Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

PHYTOCHEMICAL PROFILING AND ANTIMALARIAL EVALUATION OF *EUPHORBIA HETEROPHYLLA* EXTRACTS LEADING TO THE ISOLATION OF 3-O-DIGLUCOPYRANOSYL-7,4'-O-DIMYCAROSYL-3'-METHOXY-5-HYDROXYFLAVONE

Yabvo K. Musa[1]*, Zakari Ladan[1], Salit P. Bako[1], Abdullahi N. Maikudi[1], Gabi Baba[2,3]

[1]Department of Pure and Applied Chemistry, Kaduna State University, Kaduna
[2]Department of Biochemistry, Kaduna State University, Kaduna
[3]Department of Applied Chemistry, Kaduna Polytechnic, Kaduna

*Corresponding Author Email Address: musa201430@gmail.com

ABSTRACT
The investigation of the acclaimed traditional antiplasmodial efficacy of *Euphorbia heterophylla* extracts and the subsequent phytochemical isolation of potential bioactive natural compounds is a step in the fight against the Plasmodium parasite, which causes malaria. In this research, ethanol, chloroform, and n-hexane extracts were phytochemically screened for alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and cardiac glycosides. The results revealed the presence of all tested phytochemicals in the ethanol extract, whereas tannins and saponins were absent from the chloroform extract. The n-hexane extract of this plant confirmed the absence of alkaloids and tannins, thereby making the ethanol extract in this research of the highest quality. These extracts were further screened for antimalarial activity using the World Health Organization (WHO) in vitro (Mark III) Schizont maturation inhibition protocol 1996, modified in 2001. The ethanol extract showed the only promising result with an IC_{50} of 10 µg/ml, while the chloroform and n-hexane extracts gave an IC50 of 15.3 µg/ml and 21.0µg/ml, respectively, as compared with the positive control, which showed an IC_{50} of 2.8 µg/ml. The promising ethanol extract was used to isolate 3-O-Diglucopyranosyl-7,4'-O-dimycarosyl-3'-methoxy-5-hydroxyflavone. The research supported the traditional use of the plant for managing mild, uncomplicated malaria.

Keywords: *Euphorbia heterophylla*, antiplasmodial efficacy, phytochemically screened, phytochemical isolation.

INTRODUCTION

Euphorbia heterophylla, popularly known as Nonon kurciya in Hausa, Adimeru in Yoruba, and Egele in Igbo, has a long-standing traditional history of antimalarial efficacy across the North, West, and Eastern parts of Nigeria. According to the World Health Organization (WHO), malaria caused an estimated 610,000 deaths in 2024, with 95% of these deaths occurring in Africa, hence making it one of the deadliest diseases, especially in Africa (WHO, 2025). The disease is characterized by a high transmission rate, deadly parasite species with high resistance, a vulnerable population, and climate change, which collectively challenge the fight against this disease (WHO, 2025). Traditionally, the boiled plant extract of Euphorbia heteropylla has been used locally for the management of malaria, but with limited scientific documentation of its antimalarial activity. In a research by Clarkson *et al*. (2004), the crude extract of ethanol and chloroform solvent mixture (1:1) was investigated for activity against the chloroquine-sensitive strain D10 Plasmodium falciparum using parasite lactase dehydrogenase (pLDH) activity to measure the Plasmodium parasite viability. The *in vitro* experiment revealed a half inhibitory concentration (IC_{50}) of about 10 µg/ml, which was classified as promising because, according to the research, inhibition of the plasmodium parasite growth at lower concentration reflects selective ability as compared with higher concentration, where non-specific toxicity is often observed (Clarkson *et al*.,2004).
This research reports the evaluation of the phyto-components of the extracts and the in vitro investigation of their anti-plasmodial activity, leading to the isolation of a natural medicinal compound for future use.

MATERIALS AND METHODS

Analytical-grade chemicals and solvents (Sigma-Aldrich Chemicals Pvt. Ltd., India) were used in this research, including ethanol, methanol, chloroform, ethyl acetate, n-hexane, petroleum ether, sulfuric acid, Iron (II) sulfate, sodium hydroxide, and hydrogen chloride. The main instruments include: an optical absorption spectrum device, a Carry 630 FT-IR spectrometer by Agilent Technologies, an Analtech UV cabinet 254 nm shortwave and 366 nm long wave, a C-Parmer RV-200 rotary evaporator, a pH-tester (HANNA INSTRUMENT), an E200-LED Trinocular compound microscope, and a Bruker Avance III HD 500 MHz NMR spectrometer (Germany).

Plant Sample Collection and Preparation

Euphobia Heterophylla plant samples were collected randomly at Kujama in the Cikun Local Government Area of Kaduna State, at latitude 10.391 N and longitude 7.270 E. The plant samples were authenticated at the Biological Science Department Herbarium of Kaduna State University, with voucher number KASU/BSH/1702 deposited. The collected plant leaves were washed with distilled water and allowed to dry in the shade. The dried plant materials were pulverized into fine particles and stored in a properly covered glass bottle at 27 ^{o}C for subsequent experiment.

Extraction of Crude Plant Extracts

A 1.5 Kg of dried *Euphorbia heterophylla* powdered plant sample was carefully transferred into a 4 Ltr flat-bottom flask, and 2.4 Ltrs of ethanol solvent was added to it. The mixture was occasionally agitated daily for 7 days, and the plant was macerated. The mixture was sieved using a 150 mm filter, and the residue was carefully

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

rinsed. The collected extract was concentrated using a rotary evaporator at 50 °C and then allowed to dry. The dried crude extract was weighed and stored in a refrigerator at 4 °C for subsequent use (Bitwell *et al.*, 2023).

Qualitative Phytochemical Evaluation of Crude Extracts

The qualitative phytochemical screening of the ethanol, chloroform, and n-hexane crude extracts was conducted according to seven standard procedures described by Trease and Evans (2009) for the seven tested phytochemicals, including alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and cardiac glycosides.

***In Vitro* Antimalarial Assay Procedures**

This procedure is adopted in line with the World Health Organization antiplasmodial activity drug test, with slight modification according to the WHO Mark III 1996, modified by the 2001 protocol.

Standard RPMI-1640 medium with MOPS (PM150147) treated with Gentamicin (0.25 ml Gentamicin to 500 ml RPMI medium) was supplemented with 15 % human serum and mixed with 2 % *plasmodium falciparum* infected blood sample obtained from Kadpoly Clinic. This prepared culture media was dispensed into a 96 well microliter plate and calculated concentrations (100 ug/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, and 1..562 µg/ml) of various ethanol, chloroform and n-hexane crude extracts were added into the 96 well microliter plate as treatment and thereafter incubated at 37 °C temperature with about 5 % CO_2 in an incubator for 48 hrs. The experiment was conducted in duplicate, with Fidson Healthcare Nigeria Artemisinin as the positive control and the infected, untreated culture medium as the negative control.

