

Isolation and Characterization of Tannins from *Euphorbia hirta*

Yakubu S.; Abubakar I.; and Juth D.

Department of Science Laboratory Technology, Federal Polytechnic, Mubi, Adamawa State, Nigeria.

Corresponding author: yakubusulethliza72@gmail.com

DOI: <https://doi.org/10.62154/ajastr.2025.018.010773>

Abstract

The aim of the study is to isolate and characterize tannins from *Euphorbia hirta* using various analytical techniques. Approximately 300g sample of *E. hirta* was extracted with 70% acetone, yielding 19mg of crude extract. Column chromatography and Thin Layer Chromatography (TLC) were employed to isolate and purify the tannin fractions. UV-Visible spectroscopy and Fourier Transform Infrared (FT-IR) analysis were used to quantify and elucidate the structure of the isolated tannins. The results showed a high total phenolic content of 43.47 mg TAE/g, with the presence of functional groups typical of polyphenols (OH, C-C, C=O, C-O, C-H). Although minor impurities were detected, the findings indicated high amount of tannins present, sufficient for pharmaceutical formulations and drug development. Accurate structural determination and quantification of tannins facilitates reliable research and development, enabling in-depth studies of their pharmacological effects and potentially informing the creation of novel treatments.

Keywords: Tannins, *Euphorbia hirta* Uv-visible, Fourier Transform Infrared, FTIR, Chromatography.

Introduction

Tannins are a class of polyphenolic compounds ubiquitously found in various plant species, including monocots, dicots, and ferns. They are among the most abundant plant constituents, surpassed only by cellulose, hemicelluloses, and lignin. Tannins are secondary metabolites of higher plants, primarily consisting of galloyl esters or their derivatives. These complex molecules play a vital role in plant defense mechanisms and have been extensively studied for their diverse pharmacological properties. (Sharma K. et al., 2021). They are categorized into two main types: hydrolysable and condensed tannins: The hydrolysable tannins are economically significant and can be extracted from plant sources such as *Terminalia chebula*. The condensed tannins known as proanthocyanidins, are found in various plant-based foods and are known for their astringent properties. (Sharma K. et al., 2021)

Studies have shown that tannin-containing plant extracts exhibit anticancer and anti-tumor activities against various types of cancer, including stomach and duodenal cancers. (Ali et al., 2024). Tannin-rich plant extracts have been shown to possess anti-inflammatory and antimicrobial properties, making them effective as antiseptic agents. (Ucella-Filho et al., 2022; Maria et al., 2021 and Ashwaq, 2024) Tannins have been shown to precipitate heavy

metals such as lead, mercury, and arsenic, in the same vein it precipitate alkaloids except morphine, hence are useful in treating heavy metal and alkaloids poisonings (Sharma K. *et al.*, 2021; Lician E, 2023 and Aadil., 2022)

E. hirta, sometimes referred to as snake weed or asthma weed, belongs to the Euphorbiaceae family. It is an annual or perennial herb that grows up to 30-40 cm in height (Panda & Giri, 2024). It is a slender-stemmed, hairy plant that is reddish or purplish in colour, and has many branches from the base to the top. Its leaves are opposite, elliptic, oblong to oblong-lanceolate, acute or subacute, dark green above, pale beneath, and it is 1-2.5 cm long. Its fruits are yellow, three-celled, hairy, keeled capsules that are 1-2 mm in diameter and contain three brown, four-sided, angular, wrinkled seeds in these capsules. (Khursheed, *et al.*, 2022)

Traditional Medicinal Uses of *Euphorbia hirta*

Euphorbia hirta is a widely used herb in traditional medicine, employed for centuries to treat various ailments, including; Cough and asthma, Genitourinary infections, Bronchitis, Scalds and burn, Skin diseases. . In China, the Yeo people use the entire plant to treat bronchitis ((Tripathi *et al.*, 2022); Yi-Won *et al.*, 2024). The decoction of the dry plant is applied externally for scalds and burns, while freshly prepared leaves are used as an antidote against skin diseases ((Tripathi *et al.*, 2022); Yi-Won *et al.*, 2024). It is also interesting to know that; an infusion of the plant is used as a stimulant to boost milk secretion (Ali and Al-Snaf, 2022). It has also been effectively used to treat gonorrhea and as a potential hemostatic agent among the Nazi people (Meda *et al.*, 2023) The application of *E. hirta* as anti-inflammatory agent, antimicrobial agent and as antioxidant agent has been demonstrated by research work carried out by Kumar *et al.*, (2021).

