Jewel Journal of Scientific Research (JJSR) 10(1): 68–79, 2025
©Federal University of Kashere-Nigeria (Print ISSN: 2384 – 6267, Online ISSN 2756-651X)
https://journals.fukashere.edu.ng/index.php/jjsr

Isolation and Characterization of Flavonoids from *Myosotis scorpioides* L. (Boraginaceae) and Evaluation of Antimalarial Efficacy of Dichloromethane Extract of the Plant

Abdu, Z.[1*], Isyaka, M. S.[2], Sai'd, J.[1], Fatope, O. M.[3], Kendeson C. A.[1], Andrew, S.[4] and Sunday, D.[5]

[1]Department of Chemical Sciences, Federal University of Kashere, P.M.B 0182, Gombe – Nigeria
[2]Department of Chemistry, North Eastern University, Gombe – Nigeria
[3]Department of Chemistry, Sultan Qaboos University (SQU) Muscat - Oman
[4]Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu – Abuja, Nigeria
[5]Department of Pharmacology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu – Abuja, Nigeria
***Corresponding Author**: abdu.zakari@fukashere.edu.ng, zabdu70@gmail.com, +2348136202778

Abstract
The plant sample (*Myosotis scorpioides L (*Boraginaceae*)*) was selected based on its ethnomedicinal usage by the traditional Lala people in Adamawa State as a remedy to malarial disease. The extract was initially screened for the presence of phytocompounds and antiplasmodial assay and was run in the Vacuum Liquid Chromatography followed by Column Chromatography to obtain the pure compounds. The result of the phytochemical screening revealed that extract of the plant contained most of the important secondary metabolites such as alkaloids, flavonoids, terpenes, steroids, saponins etc. The antimalarial evaluation revealed a potential drug candidate to remedy malaria disease. The Vacuum Liquid Chromatography (VLC) followed by Column Chromatography of the plant extract afforded two pure Compounds: Ms-25-3 (**1**) and Ms-10-102 (**2**). The two Compounds were analyzed on FT-IR machine, BRUKER NMR-spectrophotometer (700MHz) and a HR-MS machine. The data obtained were used for the structural elucidation of the Compounds. 5,7-dihydroxy-2-(3-hydroxyphenyl)-3-((3,4,5,6tetrahydroxytetrahydro-2H-pyran-2-yl)methoxy)-4H-chromen-4-one (**1**) a **new flavonoid** and 2-(3,4-Dihydroxy-phenyl)-5,7-dihydroxy-3-[3,4,5-trihydroxy-6-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxymethyl)-tetrahydro-pyran-2-yloxy]-chromen-4-one (**2**). The two flavonoids might be responsible for the antimalarial activity observed in the crude extract of the plant. The result has supported the ethnomedicinal use of the plant, confirmed its activity and has also provided a baseline data for future research on the plant. However, further phytochemical, biological, pharmacological investigations and exhaustive isolation and purification methods are highly recommended on the solvent extracts of the plants.

Keywords: Isolation, 2D NMR, Structural Elucidation, Flavonoid, Ethnomedicinal
Received: 19th Feb., 2025 *Accepted:* 27th June, 2025 *Published Online:* 30th June, 2025

Introduction

Plants have great potential uses, especially as traditional medicine and pharmacopeia drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine (Abdallah *et al*, 2017). Medicinal plants have provided the modern

medicine with numerous plant- derived therapeutics agents (Evans, 2000). Many plants contain a variety of phytopharmaceuticals which have found very important applications in the field of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drugs leads for the treatment and prevention of diseases (Dave *et al*., 2012); (Kalayou *et al*., 2012). The emergence of multi drug resistance in human pathogens including the malaria parasite *Plasmodium falciparum* has triggered interest in the search for new antimalarial drug and vaccine from plants origin (Weatherall, 2008). One of the possible ways to overcome this phenomenon of multiresistance is the continual search for new bioactive molecules (Igor *et al*., 2016). With regard to the broad diversity of their secondary metabolites, medicinal plants represent undeniable sources of antibacterial agents. According to WHO (WHO, 2016), 80 % of people in Africa have used medicinal plants for their health care; it is also estimated that among medicines sold worldwide, 30 % contain compounds derived from medicinal plants (FAO, 2016). Several African medicinal plants previously investigated for biological potential showed good antibacterial activities (Mbaveng, *et al*., 2015). *Myosotis scorpioides* L (Boraginaceae) is commonly found in freshwater marshes, ditches, slow moving water and shallow pools **(**Kuete *et al*, 2013**)**. It grows best in saturated soils and is usually found growing with many obligate plant species such as water parsley (*Oenanthe sarmentosa*), and sedges (Carex species). *Myosotis scorpioides* is a stoloniferous to rhizomatous plant that grows 20-60 cm tall **(**Kamaraj *et al*, 2012**)**. There are about 100 species of *Myosotis scorpioides L* native to temperate areas **(**Fabry *et al*, 2009**)**. These species include *Myosotis scorpioides*, *Myosotis australis*, *Myosotis uniflor*, *Myosotis brevis* etc. *Myosotis* plants display an amazing amount of diversity regarding habitat, flower shape, colour, and ecology **(**Bantie *et al*, 2014**)**. *Myosotis scorpioides* L., has been studied for its potential medicinal properties, including antiplasmodial activity. Plasmodium, the genus responsible for malaria, remains a major cause of morbidity and mortality, especially in tropical regions. With the increasing resistance of Plasmodium spp. to current antimalarial drugs, the exploration of natural plant-based compounds has gained significant attention as a promising alternative source for antimalarial agents. Frank *et al.,* (2010) in an article titled 'Antibacterial activity of traditional medicinal plants' in the report *Myosotis scorpioides* L was revealed to have antibacterial efficacy. Tunon *et al.,* (1995) reported the anti-inflammatory activity of *Myosotis scorpioides L* in their work titled 'Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Abdu *et al* (2017) reported the antiplasmodial activity of the methanol extract of *Myosotis scorpioides* L. Chemically *Myosotis scorpioides L* have been shown to contain relatively large proportions of γ-linolenic and octadecatetraenoic acids as well as the more usual palmitic, linoleic and linolenic acids. The plant extract was also reported to contain some pyrolizidine alkaloids (Jaimieson *et al* 1968).

