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PHYTOCHEMICAL CONSTITUENTS AND ANTI-OXIDATIVE PROPERTIES OF *LANDOLPHIA HEUDELOTTI* ROOTS

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ABSTRACT: The roots of *Landolphia heudelotti* are employed in traditional medicine for the treatment of a plethora of ailments. In this study, the antioxidant activity and bioactive constituents of the methanol root extract were investigated. Fourteen known compounds including a lignan, neolignans, sesquilignans, a coumarin and an aromadendrane sesquiterpene were isolated from the root extract. The structure elucidation of compounds was performed based on mass spectral and NMR spectroscopic data and by comparison with literature. The crude extract had a high total antioxidant Capacity of 108.8 ± 14.52 mg/g of dried extract (ascorbic acid equivalent) and also demonstrated significant DPPH free radical scavenging activity (IC_{50} = 6.956 ± 0.8121 µg/mL). The extract also had a total phenolic content of 98.14 ± 14.70 mg/g of dried extract (tannic acid equivalent). The results of this study have given scientific credence to the use of *L. heudelotti* roots in traditional medicine. This is the first report of these phyto-constituents from *L. heudelotti*.

INTRODUCTION: Medicinal plants have over the years served as a continuous source of alternative and complementary therapies as well as novel drug lead compounds [1]. In view of this, several plants have been investigated in order to facilitate the identification of bioactive constituents which could be drugs or lead molecules for drug development. *Landolphia heudelotti* A. DC (Apocynaceae) is a climbing shrub and was at one time the main rubber producing plant widely distributed in Western tropical Africa [2].

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In many sub-Saharan countries including Ghana, the Plant is also used for the treatment of a variety of ailments; a decoction of the stems or roots is used for the treatment of enteritis, gastric ulcers and stomach cramps. The ground stem bark paste is used as a vermifuge. Latex from the young stem is instilled in the eyes to treat cataract, conjunctivitis and glaucoma. The root maceration is used as pain relief and to treat haemorrhoids. The roots are chewed as an aphrodisiac and general tonic. Despite these numerous claims of medicinal effects, very little information exists on the biological activity and phytochemistry of *L. heudelotti*.

Therefore in a continuing effort to identify the bioactive constituents from tropical medicinal plants [3-6], this study investigated the antioxidant

activity and phytochemical constituents of the roots of *Landolphia heudelotti*.

MATERIALS AND METHODS:

Chemicals: All chemicals used were purchased from Sigma-Aldrich Co Ltd. Irvine, UK. All organic solvents used were of analytical grade and obtained from BDH, Laboratory Supplies (Merck Ltd, Lutterworth, UK).

Plant material: The stem barks of *L. heudelotti* were collected in January, 2015 from Kwahu-Asakraka in the Eastern region of Ghana. The plant material was identified and authenticated by Mr. Clifford Asare of the Herbal Medicine Department, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST where a voucher specimen was also deposited (KNUST/HM/2016/12).

Extraction and isolation of constituents: The roots of *L. heudelotti* were air dried for seven days and ground to obtain 1.2 kg of dry powder. The powdered material was then cold macerated with a mixture of methanol and chloroform (4:1) for 72 hours. The crude extract obtained was then filtered and concentrated at low temperature on a rotary evaporator to give 19.2 g (yield = 1.60 $\%^{w}/_{w}$) of brown oily extract. The dried extract was subjected to chromatographic purification to yield 14 compounds labelled LH-1 to LH-14 as shown in **Fig. 1**. Purification by the HPLC was performed according to our previously described method [4].