Statement of the Problem

The growing popularity of traditional medicine, particularly in Africa, warrants scientific attention. Herbal remedies, such as *E. hirta*, have shown promise in managing ailments like diabetes, which have sometimes proven resistant to conventional treatments. However, the lack of understanding of the structure and profiles of specific antibiotic agents responsible for *E. hirta*'s hypoglycemic properties poses significant risks. Similarly, the unscientific consumption of whole-plant preparations can lead to unforeseen consequences, including adverse reactions or toxicity. To mitigate these risks, it's essential to isolate and determine the structure and quantify the tannins present in *E. hirta*, hence bridging the gap in traditional application of the plant.

Aim and Objectives

The aim of this research is to isolate, quantify and elucidate the structure of tannins from *E. hirta* plant extract. The specific objectives are to:

- Isolate and identify tannins using column chromatography, Thin Layer chromatography and ferric chloride test

- Determine the structure of tannins using FTIR and UV visible spectroscopy
- Quantify the amount of tannins present in the *E. hirta* using Uv visible spectroscopy

Significance of the Study

This study provides valuable insights into the structure and quantity of tannins present in *E. hirta*. By determining the structures of these tannins, we can understand the chemical properties and potential reactivity of the tannins. Which will make it easier to assess or evaluate its pharmaceutical potentials.

Material and Methods

Materials

The materials used in this research work include Plastic funnel, reagent bottles, measuring cylinder, pre-coated silica gel, TLC Plates, electric blender, glass Column, weighing balance, cotton wool, filter paper, development tank, iodine tank, and capillary tube. Reagents used include: n. hexane, toluene. Acetone, methanol, Acetone, distilled water, formic acid, ferric chloride solution

Collection and Authentication of Plant Material

The aerial parts of *E. hirta* were collected from Gipalma, Mubi South, Adamawa State, Nigeria. The plant material was subsequently authenticated at the Federal College of Forestry, Jos, Plateau State, Nigeria. The collection was carried out by hand-picking, after which the plant material was washed with clean water to remove dirt or debris. The cleaned material was then air-dried at room temperature.

Sample Preparation

The dried leaves of *Euphorbia hirta* were pulverized using an electric blender to obtain a uniform powder. Approximately 270 g of the powdered plant material was accurately weighed using a digital balance and transferred to a reagent bottle. The powdered material was then macerated in 750 ml of 100% methanol, allowing it to stand for 48 hours. The mixture was intermittently agitated to facilitate complete dissolution of the plant constituents. Following the maceration period, the mixture was filtered using Whitman filter paper to obtain a clear filtrate. The filtrate was then left uncovered for 10 days, allowing the methanol to evaporate and the resulting extract to dry.

Ferric Chloride Test

Approximately 1 g of the tannins fraction was dissolved in 10 mL of distilled water. A 2 mL aliquot of the prepared solution was transferred to a test tube. Two to three drops of 10% ferric chloride solution were then added. Upon addition, a distinct blue-black coloration was observed, indicating the presence of tannins. This positive result confirms the presence of tannins in the sample. The same analysis was carried out on tannic acid used as a standard

Column Chromatography

A wet packing method was employed for column packing. Approximately 60g of silica gel was transferred into a glass column, and toluene was continuously added until the column was well-packed. About 19.8g of the extract was dissolved in 15 ml of methanol and adsorbed onto 20g of silica gel. The mixture was stirred continuously until dry. The dried sample was transferred to the column and eluted with a series of toluene-acetone mixtures in the following ratios: 100:00, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 100:00 and 60:60:10. acetone: toluene: formic acid.