Jamieson *et al*., 1969 reported the lipids content of leaf of some members of the Boraginaceae family including *Myosotis scorpioides L*. A report submitted by Li-jing *et al.,* 2016 on 'Isolation and identification of flavonoids components from *Pteris vittata* L.' provided supporting data to the analysis of the present study. The numerous advantages of this substance based on its antioxidant activity seem to be the reason for the high interest in flavonoid research. The chemical properties of flavonoids are determined by their chemical structure, hydroxylation level, conjugation, other substitutions, polymerization, level of oxidation, glycosylation pattern, and other substitutions. Although flavonoids have various biochemical characteristics, their capacity to function as antioxidants is one of the best known in almost every flavonoid group. Most flavonoid research has focused on their antioxidant and anti-inflammatory properties (Haeria *et al.,* 2022).

Materials and Method

Collection and identification of plant materials

The plant under investigation (*M. scorpioides L*) was collected from Girei Local Government Area of Adamawa State. Dr Keneth S. C (Botanist) in the Department of Biological Sciences Modibbo Adama University of Technology Yola did the identification of the plants. The plants specimens and voucher number were kept in the Herbarium.

Preparation of Plant Material

The whole plant was collected and washed thoroughly with distilled water to remove soil particles and dust. The washed sample was dried under shade to avoid direct sunlight. The dried sample was ground into fine powder, weighed and recorded.

Extraction of Plant Material

The Soxhlet Apparatus was assembled by placing a thimble containing the plant material into the Soxhlet extractor. The round-bottom flask was filled with the solvent (200 mL was enough to allow repeated cycling). The Soxhlet extractor was placed between the flask and the condenser. The condenser was connected to a water source (inlet at the bottom, outlet at the top) for cooling. The solvent was heated in the round-bottom flask gently (using a heating mantle or water bath). The solvent vapor rises through the apparatus, condenses in the condenser, and drips into the thimble, soaking the plant material. As the solvent level reaches the siphon arm, it drains back into the flask, carrying extracted compounds. This cycle was repeated automatically (typically 15–30 cycles over 4–6 hours). The extraction continued until the solvent in the thimble appears clear (indicating exhaustive extraction) then the heating was stopped and allowed the system to cool. The apparatus was disassembled and the round-bottom flask was carefully removed and the solvent was evaporated using a rotary evaporator under reduced pressure to obtain the crude extract. The extract was dried further in a desiccator and weighed to determine yield. All the solvents and other reagents and chemicals used in the research were all of analytical grade. These include but not limited to Dichloromethane (DCM), n-hexane, chloroform, ethylacetate and ethanol of SIGMA-ALDRICH analytical grade. TLC of all solvent extracts was run and carefully selected based on number of spots visible after spray and/or exposure in Iodine tank. Hot DCM was used to extract the plant powder (2.0Kg) using Soxhlet apparatus.

Phytochemical Screening of Dichloromethane (DCM)

Standard method described by Usman *et al.*, (2009) was used to test for the presence of phytochemical compounds (saponins, sterols, terpenoids, glycosides, phlobatannins, resins, flavonoids, phenols, alkaloids, and carbohydrates) in the extracts.

Antiplasmodial Screening of Dichloromethane (DCM) Plant Extract

Anti-malarial Activity

The antimalarial activity was carried out using the strain parasite *Plasmodium bergei NK45*, and Swiss albino mice. A three-model antimalarial *assay* (Suppressive, Curative and Prophylactic) was adopted. The parameters evaluated included body weight, body temperature, survival time, and parasite count (Abdu *et al*., 2018).

