Physicochemical Evaluation, Fluorescence Analysis, and Preliminary TLC Analysis of *Culcasia Falcifolia* Leaves

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ABSTRACT

Aim: The present study aimed to evaluate the physicochemical properties, fluorescence characteristics, and preliminary thin-layer chromatography (TLC) analysis of *Culcasia falcifolia* leaves.

Methods: The physicochemical parameters were evaluated which includes determination of the values of total ash, acid-insoluble ash, water-soluble ash, sulphate ash, water-soluble extractive value, alcohol-soluble extractive value, and loss on drying. Fluorescence analysis of the leaf powder was performed under visible light and ultraviolet light at 254 and 366 nm following treatment with different chemical reagents. Thin-layer chromatography (TLC) of the ethanol leaf extract was carried out using ethyl acetate, formic acid, acetic acid, and water (100:11:11:26, v/v/v/v) as the mobile phase.

Results: The study showed the following values 10.3 ± 0.33%, 0.11 ± 0.05%, 5.36 ± 0.03%, and 0.19 ± 0.07% of the following parameters total ash, acid-insoluble ash, water-soluble ash, and sulphate ash values were respectively. The water-soluble and alcohol-soluble extractive values were 21.8 ± 0.24% and 6.2 ± 0.27%, respectively, while loss on drying was 5.26 ± 0.12%. Fluorescence analysis showed different color variations after the treatment of the leave of *C. falcifolia* with different reagents under visible and ultraviolet light. TLC analysis showed seven spots with retardation factor (Rf) values ranging from 0.27 to 0.94.

Conclusion: The study provides the preliminary physicochemical, fluorescence, and chromatographic data to support the quality assessment and pharmacognostic characterization of *C. falcifolia* leaves.

Keywords: *Culcasia falcifolia,* physicochemical evaluation, fluorescence analysis, thin-layer chromatography.

INTRODUCTION

Medicinal plants have been used to treat various diseases across different cultures for centuries. The bioactive compounds in medicinal plants are the important sources for the development of modern therapeutic agents. Approximately 40% of currently used drugs are reported to originate directly or indirectly from natural sources (Calixto, 2019). The use of herbs and herbal products, in both developing and developed countries, for the treatment of various diseases has increased dramatically in recent years. However, the major drawback in promoting the use of medicinal plants is the lack of standardization as well as the confusion in the identification of the plant and their substitutes or adulterants. To ensure reproducible quality of herbal plants, authentication is invaluable. The pharmacognostical studies not only give the authentication but also quality and purity standards of the plant (Annan et al. 2013).

Herbal medicines are frequently considered to be safer than conventional medicines because of their better tolerance. But there are reports of side effects and adverse reactions that have been related to herbal medicines (Abebe, 2002). Some of these side effects and adverse reactions are due to the intrinsic bioactive secondary metabolites present in the herbal materials (Siraj et. al 2020). According to WHO chapter of standardization and quality control of herbal drugs, standardization involves the physicochemical evaluation of crude drug covering aspects such as selection and handling of crude material. Most of the attention is normally paid to quality indices

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including macro and microscopic examination, ash values, moisture content, extractive values, crude fiber, qualitative and quantitative chemical evaluation and chromatographic examination (Alam and Saqib, 2015).

Fluorescence analysis of crude plant drugs further serves as a rapid and reliable diagnostic tool in pharmacognosy. Many phytoconstituents either fluoresce naturally or exhibit fluorescence upon treatment with acidic, alkaline, or oxidizing reagents, producing characteristic color changes under visible and ultraviolet light. (Chase and Pratt, 1949). The distinctive colors observed under UV light serve as a simple, rapid, and reliable pharmacognostic parameter for the identification and quality control of crude drugs (Khatoon et al. 2015).