Microscopy Analysis

After 48 hours of incubation, thin blood smears were prepared and fixed with methanol, then stained with Giemsa for 15 minutes before viewing under a microscope at ×100 magnification. Infected red blood cells were counted under the microscope, and the number of Schizonts observed per 200 trophozoites infected red blood cells (Lombard & Le Foll, 1989). The schizont count was recorded to determine the percentage of schizont inhibition as well as the corresponding percentage of schizont viability. The percentage schizont inhibition for each concentration of crude extract was calculated, and using Prism GraphPad software (version 10), the corresponding IC_{50} value, representing the concentration required to inhibit 50% of schizont growth, was determined from its nonlinear regression analysis tool. The inhibition of parasite growth by the tested crude extract (drug) was calculated for various groups using the schizont counts for treated and untreated red blood cell (RBC) as follows:

$$\% \text{ Inhibition} = \frac{\text{Schizont count for control} - \text{Schizont count for treated RBC}}{\text{Schizont count for control}} \times 100$$

$$\% \text{ Viability} = \frac{\text{Schizont count for treated RBC}}{\text{Schizont count for untreated RBC}} \times 100$$

Ethical Considerations

All experimental procedures involving human erythrocytes were conducted in accordance with the ethical guidelines approved by the Kaduna State Ministry of Health Review Board. Informed consent was also obtained from donors providing blood samples for parasite culture.

Thin Layer Chromatography Procedures

Standard thin-layer chromatographic Aluminium foil coated with silica gel adsorbent mesh 60F254 (Merck) was used for the TLC. The TLC plate was activated at 110°C for 30 minutes in an oven and allowed to cool. Using a capillary tube, the activated plate was spotted about 1cm from the bottom of the chromatographic plate, and this distance was recorded as the chromatographic starting point. The stained spot on the chromatographic plate was allowed to dry for 5-10 minutes; thereafter, the plate was developed in a developing tank using a solvent mixture of ethanol, ethyl acetate, and n-hexane at a ratio of 3:2:1. The resulting chromatogram after development was dried in an oven at a temperature of 45 °C and the component spot identified under UV at 366 nm (Mayo *et al.,* 2011).

Column Chromatography Separation Procedure

A portion of silica gel mesh 70-230 (500 g) was activated in an oven at 100 °C for 1 hour and then allowed to cool at room temperature (25 °C –27 °C) in a desiccator for about 30 minutes. A slurry of the activated silica gel (SiO_2) was prepared using a mixture of ethanol, ethyl acetate, and n-hexane in a 3:2:1 ratio to achieve full resolution. The slurry was poured carefully into a chromatographic column that was a quarter (1/4) filled with the chromatographic solvent; the packing was carried out while the column tap was opened to ensure proper packing and stabilization. The separating sample (analyte), which was about 3-5 % by mass of solid adsorbent packing was carefully loaded into the packed column and the column tap was finally opened, the flow rate was adjusted to 0.5 ml/min while the isolated component was collected as it ran down the column (Mayo *et al.,* 2011). The isolated compound was designated as compound C for further analysis.

RESULTS

Table 1: Physical properties of various crude extracts from *Euphorbia heterophylla*

Euphorbia Heterophylla (1.5Kg dry sample)				
Solvents	**Colour**	**Texture**	**Mass (g)**	**% Yield**
Ethanol	Green	Sticky solid	94.8 g	6.3 %
Chloroform	Dark green	Sticky solid	54.3 g	3.6 %
n-Hexane	Light green	Sticky oily solid	18.2 g	1.2 %

Table 2: Qualitative Phytochemical Screening of *Euphorbia heterophylla* Crude Extracts

Metabolites	**Ethanol Extraction**	**Chloroform Extract**	**n-Hexane Extract**
Alkaloids	+	+	_
C. Glycosides	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Terpenoids	+	+	+
Tannins	+	_	_
Saponins	+	_	+

Key: Present (+) and Absent (-)

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Table 3: Antimalarial Activity Investigation of Ethanol Extract

Conc.	Ave. P.Count	Ave %Viability.	Ave. %Inhibiton
1.562 µg/ml	49.0 ±1.414	96.07 ±2.764	3.92 ±2.771
3.125 µg/ml	47.0 ±1.414	92.15 ±2.778	7.84 ±52.778
6.25 µg/ml	44.5 ±0.707	87.25 ±1.385	12.75 ±1.385
12.5 µg/ml	42.0 ±0.000	82.35 ±0.000	17.64 ±0.007
25 µg/ml	38.5 ±0.707	75.49 ±1.385	24.50 ±1.393
50 µg/ml	35.5 ±0.707	69.60 ±1.393	30.39 ±1.393
100 µg/ml	33.5 ±0.707	65.68 ±1.393	34.31 ±1.393

Calculated IC_{50} =10.0 µg/ml

Table 4: Antimalarial Activity Investigation of Chloroform Extract

Conc.	Ave. P.Count	Ave. %Viability.	Ave. %Inhibition.
1.562 µg/ml	49.0 ±1.414	96.07 ±2.764	3.92±2.771
3.125 µg/ml	47.0 ±1.414	92.15 ±2.773	7.845±2.778
6.25 µg/ml	44.5 ±0.707	87.25 ±1.385	12.75±1.385
12.5µg/ml	42.0 ±0.000	82.35 ±0.000	17.64±0.007
25 µg/ml	38.5 ±0.707	75.49 ±1.385	24.5±01.393
50 µg/ml	35.0 ±50.707	69.60 ±1.393	30.39±1.393
100 µg/ml	33.5 ±0.707	65.68 ±1.393	34.31±1.393

Calculated IC_{50} value =15.3 µg/ml

Table 5: Antimalarial Activity Investigation of n-Hexane Extract

Conc.	Ave. P.Count	Ave. %Viability.	Ave. %Inhibition.
1.562µg/ml	49.5 ±0.707	97.05±1.393	2.94±1.385
3.125 µg/ml	47.5 ±0.707	93.14±1.385	6.85±1.371
6.25 µg/ml	45.5 ±0.707	89.21±1.378	10.78±1.385
12.5 µg/ml	42.0 ±1.414	82.35±2.771	17.65±2.771
25 µg/ml	37.5 ±0.707	73.38±1.598	26.47±1.385
50 µg/ml	34.0 ±0.000	66.67±0.000	33.31±0.021
100 µg/ml	30.5 ±0.707	59.8±1.385	40.20±1.385

Calculated IC_{50} Value = 21 µg/ml

Table 6: Antimalarial Activity Investigation of Artemisinin Positive Control

Conc.	Ave. P.Count	Ave. %Viability.	Ave. %Inhibition.
1.562 µg/ml	27.5 ±0.707	53.92 ±1.385	46.08 ±1.385
3.125 µg/ml	18.5 ±0.707	36.27 ±1.385	63.73 ±1.385
6.25 µg/ml	14. 0 ±1.414	27.45 ±2.771	72.55 ±2.771
12.5 µg/ml	9.0 ±1.414	17.65 ±2.771	82.35 ±2.771
25 µg/ml	4.5 ±0.707	8.82 ±1.385	91.18 ±1.385
50 µg/ml	3.0 ±0.000	5.88 ±0.000	94.12 ±0.000
100 µg/ml	1.50 ±0.707	2.94 ±1.385	97.06 ±1.385

Calculated IC_{50} value = 2.8 µg/mL

KEYS: Concentration (Conc.); Average Parasite Count (Ave. P.Count); Average Percentage Viability (Ave. %Viability); Average Percentage Inhibition (Ave. % Inhibition).