Vacuum Liquid Chromatography (VLC)

The pulverized plant sample was subjected to vacuum liquid chromatography (VLC) to obtain the fractions. The sintered glass funnel (porosity 3) used was loaded with silica gel (50-70 mesh particle size) under vacuum ensuring that it was compacted and uniformly spread. A non-polar solvent (petroleum ether) was run through the column under vacuum then the pre-adsorbed sample was evenly spread on the silica gel. Suction was applied to compress the sample to the silica gel and a piece of Whatman filter paper was used to cover the surface to prevent disturbance during the course of the elution. Gradient elution was employed starting with hexane, hexane/dichloromethane (1:2), dichloromethane, dichloromethane/chloroform (1:2), chloroform, chloroform/ethylacetate (1:2), ethylacetate, ethylacetate/ethanol (1:2) and finally ethanol. The fraction/eluates were concentrated using the rotary evaporator

under reduced pressure and were spotted on TLC (plate thickness, 1.0 mm), where fractions/spots with same Rf values were pooled together and considered for column chromatography (Adoum, 2000).

Column Chromatography of the Dichloromethane (DCM) Extract

The DCM extract (18.23g) of *Myosotis scorpioides L* was loaded on a column (60cm x 3.5cm). The column was initially stabilized by loading with silica gel (70-230 mesh ASTM - MERCH) simultaneously using n-Hexane (Analytical grade) down the column with the tap opened to collect the n-Hexane containing fatty materials and at the same time loading smoothly the column with the defatted silica gel up to 2/3 of the column length (40cm). A sand layer of 50-70 mesh particle size (SIGMA-ALDRICH) was placed on top of the silica followed by the extract (sample). Another layer of sand was placed on top of the sample for protection against the strong down pour of solvent unto the sample layer which might disturb the smooth surface. The column was eluted in the following order; n-hexane (1L), 9.5:0.5 hexane: dichloromethane (1L), 9:1 hexane: dichloromethane (1L), 8:2 hexane:dichloromethane (1L), 7:3 hexane:dichloromethane (1L), 6:4 hexane: dichloromethane (1L),1:1hexane: dichloromethane (1L), Dichloromethane (1L), 9:1dichloromethane:chloroform (1L),8:2 dichloromethane:chloroform (1L), 7:3 dichloromethane: chloroform (1L), 6:4 dichloromethane:chloroform (1L), 1:1 dichloromethane: chloroform (1L), Chloroform (1L), 9:1 chloroform:ethyl acetate (1L), 8:2 chloroform: ethyl acetate (1L), 7:3 chloroform: ethyl acetate (1L), 6:4 chloroform: ethyl acetate (1L), 1:1 chloroform: ethyl acetate (1L), ethyl acetate (1L), 9:1 ethyl acetate: ethanol, 8:2 ethyl acetate: ethanol, 7:3 ethyl acetate: ethanol, 6:4 ethyl acetate: ethanol, 1:1 ethyl acetate: ethanol, Ethanol (1L) Fractions collected were spotted, viewed under UV light, eluted in hexane: dichloromethane solvent systems. Spots were viewed under UV light, then sprayed with dodeca-molybdophosphoric acid (0.5g dissolved in 100ml ethanol) and heated using a heat gun (Abdu, 2019).

Results and Discussion

The result of the phytochemical screening in Table 1 showed that the DCM extract of the plant contained most of the secondary metabolites which include alkaloids, flavonoids, saponins, etc. These phytocompounds may be responsible for the observed bioactivity during the screening.

Table 1: Phytochemical Screening of Dichloromethane (DCM) Extract

Phytochemical Constituents	DCM	MeOH
Indole alkaloid	+	+++
Tropane alkaloids	-	++
Quinoline alkaloids	+	++
Morphane alkaloids	-	-
Steroids	+	+
Flavonoids	++	+++
Saponins	++	++
Tannins	-	++
Phenols	+	-
Carbohydrates	+++	++
Anthraquinones	-	+
Resin	-	-
Cardiac glycosides	+	+

Antimalarial Screening of Ethanol Extract

The results in table 2 above revealed the suppression of the *P. bergei* parasite by varying the concentration of the DCM extract of the plant. At higher concentration (250 $mgKg^{-1}$) the suppression was recorded to be 75.62% as compared to 98.39% for the control (CQ $5mgkg^{-1}$).

Table 3 contained the results of the curative effect of the DCM extract of the plant on *P. bergei* parasite. At a concentration of 250 $mgKg^{-1}$ the extract exhibited 83.52% chemosuppression.

The results also showed that the DCM extract of the plant has 0.07 ± 0.02 %parasitemia as compared to the CQ control with 0.07 ± 0.02 value. Table 4 revealed the results of the prophylactic effect of the DCM extract of the plant on *P. bergei* parasite. At a concentration of 250 $mgKg^{-1}$ the extract exhibited 60.59% chemosuppression. The results also showed that the DCM extract of the plant exhibited 0.07 ± 0.01 parasitemia as compared to the control (CQ) with 0.06 ± 0.01 value.

Table 5 above revealed the mean survival period of Swiss Albino Mice in dichloromethane (DCM) extract and Chloroquine in established malaria infection. The result showed that at a concentration of 250 $mlKg^{-1}$ the survival time in days was 26 as compared to the control (CQ) with 30 days.

