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Phytochemical Profiling and TLC Analysis of *Hymenocardia acida* Methanol Leaf Extract

Abdulgafar Omeiza Zubair
Department of Chemistry, Confluence University of Science and Technology, Osara-Kogi State, Nigeria.

Corresponding author*
abdulgafarzubair1@gmail.com

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Abstract

The study investigated the phytochemical constituents and chromatographic profile of *Hymenocardia acida* leaf extract obtained through methanol extraction. *Hymenocardia acida*, widely used in traditional medicine across Africa, is reputed for its therapeutic potential in treating various ailments. To explore the bioactive compounds, we conducted a series of qualitative phytochemical screenings to detect the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids. The results revealed a broad spectrum of phytochemicals known to exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Additionally, thin-layer chromatography (TLC) was performed to obtain a chromatographic profile of the methanol extract, aiming to separate and identify potential bioactive compounds. Silica gel plates were used as the stationary phase, and various solvent systems, such as hexane acetate and chloroform were optimized for effective separation of phytochemicals. The resulting TLC plates were analyzed under UV light at 254 nm and 365 nm, and retention factor (Rf) values were calculated to provide preliminary identification. Different solvent systems were used which showed different behaviors in the plant compounds. The resulting mixture between hexane and ethyl acetate at ratio 9:1, 8:2, 7:3, 6:4 and 5:5 are given as (0), (0,0.26,0.16), (0,0.95,0.94), (0) and (0,0.91,0.95) respectively. The TLC fingerprint and phytochemical profile generated from this study offer valuable insights into the potential pharmacological applications of *Hymenocardia acida*, supporting its use in traditional medicine and guiding further isolation and characterization of its active compounds for drug development.

Keywords: Phytochemical; Methanol; Chromatography; Silica gel; Retention factor.

INTRODUCTION

Herbs have always been used for therapeutic purposes. *Hymenocardia acida* belongs to the family *Hymenocardiaceae* and is a native plant of tropical Africa, often used in traditional remedies. The plant is known by various local names across different tribes in Nigeria, "*Eso-ogun*" (Yoruba), "*Ega*" (Ebira), "*Ogilisi*" (Igala), "*Daura*" (Hausa), " *Udara* " (Igbo) (Usman et al., 2021). The leaves, bark, and roots are used in the treatment of fevers, skin conditions, gastrointestinal disorders, and inflammatory diseases. It has been found to have great therapeutic relevance (Traore *et al*., 2022).

The plant indicates by some extent scientific data (Felix *et al*., 2019; Lawal *et al*., 2024). The bioactive compounds plays an important role in minimising oxidative stress (Rafi *et al*., 2020; Mathieu *et al*., 2024). However, further studies are needed to show the plant as an anticancer potential (Adedokun *et al*., 2022).

These activities demonstrate the use of the plant in the management of inflammatory challenges (Mathieu *et al*., 2024). Studies suggest its importance in the development of ant- infectious diseases drugs (Agbidye *et al*., 2020; Kebede *et al*., 2021). The present study investigates the phytochemical constituents and chromatographic profile of H. acida leaf extract obtained through methanol extraction.

MATERIALS AND MAETHODS

Materials

Apparatus included Round bottomed flask, Water-bath, Glass wool, Heating mantle, Anti-bumping chips, pre-coated silica gel plate, rotary evaporator and Weighing balance, beakers, separating funnel and thin-layer chromatographic plate.

Reagents

The solvent used were of analytical grades with no further purification obtained from GH Tech chemicals, China which include; Methanol (m.mass = 34.04, m.p=-98^{0}c, b.p=64.5^{0}c, Assay= 99.50%, Density= 0.791-0.793g/cm^3), Ethyl ethanoate(Assay=99%), Density=0.899-0.902g/cm^3, Chloroform (m.mass = 119.38g/mol, b.p=61^{0}c, Assay= 99.50%, Density= 1.49g/cm^3), n-hexane (m.mass = 86.18, m.p=94.3^{0}c, b.p=69^{0}c, Assay= 97.0%, Density= 0.059-0.663g/cm^3),

distilled water, Sodium Hydroxide, Meyer's reagent, Fehling Solution, Molisch's reagents, Wagner's reagents, Hydrochloric acid etc

Sampling

The plant was collected from Idah metropolis, by Mr Upahi Lookman from department of Biology, Confluence University of Science and Technology, Osara Kogi State.

Preparation of extract

The leafs of *H. acida* was firstly rinsed with warm water to remove any adsorbed particles and dried at (26 °C) for a month. It was grounded to a fine powder with mortar and pestle, and also with the use of a mechanical grinder and sieved. The extraction was done using cold maceration by soaking 1200 g of dried powdered sample in 1500 cm^3 of hexane for defatting. The filterate was collected, followed by continuous extraction until their was no observable colour inference from the sample. The Hexane filterate was collected, concentrated and weighed for further analysis. The dried residue was soaked in methanol for 3days. At the end of extraction, the plant extracts was filtered (whatman no 10). The filtrate was then concentrated at 40°C. The yield of *H. acida* methanol crude was also calculated.

Phytochemical Screening of H. acida

The presence of the bioactive compounds from the seeds of H. acida forms a platform for further pharmacological studies (Agbidye et al., 2020).

Fractionation of the Methanol Extract

A portion (13 g) of dried methanolic extract was transferred into a Bama bottle and about 200 cm^3 of hexane was added and shaken for 2 min and left for about 3 min, it was then filtered and the filtrate was collected using another Bama bottle. It was then concentrated at room temperature to prevent denaturation of the extract. The process was repeated multiple times until there was no observable colour from the extract on the solvent. The residue was dried and same procedure was carried out using chloroform and then followed by Ethyl acetate leaving behind the residual fraction. The solvent was evaporated from each fraction, weighed and its percentage yield was calculated (Nwauzoma *et al*., 2013).

