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RESEARCH ARTICLE

A New Isoflavone from *Lomariopsis guineensis* (Underw.) Alston

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ABSTRACT

Aim/background: *Lomariopsis guineensis* (Underw.) Alston is an epiphytic climbing fern. It is widely distributed in Africa where it is also used in traditional medicine and as food. There are no previous reports of any constituents of the plant, hence this study to isolate any phytoconstituents. **Method:** The ethyl acetate extract of the leaves was subjected to column chromatography and isolated constituents were characterized using nuclear magnetic resonance and mass spectrometry. **Results:** Three compounds were isolated and identified as cycloartenol, pheophytin A and a new isoflavone (5, 7-dihydroxy-4′ methoxy-6,8-dimethylisoflavone). **Conclusion:** Three phytochemicals including a new isoflavone are reported from the plant for the first time.

Keywords: Isoflavone; *Lomariopsis guineensis*; pheophytin A; cycloartenol; antitrypanosomal assay

INTRODUCTION

Drug discovery from medicinal plants based on ethnopharmacological practices has gained momentum in recent times (Igoli et al., 2022, Ribeiro-Filho et al., 2022, 2023). Another recent strategy is the *in silico* or Computer Aided Drug Design screening of natural compound libraries for active moieties or to identify pharmacophores responsible for drug activity (Rao et al., 2022, Wilson et al. 2020) or toxicity (Onguene et al., 2017, Roncaglioni et al., 2013). Natural product libraries are also being created and interrogated for their activities and the diverse chemical space which they easily provide. Thus the isolation of novel natural compounds to add to the compound libraries and introduce more chemical diversity is a new approach in natural products isolation and drug discovery from natural compounds. *Lomariopsis guineensis* (Underw.) Alston (Lomariopsidaceae) is an epiphytic fern growing on forest trees by flattened rhizomes clamped on to the supporting tree. It is a vegetable growing wild in the farming communities of Sub-Saharan Africa (Caldero´n et al., 2002; Global plants, 2021). It is recognized as a vital addition to food and diet in many African communities. The plant grows naturally in self-maintaining populations in natural ecosystems and can survive without any human farming activity (Nes et al., 1998). It grows freely in Angola, Benin, Central African Republic, Equatorial Guinea, Gabon, Ghana, Gulf of Guinea Island, Ivory Coast, Liberia, Nigeria, Sierra Leone, Togo and Zaïre (Ngone et al., 2016; Royal botanic gardens, 2021). It is a vegetable (Mih et al., 2017) which is also used as a medicinal plant. It is used to prepare therapies (Sobolev et al., 2005) to treat gastro-intestinal and skin disorders, cough and cold, ear problems and eye troubles. Its leaves are chewed as treatment for constipation, pneumonia, meningitis and as a first aid for epileptic attacks (Zhao et al., 2012) and other nasopharyngeal infections (Ngone et al., 2016). The fresh and tender fronds and leaves are usually cooked as soup (Zhao et al., 2012). To the best of our knowledge, no reports on the isolation or characterization of phytochemicals from the plant is available in literature, hence the plant material is hereby investigated for any known or novel compound isolation and bioassay against a disease parasite. This will not only identify the antitryparasitic activity of the plant

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but will make the isolated compounds available for other screening and add to the rapidly growing library of natural compounds for *in silico* and other screening methods. This is also an initial report of the isolation of phytochemical constituents from the plant material and antitrypanosomal assay of the plant extracts.

MATERIALS AND METHODS

NMR spectra were acquired on a JEOL Eclipse 400 spectrophotometer (400MHz) using DMSO-*d6* as solvent. HRLC-MS was run with the sample dissolved (1 mg/mL) in methanol and analyzed on an Accela 600 HPLC system combined with Q-Exactive (Orbitrap) mass spectrometer from ThermoFisher, Hemel Hempstead UK. Column chromatography was performed using silica gel MN-60 of particle size 0.063–0.200 mm (Macherey-Nagel GmbH & Co. KG). Solvents were obtained from Sigma-Aldrich or Fisher Scientific UK. UV–Vis spectra were acquired on a UNICAM UV 300 spectrophotometer.

