

Enhancing Students' Understanding of Phytochemical Analysis and TLC Using *Deinbollia pinnata* Leaves

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Abstract

The phytochemical analysis of chemical compounds produced by plants offers benefits across various fields. Students were introduced to plant constituents to provide them with a better understanding of plant science. This practical demonstration using TLC techniques supported with educational intervention has motivated twelve (12) final year students' interest in medicine/pharmaceutical, which were selected from CHEM/BIO, CHEM/MATH, CHEM/ISC and CHEM/PHY combinations. The study revealed six (6) constituents from *Deinbollia pinnata* leaves, which can be compounds from simple sugars, flavonoids, coumarins, fixed oils/fats, phenols, tannins, steroids, terpenoids, saponins, and alkaloids as identified through phytochemical analysis results. Thus, educators can help students explore immediate plant phytochemicals and evaluate their pharmacological activities to address global challenges related to health, nutrition, and environmental sustainability.

Keywords: Students, Phytochemical Analysis, Chemical Constituents, Thin Layer Chromatography, Spots, *Deinbollia pinnata*.

Introduction

Phytochemical analysis is a vital area of research in understanding the potential health benefits of plants and developing natural products for health and wellness (Shah & Singh, 2025; Yang & Ling, 2025). This type of analysis involves studying the plant's constituents that can have beneficial effects on health. Their effect are associated with some classification such as alkaloids; nitrogen-containing compounds often with pharmacological effects (Chaachouay & Zidane, 2024) (e.g., caffeine, morphine), flavonoids; antioxidants that can have anti-inflammatory properties (Jomova et al., 2025a) (e.g., quercetin, catechins), terpenoids; compounds that contribute to the aroma and flavor of plants (Siddiqui et al., 2024) (e.g., menthol, limonene), phenolic; associated with antioxidant activity (Dayal et al., 2024) (e.g., tannins, lignins) and saponins; known for their foaming properties and potential health benefits (Lv et al., 2025). These constituents were obtained through extraction methods, utilising the chromatography process and the application of spectroscopic methods. Aluminium thin layer chromatography is a versatile and accessible technique widely used in laboratories for the analysis of various compounds

across different fields (Kowalska, 2022; Urbain & Simões-Pires, 2020). TLC is based on the differential adsorption of compounds on a stationary phase and their solubility in a mobile phase (Tiwari & Talreja, 2022).

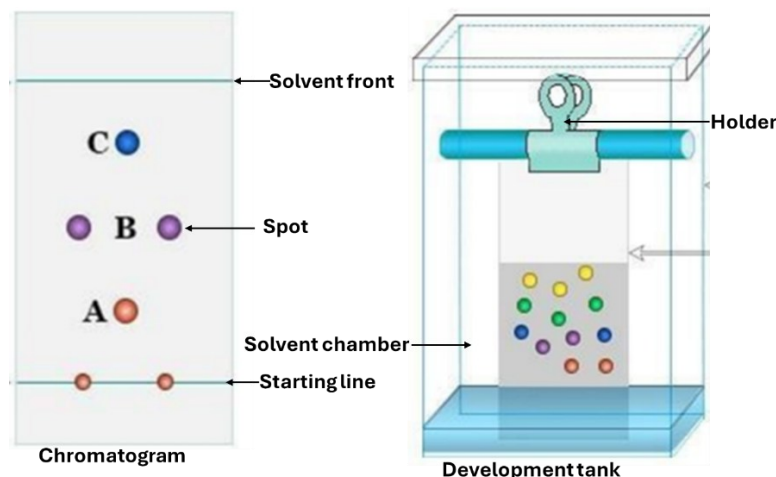


Figure 1: Development of TLC Plate

The stationary phase in aluminium TLC is an adsorbent (commonly silica gel or alumina) coated onto an aluminium plate, as shown in Figure 1. TLC is based on the differential adsorption of compounds on a stationary phase and their solubility in a mobile phase (Tiwari & Talreja, 2022). As such, the number of components present would be identified through a visualisation setup apparatus, such as a UV machine (Loconsole & Santamaria, 2021), chemical stains (Suebkhampet, 2024), or other detection methods, as part of qualitative analysis.

D. pinnata is a plant species that belongs to the family Sapindaceae (Rufai, 2021). Commonly known as bitter bark or bitter wood, this plant is typically found in tropical regions, particularly in West and Central Africa (Sangeeth Shyam Sundar et al., 2024). It thrives in forested areas and along riverbanks, growing as a shrub or small tree with distinctive pinnate leaves that reach heights of 4-10 meters (Green Institute, 2022). The leaves contain several bioactive compounds (Rufai, 2021), which serve as an excellent subject for hands-on experimentation for learning in educational institutions. However, there remains a bottleneck with students' practical comprehension of identifying the number of phytochemical constituents found in the plant matrix. Despite their well-documented medicinal properties. Challenges arise as students are not individually involved in lab practices for effective use of the TLC process to separate constituents and to identify phytochemicals from plant extracts.

Engaging in phytochemical analysis and TLC helps students bridge the theoretical knowledge and practical application. It reinforces concepts learned in lectures and enhances critical thinking and problem-solving skills. Phytochemicals are bioactive compounds found in plants that contribute to their colour, flavour, and disease resistance