Table 2: Suppressive Effect of Dichloromethane (DCM) Extract and Chloroquine against *P. bergei* Infection in Swiss Albino Mice

Treatment	% Parasitemia	%Chemo-suppression	Significance
10% saline 5 $mlkg^{-1}$	0.36 ± 0.057	0	NS
Extract 100 $mgKg^{-1}$	0.23 ± 0.03	57.92	P < 0.01
Extract 150 $mgKg^{-1}$	0.16 ± 0.06	66.62	P < 0.01
Extract 250 $mgKg^{-1}$	0.13 ± 0.07	75.62	P < 0.01
Chloroquine(CQ) $5mgkg^{-1}$	0.07 ± 0.02	98.39	P < 0.01

NS = No Significance

Table 3: Curative Effect of Dichloromethane (DCM) Extract and Chloroquine against *P. bergei* Infection in Swiss Albino Mice

Treatment	% Parasitemia	%Chemo-suppression	Significance
10% saline 5 $mlKg^{-1}$	0.45 ± 0.13	0	NS
Extract 100 $mgKg^{-1}$	0.13 ± 0.07	71.26	P < 0.01
Extract 150 $mgKg^{-1}$	0.12 ± 0.06	74.16	P < 0.01
Extract 250 $mgKg^{-1}$	0.07 ± 0.02	83.52	P < 0.01
Chloroquine 5 $mgkg^{-1}$	0.07 ± 0.02	96.44	P < 0.01

NS = No Significance

Table 4: Prophylactic Effect of Dichloromethane (DCM) Extract and Chloroquine against *P. bergei* Infection in Swiss Albino Mice

Treatment	% Parasitemia	%Chemo-suppression	Significance
10% saline 5 $mlKg^{-1}$	0.23 ± 0.02	0.00	NS
Extract 100 $mgKg^{-1}$	0.11 ± 0.02	49.17	P < 0.01
Extract 150 $mgKg^{-1}$	0.09 ± 0.02	56.56	P < 0.01
Extract 250 $mgKg^{-1}$	0.07 ± 0.01	60.59	P < 0.01
Chloroquine 5 $mgkg^{-1}$	0.06 ± 0.01	93.94	P < 0.01

Table 5: Mean Survival Period of Swiss Albino Mice in Dichloromethane (DCM) Extract and Chloroquine in Established Malaria Infection

Dose of Extract(mg/Kg/day)	Survival Time (days)
10% Saline 5 $mlKg^{-1}$ (control)	15
Extract 100 $mlKg^{-1}$	20
Extract 150 $mlKg^{-1}$	23
Extract 250 $mlKg^{-1}$	26
Chloroquine (CQ) 5 mg/Kg^{-1}	30

Column Chromatography of Dichloromethane (DCM) Extract

Fractions Ms-25-3 and Ms-50-5 were collected as solids with R_f = 0.40 and 0.45 in dichloromethane: chloroform (4:6) solvent ratio respectively. The HR-ESIMS m/z for three compounds are; 447.38 $[MNa]^+$ (calculated for $C_{21}H_{20}O_{11}Na$ 470.38), 312.10 $[MNa]^+$ (calculated for $C_{18}H_{16}O_5Na$ 335.10 and 626.15 $[MNa]^+$ (calculated for $C_{27}H_{30}O_{17}Na$ 649.15. The proton (^1H) NMR, ^{13}C NMR, and IR, suggest that the compounds are flavonoids. The signal of hydroxyl protons at δ5.0 value range and aromatic ring protons were conspicuous at δ value 4.69 and 4.68, a δ value of 4.57 corresponds to O-CH protons. A δ value of 2.3 corresponds to C=C-H protons. The carbon-13 NMR revealed 18-carbon atoms and 27-carbon atoms compound for Ms-25-3 (**1**) and Ms-10-102 (**2**) respectively. Carbonyl carbon at δ171ppm, two peaks at δ144ppm and δ139ppm corresponding to C=C aromatic ring alkene, a moderate peak at 81ppm revealed a O-CH ring carbon. The peak at 77ppm with high intensity is the solvent (deuterated chloroform). Generally, the spectroscopic analysis supported the presence of flavonoid chromophore for the two isolated compounds. The IR spectrum revealed a carbonyl (C=O) absorption band at 1732.629 cm^{-1}, C-O stretch at 1245 cm^{-1}, C-H stretch (broad) at 2942.273 cm^{-1}, C-H stretch at 3074.489 cm^{-1}, broad peak at 3300 cm^{-1} corresponding to the OH group. Tables 1.0-2.0 provide the data (carbon atom positions, ^1H multiplicity, coupling constant and ^1H-^1H COSY) for Compound 1. From these data and the library data obtained from both ChemBioDraw Ultra 3.0 and MestReNova application software, compound (**1**) was identified as a new flavonoid**.** A report submitted by Li-jing *et al.*, 2016 on 'Isolation and identification of flavonoids components from *Pteris vittata* L.' provided supporting data to the submission of this study. Plants are one of the important sources for screening active compounds. Consequently, the attempt to obtain bioactive components from *M. scorpioides* L to provide more information about its chemical constituents in this experiment became imperative. Previous works showed that flavonoids exhibited different bioactivities, such as anti-inflammatory, anti-oxidative, hypolipidemic,

or antitumor effects (Bao *et al*. 2016; Feng *et al*. 2014; Matias *et al*. 2014; Raman *et al*. 2016). Compound (**1**) is believed to be a derivative of quercetin mentioned in previous results (Imperato 2006).