Plant material

The leaves of the plant were collected from Cameroon in 2013 and identified by Mr Akeem Adeyanju, a taxonomist with the National Research Institute for Pharmaceutical Research and Development, Abuja Nigeria with the herbarium number NIPRD/H/7261. A voucher specimen was deposited at the herbarium of the Institute.

Extraction and isolation

Powdered leaves (105.0 g) was extracted successively using hexane, ethyl acetate and methanol in a Soxhlet apparatus and extracts were evaporated using a rotary evaporator. The crude extracts were examined by proton NMR and the ethyl acetate extract gave signals from which a flavonoid constituent was inferred. Thus 2.40 g of the ethyl acetate extract was subjected to column chromatography and eluted with hexane-ethyl acetate and ethyl acetate-methanol mixtures. Similar fractions based on TLC were pooled and allowed to evaporate to yield compound **2** (fraction 33-38, 23.0 mg), compound **3** (Fraction 40-52, 31.0 mg) and compound **1** (fraction 88-89, 48.4 mg). The compounds were identified using one dimensional ^{1}H, ^{13}C NMR and 2D experiments (COSY, HMQC and HMBC). Results were compared with literature to identify the compounds and elucidate their structures.

Antitrypanosomal assay drug sensitivity using blood stream forms of *trypanosoma brucei brucei* s427 lister strain

This was carried out as previously described (Igoli et al., 2011). The hexane, ethyl acetate and methanol extracts were screened for activity against blood stream form of *Trypanosoma brucei brucei* (*T.b.brucei*) S427 using an Alamar Blue™ assay. The extracts and compounds for assay were prepared and stored as 10 mg/ml solutions in 100% DMSO. 100 µL of the extracts were prepared in HMI-9 medium placed in respective wells of a 96-well plate. Initial screening was carried out at a single concentration of 20 µg/ml. The screening plate was set up to include sterility control, DMSO control and a concentration range of Suramin as positive control. Trypanosomes were counted and prepared at a density of 3×10^4 cells/ml, 100 µL of this suspension was added to each well of the assay plate with the exception of well A1, which is the sterility control. The assay plate was incubated at 37°C and 5% CO_2 under humidified atmosphere for 48 hours, after which 20 µL of Alamar blue dye was added to each well and the incubation continued for a further 24 hours. Fluorescence was then determined using the Wallac Victor microplate reader at λ 530 nm excitation and λ 590 nm emission. The results were calculated as % of the DMSO control values.

RESULTS AND DISCUSSION

Characterization of compound 1 as 5, 7-dihydroxy-4′ methoxy-6, 8-dimethylisoflavone

The UV maxima for the compound (1mg/ml concentration in methanol) was at 407 nm (abs = 0.78). The ^{1}H NMR (400 MHz) spectrum of the compound in DMSO-d6 (Fig. 1) showed signals typical of an isoflavone. The hydrogen bonded C-5-OH signal was observed at δ_H 13.14 (1H, s) and the characteristic alkene proton of isoflavones (H-2) at 8.43 (1H, s). Two AA'BB' coupled protons from a para substituted aromatic ring were observed at δ_H 7.50 (2H, d, J = 8.7 Hz) and 7.00 (2H, d, J = 8.7 Hz) ppm. The other protons were a methoxy group at 3.78 and two methyl protons at 2.06 and 2.18 ppm. The ^{13}C NMR (100 MHz) spectrum (Fig. 2) indicated the presence of 18 carbon atoms including a carbonyl at δc 181.0 (C-4), aromatic 2xCH at δ_C 130.8, 114.3 and the oxyethylenic carbon (C-2) at 157.1 ppm. The rest of the carbons were made up of two methyls at 8.68 and 8.63, one methoxy at 55.7, four phenolic carbons between 153.0 and 161.0 and five quaternary aromatic carbons. The complete chemical shift assignments were made based on examination of its 2D NMR spectra as follows: The COSY spectrum (Fig. 3) showed couplings between H-2'/6' and H-3'/5' as expected for a para substituted benzene ring. The HMQC spectrum (Fig. 4) revealed protons attached to their respective carbon atoms while the HMBC spectrum (Fig. 5) showed the attachment of side chains and functional groups as well as ^{2}J and ^{3}J long range connectivity between the protons and carbon atoms in the