(Gupta, 2023; Kumar et al., 2023). They are not essential nutrients but have been linked to various health benefits. These phytochemical constituents include Flavonoids, Carotenoids, Glucosinolates, Saponins, Terpenes, Alkaloids, Lignans, Phenolic Acids. Each of these groups contains numerous individual compounds, each with its potential health benefits (College & Pradesh, 2025). The *D. pinnata* roots and leaves are used in folkloric medicine as a remedy for numerous diseases (Lasisi et al., 2016). Reports on their essential oil bioactivity and volatile constituents were documented (Sotubo et al., 2016). Isolated compounds range from coumarins, phenolics, esters and ethers (Rufai et al., 2019a) were identified. The leaves and bark of *D. pinnata* are used for medicine due to their potential anti-inflammatory and analgesic properties (Rufai et al., 2023). Isolated compounds such as Taraxasterol, stigmasterol, betulinic acid, methyl gallate, ethyl gallate, methyl- β -D-glucopyranoside, ethyl oleate, ethyl stearate, and phytyl palmitate, myristic acid, stearic acid, Quercetin, Stigmasterol, Lupeol, γ -sitosterol, Tetrahydrospirilloxanthin (squalene), Pyrogallol, Stigmasta-5,22-diene-3-ol acetate, 4,8,12,16-tetramethylheptadecan-4-olide (also referred to as 4,8,12,16-tetramethylheptadecan), Palmitic acid, 1,4-Benzene dicarboxyl ester, 1,2-Benzene dicarboxyl ester (Lasisi et al., 2016; Rufai et al., 2019a; Rufai, Basar, Chandren, et al., 2020; Rufai et al., 2022; Sotubo et al., 2016). In the laboratory, TLC and phytochemical analysis are mostly used for the separation and analysis of various chemical compounds. It offers simplicity, speed, and cost-effectiveness, making it a popular choice in research for qualitative and quantitative analysis.

Materials and Methods

Chemicals and Reagents

Solvents would be of general-purpose grade, and the reagents of analytical grade. The solvents were n-hexane (HEX), diethyl ether (DEE), dichloromethane (DCM), chloroform (CHCl₃), ethyl acetate (EtOAc), acetone (ACE), methanol (MeOH), ethanol, TLC plate (aluminum foil coated with an adsorbent like silica gel), TLC chamber (a container with a lid), capillary tubes, developing solvent (appropriate for the compounds being separated), sample to be analyzed, visualization agent (if needed), spraying with vanillin sulphuric acid reagent majorly.

Plant Material Collection

The *D. pinnata* (Poir.) Schumach. & Thonn leaves would be collected from Okehi Local Government Area of Kogi State, Nigeria, during the dry season, early January, for one week (daytime temperature ranges from 28 °C in January to 32 °C). The plant would be identified and confirmed at the Biological Department, Federal College of Education, Okene, Kogi State, by Mrs Aniana Salome O.A., a botanist. The plant material would be authenticated at the Forestry Research Institute of Nigeria, Ibadan, through comparison with a voucher specimen. The collected leaves would be washed, air-dried at room temperature for one month and ground to a powder.

Extraction Procedure

This study is a laboratory-based activity. Three students from each course combination were selected based on their academic performance, resulting in twenty-one participants. To each of the group. powdered plant material of *D. pinnata* (Poir.) Schumach. & Thonn was extracted using the *Ruf-azah* method (Rufai et al., 2019b). Briefly, the leaves were taken into several conical flasks (30 g each) and extracted with organic solvents (150 mL) such as *n*-hexane, ethyl acetate, and methanol in a sonicator using an ultrasonic-assisted extraction method at an interval of ten minutes' agitation; filtered into a bottle and allowed for 24hr; then filtered using Whatman No. 1 filter. The extraction was repeated three times for various solvents until complete extraction. The combined extracts were filtered and concentrated in vacuo at 40°C using a rotary evaporator, the extractives were evaporated to dryness and residues were obtained in grams for all selected parts of *D. pinnata* (Poir.) Schumach. & Thonn. Phytochemical analysis was carried out with the sample extracted with methanol only. Purifying each crude extract was carried out using vacuum liquid chromatography (VLC), and spotting/identification was guided by aluminium thin-layer chromatography (TLC). The *R_f* of each identified constituent was calculated and recorded. Each course combination involved in all stages and presented their results as best collated by the lab attendant into the laboratory report.

Phytochemical Analysis Procedure

A preliminary phytochemical screening of *D. pinnata* (Poir.) Schumach. & Thonn. Leaves was conducted using standard procedures with slight modifications (Nkwocha et al., 2024).

Tests for simple sugar (Fehling's test): To conduct the Fehling's test, combine approximately 1 mL each of Fehling's solutions A and B, warmed for 1 minute. Next, add this mixture to an equal volume of the test solution and heat the solution in a boiling water bath for 5-10 minutes. A yellow precipitate formed, which turned brick red.

Test for anthraquinone glycosides (Borntrager's test): In the testing process, each sample was mixed with 3 mL of diluted H_2SO_4 , heated, and then filtered. The resulting filtration was cooled, and an equal amount of benzene was introduced. The solution was vigorously shaken to separate the organic layer. Following this, an equal amount of diluted ammonia solution was added to the organic layer. The absence of anthraquinone glycosides was indicated by the failure of the ammonia layer, which turned pink.

Test for Cardiac Glycosides (Keller-Killiani Test): To test for the presence of cardiac glycosides in the extracts, 1 mL of concentrated H_2SO_4 , 2 mL of glacial acetic acid, and 1 drop of $FeCl_3$ solution was added to 5 mL of the extracts. The formation of a brown ring indicated the presence of cardiac glycosides.

Test for Coumarins: A 10% NaOH solution was added to 2 mL of the extracts, followed by vigorous shaking for 5 minutes. The appearance of yellow colouration indicated the presence of coumarins.

Test for Saponins (foam test): To prepare the solution, 20 mL of distilled water was added to 1 mL of the extract in a measuring cylinder. Shake the mixture vigorously for 15 minutes and was carefully observed for the presence of a foam layer.

Tests for Quinone: To begin the experiment, mix concentrated sulfuric acid (H_2SO_4) with 2 mL of the extract in a test tube. The mixture was vigorous for 5 minutes. A Noticeable red colour was observed.

Test for Steroids (Salkowski test): To conduct the Salkowski test for steroids, 2 mL of concentrated H_2SO_4 with 2 mL of chloroform was added to the mixture with 2 mL of the extracts. Shaked vigorously. The chloroform layer should turn red, while the acid layer will exhibit a greenish-yellow fluorescence. This test showed the presence of steroids in a sample.