Table 6: ^{13}C and ^{1}H NMR data (Chloroform-d_1 700 MHz for ^{1}H and 176 MHz ^{13}C) for Compound (1) Ms-25-3.

Position	δC	δH	J (Hz)
O	-	-	-
2	156.4C	-	-
3	166.4C	-	-
4	158.8C	-	-
5	81.6 CH	3.60d	4.20
6	75.1CH	4.00t	3.20,3.31
7	71.5CH	3.60 t	3.40,3.51
8	76.5CH	3.70d	4.30
9	178.2C	-	-
10	135.1C	-	-
1’	161.8C	-	-
2’	98.3CH	6.02d	5.30
3’	115.8CH	7.48t	3.40,3.31
4’	94.0 CH	6.65t	4.50,4.51
5’	129.2CH	6.65t	3.32,3.20
6’	115.8 CH	7.48d	3.21
1’’	109.6CH	5.60d	3.41
2’’	157.7CH	4.50t	3.31,3.30
3’’	104.5C H	5.21t	4.21,4.20
4’’	122.9CH	5.94t	4.22,4.21
5’’	129.2CH	4.21q	4.41,4.40
6’’	62.2 CH_2	3.57d	3.06
O	-	-	
OH	-	9.68s	-
OH	-	4.77s	-
OH	-	4.88s	-
OH	-	4.71s	-
OH	-	9.68s	-
OH	-	4.77s	-
OH	-	4.88s	-

Table 7: ^{13}C and ^{1}H NMR data (Chloroform-d_1 700 MHz for ^{1}H and 176 MHz ^{13}C) for Compound (2) Ms-10-102 and Comparative Literature Data

Position	δC Expt.	δC Lit.	δH Expt.	δH Lit.	J(Hz)
O	--	-	-	-	-
2	158.4C	156.4	-	-	-
3	136.4C	133.2	-	-	-
4	178.8C	177.3	-	-	-
5	158.6 CH	156.6	11.60 (OH, s)	12.62 (OH, s)	
6	95.1CH	98.6	7.10 (1H, d)	6.19 (1H, d)	1.88
7	167.5CH	164	11.92 (OH, s)	12.62 (OH, s)	

8	96.5CH	93.6	6.51 (1H, d)	6.41 (1H, d)	1.80
9	162.2 C	161.1	-	-	-
10	105.1C	103.9	-	-	-
1'	123.8C	121.1	-	-	-
2'	118.3CH	115.2	7.73 (1H, d)	7.53 (1H, d)	8.08
3'	145.8CH	144.6	12.92 (OH, s)	12.62 (OH, s)	
4'	149.0 CH	148.3	12.42 (OH, s)	12.62 (OH, s)	
5'	119.2CH	116.2	6.55 (1H, d)	6.85 (1H, d)	7.84
6'	124.8 CH	121.6	8.10 (1H, d)	7.55 (1H, d)	7.56
O	-	-	-	-	-
O	-	-	-	-	-
2a	104.5CH	101.1	4.92 (1H, d)	5.32 (1H, d)	7.44
3a	72.9CH	73.9	3.10 (1H, d)	3.08 (1H, d)	9.28
4a	79.2CH	75.8	3.52 (1H, d)	3.23 (1H, d)	6.0
5a	68.2 CH_2	69.9	3.56-3.76 (3H)	3.26-3.36 (3H)	
6a	79.4CH	76.3	3.51 (1H, d)	3.21 (1H, d)	5.52
7a	69.4CH	66.9	3.66-3.76 (3H)	3.26-3.36 (3H)	
O	-	-	-	-	-
1B	98.6 CH	100.7	4.77 (1H, d)	4.37 (1H,d)	7.6
2B	71.1CH	70.3	3.54 (1H, d)	3.04 (1H,d)	2.68
3B	71.5CH	70.5	4.19 (1H, d)	3.69 (1H,d)	10.4
4B	74.5CH	71.8	3.66-3.76 (3H)	3.26-3.36(3H)	
5B	70.2 CH	68.2	3.89 (1H, d)	3.39 (1H, d)	1.76
6B	15.1CH	17.6	18.6-16.97 (3H, d)	15.6-15.97(3H,d)	6.12