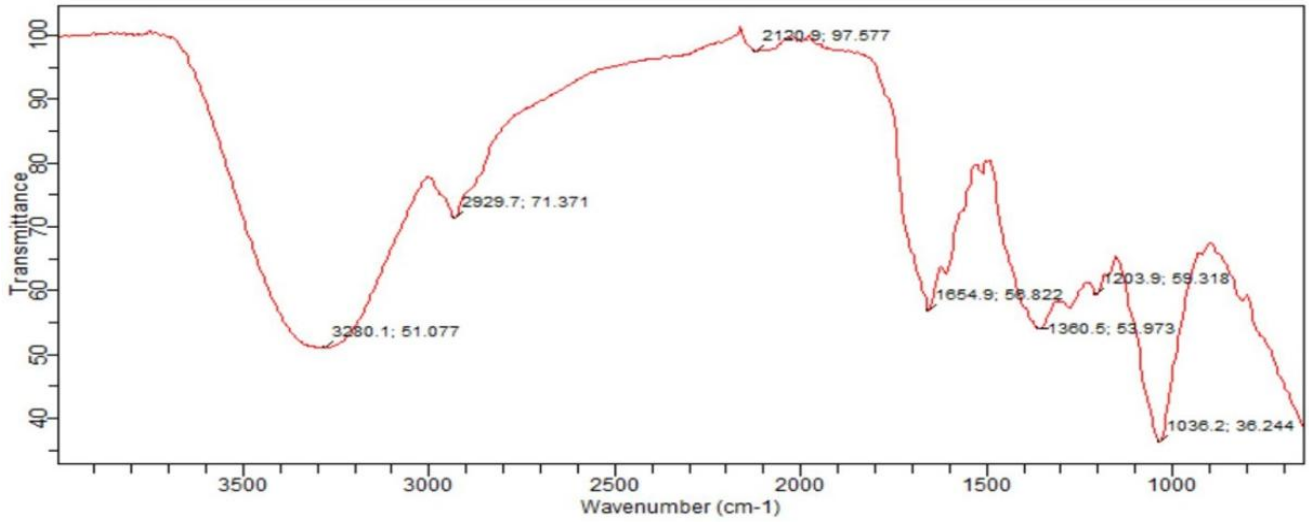


Figure 1: FTIR for Isolated Compound C

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

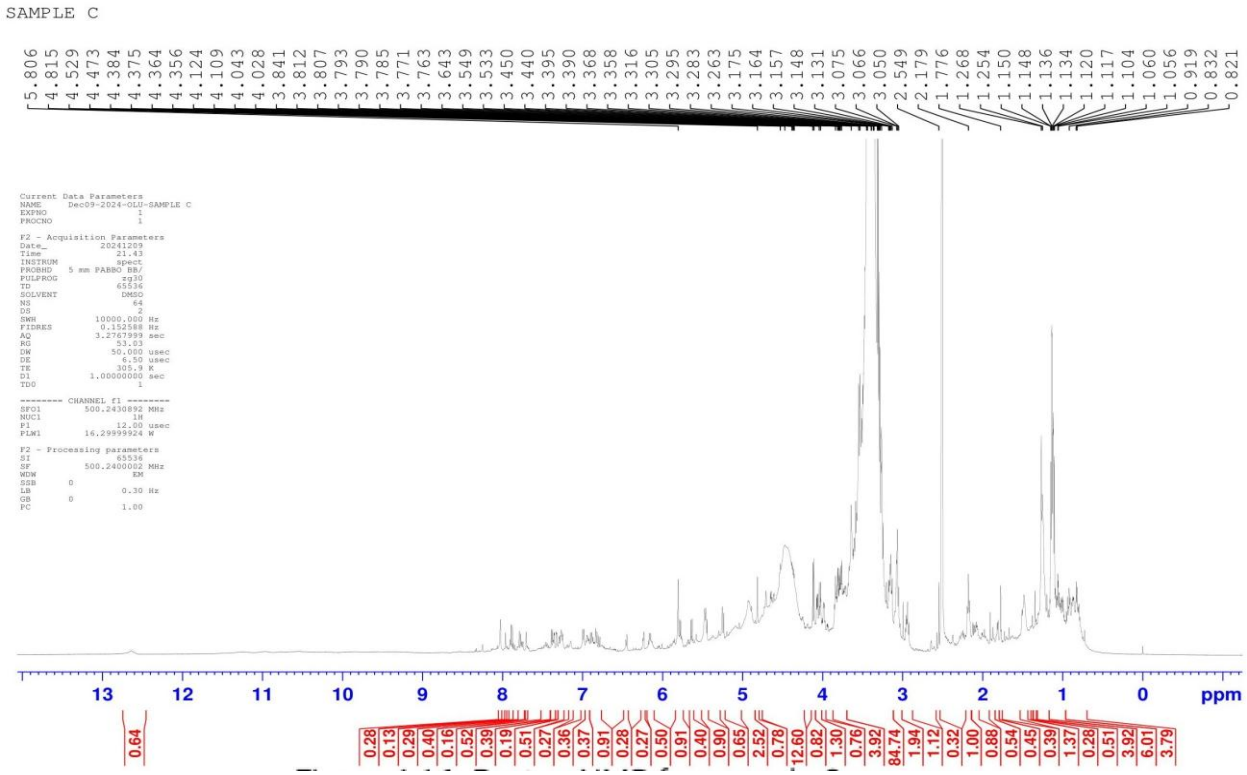


Figure 2: ^{1}H-NMR Spectrum for Isolated Compound C

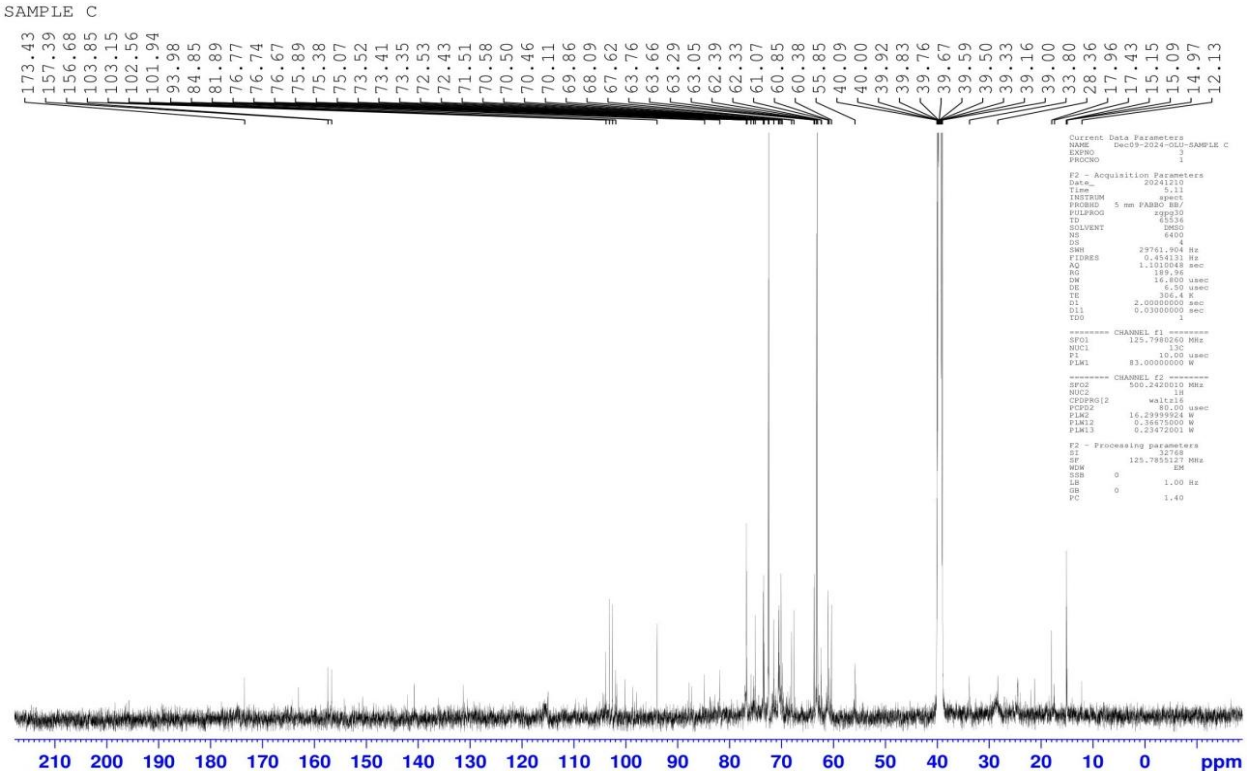


Figure 3: ^{13}C-NMR Spectrum for Isolated Compound C

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

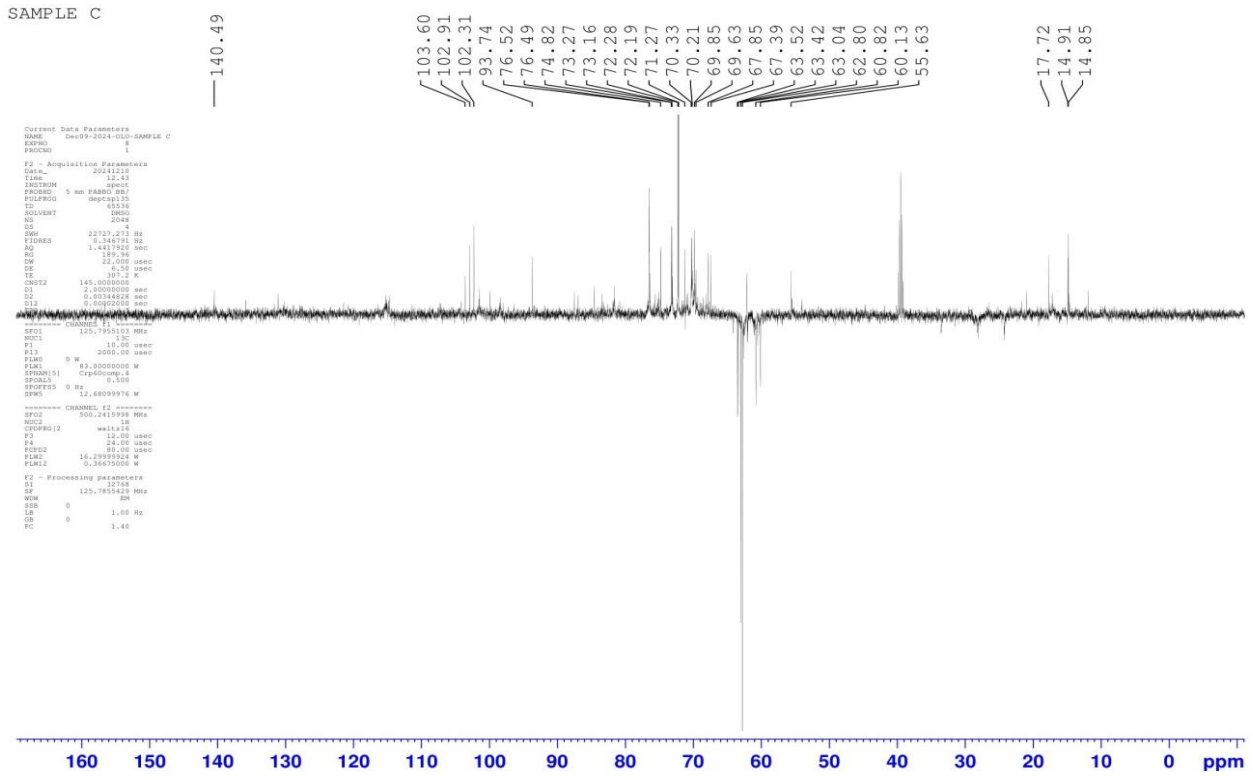


Figure 4: DEPT Spectrum for Isolated Sample C

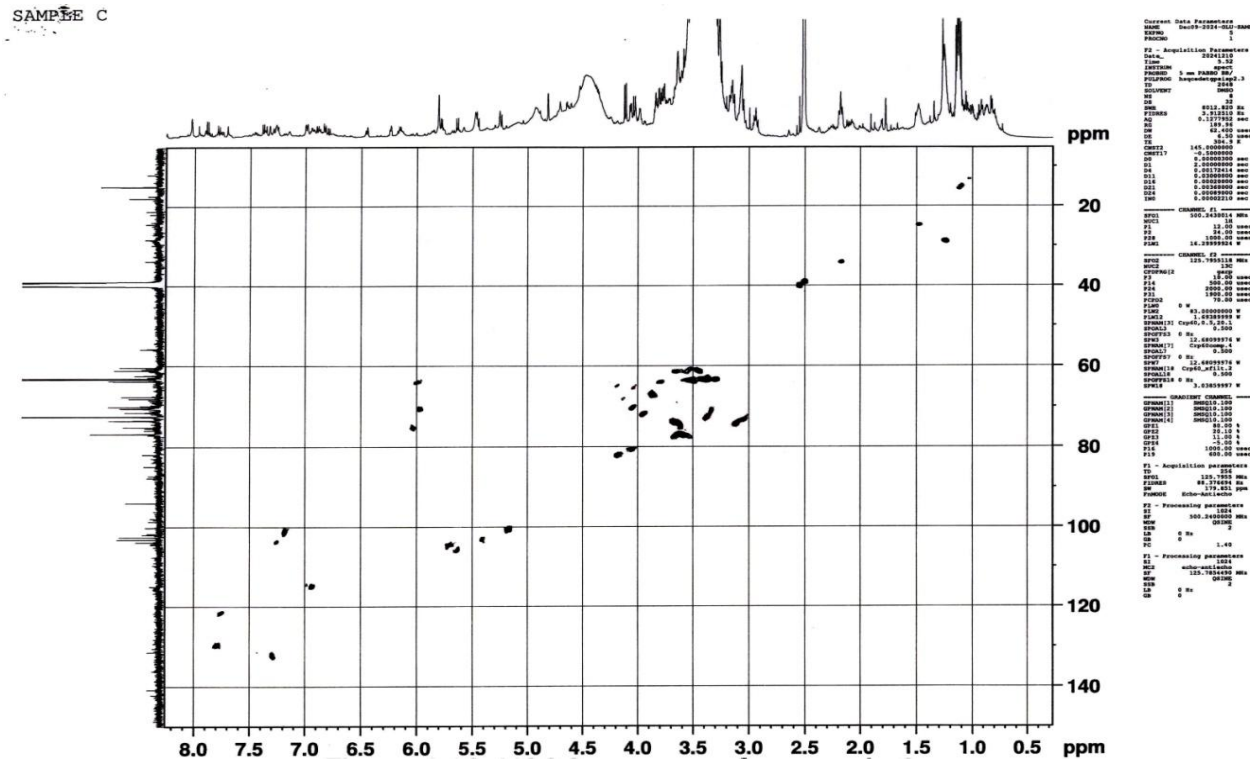


Figure 5: HSQC Spectrum for Isolated Sample C