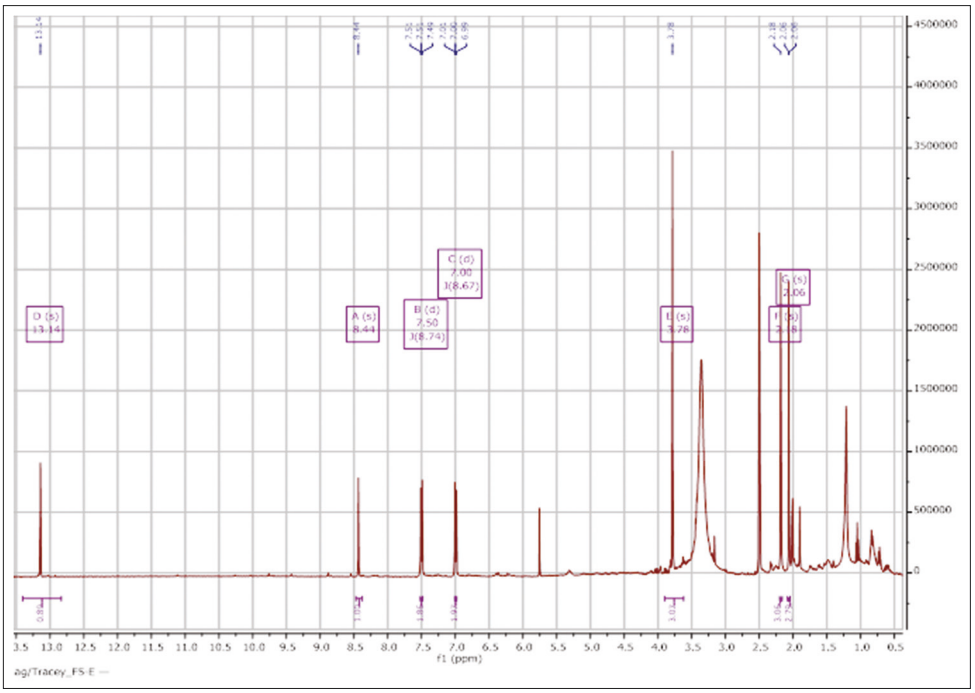

Fig 1. ^{1}H Spectrum of compound **1** in DMSO-d6.

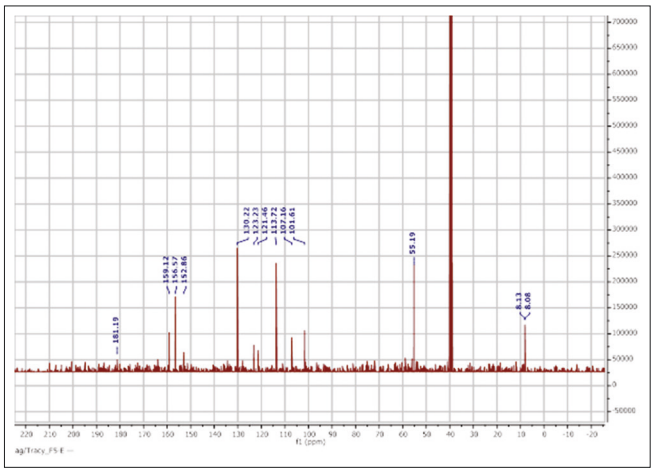

Fig 2. ^{13}C Spectrum of compound **1** in DMSO-d6.

