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Short communication

NEW PHYTOCOMPOUNDS FROM *Vernonia amygdalina* WITH ANTIMALARIAL POTENTIALS

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Abstract

Background: *Malaria being one of the diseases most predominant, especially in the African continent, has been reported to be treated with plants and also some in-vitro and in-vivo tests have supported this. Vernonia amygdalina is one of the plants widely used in Nigeria and studied for use in the treatment of malaria and some scientific researches have validated this claim.* ***Objectives:*** *In this present study, we aimed at isolation of possible compounds from the methanolic stem-bark of Vernonia amygdalina, elucidation and characterisation of the isolated compounds and carry out antimalarial evaluations of the isolated compounds.* ***Methods:*** *Isolation of compounds was done using column chromatographic technique, elucidation and characterisation was done based on IR, Mass, ^{1}H and ^{13}C NMR spectra. The in-vitro antimalarial activity was carried out on the ring stage of*

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malaria parasite cycle of chloroquine sensitive (3D7) and chloroquine resistant (Dd2) strains. ***Results:*** *Three new compounds were isolated; 1-Heneicosanol-O-β-D-glucopyranoside, 10-Geranilanyl-O-β-D-xyloside and 6β,10β,14β-Trimethyl heptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide. The compounds showed antimalarial activity with IC_{50} values of, 10.55µg/ml, 12.56µg/ml and 11.68µg/ml respectively.* ***Discussions:*** *The isolated compounds are all glycosides and produce positive results in the preliminary antimalarial test prior to IC_{50} determination for both chloroquine sensitive and resistant strains. The IC_{50} values obtained are much higher than that of chloroquine which is 0.02µg/ml.* ***Conclusions:*** *The three compounds isolated showed antimalarial activity at different levels. There are possibilities of more un-isolated compounds in this part of the plant studied and so more work need to be done.*

Keywords: *Vernonia amygdalina*; isolation; antimalarial; phytocompounds

Introduction

Nature as old as mans' existence has been a provider of medicines and agents used for the development of medicine. The most prevalent sources have been plants (Cragg *et al*., 2001). The development of most of these medicines obtained from plant stemmed from the study of the utilization of the plant in traditional medicinal practices, which gave an insight into the type of activity or likely pharmacological effect for which the products from plants could be developed for.

Vernonia amygdalina is one of the plants that is widely utilised by traditional medicine practitioners in the tropical region of the African continent for management, treatment and prevention of certain disease conditions, with some scientific researches to validate these claims. It is frequently referred to as bitter leaf as a result of its bitter taste. Throughout the cultures of the

countries in the equatorial regions of Africa, the leaf of *Vernonia amygdalina* are a staple vegetable used for making soups and stews (Ucheck 2004)

Some studies on *Vernonia amygdalina*, have reported that this plant has an antibacterial property (Newbold *et al*., 1997), antibacterial effect towards *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Escherichia coli* (Kambizi *et al*., 2001), amplification of glucose utilization of muscle and liver cell cultures but not on adipose cells (Erasto *et al.,* 2009), lowers blood glucose levels of both normoglycaemic and hyperglycaemic rats induced by alloxan (Osinubi 2007), suppresses polymorphonuclear cell and mononuclear cell activity without affecting the cell viability (Koko *et al*., 2008) and other scientifically proven effects. The plant has been reported to contain vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxyvernolide, vernodalinol, vernomenin, vernolic, 11, 13-dihydrovernodalin, 11, 13-dihydrovernorodeline, 4, 15-dihydrovernodalin, (Kupchan *et al*., 1969; Jisaka *et al.,* 1992; Koshimizu *et al.,* 1994) and some other phytocompounds

Our study was targeted at the isolation of phytocompounds from the methanolic extract of the stem-bark part of *Vernonia amygdalina* and pharmacological evaluation of the isolates for antimalarial property.

Materials and Methods

Methanolic stem-bark extract of Vernonia amygdalina (114 g), column chromatographic silica gel (60-120 mesh, Spectrochem), chromatography column (1000 mm length and 40 mm inner diameter [I.D]), Erlenmeyer flask 250 ml, thin layer chromatographic plate, hexane, ethyl acetate, methanol, TLC plate (TLC Silica gel 60 F254 [Germany]). Spots were viewed in a

CAMAG UV cabinet 3, melting point apparatus (Optic technology), FT-IR Bruker Alpha, FT-NMR (BrukerAvance II, 400 MHz), Mass spectrophotometer (Water ZQ-4000), dimethyl sulfoxide (DMSO), incomplete culture medium with the absence of Albumax-II and Hypoxanthine, 96-well flat bottom microtitre plate, malaria parasites (3D7 strain [Chloroquine sensitive strain] and Dd2 [Chloroquine resistant strain]), blood, Chloroquine diphosphate, an incubator with 5% CO2 environment at a temperature of 370C, giemsa stain, glass slides.

Isolation

First we prepared coating of the extract on the silica gel (stationary phase). This was done by making slurry of the methanolic stem-bark extract in methanol on a water bath and pouring the silica gel into it and steering with a spatula to form a non-sticky granule. The mass was spread and allowed to dry and crushed properly to ensure uniform size. The packing of the column was done by dry method. The column was eluted dropwise, first with hexane and then with increasing ratios of hexane: ethyl acetate, ethyl acetate, increasing ratios of ethyl acetate: methanol and finally with methanol. During the elutions, a size of 250 ml was collected per fraction. The isolated samples were subject to Proton NMR, Carbon-13 NMR, Mass spectroscopy, Infra-red spectroscopy. The spectra obtained were used for the structural elucidation and characterisation of the isolates.