Test for Alkaloids (Mayer's test): To conduct Mayer's test for alkaloids, add approximately 1 mL of diluted hydrochloric acid and 1 mL of Mayer's reagent was added to 2-3 mL of the filtrate. Shaked. The formation of a yellow precipitate indicated the presence of alkaloids.

Test for Flavonoids (Shinoda test): To conduct the Flavonoid Test, concentrated hydrochloric acid (HCl) was added drop by drop to the test solution. Next, magnesium turnings are introduced into the solution. The presence of flavonoids was indicated by the observation of a pink-red colour change.

Test for Tannins and Phenolic: To test for Tannins and Phenolic compounds, a solution of 5% FeCl_3 should was to 2 mL of the extracts. The presence of tannins and phenolic compounds in the test sample was indicated by a deep blue-black colouration.

Thin Layer Chromatography Procedure

A small amount of crude extract from each solvent was prepared by high dilution. Capillary tubes were used to apply a small amount of the sample solution to the bottom of an aluminium TLC plate. The spots were small and evenly spaced and allowed to dry completely before proceeding with development. The TLC plate was carefully placed in a developing chamber, ensuring that the solvent level was below the spots. The plate was covered, and the solvent will rise on the plate through capillary action. Development was stopped when the solvent front was close to the top of the plate. The plate was removed from the chamber, and the solvent front was marked with a pencil. Colored compounds were visible, and some compounds are colourless. Visualisation agents such as UV light, Iodine vapour, and chemical reagents were used. The R_f values of the compounds were calculated.

Results and Discussions

The students knew them as an entity with a single colour, but after the powdered leaves (100 g) of *D. pinnata* were extracted with *n*-hexane (Hex), followed by ethyl acetate (EtOAc) and methanol (MeOH) in succession, they found them with different colours. The process of cold solvent extraction was employed and repeated three times for each solvent, showing clearly different quantities of constituents in the leaves. A rotary evaporator was used to recover the crude extracts as reported by Okocha & Orié (2025). It was then completely evaporated in a fume hood, with the appropriate weight recorded, as shown in Table 1. Another 10 g of the powdered leaves were then extracted with methanol,

evaporated, dried, and used for preliminary phytochemical analysis only as detailed in Table 2. Students were surprised as different unimaginable colours showed during the test. The qualitative colour appearance of the leaf sample is shown in Table 3. The collected leave sample is shown in Figure 1. Further identification of the phytochemical component from the leaf's extractives was shown in Table 4. The cold plant extraction approach is important in natural product research experimentation towards drug discovery, food, and cosmetics from a plant-based product, as mentioned by Awasthi et al. (2024). The use of different polar solvents such as *n*-hexane, ethyl acetate and methanol for the extraction of phytochemicals started by (Nkwocha et al., 2024) from *D. pinnata* leaves allows distributions of the abundant components into like dissolves like as explained by (Ogechi Onyeocha & Ikejiofor Onuchukwu, 2024). The methanol solvent gave the highest yield of extractives (16 g), indicating more polar components present in the leaf sample, as shown in Table 1. This was followed by the least yield with *n*-hexane at 8.0 g and a moderate yield of 12.0 g for ethyl acetate extractives. This method helps in targeting specific phytochemicals with ease in their isolation. Research has shown that no single known solvent can efficiently extract diverse components present in a plant depleted by (Cao et al., 2025). Thus, using different solvents results in a more comprehensive identification of the plant phytochemical profile, as supported by Munuo et al. (2025). This can lead to a higher overall yield of diverse phytochemicals. Thus, it shows the highest activity for a particular purpose and influences biological activities.

Table 1: Extractive Yield for *D. pinnata* Leaves for Thin Layer Chromatography Experiment

Solvents	Hex	EtOAc	MeOH
Yield (g)	8.0	12.0	16.0
Extraction time	3 days	3 days	3 days

100 g /X mL proportion for the solvents to sample ratio was used in the experiment, and is expressed in grams.

While advancement in teaching students using aluminum TLC offers a clear identifying numerical strength of chemical components (García-Zavala et al., 2025) that is present in a particular extracts, qualitative analysis remain valuable for quick preliminary assessment of the present or absent of phytochemicals (Maheshwaran et al., 2024) such as steroids, tannins, saponins, terpenoids, alkaloids, phenols, glycosides, e.t.c. It is easier with less cost involved, and importantly, colourfully shows visible symptoms for the physiological/biochemical process taking place, leading to identification of some phytochemicals for either nutritional benefit or potential medicine (Hossain et al., 2025). The reddish-browened colour observed in the experimental test tube indicated alkaloid (Resin et al., 2025), and the mild to dark yellow colour indicated flavonoid (Jhana Rhel Plantas Dulfo et al., 2025) as shown in Table 2. Scientific validation / traditional uses of plants rely on qualitative phytochemical screening and help students/researchers understand how plants adapt to their environment with different compounds present in

them. Permitting the herbal medicine industry to align with the understanding between traditional wisdom and scientific understanding (Jumba, 2025). Therefore, identification noticed during this phytochemical analysis will further help in isolation techniques to be used and the appropriate extraction solvent to be selected.