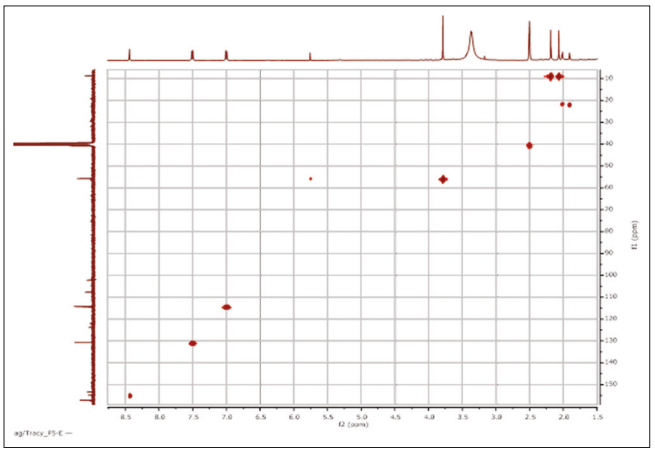

Fig 4. ^{1}H-^{13}C HMQC Spectrum of compound **1** in DMSO-d6.

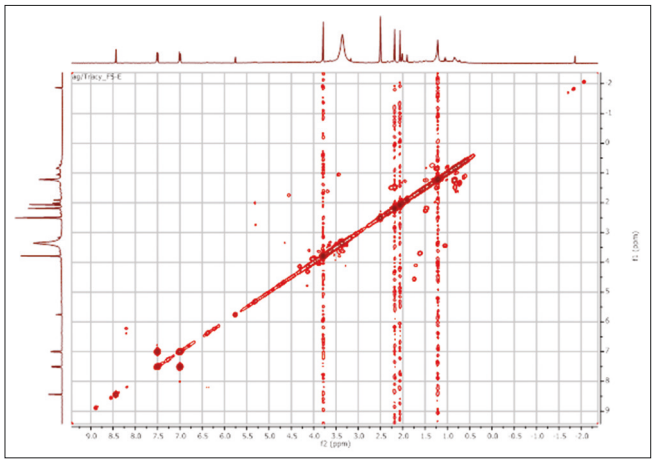

Fig 3. ^{1}H-^{1}H COSY Spectrum of compound **1** in DMSO-d6.

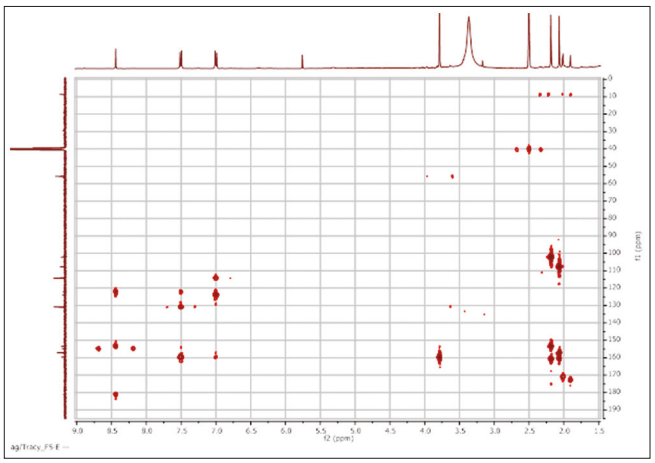

Fig 5. ^{1}H-^{13}C HMBC Spectrum of compound **1** in DMSO-d6.