Thin Layer Chromatography

TLC plates, specifically Silica gel plate gF254 (Silica gel on aluminum support), were obtained from Shijiazhuang Lemondou Chemicals Co., LTD. The plates were carefully cut into slides measuring 3 cm in length and 9 cm in height. A fraction of the sample was spotted onto the TLC plate, approximately 1 cm above the bottom edge, using a capillary tube. The spotted TLC plate was placed in a development tank containing a solvent mixture of acetone and toluene in a ratio of 2:8. The plate was allowed to develop for 10-15 minutes. The developed chromatogram was removed from the tank, and the solvent front was marked with a pencil. The spots on the chromatogram were visually observed and marked with a pencil for further analysis.

Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the characteristic functional groups present in the chromatographic fraction of the leaf extract of *Euphorbia hirta*. A small quantity (5 mg) of the tannin isolate was dispersed in dry potassium bromide (KBr) and thoroughly mixed in a mortar. The KBr disc was placed in a sample cup of a diffuse reflection accessory, and the IR spectrum was obtained using a Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 400 to 4000 cm^{-1} for 16 times to enhance the signal-to-noise ratio.

Uv-visible Spectroscopy

The extract was subjected to centrifugation at 3000 rpm for 10 minutes, followed by filtration through Whatman No.1 filter paper. The sample was then diluted 1:10 with the same solvent and scanned over a wavelength range of 200-1100 nm using a Perkin Elmer Spectrophotometer. The resulting UV-VIS spectrum revealed characteristic peaks, which were recorded and analyzed.

Determination of Tannin Concentration in Tannin Fraction

Tannic acid was employed as a standard to determine the concentration of tannins in the plant tannin fraction. A stock solution of tannic acid was prepared, and working solutions ranging from 50 ppm to 250 ppm were subsequently diluted. The UV-Visible spectrophotometer was properly calibrated, and the absorbance of each tannic acid

standard was measured at a specific wavelength. A calibration curve was generated by plotting the absorbance values against the corresponding concentrations. The absorbance of the sample extract was measured at the same wavelength, and the concentration of tannins in the tannin fraction was determined using the calibration curve. Finally, the tannin content was calculated using the determined concentration and the sample's weight. (Ningsih et al., 2022)