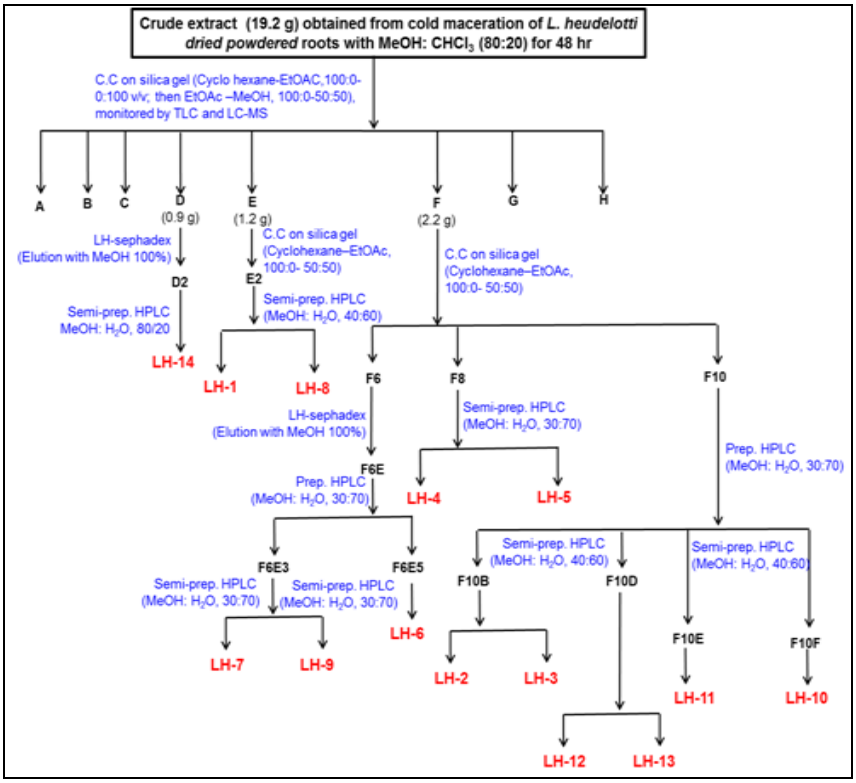

FIG. 1: SCHEMATIC PRESENTATION OF THE ISOLATION (PURIFICATION) SCHEME OF *L. HEUDELOTTI* EXTRACT

Structural elucidation: Liquid chromatography-electrospray ionization-high resolution mass spectrometry (LC-ESI-HRMS) was employed to check the purity and provide the exact mass and molecular formula of the isolated compounds. Mass fragmentation ($MS^{2/3}$) experiments were performed by collision-induced dissociation (CID) to evaluate the structural features of compounds based on the fragment information. The LC-HRMS experiments were carried out on a LTQ Orbitrap spectrometer (Thermo Fisher, USA) equipped with a HESI-II source according to our previously reported protocol [4]. Nuclear magnetic resonance (NMR) experiments [1D (^{1}H and ^{13}C) and 2D (HSQC, HMBC, COSY)] spectroscopy were measured with Varian Unity Inova spectrometer (600 MHz) using Methanol-d_4 (CD_3OD) (Deutero GmbH, Kastellaun, Germany) as NMR solvent. All spectroscopic data obtained were compared with published data for the compounds in literature.

Antioxidant activity: The methanol extract of *L. heudelotti* roots was investigated for radical scavenging activity (DPPH free radical scavenging assay), total antioxidant capacity and total phenol content (Folin Ciocalteu's reagent assay). The experiments were performed according to our previously described methods [6].

RESULTS AND DISCUSSION: The DPPH radical scavenging assay was used to evaluate the ability of the extract to mop up free radicals in a system. Different concentrations of the crude extract (0.06 - 0.1 mg/mL) were tested in this assay and the results obtained showed a concentration-dependent scavenging effect of the extract (**Fig. 2**). The IC_{50} was determined as 6.956 ± 0.8121 µg/mL for the root extract and 2.44 ± 0.0134 µg/mL for the positive control, ascorbic acid (**Fig. 3**). The MeOH extract was found to have a total phenolic content of 98.14 ± 14.70 mg g^{-1} of dried extract (expressed as tannic acid equivalent) and a total antioxidant capacity of 108.8 ± 14.52 mg g^{-1} of dried extract (ascorbic acid equivalent).