Thin-Layer Chromatography (TLC) is another indispensable technique used to separate complex mixtures of plant compounds. It provides insight into the number and nature of chemical constituents present in crude extracts and helps detect similarities across species by comparing retention factor (Rf) values (Stahl, E 2005)

Culcasia falcifolia is a perennial climber, epiphytic on trees and growing to several meters, stem with adventitious roots, penetrating bark with short clasping roots at the nodes. The leaves are lanceolate to narrowly ovate or elliptic to oblong, leathery, dark glossy green, acuminate to 30 cm long. Spathe is greenish-white, rigid and waxy, 6 cm long; spadix almost as long as spathe, cream-yellowish, foetid. Inflorescences are short separate branches, surrounded by a thick leathery, boat-shaped spade, greenish or yellowish white. Fruits are in a cluster of obovoid berries orange-red when ripe (Ghogue, 2010). The plant is native to damp evergreen forest in shade; riverine and swamp forests; marshy forest. It is found in Kenya, Ethiopia, Malawi, Tanzania, Uganda, Zambia, Zimbabwe (Ghogue, 2010). The leaves of *Culcasia falcifolia* are used as ash (internal) for dry cough, edema, and epilepsy**.** It is used as a tonic or ashes taken with porridge. *Culcasia falcifolia* is called as Chepnamobon/ Kipnamobon in Aldai division of south Nandi district (Pascaline *et al.,* 2008).

MATERIALS AND METHODS

Plant Material and Authentication

The leaves of *Culcasia falcifolia* were collected from along the river Kiŋ̀wal in Kaptildil, Nandi County, Rift Valley, Kenya. The leaves were identified and authenticated from the National Herbarium, Kenya (voucher number PS 22/05). The leaves were thoroughly washed with tap water to remove dust and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade in the laboratory for two weeks. The air dried leaves were ground to powder using laboratory electric blender to obtain a powder. The powder was further passed through a sieve to obtain finer particles. The leaf sample was stored in a clean dry labeled glass beaker until needed for extract preparation and analysis.

Extract preparation

The leaf sample was mixed and macerated with absolute ethanol at a 1:10 ratio (100 g in 1 litre of solvent) for 7 days. Then the extract was filtered through Whatman No 1 filter paper followed by evaporation of the supernatant using the BUCHI Switzerland rotary evaporator to remove the ethanol and to obtain concentrated, oily extract. The crude extract was stored in sterile universal glass bottle until further use.

Physicochemical Parameters

The following parameters were evaluated: total ash, acid-insoluble ash, water-soluble ash, sulphate ash, water-soluble extractive value, alcohol-soluble extractive value, and loss on drying.

Determination of Total Ash

Approximately 2 to 3 g accurately weighed powdered *Culcasia falcifolia* was incinerated in a tared platinum or silica dish at a temperature not exceeding 450º until the sample was free from carbon. The crucible was cooled and weighed. The total ash content was expressed as a percentage of the air-dried plant material (Government of India, 2001).

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Determination of Sulphate Ash

A silica or platinum crucible was heated to redness for 10 minutes, allow to cool in a desiccator and weigh. Accurately weighed 1 to 2 g of the powdered *Culcasia falcifolia,* was put into the crucible; gently ignited at first, until the substance is thoroughly charred. The residue was cooled, moistened with 1 ml of H_2SO_4 heat gently until white fumes were no longer evolved and ignite at 800º ± 25º until all black particles have disappeared. The ignition was conduct the ignition in a place protected from air currents. The crucible was allowed to cool, a few drops of H_2SO_4 were added and the crucible was heated. Then it was ignited as before, allow to cool and weighed. The operation was repeated until two successive weighing did not differ by more than 0.5 mg (Government of India, 2001).

Determination of Acid Insoluble Ash

To the crucible containing total ash, 25 mL of dilute HCl was added. The insoluble matter was collected on an ash less filter paper (Whatmann number 41) and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The content of the insoluble ash was calculated with reference to the air dried plant material (Government of India, 2001).