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

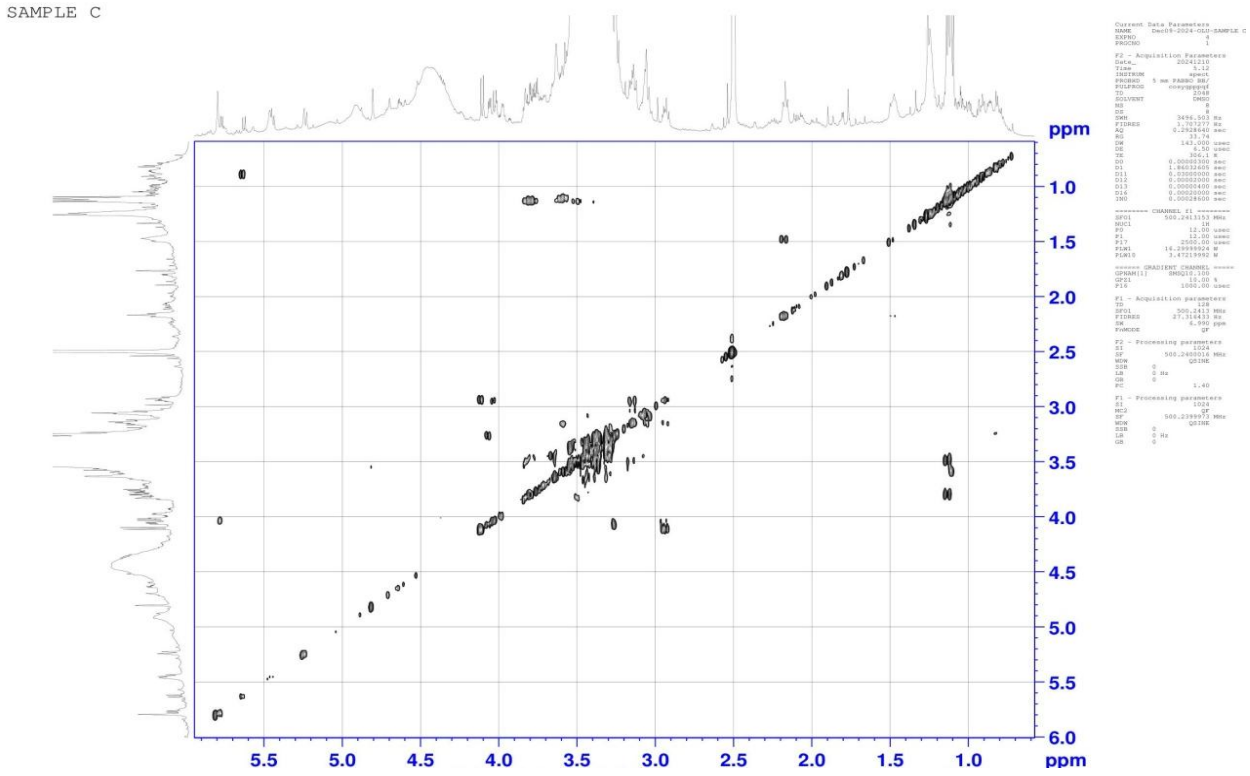


Figure 6: H-H COSY Spectrum for Isolated Compound C

DISCUSSION

Macerated Crude Extracts of *Euphorbia Heterophylla*

The macerated crude extracts of ethanol, chloroform, and n-hexane were evaluated for color, texture, mass, and calculated percentage yields, as presented in Table 1. The extracts were generally green due to the high levels of chlorophyll, a common pigment in *Euphorbia* species (Mahomoodally *et al*., 2020). Similarly, the general sticky solid texture of the extracts could be attributed to the presence of polysaccharides like mucilage or gums, sugars, glycosides, or other water soluble compounds (Harborne, 1998); while the additional oily component in the n-hexane extract is attributed to the presence of terpenes, mainly soluble in n-hexane solvent (Harborne, 1998). The mass and calculated percentage yields have proven ethanol as the preferred extracting solvent, with a percentage yield of 6.3%, followed by chloroform, and the lowest being n-hexane.