Results and Discussion

Results

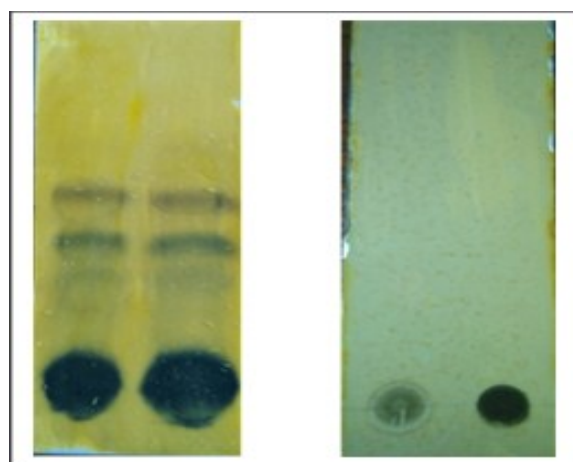


Fig. 1: Chromatogram of tannin isolate and tannic acid

FT-IR Peaks

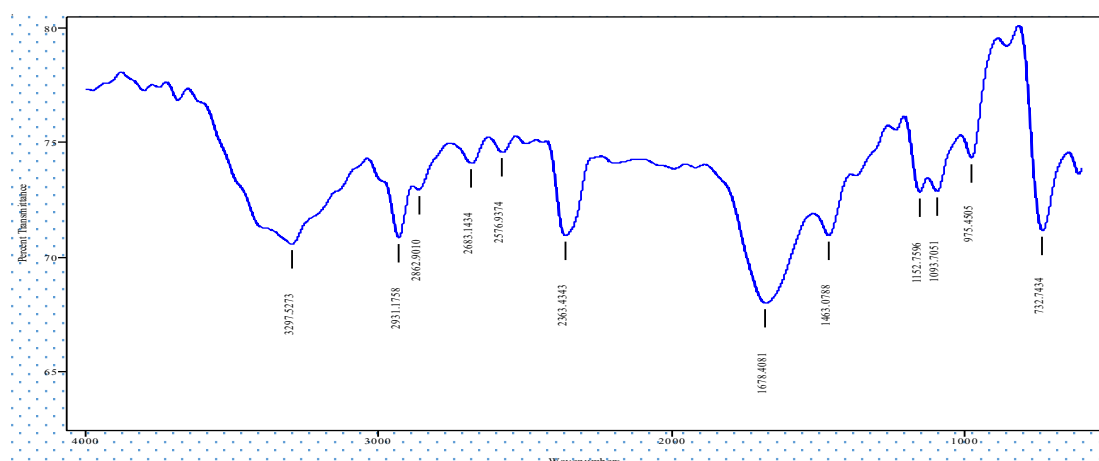


Fig. 2: FTIR Spectroscopy Analysis of tannin isolate of *E. hirta*

Table 1: Attributes of FTIR spectra acquired for tannin isolate of *E.hirta*

S/No	peak (cm ⁻¹)	Peak height	Functional Group Assignment	Predicted Compound
1	3297.5273	70.5710	O- H Stretching	Alcohol and phenols
2	2931.1758	70.8459	C – H Stretching	Alkanes
3	2862.9010	72.9412	H–C=O:C–H stretching	Carboxylic acid
4	2683.1434	74.0852	H–C=O,C-H Stretching	Carboxylic acid
5	2576.9374	74.5726	C-H Stretching	Alkanes
6	2363.4343	70.9208	C – H Stretching	aldehyde
7	1678.4081	67.9734	C = O Stretching	Carbonyl
8	1463.0788	70.9520	C – C Bending	Aromatic
9	1152.7596	72.8505	C-O Stretching	Carboxylic acid
10	1093.7051	72.8547	C – H Stretching	Alkyl halide
11	975.4505	74.3390	C- O Stretching	phenol
12	732.7434	71.1360	C-H	Aromatic

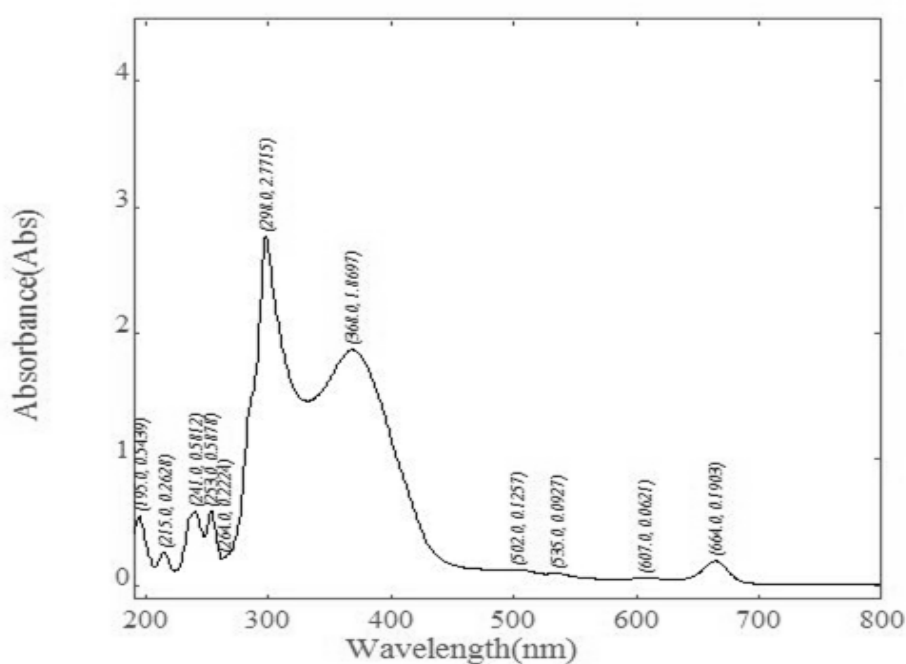
**Fig. 3: Ultra Violet-Visible Spectroscopy Analysis of tannin isolate of *E. hirta***

Table 2: UV-VIS Spectrum Peak values of E, hirta tannin Isolate

Wavelength	Abs	Fw
195.0 nm	0.5439	6.0
215.0 nm	0.2628	13.0
241.0 nm	0.5812	15.0
253.0 nm	0.5878	12.0
264.0nm	0.2224	2.0
298.0 nm	2.7715	47.0
368.0 nm	1.8697	75.0
502.0 nm	0.1257	38.0
535.0 nm	0.0927	45.0
667.0 nm	0.0621	46.0
664.0 nm	0.1903	26.0