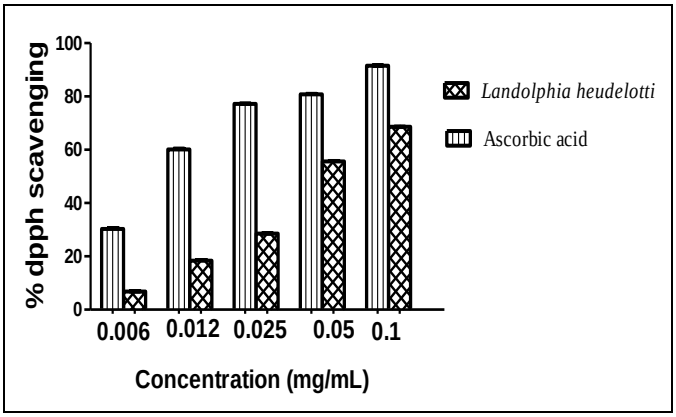

FIG. 2: PERCENTAGE DPPH SCAVENGING OF *L. HEUDELOTTI* ROOT MEOH CRUDE EXTRACT

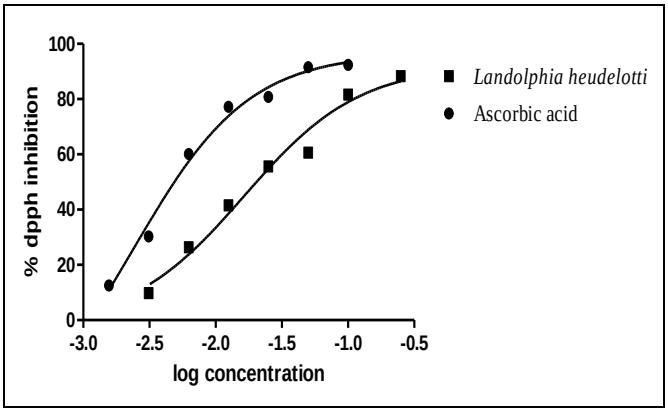

FIG. 3: LOG CONCENTRATION VERSUS % DPPH SCAVENGING EFFECT OF *L. HEUDELOTTI* ROOT EXTRACT

Repeated chromatographic purification of the MeOH root extract of *L. heudelotti* led to the isolation of fourteen known compounds including lignans, neolignans, sesquilignans, an aromadendrane and a coumarin (**Fig. 4**). On the basis of their spectroscopic data and comparison to reported literature, the compounds were identified as pinoresinol (LH-1) [7], *erythro*/*threo*-guaiacylglycerol-8-*O*-4'-coniferyl alcohol ether (LH-2/3) [8], e*rythro/threo*-guaiacylglycerol-8-*O*-4'-coniferyl aldehyde ether (LH-4/5) [9], balanophonin (LH-6) [10], 3-(α,4-dihydroxy-3-methoxybenzyl)-4-(4-hydroxy-3-methoxybenzy1) tetrahydrofuran (LH-7), capstemol (LH-8) , picrasmalignan A (LH-9) [11], Budlenol E (LH-10) [9], e*rythro/threo*-guaiacylglycerol-8-*O*-4'-pinoresinol ether (LH-11/12) [12], scopoletin (LH-13) [13] and 2,9-dihydroxy-1(10)-aromadendren-14-oic acid 2, 14-lactone (LH-14) [14].

These compounds were being identified for the first time from *L. heudelotti* and from the genus *Landolphia*. However, the general presence of phenylpropanoid derivatives and aromadendrane sesquiterpenes has been reported in previous reports for some *Landolphia* species. From the stringy seed pulp of *L. owariensis*, phenylpropanoids, phenolic acids and phytosterols were isolated as the major constituents [15].

Plant lignans and derivatives are one of the most extensively distributed constituents in the plant kingdom [16]. Several reports have demonstrated that these compounds exhibit a wide range of biological activities including cardiovascular, anti-inflammatory, antimicrobial, antiviral, anticancer, immunosuppressive, insecticidal, anti-feeding, and anti-oxidant effects [10].

The specific phenylpropanoid derivatives isolated from *L. heudelotti* in this study have been shown in previous investigations to exhibit hypoglycaemic, anti-oxidant, hepato-protective, cytotoxic, anti-inflammatory, anti-proliferative and nitric oxide inhibitory effects [7, 9, 17-19]. The isolated constituents were investigated for radical scavenging activity using the DPPH free radical scavenging assay. Some of the compounds demonstrated significant radical scavenging effect at a concentration of 0.01 mg/mL as illustrated in **Fig. 5**. Pinoresinol (LH-1) exhibited the highest scavenging effects.

