rotary evaporator, the simplicia yield was 84.35%, and 3.5% extract was recovered from 160 g of raw material. An amount of 2N HCl equal to 50 g was used to reflux a macerated ethanol extract. The ethyl acetate fraction was then extracted via fractionation, yielding 7.269 g at a percentage yield of 14.53%. The percentage yield is crucial because it indicates the number of active chemicals in a sample.

3.2 Phytochemical Screening

The phytochemical screening included examining alkaloids, flavonoids, glycosides, saponins, tannins, and steroid/triterpenoid groups. The results of the phytochemical screening can be seen in Table 1.

Table 1. Screening results for simplicia, extracts, and ethyl acetate fraction

No	Group	Simplicia	Extract	Ethyl Acetate Fraction
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Glycosides	+	+	+
4	tannins	+	+	+
5	Saponins	+	+	+
6	Triterpenoids/steroids	+	+	-

Based on the results of phytochemical screening for simplicia, extracts and fractions of *Dioscorea hispida* tubers contain secondary metabolites of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids. In contrast, the ethyl acetate fraction alone does not contain steroids/triterpenoids. This can be an initial basis for isolating flavonoids from the ethyl acetate fraction because they are more polar and diosgenin from the ethanol extract because it contains glycosides and steroids/triterpenoids [19].

3.3 Isolation of Diosgenin from Ethanol Extract

The results of TLC analysis using silica gel 60 F_{254} stationary phase with 50% H_2SO_4 spots obtained the best toluene: ethyl acetate mobile phase with a ratio (8:2) because it produced the best stain separation, as shown in Figure 1 below.

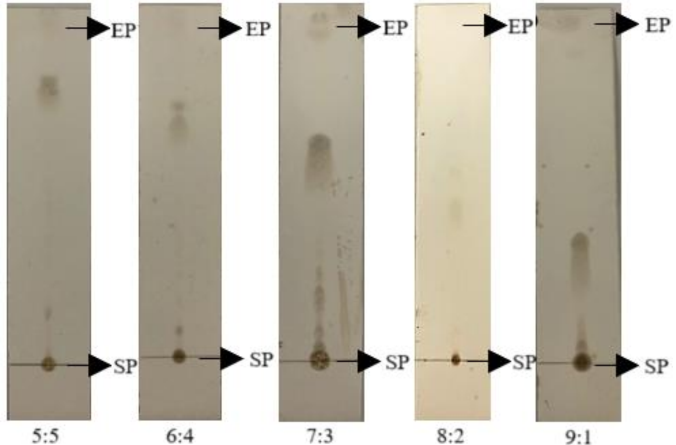

Figure 1. Thin layer chromatography of ethanol extract of *Dioscorea hispida* tubers with toluene:ethyl acetate ratio

The results of preparative thin layer chromatography with the stationary phase of silica gel F_{254} and the mobile phase of toluene: ethyl acetate (8:2) with the appearance of 50% H_2SO_4 spots from *Dioscorea hispida* tuber extract, there were two bands. The band whose Rf value corresponds to standard diosgenin, namely 0.7875, was scraped off and soaked overnight in ethyl acetate, then filtered and evaporated to obtain isolate. The results of the isolation of diosgenin compounds by preparative thin-layer chromatography can be seen in Figure 2

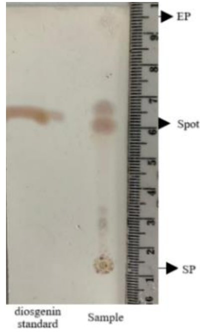

Figure 2. Diosgenin preparative TLC chromatogram

3.4 Isolation of Flavonoid Compounds from Ethyl Acetate Fraction

Isolation was carried out on the ethyl acetate fraction by paper chromatography (PC) using Whatman No.1 paper stationary phase. The eluent used in PC is a mixture of n-butanol: acetic acid: water (BAA) (5:1:4) which can provide the best separation. Because of its composition, the eluent is very polar, so it can separate flavonoid compounds which are also polar.