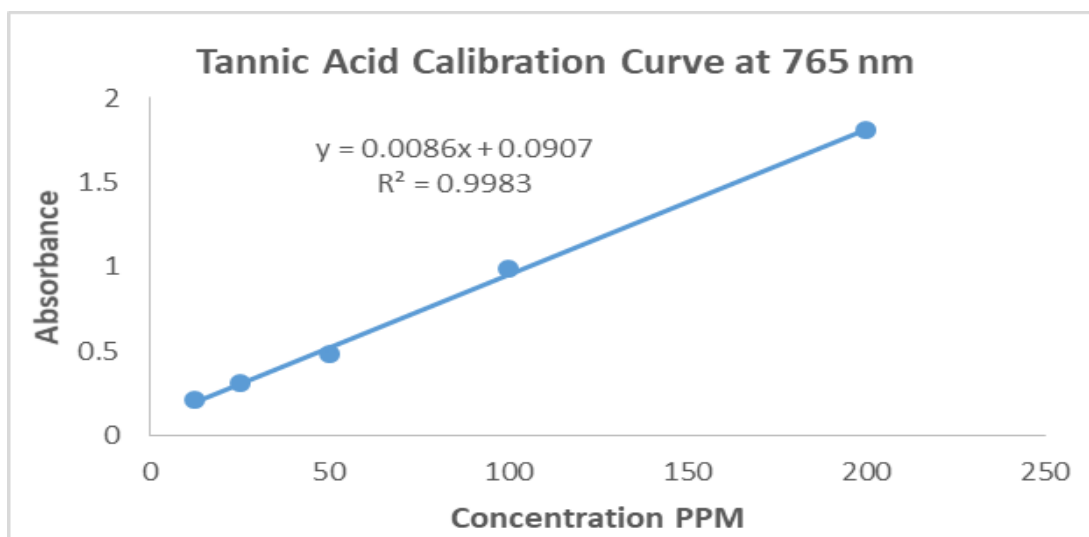


Fig. 4: tannic acid calibration curve

Table 3: Concentration and absorbance values

S/N	Absorbance	Concentration (ppm)
1	200	1.8092
2	100	0.9845
3	50	0.4802
4	25	0.3104
5	12.5	0.2115

Sample Abs - Blank

$$1.8227 - 0.9843 = 0.8384$$

$$Y = Mx + b$$

Sample Conc. 86.94 ppm of TAE

$$\text{Tannic Content} = 86.94 \times 50 \text{ ml/100mg}$$

$$= 43.47 \text{ mg TAE/g}$$

Discussion

Column fractions

A series of column chromatography fractions were collected and analyzed. Fractions 26-31 were pooled based on their similar color and retention factor profiles. The pooled fractions were subjected to chromatographic analysis, revealing a single spot at the base of the chromatogram with a blue-black color when treated with ferric chloride solution. The color observed is characteristic of tannins. To further confirm the identity of the isolated compound, a chromatogram of tannic acid, a standard tannin, was run in parallel. The standard exhibited the same blue-black color with ferric chloride, confirming that the isolated compound is indeed a tannin. (Ali et al., 2024)

FT-IT results

Peaks 1 and 9, with absorptions at $3297.5273 \text{ cm}^{-1}$ and $1152.7596 \text{ cm}^{-1}$, respectively, indicate the presence of hydroxyl (O-H) functional group and carbonyl (C=O) functional group of phenols. Peaks 2 and 8, with absorptions at $2931.1758 \text{ cm}^{-1}$ and $1463.0788 \text{ cm}^{-1}$, respectively, suggest the presence of alkane functional groups arising from C-H stretching and aromatic functional group (C=C) arising from stretching vibrations. Similarly, Peaks 3 and 4 with absorptions at $2862.9010 \text{ cm}^{-1}$, $2683.1434 \text{ cm}^{-1}$, respectively, are attributed to H-C stretching vibrations of carboxylic acid. In the same vein, Peaks 5 and 6, with absorptions at $2576.9374 \text{ cm}^{-1}$ and $2363.4343 \text{ cm}^{-1}$, respectively, are characteristic of H-C stretching vibrations in alkane and C-H vibration in aldehyde. Peak 7, with an absorption at $1678.4081 \text{ cm}^{-1}$, corresponds to C=O stretching vibrations, indicating the presence of carbonyl functional groups. Peak 10, with an absorption at $1093.7051 \text{ cm}^{-1}$, is attributed to C-O stretching vibrations, suggesting the presence of ester functional groups. Peak 12, with absorption at 732 cm^{-1} arising from C-H stretching vibration of aromatic ring.

