Selective Isolation and Characterization of *Euphorbia hirta* Extract Metabolites

Adeola A. Fadairo[1], Isiaka Mohammed[2], Pamela O. Akindeko[3], John Kennedy Onyebuchi[4], Ibrahim A. Oladosu[5]*

University of Ibadan

***Corresponding Author**

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ABSTRACT

Euphorbia hirta (family Euphorbiaceae) is a medicinal herb used traditionally for treating inflammation, respiratory conditions and gastrointestinal disorders. This study aimed to isolate and characterize the bioactive compounds in *Euphorbia hirta* extracts to validate its therapeutic potential. The plant material was collected and authenticated at the University of Ibadan Herbarium (voucher no. UIH-23576). Methanolic extraction was carried out after air-drying and pulverization. Phytochemical screening and selective isolation were performed using lead acetate precipitation, solvent partitioning and column chromatography. Characterization was achieved through FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids/steroids, phenols and reducing sugars, whereas glycosides and anthocyanins were absent. FT-IR spectra confirmed the presence of hydroxyl, carbonyl, C–O, and aromatic C=C functional groups. NMR data indicated oxygenated methines and aromatic quaternary carbons consistent with flavonoid glycosides. Two fractions (Rf = 0.4 and 0.5) were isolated as green amorphous powders containing a sugar moiety. These findings confirm *Euphorbia hirta* as a rich source of bioactive phytochemicals, supporting its ethnomedicinal use and highlighting its potential for natural product drug development.

Keywords: *Euphorbia hirta*, phytochemical screening, FT-IR, NMR, selective isolation, medicinal plants.

INTRODUCTION

Medicinal plants have long played an essential role in the development of modern pharmacology. Euphorbia hirta (commonly known as the asthma plant or milkweed) is a perennial herb of the family Euphorbiaceae widely used in traditional medicine for treating inflammation, ulcers, skin infections and respiratory diseases (Pavithra et al., 2024).

The plant's pharmacological activities i.e the antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer are attributed to the presence of secondary metabolites such as flavonoids, tannins, saponins and alkaloids (Kumar et al., 2010, Ojediran et al., 2024). Despite its ethnobotanical importance, there remains limited structural elucidation of its bioactive constituents.

This study therefore aimed to selectively isolate and characterize the phytochemical components of Euphorbia hirta extracts to establish a scientific basis for its traditional therapeutic use (Silalahi, 2021).

MATERIALS AND METHODS

Plant Material

Fresh Euphorbia hirta was collected from Agbowo community, Ibadan, Nigeria, and authenticated at the University of Ibadan Herbarium (voucher no. UIH-23576).

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Extraction Procedure

Air-dried and pulverized plant material (300 g) was macerated in 1 L of methanol for seven days. The filtrate was concentrated using a rotary evaporator to yield a thick, greenish extract (30 ml).

Phytochemical Screening

Standard qualitative tests were performed using 1 ml for each procedure to detect alkaloids, flavonoids, tannins, saponins, triterpenoids/steroids, phenols, reducing sugars, glycosides, and anthocyanins.

Selective Isolation

20 ml of lead acetate and 10 ml of glacial acetic acid were used to precipitate alkaloid-rich fractions, followed by chloroform extraction using 60 ml of chloroform. The organic phase was concentrated and subjected to silica gel column chromatography using hexane:ethyl acetate (1:1 v/v + 1 mL acetic acid). Fractions were monitored via thin-layer chromatography (TLC) and combined according to Rf values. Selected fractions (3 and 7) with Rf values 0.4 and 0.5 respectively were analyzed by FT-IR and NMR spectroscopy.

RESULTS AND DISCUSSION

Phytochemical Composition

The methanolic extract of Euphorbia hirta contained alkaloids, flavonoids, tannins, saponins, triterpenoids/steroids, phenols and reducing sugars while glycosides and anthocyanins were absent (See Table 1). These results indicate that Euphorbia hirta is rich in secondary metabolites known for their pharmacological properties.

Phytochemical Analysis	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Triterpenoids / Steroids	+
Phenols	+
Reducing Sugars	+
Glycosides	–
Anthocyanins	–

(+ = Present, – = Absent)

FT-IR Analysis

The FT-IR spectra of fractions 3 and 7 showed characteristic absorptions corresponding to hydroxyl, carbonyl and aromatic C–H groups.

Fraction 3 displayed a broad O–H stretching (3340 cm^{-1}) and strong C=O absorption (1724 cm^{-1}), confirming the presence of an oxygenated aromatic compound.

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Fraction 7 showed similar features with strong C=O stretch (1728 cm^{-1}), indicating alcohol and ester functionalities.