Table 2: Qualitative Colour Appearance Along with the Leave Sample

		
Leaves	Crude Extractives	Colour Appearance

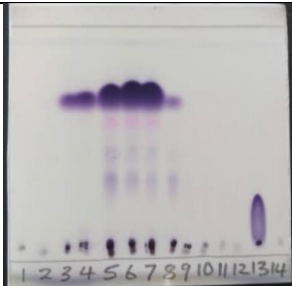
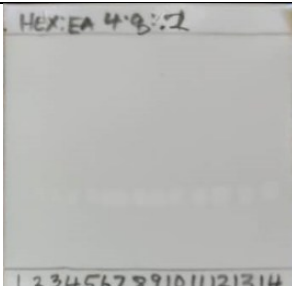
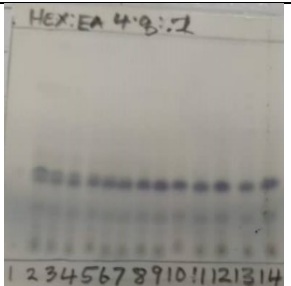
The presence of these diverse phytochemicals, as shown in Table 3, supports the traditional medicinal applications of *D. pinnata* leaves and their potential as a major source of novel therapeutic agents when researched. Phytochemical analysis shown in the following table revealed the presence of various constituents, including simple sugar, flavonoid, coumarins, fixed oil/fat, phenol, tannins, steroids, terpenoids, saponins, and alkaloids. The traditional practitioners in Africa adopt these leaves for the management of illnesses/difficulties such as cough /bronchial asthma, jaundice/infections, aphrodisiac/genital stimulants/depressants, fever (febrifuge), and fetus positioning during childbirth (Rufai, Basar, & Sani, 2020). The presence of simple sugars, which are mainly fructose and glucose, serves as a plant currency of carbon/energy (Long & Adams, 2023), directing complex developmental processes for growth/survival (Rawat & Laxmi, 2024) and enabling them to adapt to their environment. Flavonoids help neutralise harmful free radicals in the body that cause cell damage due to oxidative stress (Jomova et al., 2025b), thereby contributing to ageing and chronic diseases like neurodegenerative disorders and cancer reduction (Jomova et al., 2025b).

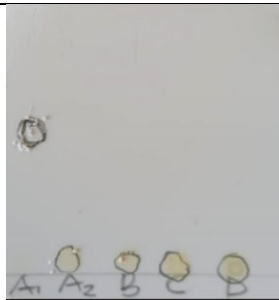
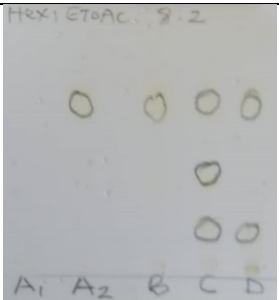
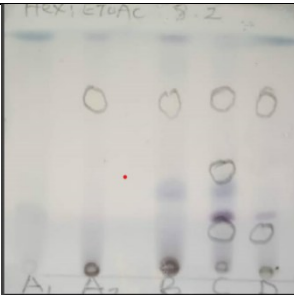
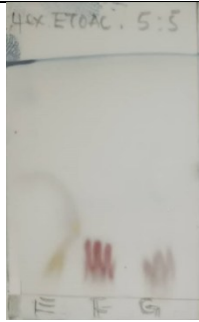
Table 3: Phytochemical Screening for Heartwood, Root Bark and Leaves of *D. pinnata*

Constituents	Reagent	LF	Phytochemical	Reagent	LF
Simple sugar	Fehling’s reagent	+	Antraquinone	Borntrager’s reagent	-
	Benedict’s reagent	+	Steriod	Salkowski’s	+
	Molisch’s reagent	+	Terpenes	Salkowski’s	+
Protein	Biuret’s reagent	–	Cardiac glycoside’s	Keller- killiani’s reagent	–
	Million’s reagent	–	Saponin	Foam	+
Flavonoids	Lead acetate	+	Quinones	Extracts + Conc. H ₂ SO ₄ (aq)	–
Coumarins	Extracts+10% NaOH _(aq)	+	Alkaloids	Hager’s reagent	+
Fixed oil	Spot test	+	Alkaloids	Dragendroff’s regent	+
Phenol	FeCl ₃ (aq)	+	Alkaloids	Mayer’s reagent	+
Tannins	Lead acetate	+	Alkaloids	Wagner’s reagent	+

The plant development and growth are not directly affected by stored compounds (Yang & Ling, 2025), but they play a vital role in their environmental interaction. They serve as strong active agents against viruses, bacteria, and fungi (Bharathi & Lee, 2024; Song, 2024; Sułkowska-Ziaja et al., 2023). Phenolic compounds are simply known for their red to dark red colored (Aaby & Amundsen, 2023; Louis et al., 2022) found in vegetables, fruits (El Rayess et al., 2024), spices, nuts/seeds, legumes, beverages, root bark of some trees, and other plant-based foods (Nardini, 2022). The rich intake of phenolics is essential for health (Hossain et al., 2025), as an antioxidant and anti-inflammatory (Jadhav et al., 2024), antimicrobial (Bouymajane et al., 2024), antiviral (Sharaf, 2023) and regulating sugar level (Golovinskaia & Wang, 2023). The presence of tannins reduces the high rate of consumption due to decreased nutrient absorption (Ozogul et al., 2025), with stomach irritation (Ojo, 2022), but serves as anticancer potential (Deb et al., 2024), antimicrobial (Widsten et al., 2014) and demonstrates cardio-protective effects (Cosme et al., 2025). Steroids help in increasing cholesterol, improving cardiovascular challenges (de Melo Junior et al., 2025), and strengthening the immune system (Ikuta et al., 2022). The four various tests for alkaloids confirmed their presence, which serve as anti-malarial, stimulants, and analgesics (Chaachouay & Zidane, 2024; Letchuman et al., 2024; Uzor, 2020). The anthraquinone, cardiac glycosides, quinones and proteins were not detected. Both phenol and tannins are present, which have anxiolytic and mood-modulating effects (Khalid et al., 2023), immunomodulatory effects, and type 2 diabetes management (Martiniakova et al., 2025). Lastly, the presence of saponins in the leaves helps in cholesterol lowering, antimicrobial/antiviral activity, antidote to heavy metal poisoning, anti-venom and skin health (Timilsena et al., 2023).