***In- vitro* antiplasmodial assay**

The isolates were solubilized in dimethyl sulfoxide (DMSO) to prepare a solution of 5 mg/ml concentration as the stock solution. A 5 µg/ml and 50 µg/ml concentrations of isolates were prepared from the stock solution using incomplete culture medium with the absence of Albumax-II and

Hypoxanthine. A 20 µl volume of each of the concentrations of the isolates was each placed in a well of a 96-well flat bottom microtitre plate in duplicates and a volume of 180 microliter of malaria parasites (3D7 strain [Chloroquine sensitive strain] and Dd2 [Chloroquine resistant strain]) contained in 3% of the volume of red blood cell to the total volume of blood (Haematocrit), having 1% malaria parasite blood infestation (Parasitaemia) was added to the 20 µl of the isolates dilutions in the microwells of the 96-well flat bottom microtitre plate. A positive control of Chloroquine diphosphate was used for the assay at its known IC_{50} concentration. A negative control containing no test sample was also used. Culture medium of 20 µl was used as negative control. The microwell plates were incubated for 24-30 hours (this depends on the maturation of the schizonts) in a modulated incubator which has 5% CO_2 environment at a temperature of 37^0C. After the incubation, on a glass slide, a blood smear was made from each of the microwells. Methanol was used for fixing, while giemsa staining was made on the slides and observation was done under a microscope. If after the assay the schizonts maturation percentage is below 20% in the negative control slide, the assay is taken to be invalid. The number of schizonts found per 200 asexual parasites and the percentage inhibition of the schizonts for each of the isolate dilutions were calculated using the formula given below. The test was performed in triplicate.

Results

The result of the isolation carried is presented in figure 1. Three compounds were isolated: 1-Heneicosanol O-β-D-glucopyranoside (**A**), 10-Geranilanyl O-β-D-xyloside (**B**), 6β,10β,14β-Trimethyl heptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide (**C**). The results of the antimalarial study is

presented in table 1. The results show that compound **A** has more schizonts inhabitation effect that the compounds **B** and **C.**

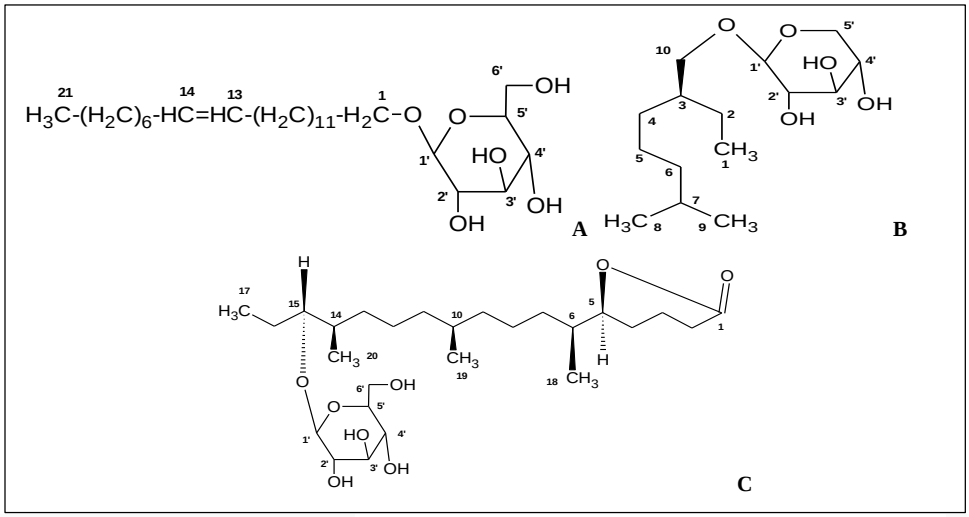

Fig 1:Isolated compounds; 1-Heneicosanol O-β-D-glucopyranoside (A), 10-Geranilanyl O-β-D-xyloside (B), 6β,10β,14β-Trimethyl heptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide (C)

Tab 1: Antimalaraial effects of the isolates

Compounds	% of Schizont Inhibition (Control-Treated / Control X 100)				IC_{50} (µg/ml)
	Chloroquine sensitive		Chloroquine resistant		
	5µg/ml	50µg/ml	5µg/ml	50µg/ml	
1-Heneicosanol O-β-D-glucopyranoside (**A**)	48	97.7	5	98	10.55
10-Geranilanyl O-β-D-xyloside (**B**)	29	96.3	0	100	11.68
6β,10β,14β-Trimethyl heptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide (**C**)	20	100	0	100	12.56

Discussions

Elution of the column with ethyl acetate-methanol (19:1) afforded a colourless crystalline mass of 10-Geranilanyl O-β-D-xyloside, R_f value 0.54 (ethyl acetate-methanol, 7:3), melting point 219 - 220^oC.Elution of the column with ethyl acetate-methanol (9:1) gave colourless amorphous powder of1-Heneicosanol-O-β-D-glucopyranoside, R_f value 0.77 (hexane: ethyl acetate, 1:1), melting point 104 - 105^oC. Further elution of the column with ethyl acetate-methanol (9:1) produced colourless amorphous powder of 6β,10β,14β-Trimethylheptadecan-15α-olyl-15-O-β-D-glucopyranosyl-1,5β-olide.R_f 0.59 (ethyl acetate), melting point 233 - 235^oC.

Conclusions

Even though three compounds were isolated, which showed different levels of antimalarial activity, more work needs to be done towards the validation of the isolation process, in-silico structure modifications of these structures for better activity, study to check for possible synergistic effects among these compounds and other possible pharmacological effects

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