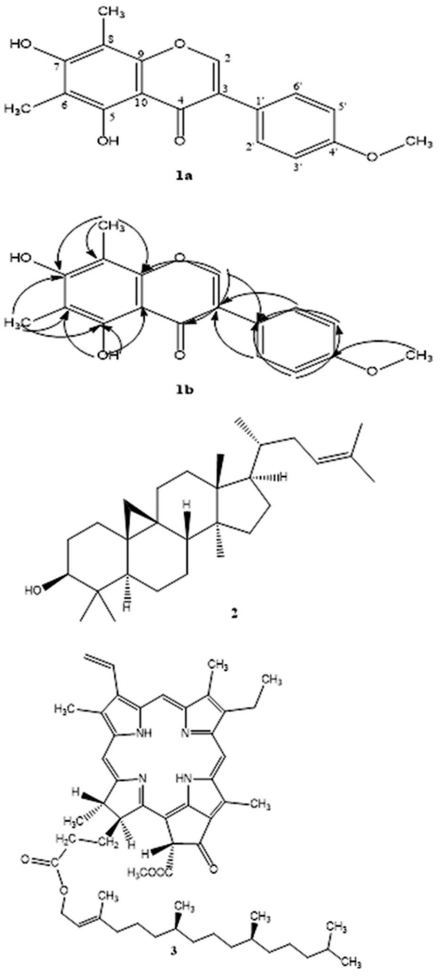

Fig 6. Structures of compounds **1**-**3**.

compound. The methyl groups showed correlations to C-6 and C-8, hence their points of attachment. They were distinguished based on their correlations to C-9 (methyl at position C-8) and C-5 (methyl at C-6) while they both showed correlations to C-7. Similarly, correlations from H-2 identified C-1', C-4, and C-9 and correlations from the aromatic protons H-2'/6' and H-3'/5' confirmed their symmetry and identified C-1', C-3 and C-4'. Finally, the long range correlation from the methoxy group to C-4' confirmed its point of attachment (Fig. 6; structure **1b**). Its positive mode HR-LC-MS spectrum (Fig. 7) gave an $[M+H]^+$ ion at m/z = 313.2364 corresponding to a molecular formula $C_{18}H_{16}O_5$. Thus compound **1** (Fig. 6) was identified as 5,7-dihydroxy-4' methoxy-6,8-dimethylisoflavone and was confirmed by comparison to reports for similar compounds (Calderon et al 2002, Zhao et al 2012). The complete chemical shift assignments for the compound are given in Table 1. Other compounds (Fig. 6) isolated were Cycloartenol **2** (Nes et al., 1998) and Pheophytin A **3** (Sobolev et al., 2005).

Antitrypanosomal activity

The hexane extract showed moderate activity (60.9%) while the ethyl acetate (73.2%) and methanol (99.2%) did not show significant antitrypanosomal activity compared to the percentage of the control DMSO. This is in agreement with a recent review (Ungogo et al., 2020) which did not identify any isoflavonoids from African medicinal plants showing antikinetoplastid activity.