Table 4: The Aluminium Thin Layers Chromatography Spots for *n*-hexane, Ethyl Acetate and Methanol Extractives from *D. pinnata*

<i>n</i> -Hexane (Hex) Extracts		
		
Hexane (1)	Hex: EA 4:8:2 (2)	Hex: EA 4:8:2 (3)
Ethyl Acetate (EtOAc) Extracts		

		
Hexane (4)	Hex: EtOAc 8:2 (5)	Hex: EtOAc 8:2 (6)
Methanol (MeOH) Extracts		
		
Hex: EtOAc 5:5 (7)	Hex: Ace 3:7 (8)	Acetone 100% (9)

Making the students involved in this physical research has made them understand the proper method for using aluminium TLC plates to effectively separate/analyse various chemical mixtures in the laboratory. Spotting and TLC development follow the same procedure except when a different solvent mixture is used. The *n*-hexane extracts were evaluated to identify spots with *n*-hexane: ethyl acetate, varying their polarity as detailed in Table 4. The TLC (1) showed a purple colour spot after spraying and heating. The R_f -value was 0.83. (*n*-hexane 100%) The identified spot was very bright with other faint spots. The base of the TLC plate clearly showed that other components present can be distributed with *n*-hexane, which is a non-polar solvent. From our review, such descriptions tend to be like squalene compound as reported (Bhat et al., 2023). The second (2) TLC plate with slightly increased polarity shows transparent spots which are more prominent for labelled 7,8,9,10,11,12,13 immediately after spraying and allowed to sit for some time. The spots are not visible under UV-light, with R_f 0.54 in *n*-Hex: EtOAc (4:1) and R_f value of 0.73 in *n*-Hex: EtOAc (4:1) indicated a straight chain ester as reported to be palmitic acid and stearic acid (Rufai et al., 2019a), showing that many non-polar compounds are colourless. But when heated, another compound was observed slightly above the colourless spots. TLC (2&3) showed four (4) compounds.

For ethyl acetate extract, the *n*-hexane extract showed five spots, where one of the spots, A₁, showed under UV-light and disappeared after spraying. The other four spots (A₂, B, C, D) demonstrated no movement after TLC development, indicating them as polar compounds. When the spot where further developed with slightly polar mixture of solvent at Hex: EA 4.8:2 as in number 5, A₂ showed only one spot, B showed similar spot at the

same distance with A₂, C depleted three spots haven one corresponded with A₁, A₂ and B. but one of the spots where completely different from the others while two spots are similar to C. all the spots where seen under the UV-light clearly and after spraying with application of heat, the spots a similar spot in B and C were noted. C showed two brownish spots as shown in (6). From the two spots, the upper spots shared similarity with B and the lower spot shared similarity with D. The brownish colored shown after heating is indicative of the presence of triterpenoids (Rufai et al., 2023). For the methanol extract, the n-hexane does not show any spots. But the mixture of Hex: EtOAc 5:5 showed a dragging spot for F and G as in (7). This was further increased in polarity of the solvent in (8), giving rise to three spots at Hex: Ace 3:7. The spots were seen under UV-light, but when sprayed and heated, a mild to more yellow colored spot was seen. E and G showed a faint yellow colour while the middle F was intense. These could be observed as a concentration difference. The 100% acetone observed in (9) showed a single spot, which was seen under UV light and after being sprayed with heat. The coloured spots in 8 & 9 TLC could be indicative of gallate compounds in the leaves such as pyrogallol, methyl gallate and ethyl gallate, as previously reported (Rufai, Basar, & Sani, 2020). The TLC experiments have shown the leaves to contain many important compounds. At the end of the work, the students were agitating for more similar practicals as they found different beneficial plants around them. They showed a potential level of exposure to real-life educational experience with scientific thinking, which can be used in many other areas of science, as well as a deeper appreciation for the natural world.

Conclusion

The presence of a medicinal chemist as a lecturer in a tertiary institution has put an increased interest in natural substances from plants for wellbeing. Emphases should be given to laboratory-based TLC and phytochemical analysis as it arouses learner inquisitiveness, allowing them to carry out their experiences with other plants now. Six (6) natural constituents were identified, and six (6) phytochemicals were analysed from *D. pinnata* leaves. More experimental designs involving different plants for students will be advised to further expose learners to unexplored natural therapeutic discoveries and more advanced methods to be used.

Ethical Considerations: None

References

- Aaby, K., & Amundsen, M. R. (2023). The stability of phenolic compounds and the colour of lingonberry juice with the addition of different sweeteners during thermal treatment and storage. *Heliyon*, 9(5), e15959. <https://doi.org/10.1016/j.heliyon.2023.e15959>
- Awasthi, N., Singh, S.K., & Yadav, P. (2024). Extraction and isolation techniques of medicinal plants: A Review. *Technische Sicherheit*, 24(7), 1–18.
- Bharathi, D., & Lee, J. (2024). Recent Advances in Marine-Derived Compounds as Potent Antibacterial and Antifungal Agents: A Comprehensive Review. *Marine Drugs*, 22(8). <https://doi.org/10.3390/md22080348>