Determination of Water Soluble Ash

The ash was boiled for 5 minutes with 25 ml of water; insoluble matter was collected in a Gooch crucible or on an ash less filter paper, wash with hot water, and ignited for 15 minutes at a temperature not exceeding 450Ċ. The difference in the weight of the insoluble matter and the weight of ash represented the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried plant material (Government of India, 2001)

Determination of Moisture Content (Loss on Drying)

About 10 g of plant material was placed (without preliminary drying) after accurately weighing it in a tarred evaporated dish. This was dried at 105°C for 5 h and weighed. Drying and weighing was continued at 1 h interval until we got the constant weight. Constant weight was reached when two consecutive weights, after drying for 30 min. and cooling for 30 min. in a desiccator, showed not more than 0.1 g difference (Government of India, 2001).

Determination of water-soluble extractive value

5 g of air-dried, coarsely powdered *Culcasia falcifolia* leaves was accurately weighed and transferred into a clean, dry, glass-stoppered conical flask containing 100 mL of distilled water and shaken thoroughly. The mixture was allowed to macerate for 24 hours with frequent shaking during the first 6 hours, and followed by undisturbed standing for the remaining 18 hours. The mixture was then filtered through dry filter paper. 25 mL aliquot of the filtrate was transferred into a previously weighed, flat-bottomed evaporating dish and evaporated to dryness on a water bath. Then the residue was dried at 105°C until a constant weight was obtained. The dish was cooled in a desiccator and weighed. The water-soluble extractive value was calculated as a percentage of the air-dried plant material (Khandelwal 2008).

Determination of alcohol-soluble extractive value

5 g of air-dried, coarsely powdered *Culcasia falcifolia* leaves was accurately weighed and transferred into a clean, dry, glass-stoppered conical flask. Ethanol 100 mL of specified concentration (95%) was added, and the mixture was shaken thoroughly and was allowed to macerate for 24 hours with frequent shaking during the first 6 hours, and followed by undisturbed standing for the remaining 18 hours. The mixture was then filtered through dry filter paper. 25 mL aliquot of the filtrate was transferred into a previously weighed, flat-bottomed evaporating dish and evaporated to dryness on a water bath. Then the residue was dried at 105°C until a constant

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weight was obtained. The dish was cooled in a desiccator and weighed. The alcohol-soluble extractive value was calculated as a percentage of the air-dried plant material (Khandelwal 2008).

Fluorescence analysis

Air-dried powdered sample of *C. falcifolia* leaf was used for fluorescence analysis. The sample was treated with different reagents like 5% aqueous sodium hydroxide, 60% aqueous sulphuric acid, glacial acetic acid, 50% nitric acid, 25% ammonia solution, 5% ferric chloride, picric acid, and iodine solution (Chase and Pratt 1949). To 0.5gm of the powdered sample of *C. falcifolia* a few drops of the following reagents and mixed well with glass rod. Then the treated and untreated powder sample was observed under visible light, Ultraviolet light longwave 365 nm and Ultraviolet short wavelength 254 nm. The specific color changes, fluorescence colors produced by each reagent were noted and recorded.

Thin Layer Chromatography (TLC) analysis

Ethanol leaf extract of *C. falcifolia* was applied on pre-coated silica gel, Ethyl acetate: formic acid: acetic acid: water (100:11:11:26 v/v/v/v) mixture was prepared as mobile phase for separating the compounds. The development chamber was lined with the mobile phase before the plate was placed in the chamber. The plate was placed in the chamber and the solvent was allowed to migrate up the plate. the solvent front had travelled 5cms from the origin. After development, the plates were removed from the developing chamber, the solvent was marked and the plate was allowed to dry. The chromatograms were examined under ultraviolet (UV) light at 366 nm using a UV TLC viewer and the positions and fluorescence characteristics of the spots were recorded. The retention factor (Rf)) of each spot was calculated by dividing the distance travelled by the spot/ from the origin by the distance travelled by the solvent front (Harborne, 1973).