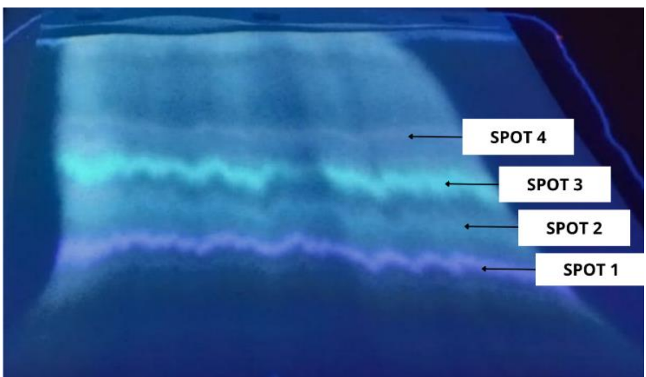

Figure 3. Chromatogram of preparative PC results of the ethyl acetate fraction

After being irradiated with a UV lamp with a wavelength of 366 nm, four bands were obtained with each Rf value whose chromatogram pattern can be seen in Figure 3. These four bands were cut out based on the color of the band and the Rf value, which can be seen in Table 2 below. This band is then soaked in methanol and evaporated to obtain isolates that can be identified.

Table 2. Results of isolation of flavonoid compounds by preparative paper chromatography

Spot/Isolate	Rf values	UV 366 nm
1	0.28	Purple
2	0.4	Blue
3	0.54	Green
4	0.62	Yellow

3.5 Purity Test for Isolates

3.5.1 Diosgenin Isolate Purity Test by One-Way Thin Layer Chromatography

Diosgenin isolates were tested for purity by thin-layer chromatography using toluene: ethyl acetate (8:2) as the mobile phase. The stationary phase used was silica gel 60 F254 with 50% H_2SO_4 specks. The results obtained that the isolates showed one stain can be seen in Figure 4.

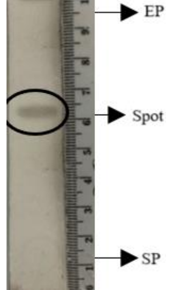

Figure 4. Results of the purity of diosgenin isolate by thin-layer chromatography

3.5.2 Test for Purity of Flavonoid Isolates by Two-Way Paper Chromatography

Flavonoid isolates were tested for purity by 2-way PC. The elution process was carried out twice with different mobile phases. Before the second elution, the paper's position is rotated 90° from the initial position of the first elution. If the two elution processes only show one point, then it can be said that the isolate obtained is a single component. Two-way paper chromatography test results, Rf values, and stain colors have been obtained. The results from the 2-way PC showed all pure or single isolates, which can be seen in Table 3 below.

Table 3. Two-way PC yield data of flavonoid isolates

Spot/Isolate	Rf value in First Eluent	Rf value in Second Eluent	Color in UV 366	Purity
1	0.28	0.34	Purple	+
2	0.42	0.42	Blue	+
3	0.51	0.65	Green	+
4	0.6	0.45	Yellow	+

3.6 Identification of Diosgenin Isolates

3.6.1 Results of Identification of Isolates with UV-Vis Spectrophotometry

The results of the spectrum of pure diosgenin isolates from *Dioscorea hispida* bulbs and standard diosgenin in the UV region (200-400 nm) and visible region (400-800 nm) can be seen in Figure 5.

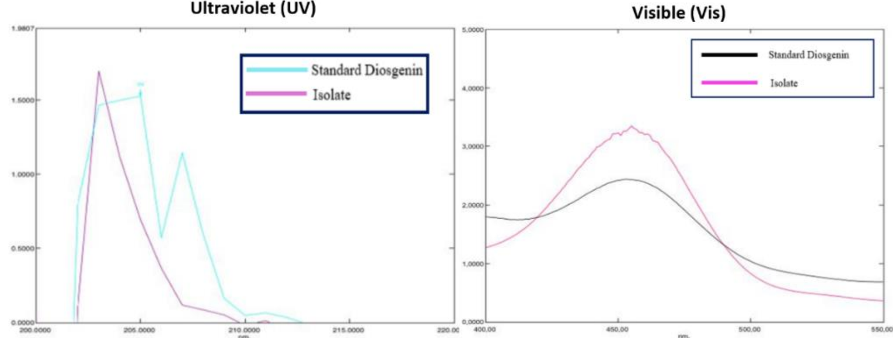

Figure 5. The results of standard diosgenin spectra and isolates for UV and visible regions