- Bhat, M. P., Rudrappa, M., Hugar, A., Gunagambhire, P. V., Suresh Kumar, R., Nayaka, S., Almansour, A. I., & Perumal, K. (2023). In-vitro investigation on the biological activities of squalene derived from the soil fungus *Talaromyces pinophilus*. *Heliyon*, 9(11), e21461. <https://doi.org/10.1016/j.heliyon.2023.e21461>
- Bouymajane, A., Filali, F. R., Moujane, S., Majdoub, Y. O. El, Otzen, P., Channaoui, S., Ed-Dra, A., Bouddine, T., Sellam, K., Boughrou, A. A., Miceli, N., Altemimi, A. B., & Cacciola, F. (2024). Phenolic Compound, Antioxidant, Antibacterial, and In Silico Studies of Extracts from the Aerial Parts of *Lactuca saligna* L. *Molecules*, 29(3), 1–15. <https://doi.org/10.3390/molecules29030596>
- Cao, S., Liang, J., Chen, M., Xu, C., Wang, X., Qiu, L., Zhao, X., & Hu, W. (2025). Comparative analysis of extraction technologies for plant extracts and absolutes. *Frontiers in Chemistry*, 13(March), 1–16. <https://doi.org/10.3389/fchem.2025.1536590>
- Chaachouay, N., & Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, 3(1), 184–207. <https://doi.org/10.3390/ddc3010011>
- College, K. P., & Pradesh, B. U. (2025). A Comprehensive Review of Phytochemicals: Health Benefits and Therapeutic. *International Journal of Pharmaceutical Research and Applications Volume*, 9(6), 2456–4494. <https://doi.org/10.35629/4494-090613731379>
- Cosme, F., Aires, A., Pinto, T., Oliveira, I., Vilela, A., & Gonçalves, B. (2025). A Comprehensive Review of Bioactive Tannins in Foods and Beverages: Functional Properties, Health Benefits, and Sensory Qualities. *Molecules*, 30(4), 1–28. <https://doi.org/10.3390/molecules30040800>
- Dayal, B., Kulkarni, A., Lea, M., Kaur, G., Karani, W., & Singh, J. (2024). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Asian Journal of Organic & Medicinal Chemistry*, 8(3), 29–34. <https://doi.org/10.14233/ajomc.2023.ajomc-p28926>
- de Melo Junior, A. F., Escouto, L., Pimpão, A. B., Peixoto, P., Brasil, G., Ronchi, S. N., Pereira, S. A., & Bissoli, N. S. (2025). Anabolic-androgen steroids: A possible independent risk factor for Cardiovascular, Kidney and Metabolic Syndrome. *Toxicology and Applied Pharmacology*, 495(117238), 1–14. <https://doi.org/10.1016/j.taap.2025.117238>
- Deb, S., Principal, R., Shil, D., Gujri, M., Chakraborty, J., Paul, A., Roy, S., Jit, T., Roy, D., & De, D. (2024). The potential of tannins from medicinal plants as anti-cancer agents. *Journal of Medicinal Plants Studies*, 12(4), 414–423. www.plantsjournal.com
- El Rayess, Y., Nehme, N., Azzi-Achkouty, S., & Julien, S. G. (2024). Wine Phenolic Compounds: Chemistry, Functionality and Health Benefits. *Antioxidants*, 13(11), 1–21. <https://doi.org/10.3390/antiox13111312>
- García-Zavala, A., Jiménez, C. C., Martínez-Bourget, D., & Romero-Ávila, M. (2025). Exploring Thin-Layer and Column Chromatography Fundamentals via Experiential Learning with Simple and Affordable Materials. *Journal of Chemical Education*, April, 1–10. <https://doi.org/10.1021/acs.jchemed.4c00957>
- Golovinskaia, O., & Wang, C. K. (2023). The hypoglycemic potential of phenolics from functional foods and their mechanisms. *Food Science and Human Wellness*, 12(4), 986–1007. <https://doi.org/10.1016/j.fshw.2022.10.020>
- Green Institute. (2022). *Deinbollia pinnata*. In *The Green Institute and the Green Campus Initiative* (pp. 1–3).
- Gupta, C. (2023). Bioactive phytochemicals on humanoid endurance & advancement of herbal medicine. *Journal of Microbiology & Experimentation*, 11(5), 125–135. <https://doi.org/10.15406/jmen.2023.11.00400>
- Hossain, M. S., Wazed, M. A., Asha, S., Amin, M. R., & Shimul, I. M. (2025). Dietary Phytochemicals in Health and Disease: Mechanisms, Clinical Evidence, and Applications—A Comprehensive Review. *Food Science and Nutrition*, 13(3), 1–23. <https://doi.org/10.1002/fsn3.70101>
- Ikuta, K., Ejima, A., Abe, S., & Shimba, A. (2022). Control of immunity and allergy by steroid hormones. *Allergy International*, 71(4), 432–436. <https://doi.org/10.1016/j.alit.2022.07.006>