Statistical analysis

The results of the physicochemical parameter were determined using descriptive statistics and expressed as mean ± standard error of the mean (SEM). Each parameter was determined using three measurements (n=3). The Rf values were obtained from three trials and were analyzed using descriptive statistics. The arithmetic mean was calculated for each spot to summate the results.

RESULTS AND DISCUSSION

Physiochemical parameters

The results of the physicochemical parameters are presented in table 1. The results revealed total ash value of *Culcasia falcifolia* was10.3 ±0.33 %, represents the total inorganic residue remaining after incineration of the plant material. The acid-insoluble ash value was 0.11 ± 0.05% indicate relatively low inorganic residues of siliceous materials. The reported acid-insoluble ash values were (2.726 ± 0.080%) w/w for C. sativa leaves and 3.14% for P. cineraria material (Singh et al., 2022). The water-soluble ash value was 5.36 ± 0.03% which shows that measurable portion of inorganic residue was water soluble. The sulphated ash value was 0.19 ± 0.07% which indicates residue is low after treatment with sulphuric acid. The water-soluble extractive value was 21.8 ± 0.24% whereas the alcohol-soluble extractive value was 6.2 ± 0.27% indicates that greater portion of waster was used for extraction than alcohol. A comparable investigation of *P. cineraria* reported water-soluble extractive values of 24.82% and ethanol-soluble extractive values of 12.19% (Singh et al., 2022). The loss on drying was used to estimate the moisture content and the value was 5.26 ± 0.12 indicates low portion of mass lost during the drying procedure. Limpabandhu et al. (2024), in their pharmacognostic standardization study of *Ficus rumphii* leaves, reported a loss-on-drying value of (6.87 ± 0.79%), while a study of Cannabis sativa leaves reported (4.068 ± 0.084%) w/w. These findings provide a preliminary physicochemical profile that may be useful for the quality assessment and future standardization of the plant material.

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Table No: 1. Physicochemical parameters of the leaves of *culacasia falcifolia*

Parameters	Values% (w/w) * (SEM±)
Total ash	10.3±0.33%
Acid insoluble ash	0.11±0.05%
Water soluble ash	5.36±0.03 %
Sulphated ash	0.19±0.07%
Water soluble extractive value	21.8±0.24 %
Alcohol soluble extractive value	6.2±0.27 %
Loss on Drying/Moisture Content	5.26±0.12 %

Note: Values are presented as mean ±standard error of the mean (SEM), n=3

Fluorescence analysis

The leaf powder of *C. falcifoli*a were studied under visible light, and ultra violet short (254nm) and long wavelength (366nm) light for fluorescence characters following treatment with different reagents including NaOH, Sulphuric acid, Acetic acid, HNO3 (FeCl3), NH3 solution, Iodine, and Picric Acid. The results are presented in table 2. The powdered sample showed various color changes including dark green, black, dark brown, yellowish brown, purple, light brown, light green. These variations in color and fluorescence characteristics may reflect differences in the chemical compounds and fluorescence substances of the plant material. Biswal et al. (2011) also have similar statement, that fluorescence is important to detect the presence of phytoconstituents and fluorescent compound in crude powder when shows color changing with UV and various reagent. The fluorescence analysis serves as a preliminary method for the identification and standardization of medicinal plant. The variations in fluorescence responses of the studied plant confirm the presence of diverse groups of phytochemicals and demonstrate their diagnostic value in plant identification and standardization studies (Patel and Thakur 2023). Therefore, the fluorescence analysis provides a simple preliminary method for characterization of powdered *C. falcifolia* leaves and may contribute to future pharmacognostic standardization.