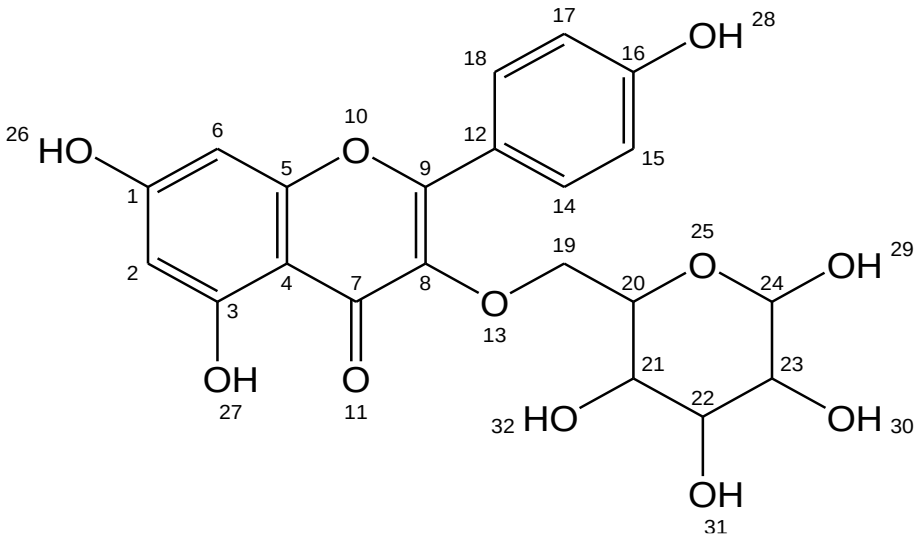
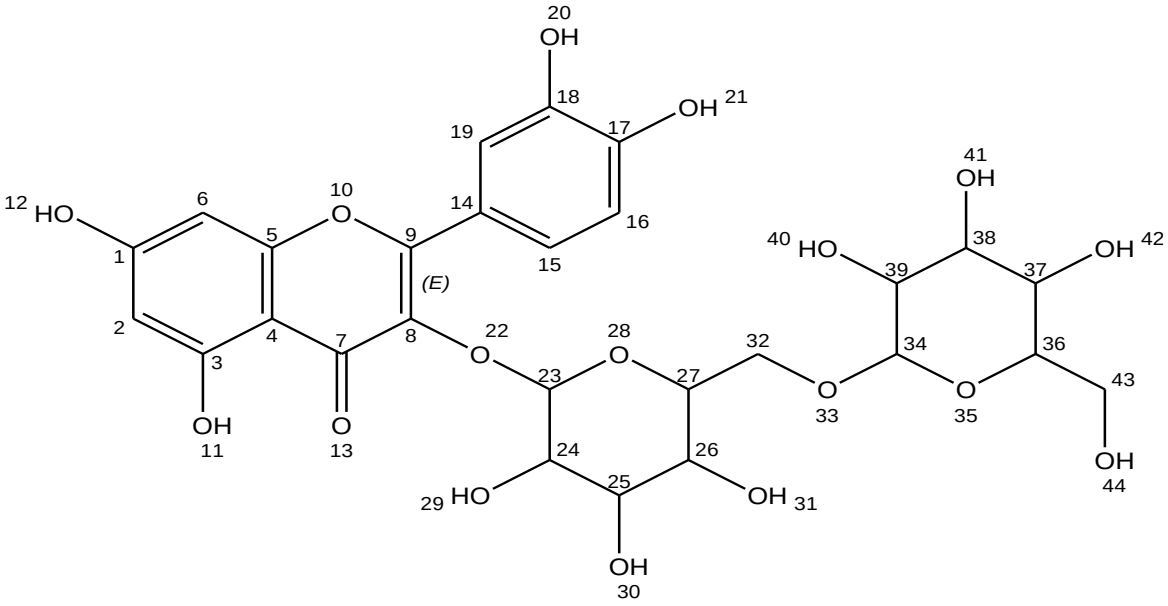


Figure 1: Atomic Labels of Compound 1

Figure 2: 3D Model of Compound 1



2-(3,4-Dihydroxy-phenyl)-5,7-dihydroxy-3-[3,4,5-trihydroxy-6-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxymethyl)-tetrahydro-pyran-2-yloxy]-chromen-4-one

Figure 5: Atomic Labels of Compound 2

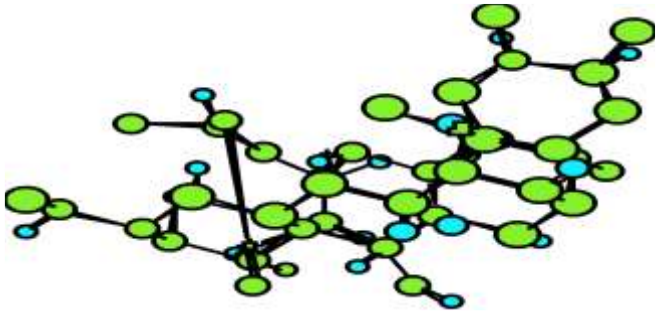


Figure 6: 3D Model of Compound 2

Discussion

Myosotis scorpioides presented a promising candidate for the development of new antimalarial drug, especially in light of increasing drug resistance. The plant's rich array of bioactive phytochemicals as demonstrated in the results of preliminary phytochemical screening and the antimalarial investigation conducted in this study suggested that it could serve as a valuable

