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Antiproliferative Potential of Root Bark Extract of *Adenodolichos Paniculatus* (Hua & Hutch) against Ovarian Human Cancer Cell Lines (A2780)

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Abstract

Herbal medicines remain a major birthplace of lead compounds for drug discovery and development. Adenodolichos paniculatus Hua & Hutch (Fabaceae) is a plant whose root bark was claimed to be used against diabetes mellitus and cancer in traditional medicine. This study aimed to isolate antiproliferative compounds from root barks of Adenodolichos paniculatus through bioassay-guided fractionation and to investigate the antiproliferative potentials of the isolated compounds against ovarian human cancer cell lines (A2780). The powdered root bark of Adenodolichos paniculatus was extracted with dichloromethane for 72 h to afford dichloromethane extract (DCM). The DCM was solvent-partitioned into hexane and aqueous methanol fractions. Based on the results of preliminary studies against the A2780 ovarian cancer cell line, the bioactive fraction (Hexane) was subjected to various chromagraphic techniques leading to the isolation of a triterpenoid saponin. The triterpenoid was screened against A2780 and subjected to various spectral analyses such as ^{1}H, ^{13}C NMR and MS to elucidate and characterise its structure. A triterpenoid saponin, 3,22-dihydroxy-3-O-β-D-glucosyloleanane isolated displayed antiproliferative effect against the A2780 ovarian cancer cell line, with IC_{50} value of 0.033 μM when compared with the standard Paclitaxel (IC_{50} = 0.013μM). The results significantly support the traditional use and the study revealed that the isolated compound possesses potential health benefits which might serve as a good molecular template in drug design for the development of new antiproliferative agents.

Keywords: *Adenodolichos paniculatus,* antiproliferative activity, Fabaceae, Ovarian cancer cell line, triterpenoid.

INTRODUCTION

Cancer is an uncontrollable growth of mammalian cells that can result to death of the host (Unnati *et al*., 2013). Cancer has remained a leading cause of death worldwide with an alarming increase in mortality (Roleira *et al*., 2015; Li *et al*., 2018). It is projected that by 2030 according to cancer statistics 26 million new cases are expected while 17 million deaths were projected (Rai *et al*., 2016). Despite the rigorous efforts by scientists and several pharmaceutical companies in providing potent chemotherapeutic anticancer agents, to solve the menace of death; cancer still remains a major cause of death in several countries (Khazir *et al*., 2014). The

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management of cancer in the medical community has remained a nightmare due to the lack of selectivity against cancer cells because of the narrow therapeutic window displayed by the anticancer agents and the unwanted side effects of the anticancer agents (Khazir *et al*., 2014). Several potent anticancer agents including paclitaxel, vincristine, and irinotecan are presently in clinical use for the management of different types of cancer. Unfortunately, these agents demonstrate unfavourable side effects ranging from mild gastrointestinal alterations (ulceration, pain, diarrhea, constipation) and nausea to severe gut mucosa dysfunction, cardiovascular toxicity or immunity disorders. (Garcia-Oliveira *et al*., 2021). This high venomousness of some cancer chemotherapy drugs, as well as their hostile side effects and drug resistance, initiates the high demand for alternative medicines from medicinal plants as a source of new anticancer drugs (Siddiqui *et al*., 2022). Natural products remain valuable sources for the new therapeutic drug templates from where most of the anticancer drugs were generally developed (Sen *et al*., 2016). One of such plants is *Adenodolichos paniculatus* which could serve as a good source of anticancer agents.