The interpretation of the UV and Vis spectra with methanol (Figure. 5) gives the maximum wavelengths of standard diosgenin (203 nm and 455 nm) and isolate diosgenin (205 nm and 453), respectively. Ultraviolet and visible light will cause electrons to be excited to higher orbitals. The system responsible for the absorption of light is called a chromophore. Based on this, diosgenin does not have a chromophore group that can cause it to be measured in the UV region (200-400 nm). However, the spectrum of the isolate obtained has two peaks similar to the diosgenin spectrum.

3.6.2 Identification of Isolates with FT-IR Spectrophotometer

The infrared spectrum data, as depicted in Figure 6, reveals a distinct absorption at the wave number 3448.72 cm-1, indicating the presence of an OH group. This assumption is reinforced by absorption at wave numbers 1058.82 cm-1, 1174.65 cm-1, and 1244.09 cm-1, which is absorption from the C-O alcohol group. The sharp absorption in the wave number region of 1660.71 cm-1 is thought to be absorption from aromatic C=C. This assumption is strengthened by the appearance of absorption at wave number 3026.31 cm-1, which is absorption from sp2-H. The sharp absorption in the wave number region of 2943.37 cm-1 is considered sp3-H absorption. This assumption is strengthened by the appearance of absorption at wave number 1375.25 cm-1, which is suspected to be CH_3 absorption [20].

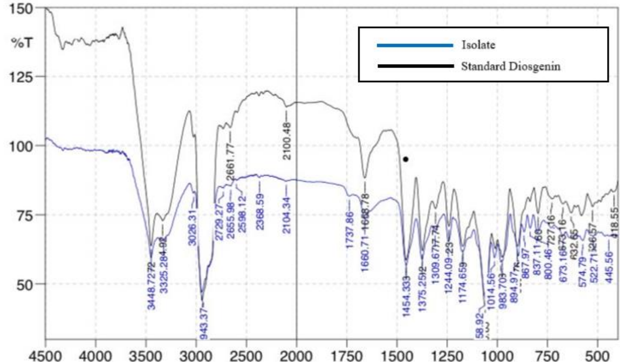

Figure 6. Results of the infrared spectrum of diosgenin isolates and diosgenin standards

3.7 Identification of Flavonoid Isolates

3.7.1 Results of Identification of Isolates by UV Spectrophotometry

Flavonoids have a maximum wavelength in the range of 200-400 nm. The maximum wavelength varies depending on the specific structure of the flavonoid, measurement conditions, and environmental influences. The results of the UV spectrum analysis of isolates 1 and 2 show that the absorption peaks are 264 nm and 265 nm. The peak wavelength I enter the UV absorption range for the flavone group, which is 250-270 nm. While for isolates 3 and 4, the absorption peaks were seen at 276 nm and 272 nm. The wavelength of this peak is within the range for the flavonol group [21–23]. The spectrum of isolates 1-4 can be seen in Figure 7.