Qualitative Phytochemical Evaluation

In view of the fact that plant metabolites are of different polarities and the principle governing phytochemical extraction is that of " like dissolves like," the extraction efficiency and composition depends strongly on solvent polarity (Do et al., 2014) and (Azeonu & Ejikeme, 2016), hence explaining the reason why ethanol, chloroform and n-hexane confirmed the presence of diverse classes of compounds in this experiment. The polar ethanol solvent extracted alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and cardiac glycosides, consistent with broad-spectrum extraction by highly polar solvents (Do *et al*., 2014). Chloroform failed to extract any tannin or saponin components, which is consistent with the fact that chloroform is not expected to dissolve highly polar tannins and saponins but does dissolve and extract moderately polar alkaloids and flavonoids (Do *et al*., 2014). The non-polar n-hexane fraction confirmed flavonoids, terpenoids, phenols, saponins, and cardiac glycosides, but revealed the absence of tannins and alkaloids; this is because the missing tannins are known to be quite polar, hence not expected to be extracted by non-polar n-hexane solvent, while the alkaloids were found absent due to their basic and polar nature (Do *et al*., 2014). It is important to note that n-hexane extracts are lipophilic compounds like terpenes, terpenoids, and some flavonoids, as well as extract very few phenolic polar compounds (Trease & Evans, 2009). Qualitatively, the broad spectrum of ethanol phytocomponents, compared with other extracts in Table 2, showed more classes of phytochemicals, hence was medicinally superior to the other extracts (Azawanida, 2015).

Antimalarial Activity Investigation

The Antimalarial activity experiment was conducted in duplicate, with Fidson Health Care Artemisinin malaria drug as a positive control, and untreated RPMI 1640 culture medium as a negative control. Parasite count, percentage viability and percentage inhibitions were determined for various (drug) concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, and 1..562 µg/ml) for ethanol, chloroform, and n-hexane extracts; while the results were presented as shown in table 3,4 and 5 respectively, with additional table 6 for the standard positive control. The *in vitro* antimalarial evaluation showed that the ethanol, chloroform, and n-hexane extracts had IC50 values of 10 µg/ml, 15.3 µg/ml, and 21.0 µg/ml, respectively, while Artemisinin,

the positive control, had an IC50 of 2.8 µg/ml. This indicates that the ethanol extract has the highest activity, followed by the chloroform extract, with the n-hexane extract showing the least activity. Comparing all the results with Artemisinin, the positive control drug (IC50 = 2.8 µg/ml), it is obvious that the tested plant extracts have fallen short of the standard requirement for good activity because, according to Rasoanaivo *et al*. (2004 the antimalarial crude extract threshold for good activity is IC_{50} < 5 µg/ml. However, the Rasoanaivo et al. (2004) threshold considers IC50 < 10 µg/ml a promising criterion for antimalarial crude extracts, indicating that the ethanol extract in this research is promising, unlike the chloroform and n-hexane extracts. It is important to note that the earlier phytochemical discussion showed ethanol extract processing to be the best phytochemical quality, confirming positive for alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and cardiac glycosides; hence, it is not surprising that it gave the highest activity when compared with chloroform or the n-hexane extracts. The better activity of ethanol extract could be attributed to the presence of multiple bioactive components: where the alkaloids are generally for possible quinine-like effect, terpenoids known for Artemisinin-type mechanism, while flavonoids are popular in oxidative pathways; the combination of phenols and tannins acts as redox modulators and protein binders, while the saponins are a membrane permeability enhancement component (Saxena *et al*., 2003). The superior antimalarial activity is generally linked to its broad spectrum of phytochemicals, with synergistic interactions among alkaloids, flavonoids, terpenoids, and others, thereby highlighting its potential as a promising extract for further malaria research.

FT-IR Spectrum Analysis for Isolated Sample C

Fourier Transform Infrared spectroscopic analysis was performed using an Agilent Technologies spectrometer, and the major absorption band results are shown in Table 7

Table 7: FT-IR characteristics bands for sample C

Bands(cm^{-1})	Bonds	Characteristics Inference
3280.1	O-H (broad)	Hydroxyl stretch absorption
2929.7	C-H	Alkane stretch absorption
1654.9	C=C-C=C	Conjugated double bond absorption
1203.9	C-O	Carbon-oxygen stretch absorption

Inference: Dudley and Ian (2006)

The inferences in Table 7 were drawn from characteristic absorption bands by Dudley and Ian (2006 and supported by Field et al (2007. The major absorption bands for some major bond types in this compound include a broad hydroxyl (O-H) stretch absorption band at 3280.1 cm-1, a conjugated carbon double bond (C=C-C=C) stretch absorption at 1654.9 cm-1, and the carbon-oxygen (C-O) stretch absorption within the fingerprint region at 1203.9 cm^{-1}. Further characterization of this compound with respect to NMR spectra was carried out as presented below.

Nmr Spectra Analysis of Isolated Sample C

The NMR analysis was carried out on Bruker Avance III HD 500 MHz spectrometer and the various categories of chemical shifts were observed for the protons and carbons present in this compound as shown: ^{13}C-NMR (DMSO-d_6, 126 MHz) δ_C, 173.43, 164.04, 163.11, 156.68, 151.01, 150.50, 141.00, 136.38, 121.50, 117.10, 115.86, 115.11, 103.85, 103.15, 102.91, 102.31, 100.16, 81.89, 76.77, 76.52, 76.49, 75.89, 75.07, 71.51, 70.50, 70.46, 70.11, 68.09, 67.62, 63.05, 62.33, 55.85, 45.00, 42.01, 28.36, 25.07, 17.96, and 15.15. Similarly the following proton chemical shifts were observed with corresponding couplings (J): ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H, 7.885d, J=8Hz, 7.775d, J=8.5, 7.390d, J=7Hz, 6.247d, J=3Hz, 6.149d, 5.780d, J=5.5Hz, 5.643d, J=8Hz, 5.461 d, J=7Hz, 5.243 d, J=8.5Hz, 4.374m, 4.351, 3.841, 3.798, 3.316, 3.066, 3.050, 2.179, 1.779, 1.501, 1.484, 1.268, 1.125, 1.117, 1.100 and acknowledging the presence of overlapping peaks.