Adenodolichos paniculatus (Hua) Hutch, locally known as Kwiwaa, wáákén wuta in Hausa, or "fire bean" in English, is a shrub belonging to the family Fabaceae/Leguminosae and it is widely distributed in Benin, Ghana, Northern Nigeria, Cameroon, Sudan, Guinea and Democratic Republic of Congo. Out of the 22 species of this genus widely distributed in tropical Africa, 15 species have been reported in the Democratic Republic of Congo while *Adenodolichos paniculatus* is available in Northern Nigeria. Ethnomedically, the leaves are used for treating wounds, toothache, and heartburn. The roots are used for the treatment of dysentery, and liver problems while the stems are employed for the treatment of diarrhea and blennorrhoea (Isyaku *et al*., 2020). The methanolic extract of the leaf showed significant analgesic and anti-inflammatory activities (Sani et al., 2012). Stigmasterol, β-sitosterol and nonanoic acid were isolated from the leaves (Isyaku *et al*., 2020; Isyaku *et al*., 2018) while some fatty acids were identified from the root with antibacterial activity (Kyahar *et al*., 2020). However, isolation of antiproliferative compounds from root bark of this plant has not been reported previously. Therefore, the study aimed to carry out bioassay-guided isolation of antiproliferative compounds using A2780 ovarian cancer cell line and evaluate their *in vitro* antiproliferative activity.

Leaves of *Adenodolichos paniculatus*

Root of *Adenodolichos paniculatus*

MATERIAL AND METHODS

Plant collection and identification.

The roots of *Adenodolichos paniculatus* were collected from mature shrubs in bushes around Binchi village, Bassa Local Government, Jos, Plateau, Nigeria between September and November, 2017. The plant was identified and authenticated in the Herbarium Unit of Department of Biological Sciences, Ahmadu Bello University, Zaria with a voucher specimen number (ABU 3107) and likewise confirmed with the Herbarium Section of Forestry Research Institute of Nigeria (FRIN) with a voucher specimen FHI 0045485-0. The material was air-dried to a constant weight chopped into smaller pieces and ground to coarse powder. The dried powdered root bark was stored in cool and dried conditions.

Extraction

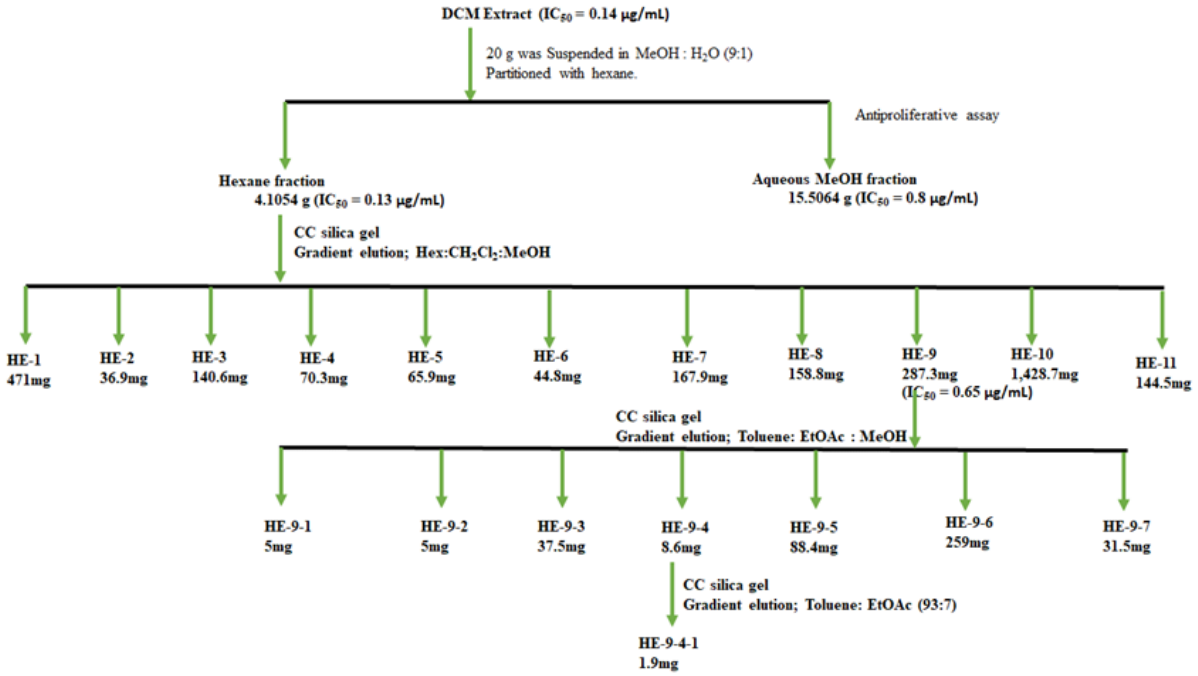
Extraction was carried out according to the procedure described by Ojerinde *et al* (2022). The powdered root barks of *Adenodolichos paniculatus* (2.5 kg) was extracted twice with dichloromethane (DCM) at room temperature for three days. The extract (DCM) was filtered using Whatman filter paper number 1 and the filtrate was dried *in vacuo* using a rotary evaporator to give dichloromethane (DCM) extract which was kept in the freezer until further use.