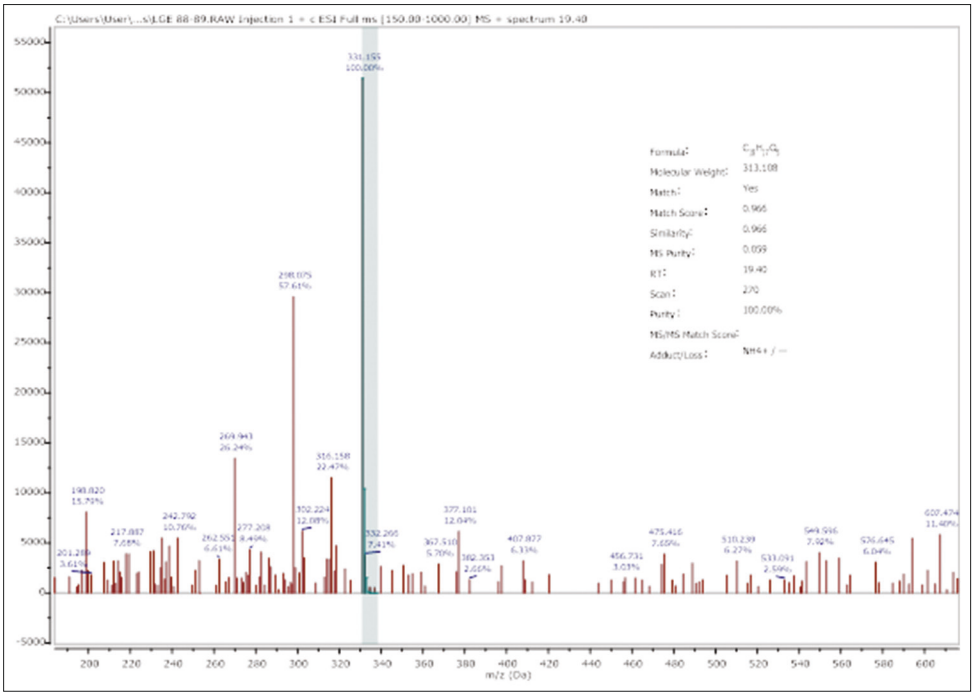

Fig 7. High resolution LC-MS Spectrum of compound **1**; $[M+H]^+$ = 313.1080 (Calc. for $C_{18}H_{17}O_5$, 313.1076).

Table 1: ^{1}H and ^{13}C NMR Spectral Data for Compound 1

Position	Experimental		Literature (ref*)		2D NMR
	^{1}H (δ ppm)	^{13}C (δ ppm)	^{1}H (δ ppm)	^{13}C (δ ppm)	HMBC
2	8.43 (1H, s)	157.1 (C)	8.35 (s, 1H)	153.8	C-1', C-4, C-9
3	-	123.8 (C)	-	121.4	-
4	-	181.0 (C)	-	180.5	-
5	-	157.1 (C)	-	152.8	-
6	-	102.2 (C)	-	107.0	-
7	-	160.6 (C)	-	159.9	-
8	-	107.7 (C)	-	101.5	-
9	-	153.4 (C)	-	156.5	-
10	-	104.9 (C)	-	104.3	-
1'	-	122.0 (C)	-	121.8	-
2'	7.50 (2H, d, *J* = 8.7 Hz)	130.8 (CH)	7.36 (d, 2H, *J* = 8 Hz)	130.1	C-3, C-4', C-6'
3'	7.00 (2H, d, *J* = 8.7 Hz)	114.3 (CH)	6.80 (d, 2H, *J* = 8 Hz)	115.0	C-1', C-5'
4'	-	154.8 (C)	-	156.5	-
5'	7.00 (2H, d, *J* = 8.7 Hz)	114.3 (CH)	6.80 (d, 2H, *J* = 8 Hz)	115.0	C-1', C-3'
6'	7.50 (2H, d, *J* = 8.7 Hz)	130.8 (CH)	7.36 (d, 2H, *J* = 8 Hz)	130.1	C-2', C-3, C-4'
6-CH_3	2.06 (s)	8.63 (CH_3)	2.17	7.9	C-5, C-6, C-7
8-CH_3	2.18 (s)	8.68 (CH_3)	2.04	8.0	C-7, C-8, C-9
4'-OCH_3	3.79 (s)	55.7 (CH_3)	-	-	C-4'
5'-OH	13.14 (1H, s)	-	13.13	-	

CONCLUSION

A new isoflavone (5, 7-dihydroxy-4' methoxy-6, 8-dimethylisoflavone) as well as cycloartenol and pheophytin A have been isolated from the leaves of *Lomariopsis guineensis*. The hexane extract of the plant showed moderate antitrypanosomal activity.

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Authors' contributions

Conceptualization, N.F.T., A.I.G. and J.O.I.; methodology, N.F.T. and J.O.I.; software, N.F.T. and J.O.I.; validation, N.F.T. and J.O.I.; formal analysis, N.F.T. and J.O.I.; investigation, N.F.T. and J.O.I; re-sources, N.F.T., A.S.A, E.Y.S, C.J.C, A.I.G. and J.O.I.; data curation, N.F.T. and J.O.I.; writing—original draft preparation, N.F.T. and J.O.I.; writing—review and editing, N.F.T. A.S.A, E.Y.S, and J.O.I.,; visualization, N.F.T. and J.O.I.; supervision, N.F.T. and J.O.I.; project administration, N.F.T. and J.O.I. All authors have read and agreed to the published version of the manuscript.

Declaration of interest

None declared.

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