Table 2: Fluorescence Analysis of *Culcasia falcifolia* leaves

S. No	Test	Visible Light	Under UV light short wavelength (254 nm)	Under UV light Long wavelength (366 nm)
1	Powder as such	Dark green	Dark green	Black
2	Powder + 5% Aqueous NaOH	Dark green	Black	Black
3	Powder + Aqueous 60% H_2SO_4	Dark Brown	Black	Black
4	Powder + Glacial Acetic acid	Yellowish Brown	Light Green	Purple
5	Powder + 50% HNO_3	Yellowish Brown	Dark Green	Purple
6	Powder + Ammonia solution 25% v/v	Dark Brown	Dark Green	Black

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7	Powder + 5% Ferric chloride	Light Brown	Light Green	Black
8	Powder + Picric acid	Yellowish Brown	Light Green	Brown
9	Powder + Iodine Solution	Black	Light Green	Black

Thin layer chromatography

TLC analysis of the ethanol extract of *Culcasia falcifolia* was performed in three separate runs. Seven spots A-G were observed with mean Rf values ranging from 0.27 to 0.94 as shown in table 3. Following the exposure to ammonia vapor and examination *under UV light (366nm), s*pots B–G exhibited bright yellow coloration, whereas the color of spot A was not recorded. The mean Rf values of spot A was 0.27, spot B was 0.36, spot C was 0.45, spot D 0.54, spot E was 0.65, spot F 0.85 and spot G was 0.94. Differences in the observed Rf values and fluorescence characteristics suggest the presence of several chromatographically different constituents. These observations are not enough to confirm the identities of individual compounds as different phytoconstituents may exhibit similar chromatographic migration and fluorescence response. Moreover, Rf values and fluorescence characteristics can vary with different conditions and detection methods. Therefore, the compounds observed as spots cannot be assigned specific identities based only on TLC results.

A study of *Dimocarpus longan* leaf extract using ethyl acetate–formic acid–acetic acid–water (100:11:11:26, v/v/v/v) reported multiple chromatographic fractions and distinct fluorescence responses under UV illumination at 254 and 365 nm. This study supports the use of this solvent system for preliminary chromatographic profiling of plant constituents (Pimjai et al. 2023). The findings of TLC should be regarded as a preliminary basis for chromatographic profile. A limitation of the TLC was the absence of reference standards for comparison. Further investigation using authenticated standards and complementary analytical methods such as high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) would be required to establish compound identification.

Table 3: TLC profile of the ethanol leaf extract of *Culcasia falcifolia*

Spot	**Trail**	**Trial 2**	**Trial 3**	**Mean Rf values**	**Color in UV + Ammonia**
A	0.27	0.27	0.27	0.27	
B	0.36	0.36	0.36	0.36	Bright Yellow
C	0.45	0.44	0.45	0.45	Bright Yellow
D	0.54	0.54	0.54	0.54	Bright Yellow
E	0.65	0.65	0.65	0.65	Bright Yellow
F	0.85	0.86	0.85	0.85	Bright Yellow
G	0.96	0.92	0.96	0.94	Bright Yellow

Note: Rf values are averages from three separate measurements, mobile phase ethyl acetate: acetic acid: formic acid: water 100:11:11:26 (v/v); UV light 366nm.

CONCLUSION

The present study provided a preliminary assessment on the physicochemical parameter, fluorescence analysis and TLC analysis of *Culcasia falcifolia* leaves. The findings of the present study provide a baseline data that may support the quality assessment and pharmacognostic characterization of *C. falcifolia*. However, the study was limited by the absence of reference standards. Therefore, further studies using authenticated reference

standards and validated analytical techniques are recommended to confirm the phytoconstituents present in the plant extract.