addition to the arsenal against malaria. However, more extensive clinical trials and research into its pharmacokinetics, safety profile, and potential for drug synergy are needed to fully realize its therapeutic potential. Previous works on the plant mainly covered bioactivity of different solvent extracts of the plant (Abdu *et al*, 2018). However this research is in agreement with several works on isolation of flavonoids from plants extract. Specific flavonoids that have been isolated from plants extract and reported by researchers include but not limited to; Quercetin, isolated from *Chenopodium album* via acetone extraction and flash chromatography (Arora *et al*, 2018; Chludil *et al*, 2008; and Choudhary *et al*, 2021). Biflavonoids, isolated from *Garcinia dulcis* leaves, purified using column chromatography(Abdullah *et al*, 2018; Khamthong *et al*, 2017 and Harrison *et al*, 1994). Unique flavonoids isolated from *Euphorbia neriifolia*, identified via HPTLC and NMR(Sharma, V., & Janmeda, P. 2017; Sultana *et al*, 2022 and Mali *et al*, 2017).

Future research should focus on isolating and characterizing the specific compounds responsible for the antiplasmodial activity of *Myosotis scorpioides*, as well as investigating their potential as leads for novel drug development. Additionally, exploring the plant's traditional medicinal uses in various cultures could offer insights into its broader pharmacological applications.

Acknowledgement

Authors wish to acknowledge TETFUND - Nigeria for the research grand. The management of Federal University of Kashere provided conducive atmosphere for the research to take place. The contribution of the staff of NIPRD was immeasurable. The contribution rendered by Professor Majek O. Fatope of the Department of Chemistry Sultan Qaboos University – Oman in this work is quite indispensable. Staff of the Department of Chemical Sciences are appreciated for their moral support.

Conflict of Interest

The authors wish to state that there is no conflict of interest in the present research.

Additional files

The IR, MS and NMR (13C and 1H) spectra data of the compounds can be found as additional files for the present article.

References

Abdu Z. (2019): Extraction and Characterization of Medicinal Plants used for the Treatment of Malaria in Adamawa State – Nigeria. Unpublished PhD Thesis of Modibbo Adama University of Technology, Yola.

Abdu, Z., Kubmarawa D, Isyaka S. M, Kendeson C. A, Baba E. B (2018): *In vivo* Evaluation of Antiplasmodial Properties of *Myosotis scorpioides L.* (*Boraginaceae*) Extract in Albino Mice Infected with *Plasmodium bergei*. *Trop J Nat Prod Res.* 2(4): 198-202. doi.org/10.26538/tjnpr/v2i4.8.

Abdullah, I., Phongpaichit, S., Voravuthikunchai, S. P., & Mahabusarakam, W. (2018). Prenylated biflavonoids from the green branches of Garcinia dulcis. *Phytochemistry Letters*, *23*, 176-179.

Arora, S., & Itankar, P. (2018). Extraction, isolation and identification of flavonoid from Chenopodium album aerial parts. *Journal of traditional and complementary medicine*, 8(4): 476-482.

Bantie L, Assefa S, Teklehaimanot T and Engidawork E. (2014). In vivo antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. *BMC Complement Altern Med.* 14: 79.

Bao L, Hu L, Zhang Y and Wang YI (2016). Hypolipidemic effects of flavonoids extracted from *Pteris vittata*. *Exp Ther Med* 11(4): 1417–1424

Chludil, H. D., Corbino, G. B., and Leicach, S. R. (2008). Soil quality effects on Chenopodium album flavonoid content and antioxidant potential. *Journal of agricultural and food chemistry*, 56(13): 5050-5056.

Choudhary, N., Prabhakar, P. K., Khatik, G. L., Chamakuri, S. R., Tewari, D. and

Suttee, A. (2021). Evaluation of Acute toxicity, In-vitro, In-vivo Antidiabetic Potential of the Flavonoid Fraction of the plant Chenopodium album L. *Pharmacognosy Journal*, 13(3):

Dave H, Ledwani L. (2012): A review on anthraquinones isolated from Cassia species and their applications. *Indian J Nat Prod Resour*. 3(3): 291–319.

Fabry, W., Okemo, P. O. and Ansorg, R. (2009). Antibacterial activity of East African medicinal plants. *J Ethnopharmacol*. 60: 79–84.

FAO (2004). Trade in medicinal plants ftp://ftp.fao.org/docrep/fao/008/af285e/af285e00.pdf. Accessed 25 December, 2019.

Feng Z, Hao W, Lin X, Fan D, Zhou J (2014). Antitumor activity of total flavonoids from *Tetrastigma hemsleyanum* Diels et Gilg is associated with the inhibition of regulatory T cells in mice. *Onco Targets Ther* 7:947–956

Frank, M. F. and Ryan M. (2010). Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. *BMC Complementary Altern Med*. 10: 64. doi: 10.1186/1472-6882-10-64 Retrieved 29/6/2019

Harrison, L. J., Leong, L. S., Leong, Y. W., Sia, G. L., Sim, K. Y., & Tan, H. T. W. (1994). Xanthone and flavonoid constituents of Garcinia dulcis (Guttiferae). *Natural Product Letters*, *5*(2), 111-116.