The overall FT-IR results revealed the presence of polyphenolic functional groups, confirming that the isolate contains tannins. However, the spectra also indicated the presence of additional functional groups not characteristic of tannins, suggesting that the isolate is not exclusively composed of tannins, but also contains other compounds.

According to Tripathi et al., (2022) the shape of the OH-stretching band provides preliminary information on the occurrence of a polymerization process in condensed

tannins. These compounds exhibit a varying degree of polymerization, resulting in a broad spectral range of 3,700-3,000 cm^{-1} .

(Isler S et al., 2025) Conducted a study on Mechanisms of gold recovery from aqueous solutions using a novel tannin gel adsorbent synthesized from natural condensed tannin which involved the analysis of spectral patterns for condensed tannins. The researchers identified characteristic infrared (IR) absorption bands indicative of proanthocyanidins in complex matrices: The results he obtained agrees with the results of this current study having similar absorption bands

The identification of tannins in this study showed similarities with the report by (Akiode et al., 2025). Their work compared the FT-IR spectrum of tannins with that of tannic acid, used as a standard reference compound. The alignment of the FT-IR spectra of tannic acid with the standard, confirms the accuracy of the tannin identification in this study.

The consistency between the FT-IR spectra of tannins in this study and those reported in other studies, as well as the standard tannic acid reference compound, supports the reliability and validates the use of FT-IR spectroscopy for tannin identification. This concurrence confirms the effectiveness of FT-IR spectroscopy as an analytical tool for tannin identification, reinforcing its utility in various applications.

Uv-vissible Analysis of tannin Isolate

The spectrum shows several absorption peaks across the UV-Visible region. The peak at 298 nm has the highest absorbance value (2.7715), indicating a strong absorption. The peak at 368 nm has absorbance value of (1.8697) and a broad bandwidth (75.0). The absorption peaks at 195 nm, 215 nm and 241 nm suggest the presence of aromatic rings in the tannin molecule. Absorption at 253 nm may be attributed to transition of carbonyl group in the tannins molecules. The strong absorption at 298 nm is characteristic of phenolic compounds. The broad absorption band at 368 nm may indicate the presence of conjugated systems. The multiple absorption peaks suggest a complex molecular structure with various functional groups. Similarly, weak absorption at 502 nm and 535 nm may indicate the presence of trace amounts of other compounds. The UV-Visible spectrum suggests a complex molecular structure with various functional groups, including aromatic, phenolic, and conjugated systems which are characteristics of tannin molecule. Similar investigation was conducted by Patile et al (2020), the results showed, higher amount of total flavonoid compounds.

Estimation of tannins

The amount of tannins in *E. hirta* isolate was determined using the calibration curve obtained from concentration and absorbance of tannic acid. The calibration curve demonstrates a direct proportional relationship between absorbance and concentration of tannic acid. This relationship allows for the estimation of total tannins in a sample by measuring its absorbance. The phenolic content was expressed as mg tannic acid equivalent per gram of dry weight basis of powdered plant sample (mg TAE/g sample) as

given in table 2. The total phenolic content was expressed in terms of TAE using the standard curve equation ($y = 0.0086x + 0.0907$) with a correlation coefficient (R^2) of 0.9983. From the results, the concentration of tannin in the tannin isolate is 43.47 mg TAE/g. The concentration of tannins in the sample is appreciable and may have significant implications for its use.