RESULTS AND DISCUSSIONS

Results

Table 1. Percentage yield of *H. acida* Methanol Extract.

Extracts	Yield (G)	Percentage Yield (%)
Methanol	13.5	5.97
Hexane	5.5	2.4
Chloroform	0.1	0.04
Ethyl acetate	1.9	3.11

Table 2. Phytochemical screening of H. acida methanol extract.

Phyto-compounds	Inference (Crude Extract)
Alkaloid	+ve
Carbohydrates	-ve
Reducing Sugars	-ve
Protein	+ve
Flavonoid	+ve
Phenols	+ve
Tannins	+ve

Table 3. Phytochemical screening of *H. acida* hexane extract.

Phyto-compounds	Inference (Crude Extract)
Alkaloid	-ve
Carbohydrates	-ve
Reducing Sugars	-ve
Protein	-ve
Flavonoid	-ve
Phenols	-ve
Tannins	+ve

Table 4. Phytochemical screening of *H. acida* chloroform extract.

Phyto-compounds	Inference (Crude Extract)
Alkaloid	-ve
Carbohydrates	-ve
Reducing Sugars	-ve
Protein	-ve
Flavonoid	-ve
Phenols	-ve
Tannins	-ve

Table 5. Phytochemical screening of *H. acida* ethyl acetate extract.

Phyto-compounds	Inference (Crude Extract)
Alkaloid	+ve
Carbohydrates	-ve
Reducing Sugars	-ve
Protein	+ve
Flavonoid	+ve
Phenols	+ve
Tannins	-ve

Table 6. TLC Values for each fraction of *H. acida* methanol extract.

System	Rf (hexane,chloroform,ethylacetate)	No of Spot
9:1	-	0
8:2	0,0.26,0.16	3
7:3	0,0.95,0.94	2
6:4	-	0
5:5	0,0.91,0.95	2

CONCLUSION

The phytochemical screening of *H. acida* methanol leaf extract revealed the presence of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, proteins. Fractionated screening further indicated some of the

bioactive compounds present in the ethyl acetate extract. This extract was subsequently analyzed using thin-layer chromatography (TLC) which revealed numbers of spots when exposed to different solvent systems starting from the 10:0 to 5:5 ratio. These results served as a guide for selecting an appropriate solvent system for isolation and characterization. This shows that most of the polar compounds detected in the phytochemical screenings are present in the chloroform fraction, albeit in low yield, as well as in the ethyl acetate fraction. This reinforces the plant's relevance in herbal medicine and its potential for further pharmacological applications.

Recommendation: Further studies should be focused on the isolation of ethyl acetate extract for phenolics and alkaloids bio guided assay.

REFERENCES

Adedokun, O., Ntungwe, E. N., Viegas, C., Adesina Ayinde, B., Barboni, L., Maggi, F., Saraiva, L., Rijo, P., and Fonte, P. (2022). Enhanced anticancer activity of *Hymenocardia acida* stem bark extract loaded into PLGA nanoparticles. *Pharmaceuticals (Basel, Switzerland)*. **15**(5).

Agbidye, I. G., Amua, Q. M., Nwankwo, J. C., and Iortyom, S. D. (2020). Phytochemical screening and antimicrobial analysis of *Hymenocardia acida*. *Chemistry Research Journal*. **5**(3): 81–93.

Felix, A. D., Victoria, M. A., Sustain, K. O., Masaudat, A. A., and Funmilola, B. (2019). In-vitro antioxidant analysis and phytochemical evaluations of the aqueous extracts of *Hymenocardia acida* (Orupa) leaf, stem, root & bark. *International Conference of Science, Engineering & Environmental Technology*. **4**(2): 8–15.

Kebede, T., Gadisa, E., and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS ONE*. **16**(3).

Lawal, S. M., Inabo, H. I., Ella, E. E., and Okubanjo, O. O. (2024). In vitro anti-trypanosomal activity of ethanolic and n-hexane extracts of *Hymenocardia acida* Stem Bark against Trypanosoma bruceibrucei and Trypanosoma congolense. *UJMR*: 560–566.

Mathieu, M. E. J., Lucie, O., Omam, Elisabeth, N. B., Achick, T. E., Roger, A., Hermann, C., Nina, N., Pamela, T., Marie-Claire, F., Wilfred, M., and Charles, F. (2024). Evaluation of the antioxidant capacity and studies of the anticonvulsant properties of *Hymenocardia acida*. *JABB*. **27**(3): 129–139.

Nwauzoma, A.B., Chondhary, M.I., Ali, Z. and Olaiya, O, C. (2013). Biological activity of crude extracts of citrus species from Nigeria. Nature and Science: 11(7): 135-139.

Rafi, M., Meitary, N., Septaningsih, D. A., and Bintang, M. (2020). Phytochemical profile and antioxidant activity of Guazuma ulmifolia leaves extracts using different solvent extraction. *Indonesian Journal of Pharmacy*. **31**(3): 171–180.

Traore, M. S., Camara, A., Balde, M. A., St. Diallo, M., Barry, N. S., Balde, E. S., and Balde, A. M. (2022). Ethnobotanical survey of medicinal plants used to manage hypertension in the Republic of Guinea. *J Pharm Pharmacogn Res*. **10**(5): 938–951.

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