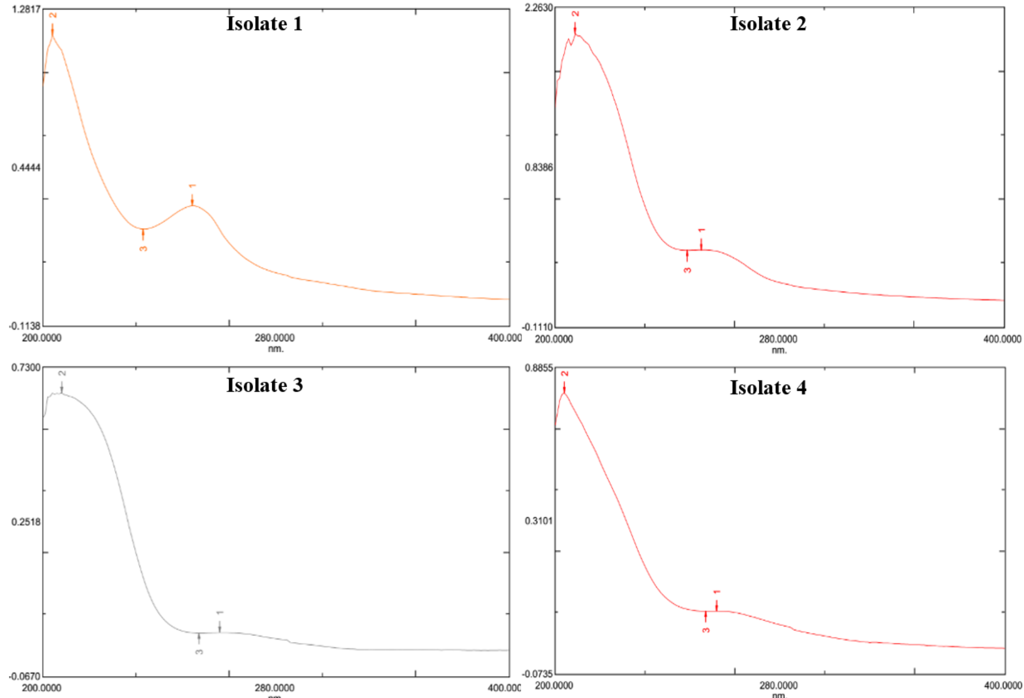

Figure 7. The spectrum of Isolates by UV Spectrophotometry

3.7.2 Isolate Identification Results with FT-IR Spectrophotometry

Isolates suspected of containing flavonoids were crushed with KBr, and then their IR spectra were measured. This spectrum can provide information about the functional groups of the isolated compounds. The FTIR spectra of the four isolates can be seen in Figure 8 below.