Antiproliferative assay using A2780 ovarian cancer line

The A2780 cell line is a drug-sensitive ovarian cancer cell line with which antiproliferative bioassay was performed at Virginia Tech (Kingston *et al*., 2007 & Kingston *et al*., 2010)

Fractionation and Isolation

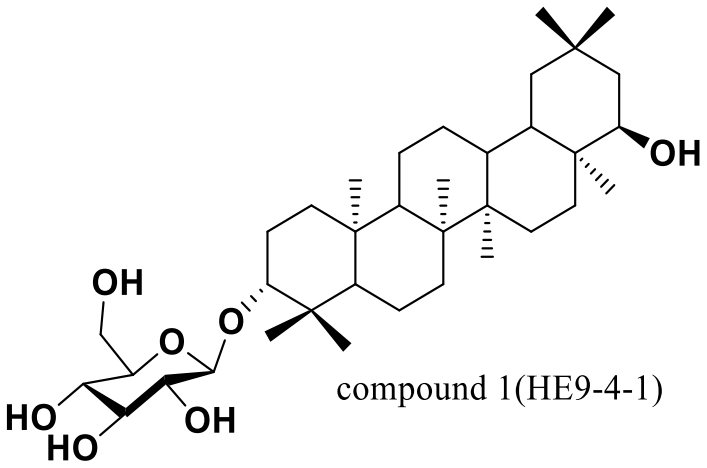
A 20.0 g sample of DCM extract was suspended in aqueous MeOH (MeOH: H_2O, 9:1, 2000 mL) and extracted with hexane (8 X 500 mL) portions to afford hexane soluble fraction and aqueous MeOH fraction. The hexane fraction was evaporated *in vacuo* to afford Hexane fraction with an IC_{50} value of 0.13µg/mL and the aqueous MeOH fraction had an IC_{50} value of 0.8µg/mL. Following bioactivity guided fractionation, HE fraction (4.0 g, IC_{50} = 0.13µg/mL) was chromatographed on Column Chromatography (CC) using silica gel 60 230-400 mesh (Merck, Darmstadt, Germany) and eluted with a gradient of hexane-dichloromethane-methanol (100:0:0 – 0:90:10) to yield eleven fractions (HE1-HE11). The most active fraction HE 9 (287.3 mg) with an IC_{50} value of 0.65µg/mL was further chromatographed on CC silica gel 60 and eluted with a gradient elution of Toluene:Ethyl acetate (100:0 – 90:10) to afford seven fractions (HE-91 – HE-97). Fraction HE 9-4 (8.6 mg) was chromatographed on CC silica gel 60 using Toluene:Ethyl acaetate (93:7) to give compound HE-9-4-1 **1** (1.9 mg) (Scheme 1). The compound was subjected to spectroscopic analysis, ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 Spectrometer in Chloroform-d, $CDCl_3$ with Trimethylsilane (TMS) as internal standard. An agilent 6220 LC-TOF-MS mass spectrometer was used to obtain high-resolution mass spectra (HR)-ESI-MS in the positive ion mode.



Scheme 1: Fractionation of DCM Extract and Isolation of Compound HE-9-4-1 from Sub-fraction HE-9-4 of Fraction HE.