Four proton peaks at 5.780 ppm, 5.643 ppm, 5.461 ppm, and 5.243 ppm correlating with carbon peaks at 102.91, 103.60, 102.31, and 100.16 ppm in HSQC on figure 5 were analyzed on 1D and 2D spectra and subsequently confirmed as anomeric (protons and carbons) as shown in table 8. The large number of oxygenated carbon peaks between 81.89 ppm and 60.13 ppm with corresponding proton peaks as shown on HSQC between 3.033 ppm and 4.200 ppm (acknowledging the overlapping peaks), were observed to be from sugar ring moieties bonding through a glycosidic bond to their aglycone or other sugar substituent (Davis & Presgard, 2023), as the case may be. A critical analysis of the glycosidic sugar component in ^{1}H-^{1}H COSY on figure 6 revealed the presence of a proton doublet (J=7 Hz) at 1.100 ppm having cross peaks correlating with protons at 3.841 ppm on mycarospyranosyl sugar ring (1) while the doublet peak at 1.090 ppm correlates with proton at 3.798 ppm on ^{1}H-^{1}H COSY for mycarosyl sugar ring (2) both indicating the deoxygenated mycarosyl sugar ring moieties according to Bruheim *et al*., 2004 and Kobayashi *et al*., 2000. Further confirmation of the mycarosyl moiety was revealed in the methylene carbon at position 2Cm and 2'Cm, shown in Table 9, with diastereotopic proton peaks for their carbons at 40.900 ppm and 45.010 ppm, respectively, corresponding to proton peaks at 2.179/1.501 ppm and 1.779/1.484 ppm, respectively, as analyzed on HSQC. Other sugar moieties with anomeric data at 100.16 ppm (doublet, 5.461 ppm, J=8Hz) and 102.31 ppm (doublet, 5.461 ppm, J=7Hz) for 1Cgp and 1'Cgp, respectively, correspond to and confirm β-glucopyranosyls according to Richter and Berger, 2013, and Huang et al., 2011. A careful analysis and evaluation of the downfield peaks beyond the anomeric regions for both proton and carbon NMR showed a correlation with those of the isorhamnetin-flavone core, as seen in flavonoid datasets according to Park *et al*., 2007, and Park *et al*., 2006. The overall analysis and assignments of observed chemical shifts were carried out on various carbons and protons in line with documented literature dataset values for hydroxyflavone derivatives by Park *et al*., 2007, supported by Kulic *et al*., 2026, for the aglycone, whereas the mycarosyls were assigned according to Bruheim *et al*., 2004, and Kobayashi *et al*., 2000; in addition, the glucopyranosyls were assigned with reference to Huang *et al.,* 2011, and Richter and Berger, 2014. The overall assignment table of the isolated compound is as shown in Table 9, and the compound structure elucidated as 3 – O - (β - (1-4) - diglucopyranosyl) - 7 – O - (β- mycarosyl) - 4 -O- (α - mycarosyl) - 3' – Methoxy – 5 –hydroxyflavone.

Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.57
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Table 8: Anomeric Carbons and Protons Analysis for 3 – O - (β - (1-4) - diglucopyranosyl) - 7 – O - (β- mycarosyl) - 4 -O- (α - mycarosyl) - 3' – Methoxy – 5 –hydroxyflavone

^{13}C-NMR (ppm)	^{1}H-NMR (ppm)	Coupling Constants ($^{3}J_{HH}$)
102.91	5.780 d	5.5Hz
103.60	5.643 d	8 Hz
102.31	5.461 d	7 Hz
100.16	5.243 d	8.5 Hz

Table 9: NMR spectra assignment analysis for 3 – O - (β - (1-4) - diglucopyranosyl) - 7 – O - (β- mycarosyl) - 4 -O- (α - mycarosyl) - 3' – Methoxy – 5 – hydroxyflavone

Position	^{13}C – NMR (ppm)	Literature values (ppm)	^{1}H – NMR (ppm)
C2	141.00	143-148	------
C3	136.38	135-137	------
C4	173.43	175-177	------
C5	164.09	160-165	------
C6	98.08	94-100	6.149
C7	163.11	160-165	------
C8	93.98	94-100	6.247 d, J=3Hz
C8a	156.68	155-158	------
C4a	103.15	103-115	------
C1'	121.01	121-123	------
C2'	115.86	115-118	7.885 d, J=8Hz
C3'	151.01	145-150	------
C4'	150.50	145-150	------
C5'	117.10	117-120	7.390 d, J=7.5Hz
C6'	115,11	115-118	7.775 d, J=8.5Hz
4'-OCH$_3$	55.8	55-57	3.274

α- Mycarosyl (1) Bruheim *et al.,* 2004

Position	^{13}C – NMR (ppm)	Literature values (ppm)	^{1}H – NMR (ppm)
1Cm	102.91	104.9	5.780 d, J=6Hz
2Cm	40.90	41.5	2.179/1.501
3Cm	70.50	70.03	-
4Cm	76.77	78.01	3.066
5Cm	67.62	65.09	3.841
6Cm	15.15	17.30	1.100 d, J=7Hz
7Cm	25.07	25.21	1.261s

β- Mycarosyl (2) Kobayashi *et al*., 2000

Position	^{13}C – NMR (ppm)	Literature values (ppm)	^{1}H – NMR (ppm)
1'Cm	103.60	103.0	5.643 d, J=8Hz
2'Cm	45.00	44.50	1.779/1.484
3'Cm	70.46	70.41	-
4'Cm	76.49	76.50	3.050
5'Cm	68.09	68.10	3.798
6'Cm	17.96	17.91	1.090 d, J=7Hz
7'Cm	28.36	28.30	1.261s

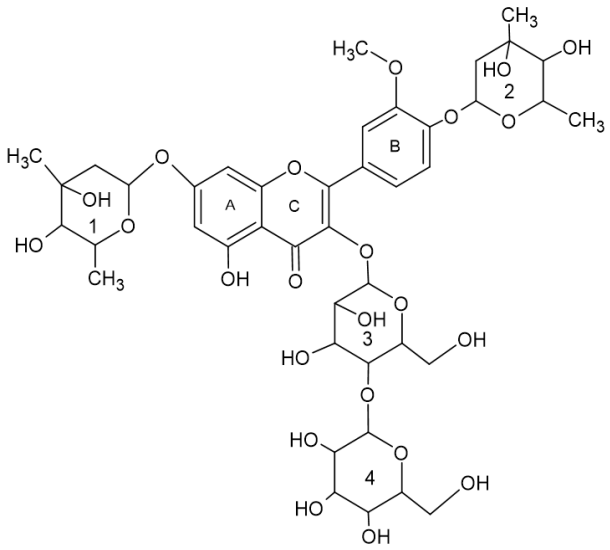
β- Glucopyranosyl (3), Richter and Berger 2013

Position	^{13}C – NMR (ppm)	Literature values (ppm)	^{1}H – NMR (ppm)
1Cgp	100.16	98.2	5.461 d, J=8.5Hz
2Cgp	81.89	79.1	4.043
3Cgp	76.77	77.4	3.3 – 3.8*
4Cgp	75.07	74.1	3.3 – 3.8*
5Cgp	70.11	71.1	3.3 – 3.8*
6Cgp	62.33	62.0	4.351 m

β- Glucopyranosyl (4), Huang *et al*.,2011

Position	^{13}C – NMR (ppm)	Literature values (ppm)	^{1}H – NMR (ppm)
1'Cgp	102.31	104.9	5.461 d, J=7Hz
2'Cgp	75.89	76.0	4.061
3'Cgp	76.52	77.8	3.316
4'Cgp	71.51	71.8	3.5 – 4.0*
5'Cgp	81.89	79.2	3.5 – 4.0*
6'Cgp	63.05	63.0	4.374 m

Overlaping peaks = (*); Glucopyranosyl = gp; Mycarosyl = m and Carbon = C



3 - O - (β - (1-4) - Diglucopyranosyl) - 7 - O - (β- mycarosyl) - 4 -O- (α - mycarosyl) - 3' - methoxy - 5 –hydroxyflavone