Phenolic and flavonoid compounds possess a unique property that enable them to accept electrons from reactive oxygen species (ROS), forming relatively stable phenoxyl radicals. This capacity to neutralize ROS allows these compounds to disrupt chain reactions that can damage cellular mechanisms. (Di Sotto & Di Giacomo 2023). Consequently, phenolic and flavonoid compounds can safeguard the human body against oxidative stress-induced harm. The antioxidant properties of phenolic compounds and flavonoids confer numerous health benefits, including: cardio-protection, anti-cancer properties, anti-diabetic effects, anti-aging benefits, neuro-protection, etc. Given the significance of phenolic compounds, it is essential to determine their presence and quantity in plant samples. This knowledge can facilitate the development of plant-based remedies and dietary interventions that leverage the antioxidant properties of these compounds to promote human health and well-being. (Patle et al., 2020)

Conclusion

This study aimed to quantify and elucidate the structure of tannin isolates obtained from *E. hirta* plant using a combination of preliminary and analytical methods. The chromatographic profile of the isolate was compared to tannic acid standard, exhibiting a characteristic blue-black coloration with 10% ferric chloride solution, a reaction distinctive to tannins. Further analysis using FT-IR spectroscopy revealed absorption peaks indicative of tannin structures. Consistent with these findings, UV-Visible spectroscopy showed absorption peaks common to tannins, providing additional evidence for the presence of tannin compounds in the isolate. Quantification of total tannins in the isolate revealed significantly high concentration, suggesting a negligible presence of non-tannin compounds. These results collectively confirm the successful isolation and characterization of tannins from *E. hirta* plant, highlighting the potential of this plant as a rich source of tannins. Which could be investigated for sugar lowering property and harnessed for the production of anti-diabetic agents and other pharmaceutical formulations.

Recommendations

- Further research can be carried out on High pressure liquid chromatography to ascertain the purity of the tannins.
- Comprehensive structural elucidation can be carried out using Nuclear Magnetic Resonance spectroscopy (NMR)
- Drug design and development could be done on the plant considering the wide application of the plant in traditional medicine across different communities.