RESULTS

In this study, a triterpenoid saponin was isolated from the active hexane fraction, sub-fraction of dichloromethane extract of root bark of *Adenodolichos paniculatus.* The isolated compound, HE 9-4-1 was analyzed by spectroscopic methods (^{1}H &^{13}C NMR and Mass spectrometric) and its data were analyzed in comparison with those reported in the literature (Dai et al., 2013). The compound was obtained as a white amorphous powder. The molecular formula $C_{36}H_{62}O_7$ was determined by HRESIMS (m/z 607.4574 $[M + H]^+$, calculated for 607.4576 and supported by the NMR spectroscopic data. The positive HRESI-MS showed the nature of the sugar present, showing ions at m/z 607 $[M + H]^+$; 445 $[M + H - 162 (Glu)]^+$. The ^{1}H NMR spectrum of compound HE 9-4-1in $CDCl_3$ showed the following methyl signals (δ_H 0.89, 0.92, 0.92, 0.94, 0.95, 0.97, 1.00 and 1.10, 3H each). Additional resonances observed included those assigned to two oxymethylene protons at δ_H 3.78 and 3.90 (1H each, d,, J = 12.3Hz) and one anomeric proton signal at 4.06 (1H, d, J = 7.0Hz) corresponding to the anomeric carbon at δc 102.7 in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum HE 9-4-1 displayed 36 carbon signals, of which 6 carbons were assigned to the sugar moiety and the remaining 30 to the aglycone. These data suggested that the aglycone part of HE 9-4-1 was likely to be a pentacyclic triterpenoid with two hydroxyl groups at C-3 and C-22. A comparison of the NMR spectroscopic data with the literature shows that HE 9-4-1 is the same as reported previously in the literature (Dai et al., 2013) and that it belongs to the oleanane series. Compound HE 9-4-1 was identified as 3,22-dihydroxy-3-O-β-D-glucosyloleanane. (Figure 1)



compound 1(HE9-4-1)