Evidently, the observed hydroxyflavone aglycone core structure was confirmed by prominent ^{13}C-NMR peaks at 93.98 ppm for carbon C8 and 98.08 ppm for carbon C6, with corresponding proton NMR peaks at 6.247d, J=3Hz and 6.149 ppm respectively, the coupling constant of J=3Hz observed for the proton revealed a long 4 bond coupling characteristic, these are all confirmed properties of the hydroxyflavone moiety (Park *et al.,* 2006). It is important to note that the presence of one broad hydroxyl proton peak at 12.650 ppm in the ^{1}H-NMR spectra conforms with the hydroxyl at position C5 (Park *et al.,* 2007), while the absence of any singlet broad peaks approximately between 9 ppm – 11 ppm confirms the methoxy and glycosylated substitution of all other hydroxyl protons on the isolated hydroxyflavone derivative (Park *et al.,* 2007). The assigned carbon skeleton core showed consistency with isorhamnetin hydroxyflavone, even the carbonyl carbon C4 with its peak at 173.45 ppm appeared slightly below the expected

175 ppm - 176ppm due to conjugation, substitution, and hydrogen bonding effects with respect to the type of NMR solvent used (Kulic *et al*., 2026). The assignment shown in table 9 was discreetly carried out also to identify some few low intensity signals hidden among impurities, artifacts or noise which is a common practice in natural products spectra: hence, some resonating peaks within 6.0ppm – 8.0ppm can be attributed to co-extracted constituents of phenols usually found co-existing in some plants' matrices especially in the *Euphorbia* species like this (Mahomoodally *et al*., 2020).

Acknowledgement
The authors acknowledge Dr. Ibrahim Didamson for the collaboration effort with the University of Johannesburg in South Africa for the NMR spectra.

REFERENCES

Bitwell, C., Indra, S.S., Luke, C., & Kakoma, M.K. (2023). A review of modern and conventional extraction techniques and their application for extracting phytochemicals from plants. *Scientific African*, 19, e01585.

Bruheim, P., Borgos, E. F. S., Tsan, P., Ellingsen, E. T., Lancelin, J. M., & Zotchev, S. B. (2004). Chemical diversity of polyene macrolides produced by Streptomyces noursei ATCC 11455 and recombinant strain ERD44. *Antimicrobial Agents and Chemotherapy*. 48(11), 4120–4129.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O. M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., & Folb, P. I. (2004). *In vitro* antiplasmodial activity of medicinal plants native to South Africa. *Journal of Ethnopharmacology*. 92 pp177-191

Davis, B.G., & Prestegard, J. H. (2023). Primary Structure of Glycans by NMR Spectroscopy, *American Chemical Society; Chemical Reviews*, 123, pp 1040–1102. DOI: https://doi.org/10.1021/acs.chemrev.2c00580

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenolic content and antioxidant activity. *Food and Drug Analysis*, 22(3), 296–302.

Dudley, W. & Ian, F. (2006). *Spectroscopic Methods in Organic Chemistry,* 6th edition, McGraw-Hill Education, Berkshire, UK, 2006

Ezeonu, C. S. & Ejikeme, C. M. (2016). Phytochemical and antimicrobial studies of Euphorbia heterophylla L. *Medicinal Plants Research,* 10(28), 409–415. doi: 10.5897/JMPR2016.6251.

Field, L.D., Sternhell, S., and Kalman, J.R (2007) *Organic structures from spectra*, 4th edition, John Wiley and Sons Limited. Chichester, New York, Brisbane, Toronto.

Harborne, J. B. (1998). *Phytochemical Methods*: *A Guide to Modern Techniques of Plant Analysis.* 3rd edition Springer. doi: 10.1007/978-94-009-1111-3.

Huang, X. A., Shen, X., Hu, Y.J., Liu, Y-M, Lui, K.-L., Zhang, F.-X., & Zhou, X-X. (2011). Two new triterpenoids from Lysimachia heterogenea Klatt and evaluation of their cytotoxicity. *Molecules* volume 16; 8076-8082

Kirsten, M. Inger, L. Hedvig, P. Artur, S. & Cooke, B.M. (2008). *Methods in Malaria Research.* MR4 American Type Culture Collection. 5th edition. University Boulevard, Manassas.

Kobayashi, W., Miyase, T., Suzuki, S., Noguchi, H., and Chen, X. M. (2000). Structure of new glycosides from plant sources determined by NMR spectroscopy. *Natural Products*, 63(8), 1121–1126. https://doi.org/10.1021/np000048q

Kulic, Z., Steiner, V. J. N., & Butterer, A. (2026). NMR chemical shifts of common flavonoids. *Planta Medica*, 92(1), 58–80. https://doi.org/10.1055/a-2706-7473

Lombard, M., & Le Foll, A (1989). *In vitro* sensitivity of Plasmodium falciparum: relationship to *in vivo* response. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83(5), 592–598. Doi;10.1016/0035-92039(89)90341-1

Lombard, M., & Le Foll, A. (1989). In vitro drug sensitivity of Plasmodium falciparum: relation to in vivo response. Transactions of the Royal Society of Tropical Medicine and Hygiene, 83(5), 595–598. doi: 10.1016/0035-9203(89)90341-1.

Mahomoodally, M. F., Dall'Acqua, S., Sinan, K. I., Sut, S., Ferrarese, I., Etienne, O. K., Sadeer, N. B., & Zengin, G. (2020). Phenolic compounds analysis of three Euphorbia species by LC-DAD-MS and their biological properties. *Journal of Pharmaceutical and Biomedical Analysis,* 189, 113477. DOI: 10.1016/j.jpba.2020.113477 ScienceDirect

Mayo D.W., Pike, R.M. & Forbes D.C. (2011). *Microscale Organic Laboratory*: With Multistep and Multiscale Synthesis, 5th edition, J. Wiley & Sons. Hoboken New Jessy.

Park, Y., Lee, Y.U.K., Kim, H., Lee, Y., Ah-Yoon, Y., Moon, B., Chong, Y., Ahn, J.H., Shim, Y.H., & Lim, Y. (2006). NMR data of flavone derivatives and their anti-oxidative activities: *Bulletin Korean chemical society;* Volume 27 No. 10 ISSN1537

Park, Y., Moon, B. H., Lee, E., Lee, Y., Yoon, Y., Ahn, J. H., & Lim, Y. (2007). 1H and 13C-NMR data of hydroxyflavone derivatives. *Magnetic Resonance in Chemistry*, 45(8), 674–679. doi: [10.1002/mrc. 2022]

Richter, T. & Bergers, S. (2013). An NMR method to determine the anomeric specificity of glucose phosphorylation. *Bio-Organic and Medicinal Chemistry*. Volume 21, Issue 10 pp 2710 – 2714.

Saxena, S., Pant, N., Jain, D. C., & Bhakuni, R. S. (2003). Antimalarial agents from plant sources. *Current Science*, 85(9), 1314–1329.

World Health Organization. (2024). World Malaria Report 2024. Geneva: World Health Organization.