- Jadhav, P., Kadam, I., Dhamne, S., Jagtap, A., & Narayankar, J. (2024). Use of Phenolic Compounds as Anti-Inflammatory. *International Journal For Multidisciplinary Research*, 6(2), 1–10. <https://doi.org/10.36948/ijfmr.2024.v06i02.18236>
- Jhana Rhel Plantas Dulfo, Meybelle Onyot Gamutan, Jeffryll Rhome Cabusca Ibarra, Ella Jean Ampong Magpayo, Klaire Jane Akut Sabuga, Shienna Margareth Benedicto Sabijon, Justin Dave Magracia Manantan, Jan Karlo Tionson Ecalne, & Mark Wilson Dy Lagamayo. (2025). Determination of total flavonoid content and antibacterial activity of solvent fractions from 'Cacao' (*Theobroma cacao* L.) leaves. *GSC Biological and Pharmaceutical Sciences*, 31(2), 040–047. <https://doi.org/10.30574/gscbps.2025.31.2.0167>
- Jomova, K., Alomar, S. Y., Valko, R., Liska, J., Nepovimova, E., Kuca, K., & Valko, M. (2025a). Flavonoids and their role in oxidative stress, inflammation, and human diseases. *Chemico-Biological Interactions*, 413(2), 111489. <https://doi.org/10.1016/j.cbi.2025.111489>
- Jomova, K., Alomar, S. Y., Valko, R., Liska, J., Nepovimova, E., Kuca, K., & Valko, M. (2025b). Flavonoids and their role in oxidative stress, inflammation, and human diseases. *Chemico-Biological Interactions*, 413(February), 1–44. <https://doi.org/10.1016/j.cbi.2025.111489>
- Jumba, K. (2025). The Evolution of Herbal Medicine: From Traditional Practices to Scientific. *Eurasian Experiment Journal of Biological Sciences*, 6(1), 10–17.
- Khalid, A. A., Jabeen, Q., & Javaid, F. (2023). Anxiolytic and Antidepressant Potential of Methanolic Extract of *Neurada procumbens* Linn. in Mice. *Dose-Response*, 21(2), 1–9. <https://doi.org/10.1177/15593258231169584>
- Kowalska, T. (2022). Thin-Layer Chromatography (TLC) in the Screening of Botanicals—Its Versatile Potential and Selected Applications. *Molecules*, 27(19), 6607.
- Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, V., & Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Methods. In *Molecules* (Vol. 28, Issue 2). <https://doi.org/10.3390/molecules28020887>
- Lasisi, A. A., Bamidele, M. O., Balogun, S., & Adebisi, S. A. (2016). Chemical Constituents and Antibacterial Evaluation of *Deinbollia pinnata* (Schum. and Thonn) Sapindaceae. *The Pacific Journal of Science and Technology*, 17(1), 183–199.
- Letchuman, S., Madhuranga, H. D. T., Kaushalya, M. B. L. N., Premarathna, A. D., & Saravanan, M. (2024). Alkaloids Unveiled: A Comprehensive Analysis of Novel Therapeutic Properties, Mechanisms, and Plant-Based Innovations. *Intelligent Pharmacy*, 12(3), 1–9. <https://doi.org/10.1016/j.ipha.2024.09.007>
- Loconsole, D., & Santamaria, P. (2021). UV lighting in horticulture: A sustainable tool for improving production quality and food safety. *Horticulturae*, 7(1), 1–13. <https://doi.org/10.3390/horticulturae7010009>
- Long, R. W., & Adams, H. D. (2023). The osmotic balancing act: When sugars matter for more than metabolism in woody plants. *Global Change Biology*, 29(7), 1684–1687. <https://doi.org/10.1111/gcb.16572>
- Louis, K. C., Abdul Hamid, S. H. F. W., Raffay, R., & Omar, A. F. (2022). Specialized Colorimeter for Phenol Red pH Measurement. *Journal of Physics: Conference Series*, 2411(1), 1–12. <https://doi.org/10.1088/1742-6596/2411/1/012020>
- Lv, J., Liu, S., Hu, C., Ding, L., Wang, H., Li, X., Yang, F., Shen, Q., Zhang, H., Ma, G., Zhang, S., & Bai, Y. (2025). Saponin content in medicinal plants in response to application of organic and inorganic fertilizers: a meta-analysis. *Frontiers in Plant Science*, 16(February), 1–23. <https://doi.org/10.3389/fpls.2025.1535170>
- Maheshwaran, L., Nadarajah, L., Senadeera, S. P. N. N., Ranaweera, C. B., Chandana, A. K., & Pathirana, R. N. (2024). Phytochemical Testing Methodologies and Principles for Preliminary Screening/

- Qualitative Testing. *Asian Plant Research Journal*, 12(5), 11–38. <https://doi.org/10.9734/aprj/2024/v12i5267>
- Martiniakova, M., Sarocka, A., Penzes, N., Biro, R., Kovacova, V., Mondockova, V., Sevcikova, A., Ciernikova, S., & Omelka, R. (2025). Protective Role of Dietary Polyphenols in the Management and Treatment of Type 2 Diabetes Mellitus. In *Nutrients* 17(2). <https://doi.org/10.3390/nu17020275>
- Munuo, M. S., Martin, M. J., Shechambo, L. F., & Gervas, I. (2025). Effect of solvent extraction on phytochemical profile and quantification of bioactive compounds in *Ocimum suave* (wild). *Journal of Current Opinion in Crop Science*, 6(1), 27–41. <https://doi.org/10.62773/jcocs.v6i1.304>
- Nardini, M. (2022). Phenolic Compounds in Food: Characterization and Health Benefits. *Molecules*, 27(3), 10–13. <https://doi.org/10.3390/molecules27030783>
- Nkwocha, C. C., Felix, J. O., Michael, L. O., & Ale, B. A. (2024). Phytochemical screening and GC-FID identification of bioactive compounds in *n*-hexane, ethyl acetate and methanol fractions of the methanolic extract of *Azanza garckeana*. *Food Chemistry Advances*, 4(April), 100712. <https://doi.org/10.1016/j.focha.2024.100712>
- Ogechi Onyeocha, V., & Ikejiofor Onuchukwu, A. (2024). A Review on Solvents - Dilute, Dissolve and Disperse. *Journal of Material Sciences & Manufacturing Research*, 5(2), 1–6. [https://doi.org/10.47363/jmsmr/2024\(5\)167](https://doi.org/10.47363/jmsmr/2024(5)167)
- Ojo, M. A. (2022). Tannins in Foods: Nutritional Implications and Processing Effects of Hydrothermal Techniques on Underutilized Hard-to-Cook Legume Seeds- A Review. *Preventive Nutrition and Food Science*, 27(1), 14–19. <https://doi.org/10.3746/pnf.2022.27.1.14>
- Okocha, B. I., & Orie, K. J. (2025). Characterization and Biological Activities of Ethanol Extract of *Justicia carnea*. *International Journal of Research and Technopreneurial Innovations*, 2(1), 16–24.
- Ozogul, Y., Ucar, Y., Tadesse, E. E., Rathod, N., Kulawik, P., Trif, M., Esatbeyoglu, T., & Ozogul, F. (2025). Tannins for food preservation and human health: A review of current knowledge. *Applied Food Research*, 5(1), 100738. <https://doi.org/10.1016/j.afres.2025.100738>
- Rawat, S. S., & Laxmi, A. (2024). Sugar signals pedal the cell cycle! *Frontiers in Plant Science*, 15(March), 1–13. <https://doi.org/10.3389/fpls.2024.1354561>
- Resin, P., Polytechnic, A., State, E., & State, E. (2025). Assessing the Minerals, Phytochemical and Proximate Compositions of *Ficus ovata* leaves in Nigeria. *Nigerian Research Journal of Chemical Sciences (ISSN: 13(i))*, 21–32.
- Rufai, Y. (2021). Phytochemicals, Antioxidant Studies of *Deinbollia pinnata* (Poir) Schumach. & Thonn. and its Uses in the Synthesis of Titania Nanoparticles (Issue August).
- Rufai, Y., Basar, N., Chandren, S., Suleiman, K., & Yinusa, I. (2020). Antiradical Activity of 1,4-and 1,2-Benzene Dicarboxyl Esters from *Deinbollia Pinnata* Leaves. *Journal of Chemical Society of Nigeria*, 45(6), 1177–1187. <https://doi.org/10.35393/1730-006-002-014>
- Rufai, Y., Basar, N., & Kabiru, S. (2022). Triterpenoids Characterization with Antioxidant Activity from the Aerial Part of *Deinbollia pinnata*. *Fane-Fane International Multidisciplinary Journal*, 6(2), 13–28.
- Rufai, Y., Basar, N., & Sani, A. (2019a). Optimization and Isolation of 4,8,12,16-Tetramethylheptadecan-4-olide from *Deinbollia pinnata*. *Asian Journal of Chemistry*, 31(11), 2503–2511. <https://doi.org/10.14233/ajchem.2019.22165>
- Rufai, Y., Basar, N., & Sani, A. (2019b). Optimization and Isolation of 4,8,12,16-Tetramethylheptadecan-4-olide from *Deinbollia pinnata*. *Asian Journal of Chemistry*, 31(11), 2503–2511.
- Rufai, Y., Basar, N., & Sani, A. (2020). *Deinbollia pinnata*: Isolation and Characterisation of Some Phytochemicals from Aerial Parts and Their Antioxidant Potential. *The Natural Products Journal*, 10, 1–12. <https://doi.org/10.2174/2210315510666200130110420>
- Rufai, Y., Basar, N., & Suleiman, K. (2023). Anti-inflammatory activity of *Deinbollia pinnata* leaves with isolation of triterpenoids. *Journal of Chemical Society of Nigeria*, 48(4), 751–766.