Figure 1 Structure of isolated compound HE 9-4-1

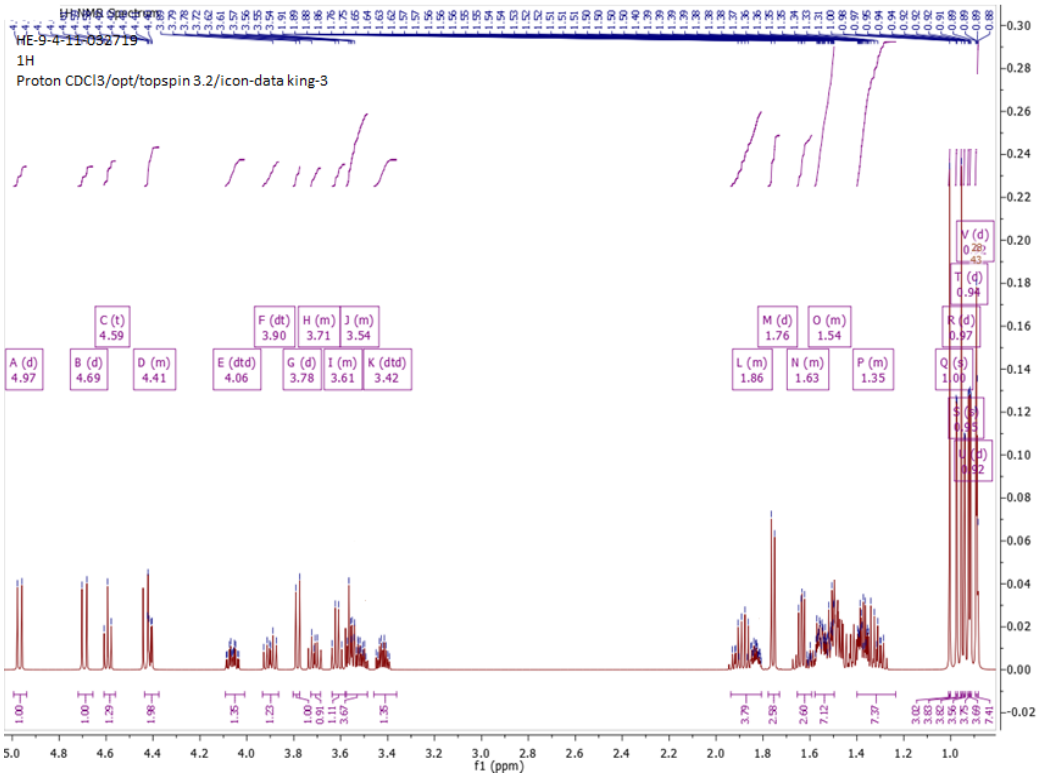


Figure 2 ^{1}H NMR Spectrum for Compound HE 9-4-1

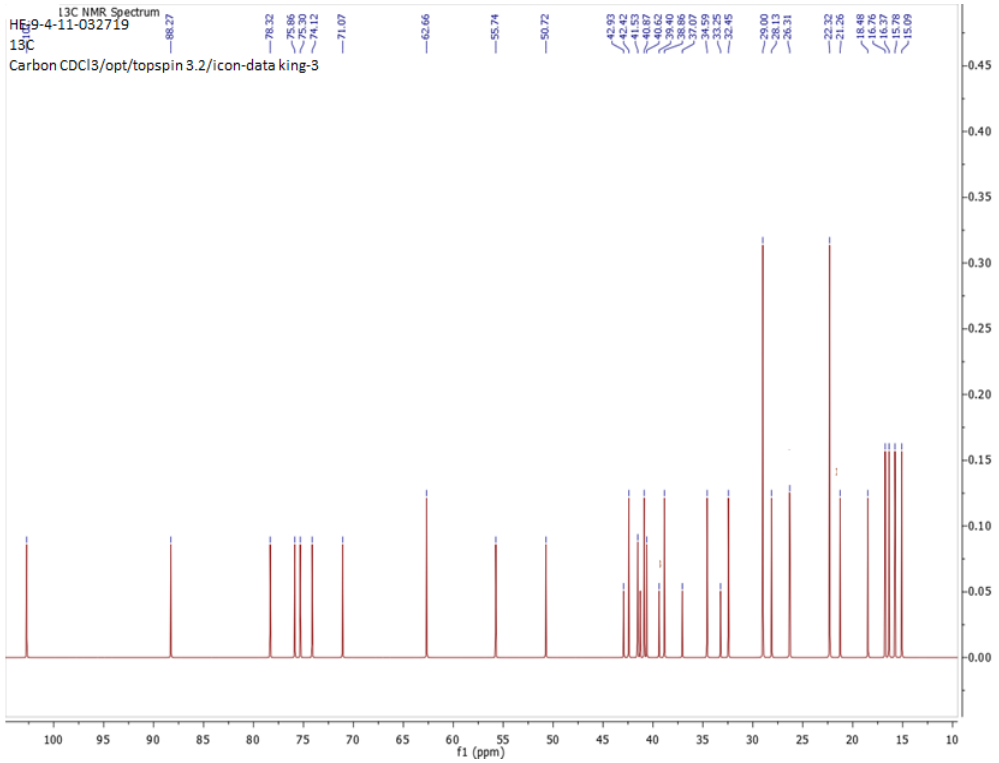


Figure 3 ^{13}C NMR Spectrum for Compound HE 9-4-1

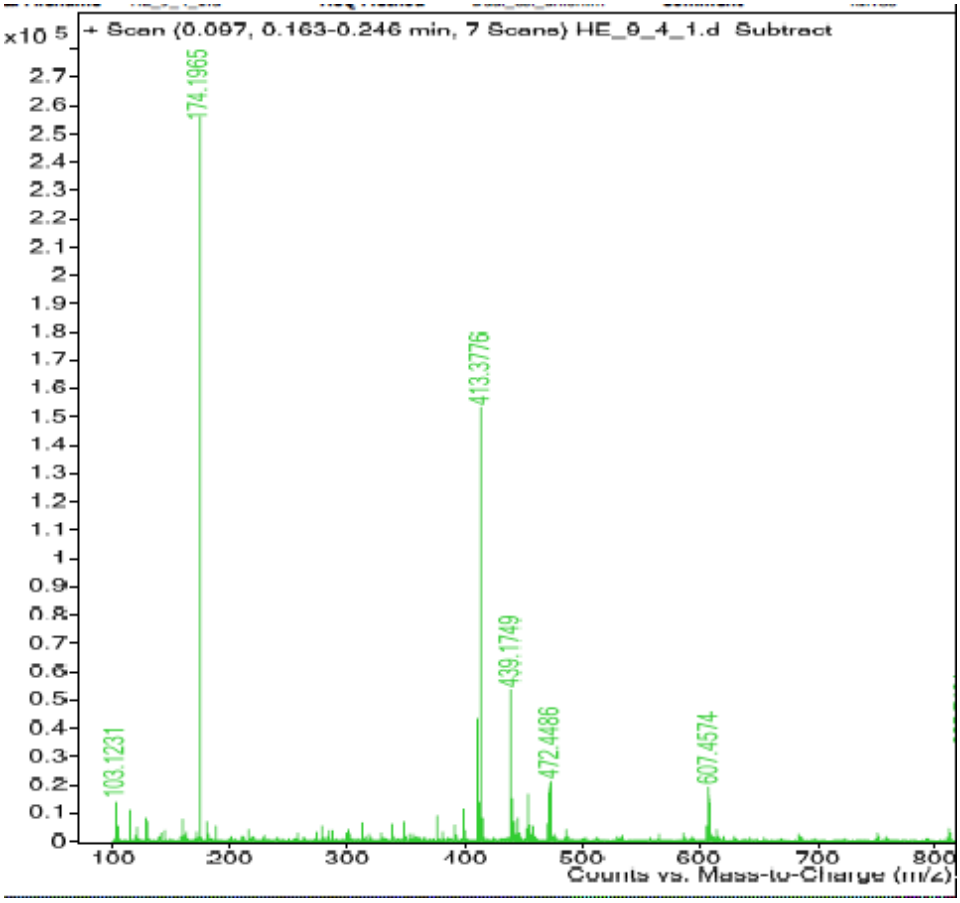


Figure 4 Mass Spectrum for Compound HE 9-4-1

Table 1. Antiproliferative Activity of the Fractions and Isolated Compound against A2780[b] cell line from the Root Bark of *Adenodolichos paniculatus* (IC_{50}, µg/mL)

Fractions/Compound	IC_{50}, µg/mL
Dichloromethane extract	0.14
Hexane Fraction	0.13
Aqueous MeOH fraction	0.80
HE 9 Fraction	0.65
HE 9-4-1	20.0
Paclitaxel[a] (Standard)	0.013µM

a Paclitaxel was used as positive control for antiproliferative; b A2780 ovarian cancer cell line used

As shown in Table 1, the isolated compound HE 9-4-1 displayed antiproliferative activity against the A2780 cell line. HE 9-4-1 exerted a moderate antiproliferative activity with an IC_{50} value of 20 µg/mL compared with the standard, paclitaxel (IC_{50} = 0.013µM)

DISCUSSION

A preliminary study of the pharmacological activity (antiproliferative, antiplasmodial, and Brine Shrimp assay) of the root barks of *Adenodolichos paniculatus* showed that the dichloromethane extract exhibited potential antiproliferative activity against A2780 ovarian cancer cell line with an inhibitory concentration (IC_{50}) of 0.14 µg/mL in the previous study (Ojerinde *et al*., 2022). Following the preliminary study, dichloromethane extract was suspended in