References

- Aadil K., Vikrant J., & Rouf W. (2022). *Euphorbia hirta* as a gold mine of high-value phytochemicals: A comprehensive review of its pharmacological activities and possible role against SARS-CoV-2. *Vietnamese Journal for Medical Biotechnology and Medicine Incorporating Advances in Regenerative Medicine*. Vol 9 No 2 (2022) / 4930-4949. DOI : [10.15419/bmrat.v9i2.728](https://doi.org/10.15419/bmrat.v9i2.728)
- Akiode, S. O., Fadeyi, A. E., Fenu, M. J., Johnwara, E. O., Asemota, U. K., & Akiode, T. F. (2025). Characterization and antibacterial activity of tannin from selected food and agricultural wastes. *African Journal of Biological, Chemical and Physical Sciences*, 2790-9530. Website: <https://journals.jozacpublishers.com/ajbcps/index>
- Ali Esmail Al-Snaf (2022) Medicinal plants with lactogenic effect: A review. *GSC Biological and Pharmaceutical Sciences* DOI: [ISSN: 2581-3250https://doi.org/10.30574/gscbps.2022](https://doi.org/10.30574/gscbps.2022).
- Ali, M. A., Al-Bedhawi, R. A., & Dakheel, M. M. (2024). The anti-cancer activity of purified tannins extracted from blackcurrant flower on the proliferation and the migration of cancer cells. *African Journal of Biomedical Research*, 27(4S). <https://doi.org/10.53555/AJBR.v27i4S.3677>
- Ashwaq T. K., and Enas J. K. (2024). Psidium guajava: A Review on Its Pharmacological and Phytochemical Constituents *Biomedical & Pharmacology Journal*, June 2024. Vol. 17(2), p. 1079-1090. DOI:[10.13005/bpj/2924](https://doi.org/10.13005/bpj/2924)
- Di Sotto A. and Di Giacomo S. (2023) Plant Polyphenols and Human Health: Novel Findings for Future Therapeutic Developments. *Nutrients* 2023, 15(17), 3764; <https://doi.org/10.3390/nu15173764>
- [E. Lecian E.](#) (2023)Tannin as an antidote in alkaloid intoxication]. *Scr Med (Brno)*. 1954;27 (7-8):271-6. Czech. PMID: 14385841.
- Isler S, Haykiri-Acma H, Özbek N., Saygili G. N. & Yaman S. (2025) Recovery of gold and silver from concentrated solutions using tannin-based hydrophobic biosorbents. *Microchemical Journal*.Volume 208, January 2025, 112473. <https://doi.org/10.1016/j.microc.2024.112473>
- Khursheed, A., Jain, V., & Wani, A. R. (2022). *Euphorbia hirta* as a gold mine of high-value phytochemicals: A comprehensive review of its pharmacological activities and possible role against SARS-CoV-2. *Biomedical Research and Therapy*, 9(2), 4930-4949. <https://doi.org/10.15419/bmrat.v9i2.72N8>
- Kumar, M.; Tomar, M.; Punia, S.; Amarowicz, R.; Kaur, C. Evaluation of cellulolytic enzyme-assisted microwave extraction of *Punica granatum* peel phenolics and antioxidant Activity. *Plant Foods Hum. Nutr.* 2020, 75, 614–620. DOI: [10.1007/s11130-020-00859-3](https://doi.org/10.1007/s11130-020-00859-3)
- Maria, F., Paz, Otero., Lucia, C., Javier, E., Paula, G., Maria, C., , Franklin, C., Catarina, L., Miguel A., & Jesus S. (2021) Traditional Applications of Tannin Rich Extracts Supported by Scientific Data: Chemical Composition, Bioavailability and Bioaccessibility. *Foods* 2021, 10, 251. <https://doi.org/10.3390/foods10020251>
- Meda, R.N.-T., Kam, S.E., Kagambega, W., Zongo, E., Ouedraogo, C., Segda, A., Koama, B.K., Somda, F.T., Zongo, E. and Ouedraogo, G.A. (2023) A Review on Bioactive Compounds Isolated from *Euphorbia hirta* L. *American Journal of Plant Sciences*, 14, 710-726. <https://doi.org/10.4236/ajps.2023.146048>.
- Ningsih, P., Pratiwi N., Supriadi , Ahmar, D. S., Tiwow, V.M., Virgianti S.H., & Rahmawati, P. S. (2022) Quantification of Tannin in Chromolaena Odorat (Kirinyu) Leaf Extract. *International Journal of Design & Nature and Ecodynamics* Vol. 19, No. 4, August, 2024, pp. 1341-1346. DOI: <https://doi.org/10.18280/ij dne.190426>
- Panda, D. S., & Giri, R. K. (2024). Medicinal plant cultivation: Current scenario, challenges, and opportunities from Indian farmer's perspective. *Journal of Health Science and Medicine Research*, 42(3). Vol 42, No 3 (2024) ISSN : 3620-0559
- Patle, T. K., Shrivastava, K., Kurrey, R., Upadhyay, S., Jangde, R., & Chauhan, R. (2020). Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV-vis and FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. DOI: [10.1016/j.saa.2020.118717](https://doi.org/10.1016/j.saa.2020.118717)

- Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., Gat, Y., & Kumar, A. (2021). Health effects, sources, utilization and safety of tannins: A critical review. *Toxin Reviews*. DOI: 10.1080/15569543.2019.1662813
- Tripathi, A. N., Sati, S. C., & Kumar, P. (2022). *Euphorbia hirta* Linn. – An invasive plant: A review of its traditional uses, phytochemistry and pharmacological properties. *International Journal of Pharmaceutical Sciences and Research*, 13(1), 13-24. DOI:[10.13040/IJPSR.0975-8232.12\(12\).6189-01](https://doi.org/10.13040/IJPSR.0975-8232.12(12).6189-01)
- Ucella-Filho, J. G. M., Freire, A. da S. M., Carréra, J. C., Lucas, F. M. F., Zucolotto, S. M., Dias Júnior, A. F., & Mori, F. A. (2022). Tannin-rich bark extract of plants as a source of antimicrobial bioactive compounds: A bibliometric analysis. *South African Journal of Botany*, 150, 1038-1050. DOI:[10.1016/j.sajb.2022.09.018](https://doi.org/10.1016/j.sajb.2022.09.018)
- Yi-Won, A., Ren, Z.-X., Rutherford, S., Ding, X.-Y., Guo, C.-A., Zhang, X., Zhang, S., Liao, H., & Wang, Y. (2024). Ethnobotanical study on medicinal plants used by the Yi people in Xiaoliangshan, Yunnan Province, SW China. *Journal of Ethnopharmacology*, 323, 117683. <https://doi.org/10.1016/j.jep.2023.117683>