REFERENCES

1. Abebe, W. (2002). Herbal medication: Potential for adverse interactions with analgesic drugs Journal of Clinical Pharmacy and Therapeutics, 27(6), 391–401. https://doi.org/10.1046/j.1365-2710.2002.00444.x.
2. Alam, F., & Us Saqib, Q. N. (2015). Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of Zanthoxylum armatum (Rutaceae). Ancient Science of Life, 34(3), 147–155. https://doi.org/10.4103/0257-7941.157159.
3. Annan, K., Dickson, R. A., Amponsah, I. K., Jato, J., & Nooni, I. K. (2013). Pharmacognostic evaluation and physicochemical analysis of Paullinia pinnata L. (Sapindaceae). Journal of Pharmacognosy and Phytochemistry, 2(2), 203–208.
4. Biswal, B., Saha, D., Beura, S., Jana, S. B., Koley, A., Sur, D., et al. (2011). Pharmacognostic studies of leaves of Derris indica. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(1), 294–296.
5. Calixto, J. B. (2019). The role of natural products in modern drug discovery. Anais da Academia Brasileira de Ciências, 91(Suppl. 3), Article e20190105. https://doi.org/10.1590/0001-3765201920190105.
6. Chase, C. R., & Pratt, R. J. (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of the American Pharmaceutical Association, 38(6), 324–331.
7. Do, T. K. T., Trettin, I., de Vaumas, R., Cañigueral, S., Valder, C., & Reich, E. (2021). Proposal for a standardized method for the identification of essential oils by HPTLC. Pharmeuropa Bio & Scientific Notes, 157–166.
8. Ghogue J.P. & Ali M. 2010. *Culcasia falcifolia*. The IUCN Red List of Threatened Species 2010: http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS
9. Government of India, Department of Indian Systems of Medicine and Homoeopathy. (2001). The Ayurvedic pharmacopoeia of India (Part I, Vol. III). Ministry of Health and Family Welfare.
10. Khandelwal, K. R. (2008). Practical pharmacognosy: Techniques and experiments (19th ed.). Nirali Prakashan.
11. Klein-Junior, L. C., de Souza, M. R., Viaene, J., Bresolin, T. M. B., de Gasper, A. L., Henriques, A. T., et al. (2021). Quality control of herbal medicines: From traditional techniques to state-of-the-art approaches. Planta Medica, 87, 964–988. https://doi.org/10.1055/a-1529-8339.
12. Limpabandhu, T., Suwatronnakorn, M., Widoyanti, A. A. E., Issaravanich, S., Zongrum, O., & Prasansuklab, A. (2024). Pharmacognostic standardization and phytochemical evaluation of Ficus rumphii Blume leaves in Thailand. Phytomedicine Plus, 4(4), Article 100661.
13. Khatoon, S., Irshad, S., Rawat, A. K. S., & Misra, P. K. (2015). Comparative pharmacognostical studies of blue and white flower varieties of Clitoria ternatea L. Journal of Pharmacognosy & Natural Products, 1, 109. https://doi.org/10.4172/jpnp.1000109.
14. Patel, R., & Thakur, M. (2023). Fluorescence analysis and phytochemical screening for authentication of medicinal plants. South African Journal of Botany, 157, 398–405.
15. Pimjai D., Sunee C., Worrapan P., Busaban S., Siripat C. Pimporn L. (2023). Selection of bioactive chemical markers for anti-aging skin assessment of *Dimocarpus longan* Lour. leaf extract. Arabian Journal of Chemistry 16, 104954. doi: 10.1016/j.arabjc.2023.104954.
16. Reich, E., Schibli, A., & DeBatt, A. (2008). Validation of high-performance thin-layer chromatographic methods for the identification of botanicals in a cGMP environment. Journal of AOAC International, 91(1), 13–20. https://doi.org/10.1093/jaoac/91.1.13.
17. Siraj, J., Belew, S., & Suleman, S. (2020). Ethnobotanical assessment and physicochemical properties of commonly used medicinal plants in Jimma Zone, Southwest Ethiopia: Traditional healers based cross-sectional study. Journal of Experimental Pharmacology, 12, 665–681. https://doi.org/10.2147/JEP.S267903.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue X October 2026

18. Singh, V., Sara, U. S., & Rizvi, A. (2022). Pharmacognostic evaluation and establishment of quality parameters of leaves of Prosopis cineraria. Research Journal of Pharmacy and Technology, 15(2), 621–627. https://doi.org/10.52711/0974-360X.2022.00102.
19. Stahl, E. (2005). Thin-layer chromatography: A laboratory handbook. Springer-Verlag.