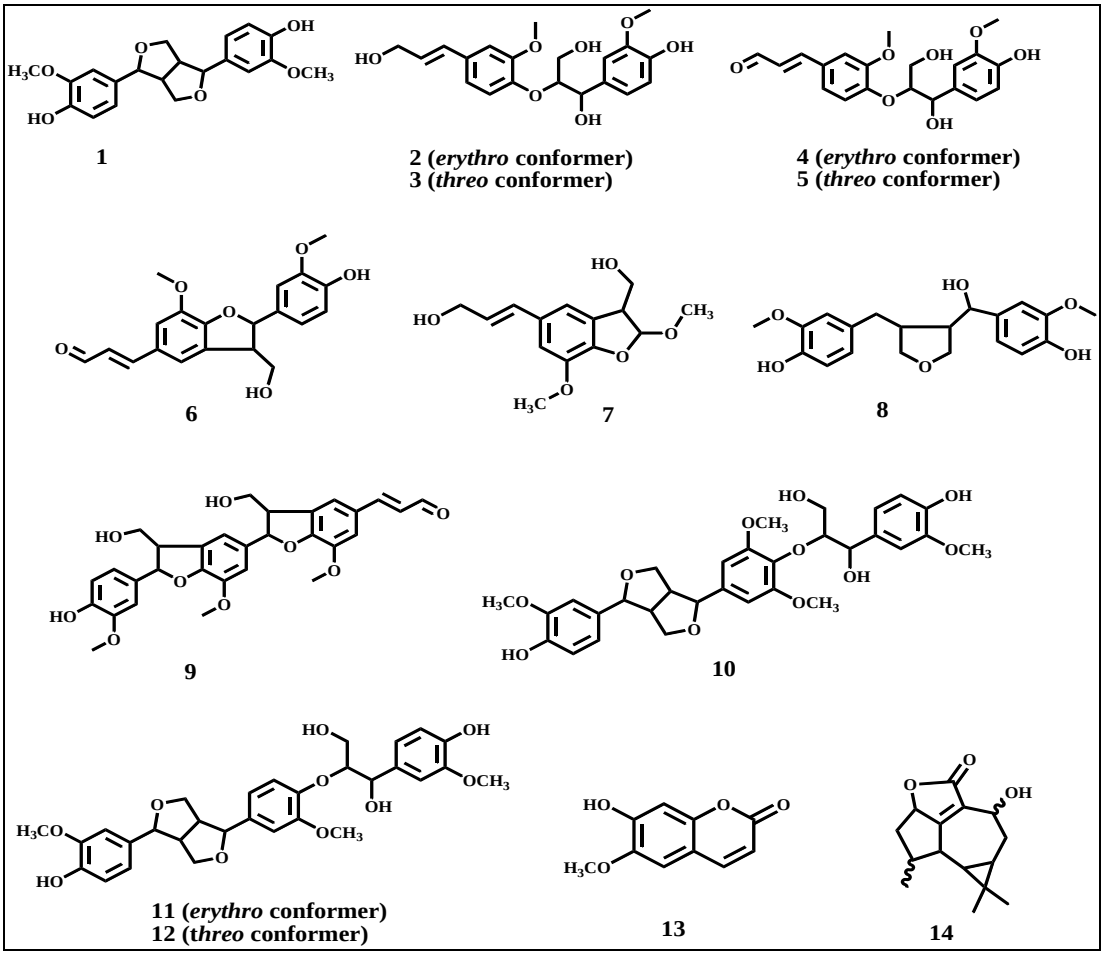

FIG. 4: COMPOUNDS ISOLATED AND CHARACTERIZED FROM *L. HEUDELOTTI*

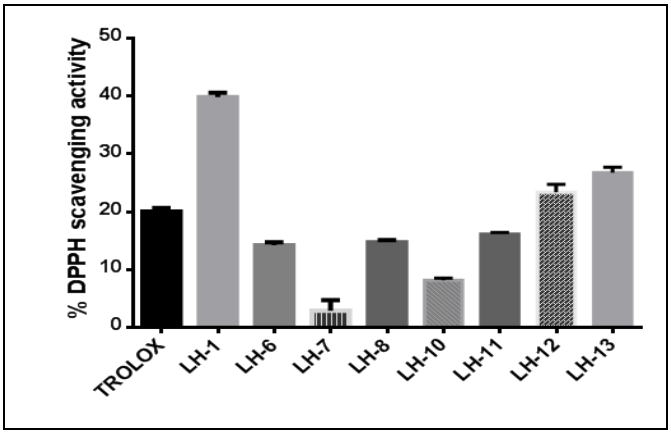

FIG. 5: PERCENTAGE DPPH FREE RADICAL SCAVENGING OF COMPOUNDS

CONCLUSION: This study has successfully identified some active principles of *L. heudelotti* A. DC. The results of biological activity screening and phytochemical investigation of *L. heudelotti* root extract give scientific justification to the use of this plant in traditional medicine. The bioactive compounds in the root extract of *L. heudelotti* may therefore contribute to the overall biological effects of the plant.

CONFLICT OF INTEREST: The authors declare no conflict of interest

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