aqueous methanol and partitioned with hexane to afford hexane and aqueous MeOH fractions. Following bioassay-guided isolation, hexane fraction showed activity with IC_{50} value of 0.13 µg/mL and was further subjected to several chromatographic separation steps leading to the isolation of compound HE9-4-1. The isolated compound HE 9-4-1 was analysed by spectroscopic methods (^{1}H & ^{13}C NMR and Mass spectrometric) and the data were compared with those reported in the literature (Dai et al., 2013)..

CONCLUSION

Several compounds, including triterpenoids and triterpenoid saponins, have been reported in the Fabaceae family but interestingly none has been identified from the genus *Adenodolichos*. The present investigation on *Adenodolichos paniculatus* has shown that the *Adenodolichos* genus is a good source of cytotoxic phytochemicals. More exploitation of this genus will make available antiproliferative agents as leads both for the treatment of cancer and drug development. An active compound from the active sub-fraction of dichloromethane extract of the plant was isolated and the structure of the compound was elucidated using spectroscopic methods. 3,22-dihydroxy-3-O-β-D-glucosyloleanane was isolated for the first time from the root barks of *Adenodolichos paniculatus* in the present study.

This study therefore corroborates and justifies the ethnomedical uses of *Adenodolichos paniculatus* for the treatment of chronic diseases including cancer.

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