- Sangeeth Shyam Sundar, S. S., Rajamanickam, C., Saraswathy, S., Venkatesan, K., Balakumbahan, R., Vijayasamundeeswari, A., & Sankar, C. (2024). Sapindaceae Fruits: A Comprehensive Overview on Phytochemicals, Nutraceuticals and Health Benefits Application. *Plant Science Today*, 11(4), 1–15. <https://doi.org/10.14719/pst.5065>
- Shah, S., & Singh, S. K. (2025). Phytochemical Analysis and Characterisation of Cancer-Cure Medicinal Plants: A Comprehensive Review. *International Journal of Environmental Sciences*, 11(10), 592–606.
- Sharaf, M. (2023). Phenolic Compounds: Promising Anti-Viral Agents: A Review. *Egyptian Journal of Chemistry*, 66(13), 1711–1718. <https://doi.org/10.21608/EJCHEM.2023.221988.8239>
- Siddiqui, T., Khan, M. U., Sharma, V., & Gupta, K. (2024). Terpenoids in essential oils: Chemistry, classification, and potential impact on human health and industry. *Phytomedicine Plus*, 4(2), 100549. <https://doi.org/10.1016/j.phyplu.2024.100549>
- Song, X. (2024). Antibacterial, Antifungal, and Antiviral Bioactive Compounds from Natural Products. *Molecules*, 29(4), 4–8. <https://doi.org/10.3390/molecules29040825>
- Sotubo, S. E., Lawal, O. A., Osunsami, A. A., & Ogunwande, I. A. (2016). Constituents and insecticidal activity of *Deinbollia pinnata* essential oil. *Natural Product Communications*, 11(12), 1889–1890. <https://doi.org/10.1177/1934578x1601101228>
- Suebkhampet, A. (2024). Using Dye Plant Extract for Histological Staining. *Journal Of Mahanakorn Veterinary Medicine*, 9(1), 1–17.
- Sułkowska-Ziaja, K., Trepka, M., Olechowska-Jarząb, A., Nowak, P., Ziaja, M., Kała, K., & Muszyńska, B. (2023). Natural Compounds of Fungal Origin with Antimicrobial Activity—Potential Cosmetics Applications. *Pharmaceutics*, 16(9), 1–32. <https://doi.org/10.3390/ph16091200>
- Timilsena, Y. P., Phosanam, A., & Stockmann, R. (2023). Perspectives on Saponins: Food Functionality and Applications. *International Journal of Molecular Sciences*, 24(17), 1–22. <https://doi.org/10.3390/ijms241713538>
- Tiwari, S., & Talreja, S. (2022). Thin Layer Chromatography (TLC) VS. Paper Chromatography: A Review. *Acta Scientific Pharmaceutical Sciences*, September, 05–09. <https://doi.org/10.31080/asps.2022.06.0894>
- Urbain, A., & Simões-Pires, C. A. (2020). Thin-Layer Chromatography for the Detection and Analysis of Bioactive Natural Products. *Encyclopedia of Analytical Chemistry*, December 2020, 1–29. <https://doi.org/10.1002/9780470027318.a9907.pub2>
- Uzor, P. F. (2020). Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. *Evidence-Based Complementary and Alternative Medicine*, 2020(8749083), 1–17. <https://doi.org/10.1155/2020/8749083>
- Widsten, P., Cruz, C. D., Fletcher, G. C., Pajak, M. A., & McGhie, T. K. (2014). Tannins and extracts of fruit byproducts: Antibacterial activity against foodborne bacteria and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 62(46), 11146–11156. <https://doi.org/10.1021/jf503819t>
- Yang, Y., & Ling, W. (2025). Health Benefits and Future Research of Phytochemicals: A Literature Review. *Journal of Nutrition*, 155(1), 87–101. <https://doi.org/10.1016/j.tjnut.2024.11.007>