Igor, K. V., Veronique, P. B. and Victor K. (2016) Antibacterial activity of six medicinal Cameroonian plants against Gram-positive and Gram-negative multidrug resistant phenotypes *BMC Complementary and Alternative Medicine* 16:388 DOI 10.1186/s12906-016-1371-y

Imperato, F. (2006). The new flavones ester Apigenin-7-O-oxy-p-hydroxybenzoate and 3-Di-C-glycosyl flavones from *Pteris vittata*. *Am Fern J*. 96(2): 62–65

Jamieson G. R. and E.H. Reid (1969): The leaf lipids of some members of the Boraginaceae family *Phytochemistry* 8(8):1489-1494 DOI: 10.1016/S0031-9422(00)85918-X

Kalayou, S., Haileselassie, M., Gebre-Egziabher, G., Tikue, T., Sahle, S., Taddele, H. and Ghezu, M. (2012). In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. *Asian Pac J Trop Biomed*. 2(7):516–22.

Kamaraj, C., Rahuman, A. A., Siva, C., Iyappan, M. and Kirthi, V. A. (2012): Evaluation of antibacterial activity of selected medicinal plant extracts from south India against human pathogens. *Asian Pac J Trop Biomed*. 2(1): 296–301.

Khamthong, N., & Hutadilok-Towatana, N. (2017). Phytoconstituents and biological activities of Garcinia dulcis (Clusiaceae): A review. *Natural product communications*, *12*(3), 1934578X1701200337.

Kuete, V., Voukeng, K. I., Tsobou, R., Mbaveng, T. A., Wiench, B., Penlap, B. V. and Efferth, T. (2013). Cytotoxicity of *Elaoephorbia drupifera* and other Cameroonian medicinal plants against drug sensitive and multidrug resistant cancer cells. BMC *Complement Altern Med.* 13: 250.

Lin, L., Xiao-bing, H. and Zhen-cheng L. (2016): Isolation and identification of flavonoids components from *Pteris vittata* L. *SpringerPlus* 5:1649 https://doi.org/10.1186/s40064-016-3308-9

Mali, P. Y., and Panchal, S. S. (2017). *Euphorbia neriifolia* L.: Review on botany, ethnomedicinal uses, phytochemistry and biological activities. *Asian Pacific journal of tropical medicine*, 10(5): 430-438.

Matias, A., Nunes, S. L., Poejo, J., Mecha, E., Serra, A. T., Madeira, P. J. and Bronze, M. R. and Duarte, C. M. (2014) Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate

recovered from *Opuntia ficus-indica* juice. *Food Funct* 5(12): 3269–3280

Mbaveng, T. A., Sandjo, L. P., Tankeo, S. B., Ndifor, A. R., Pantaleon, A., Nagdjui, T. B. and Kuete, V. (2015). Antibacterial activity of nineteen selected natural products against multi-drug resistant Gram-negative phenotypes. *SpringerPlus*. 4: 823

New flavonoid from *Pteris vittata*. *Am Fern J*. 96(1):42–47

Raman, S. T., Ganeshan, A. K., Chen, C., Jin, C., Li, S. H., Chen, H. J. and Gui, Z. (2016). In vitro and in vivo antioxidant activity of flavonoid extracted from mulberry fruit (*Morus alba* L.). *Pharmacogn Mag* 12(46): 128–133

Sharma, V., & Janmeda, P. (2017). Extraction, isolation and identification of flavonoid from Euphorbia neriifolia leaves. *Arabian Journal of Chemistry*, 10(4): 509-514.

Sultana, A., Hossain, M. J., Kuddus, M. R., Rashid, M. A., Zahan, M. S., Mitra, S. and Naina Mohamed, I. (2022). Ethnobotanical Uses, Phytochemistry, toxicology, and pharmacological properties of *Euphorbia neriifolia* Linn. against infectious diseases: A comprehensive review. *Molecules*, 27(14): 4374.

Tunón H., Olavsdotter C., and Bohlin L., (1995). Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis *Journal of Ethnopharmacology* 48(2): 61-76 https://doi.org/10.1016/0378-8741(95)01285-L

Usman H., Abdulrahman F. I, and Usman A. (2009). Qualitative Phytochemical Screening and *In Vitro* Antimicrobial Effects of Methanol Stem Bark Extract of *Ficus thonningii* (Moraceae) *Afr J Tradit Complement Altern Med*. 6(3): 289–295.

Weatherall DJ (2008). "Genetic variation and susceptibility to infection: The red cell and malaria". *British Journal of Haematology* 141(3): 276–86. doi:10.1111/j.1365-2141.2008.07085.x. PMID 18410566.

WHO. Traditional medicine. (2003). http://www.who.int/mediacentre/ factsheets/2003/fs134/en/. Accessed 25 Decenber, 2019.