These findings suggest that both fractions possess polyhydroxylated and carbonyl groups, consistent with flavonoid derivatives.

NMR Analysis

The ^{1}H and ^{13}C NMR spectra confirmed the presence of oxygenated methines, aromatic quaternary carbons and sugar moieties.

Fraction 3 displayed an aldehydic proton at δ 9.79 (^{1}H) and carbonyl carbon at δ 203 (^{13}C), indicating a formyl-substituted phenolic compound.

Fraction 7 exhibited hydroxylated aromatic signals and oxygenated carbons between δ 70–75 ppm, consistent with flavonoid glycoside structures.

These results align with known Euphorbia hirta flavonoid derivatives such as quercetin, afzelin, and euphorbin, known for antioxidant and anti-inflammatory effects.

Proposed Structures

Two compounds were isolated as green amorphous powders with Rf values of 0.4 and 0.5 (Hexane:EtOAc, 6:4). A positive glucose test confirmed the presence of sugar moieties.

Figure 1: Proposed structure of isolated compound for fraction 3.

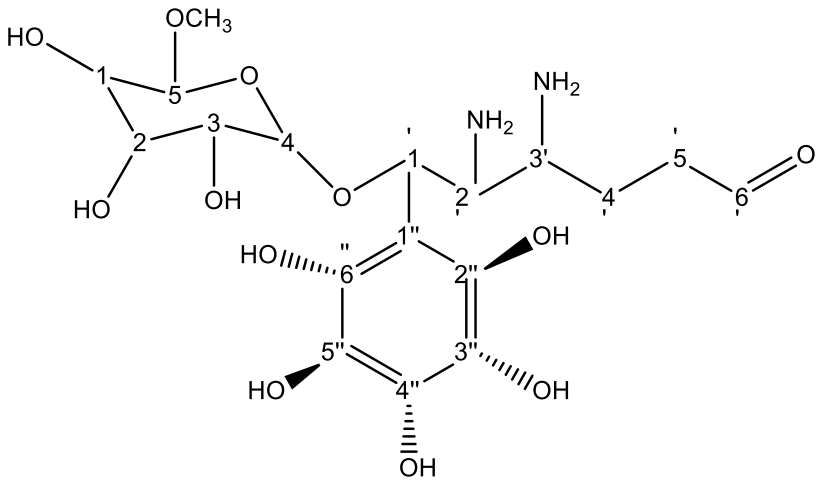
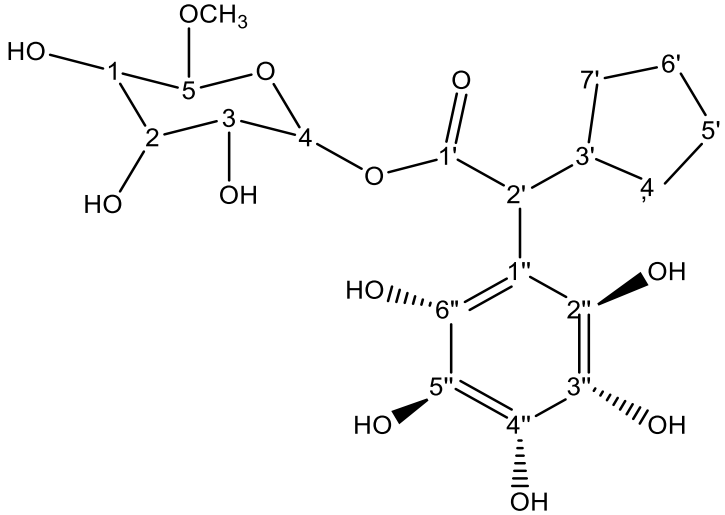


Figure 2: Proposed structure of isolated compound for fraction 7.



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CONCLUSION

This study successfully achieved the cold methanolic extraction, fractionation, isolation and characterization of bioactive compounds from Euphorbia hirta. The isolated fractions (Rf 0.4 and 0.5) contained –OH, C=O, C–O and C=C functional groups, and a sugar moiety confirmed by glucose tests.

Spectroscopic analyses (FT-IR, ^{1}H NMR, ^{13}C NMR) confirmed that the compounds are likely flavonoid glycosides. These findings substantiate the ethnomedicinal use of Euphorbia hirta and establish a scientific basis for its pharmacological applications.

RECOMMENDATION

Further biological assays such as antimicrobial, antiviral, anti-inflammatory and cytotoxicity tests are recommended to determine the pharmacological potential of the isolated compounds.

Advanced spectroscopic studies (such as LC-MS, 2D NMR, and X-ray crystallography) should be performed to establish the full structural identity of the isolates and to support drug discovery research.

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