Figure 8. Infrared Spectrum Isolates

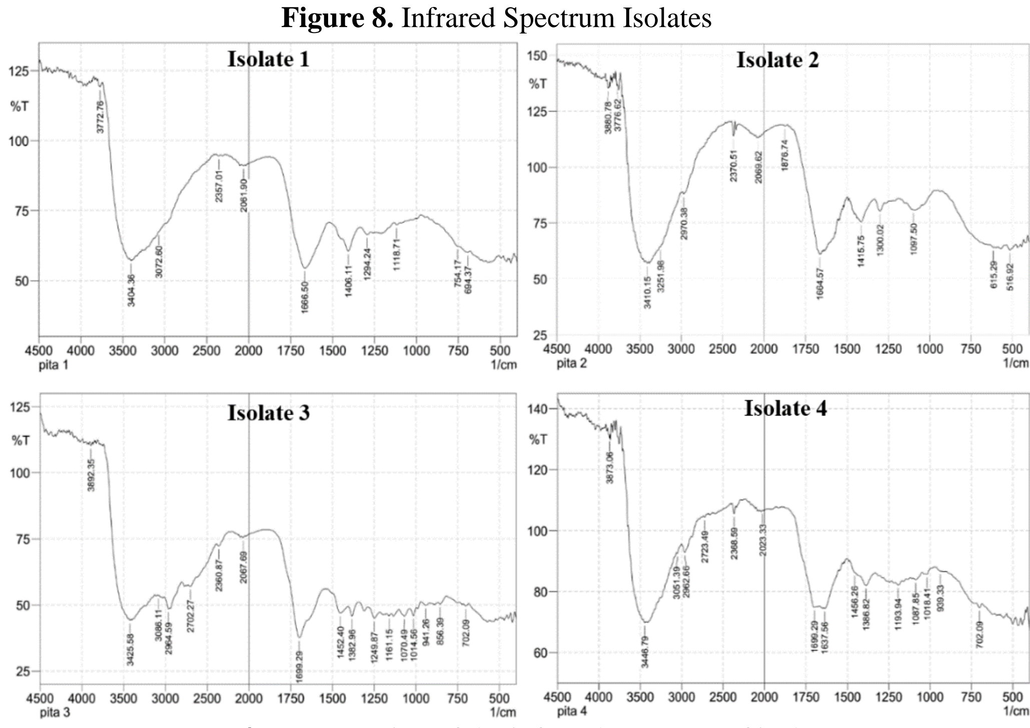

Table 4. Interpretation of the infrared spectrum of isolate

Wave Number (cm-1)				Functional Group Interpretation
Isolate 1	**Isolate 2**	**Isolate 3**	**Isolate 4**	
3404.36	3410.15	3425.58	3446.79	OH
3072.6	-	3086.11	3051.39	aromatic CH
-	2970.38	2964.59	2962.66	Aliphatic CH
1666.5	1664.57	1699.29	1699.29	C=O
1406.11	1415.75	1452.4	1456.26	C=C aromatic
-	-	1382.96	1386.82	Aliphatic CH
1294.24	1300.02	1249.87	1193.94	C-O
1118.71	1097.5	1070.49	1087.85	C-O
-	-	1014.56	1018.41	C-O
754.17	-	856.39	-	aromatic CH
694.37	615.29	702.09	702.09	aromatic CH

Identification results with FT-IR isolates 1-4 showed that the isolates contained several functional groups, as shown in Figure 8 and Table 4. The identification of the isolate is indicated by the presence of functional groups such as hydroxyl (OH), aromatic carbon-hydrogen (CH aromatic), carbonyl (C=O), aromatic carbon-carbon double bond (C=C aromatic), and aliphatic carbon-oxygen (C-O) and carbon-hydrogen (C-H) bonds. This is a flavonoid compound with a C=O carbonyl group as a general characteristic of the flavonoid compound [21–23].

4. Conclusion

The results obtained from the tests indicate that diosgenin can be successfully isolated from the ethanol extract of *Dioscorea hispida* tuber using preparative thin-layer chromatography (TLC). UV spectrophotometry identified pure isolates with 205 nm UV and 453 nm visible wavelengths. In comparison, using FT-IR spectrophotometry to identify isolates revealed the presence of functional groups -OH, aromatic C=C, and aromatic C-O. For the flavonoids, preparative paper chromatography can separate them from the ethyl acetate fraction of the *Dioscorea hispida* tubers. UV spectrophotometry was used to identify pure isolates. The first isolate had maximum absorbance at 264 nm (I) and 204 nm (II), the second at 265 and 209, the third at 276 and 210, and the fourth at 272 and 204. FT-IR spectrophotometry identified isolates with O-H, aromatic C-H, aliphatic C-H, C=O, C=C, and C-O groups

5. Acknowledgements

We would like to thank Universitas Sumatera Utara for funding this TALENTA-supported research project.

References

[1]. Estiasih T, Ahmadi K, Sari INI, Kuliahsari DE, Martati E. Traditional detoxification of wild yam (Dioscorea hispida Dennst) tuber in chips processing at East Java, Indonesia. *Journal of Ethnic Foods*. 2022;**9**(1). doi:10.1186/s42779-022-00164-1

[2]. Ahmad R. Dioscorea hispida: economic potential and applications. *International Conference On Agriculture and Applied Science (ICoAAS) 2021*. Published online 2021.

[3]. Adomėnienė A, Venskutonis PR. Dioscorea spp.: Comprehensive Review of Antioxidant Properties and Their Relation to Phytochemicals and Health Benefits. *Molecules*. 2022;**27**(8). doi:10.3390/molecules27082530

[4]. Hazrin-Chong NH, Azeem AM, Mat Zin NNIN, Mat Lazim MAS. Effectiveness of Dioscorea hispida Dennst as antibacterial and antibiofilm agent. *Malays J Microbiol*. 2018;**14**(Specialissue6). doi:10.21161/mjm.1461803

[5]. Estiasih T, Ahmadi K, Sari INI, Kuliahsari DE, Martati E. Traditional detoxification of wild yam (Dioscorea hispida Dennst) tuber in chips processing at East Java, Indonesia. *Journal of Ethnic Foods*. 2022;**9**(1). doi:10.1186/s42779-022-00164-1

[6]. Raina AP, Misra RC. Evaluation of diosgenin, a bioactive compound from natural source of *Dioscorea* species: A wild edible tuber plant. *J Pharmacogn Phytochem*. 2020;**9**(1).

[7]. Huang N, Yu D, Wu J, Du X. Diosgenin: an important natural pharmaceutical active ingredient. *Food Science and Technology (Brazil)*. 2022;**42.** doi:10.1590/fst.94521

[8]. Cheng J, Chen J, Liu X, et al. The origin and evolution of the diosgenin biosynthetic pathway in yam. *Plant Commun*. 2021;**2**(1). doi:10.1016/j.xplc.2020.100079

[9]. Zawawi N, Chong PJ, Tom NNM, et al. Establishing relationship between vitamins, total Phenolic and total flavonoid content and antioxidant activities in various honey types. *Molecules*. 2021;**26**(15). doi:10.3390/molecules26154399

[10]. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem*. 2006;**99**(1). doi:10.1016/j.foodchem.2005.07.042

[11]. Jones WP, Kinghorn AD. Extraction of plant secondary metabolites. *Methods in Molecular Biology*. 2012;**864**. doi:10.1007/978-1-61779-624-1_13

[12]. Nugraha SE, Suryadi Achmad, Erly Sitompul. Antibacterial Activity of Ethyl Acetate Fraction of Passion Fruit Peel (Passiflora Edulis Sims) on Staphylococcus Aureus and Escherichia Coli. *Indonesian Journal of Pharmaceutical and Clinical Research*. 2019;**2**(1). doi:10.32734/idjpcr.v2i1.972

[13]. Sheikh N, Kumar Y, Mishra a K, Pfoze L. Journal of Medicinal Plants Studies Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of. *Journal of Medicinal Plant Studies*. 2013;**1**(6).

[14]. Munusamy Baskar, Akanksha Singh, Bharti Arya. Identification and isolation of phytochemical constituents from the Syzygium cumini (L.) Skeels. *GSC Biological and Pharmaceutical Sciences*. 2022;**20**(3). doi:10.30574/gscbps.2022.20.3.0317

[15]. Ali Z, Smillie TJ, Khan IA. Cholestane steroid glycosides from the rhizomes of Dioscorea villosa (wild yam). *Carbohydr Res*. 2013;**370**. doi:10.1016/j.carres.2012.12.022

[16]. Saptarini NM, Mustarichie R, Herawati IE, Hadisoebroto G. Isolation, Identification, And Quantification Of Major Flavonoid In Leaves Of Pereskia Bleo (Kunth) Dc. *International Journal of Applied Pharmaceutics*. 2022;**14**(Special Issue 4). doi:10.22159/ijap.2022.v14s4.PP19

[17]. Krishnadhas L, Santhi R, Annapurani S. Isolation and identification of flavonoid fractions from the leaves of Volkameria inermis and its in-vitro cytotoxic study. *International Journal of Pharmaceutical and Clinical Research*. 2016;**8**(12).

[18]. Rambwawasvika H, Parekh CT, Naidoo B, Chiririwa H. *Extraction and Characterisation of Mucilage from the Herb Dicerocaryum Senecioides and Its Use a Potential Hair Permanent*. Vol 13.; 2017.

[19]. Cui KMR, Tonog MN, Delorino R. Studies on phytochemical screening and antimicrobial activity of Dioscorea hispida Dennst. (kurot) tuber extract. *Journal of Applicable Chemistry*. 2014;**3**(6).

[20]. Verma SK, Deb MK. Direct and rapid determination of sulphate in environmental samples with diffuse reflectance Fourier transform infrared spectroscopy using KBr substrate. *Talanta*. 2007;**71**(4). doi:10.1016/j.talanta.2006.07.056

[21]. Nukmal N, Pasutri AY, Pratami GD. Characterization of flavonoid compounds from polar leaf gamal cultivar extract North Lampung and test its activity against cocoa white mites (Planococcus minor, Hemiptera: Pseudococcidae). *Bioma*. 2019;**21**(1).

[22]. Dhivya SM, Kalaichelvi K. Uv-Visible Spectroscopic And Ftir Analysis Of Sarcostemma Brevistigma, WIGHT. AND ARN. *Int J Curr Pharm Res*. 2017;**9**(3). doi:10.22159/ijcpr.2017.v9i3.18890

[23]. Louis H MO, Akakuru OU BJ, G B. Synthesis and Characterization of Some Metal Complexes Using Herbal Flavonoids. *Nat Prod Chem Res*. 2018;**06**(03). doi:10.4172/2329-6836.1000314