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Full Length Research Paper

Some chemical constituents of the leaves of *Cassia nigricans* Vahl.

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The methanol extract of the leaves of *Cassia nigricans* Vahl. (family; Leguminosae) was investigated. Chromatographic techniques were used to purify the crude extract. The gas chromatography/mass spectrometric analysis of the extract revealed the presence of five compounds, identified as 4-hydroxyanthraquinone-2-carboxylic acid; heptadecanoic acid,14-methyl-,methyl ester; bis (2-ethylhexyl) phthalate; β-cholest-3-ene and β-sitosterol acetate.

Key words: *Cassia nigricans*, methanol extract, anthracene derivatives, steroids, ester, bis (2-ethylhexyl) phthalate.

INTRODUCTION

Medicinal plants are used traditionally to prevent or cure diseases all over the world (Nair et al., 2005). The medicinal values of these plants lie in bioactive phytochemical constituents that produce definite physiological actions on the human body (Akinmoladun et al., 2007). The bioactive phytochemical constituents in medicinal plants include flavanoids, phenolic compounds, tannins, anthracene derivatives and essential oils (Edeoga et al., 2005; Krishnaiah et al., 2009). Plants in the Leguminosae - Caesalpiniacea - family are increasingly being used not only as herbal remedies in complementary and alternative medicine, but also in conventional therapy in many parts of the world for many years, especially in Africa and India where they are widely distributed (Kaey, 1989) They have been shown to possess antibacterial, antifungal (Abo et al., 2000; Nebedum et al., 2009), antiprotozoal (Obodozie et al., 2004; Moo-Puc et al., 2007) and antidiabetic activities (Jalalpure et al., 2004), and larvicidal activity against mosquito species (Yang et al., 2003; Georges et al., 2008).

C*assia* species are rich sources of polyphenols, anthraquinone derivatives, flavanoids and polysaccharides (Singh et al., 1980; Yen et al., 1998; Ayo et al., 2007), saponins, tannins and steroids (Toma et al., 2009). Some of the *Cassia* species are rich in glycerides with linoleic, oleic, stearic and palmitic acids (Jalalpure et al., 2004; Bahorun et al., 2005). *Cassia* species are well known for their laxative and purgative constituents, and are also used for the treatment of skin diseases (Dalziel, 1956). Their medicinal properties are due mainly to the content of hydroxyanthraquinone derivatives (Yang et al., 2003). Although *Cassia* species have been used widely to treat diseases, they have shown marked toxicity to man and livestock resulting in fatalities following overdoses of remedies involving the plants (Nwude and Parsons, 1977).

Cassia nigricans Vahl. is one of the herbaceous plants that grow extensively in Africa including Nigeria, especially in the northern part of the country. The plant is widely used for treating skin diseases such as ringworm, scabies and eczema (Dalziel, 1956). The aqueous extract of the leaves is used by traditional healers in Nigeria for the treatment of peptic ulcer and other gastro-intestinal disorders (Akah et al., 1998; Nwafor and Okwuasaba, 2001). The pharmacological activity of the *C. nigricans* leaves was investigated in rats, rabbits and mice (Chidume et al., 2001). The extract was found to show good analgesic and anti-inflammatory effects, and it protected rats against gastric mucosal damage. The crude methanol extract of the leaves showed strong inhibition against *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium pyogenes, Salmonella typhi, Pseudomonas aeruginosa,* and *Escherichia coli* (Ayo and Amupitan, 2004).

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C. nigricans leaves have been reported to contain active substances, including emodin, emodic acid, citreorosein and luteonin (Obodozie et al., 2004; Ayo et al., 2007; Georges et al., 2008).

In the present paper, we report five bioactive compounds found to be present in the methanol extract of the leaves of *C. nigricans* using gas chromatography/mass spectrometric (GC/MS) analysis.

MATERIALS AND METHODS

General experimental procedures

Solvents for extraction and chromatography were distilled and dried by standard methods (Perrin et al., 1966). Thin layer chromatography was carried out on plates coated with silica gel with gypsum binder and fluorescent indicator viewed under ultraviolet lamp (254 and 366 nm). Silica gel pre-coated plates (Merck, Germany) were also used. Column chromatography was performed using silica gel (60 - 120 meshed). Infra-red (IR) spectrum was recorded on Perkin-Elmer IR. The GC/MS spectra were recorded on GC/MS ATURIN 2000R mass spectrometer (Varian), interphased with Varian Model 3800 GC/MS. Conditions: capillary column: DB5-MS, 30 m length, 0.25 mm ID, 0.5 µm film thickness, injector 250°, temp. prog.: 60 - 300°C, 15° min^{-1}; split ratio: 1:20; carrier gas He, mass spec.: 6 - 30 min, mass range: 55 - 450 m/z. The m/z parameters of the spectra were matched on the already existing computer library coupled to the analyzer.

Plant material

The leaves of *C. nigricans* Vahl. (Called *shuwakan gargari* in Hausa) were collected from Jama'a village, near the Ahmadu Bello University Dam, Zaria (11° 10'N, 07°38'E), Nigeria in September, 2006. The plant was identified by Mallam Mohammed Musa at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (No. 613) was deposited in the Herbarium of the Department.

Extraction and isolation

The leaves of the *C. nigricans* Vahl. were air-dried and powdered. The powdered leaves (500 g) were successively extracted with petroleum ether (60 - 80°C) and methanol. The solvent was removed with the rotary evaporator and the residue was dried under vacuum (Harbone, 1984).

The methanol extract was hydrolysed with 3.0 M HCl (300 cm^3). The hydrolysate was extracted with chloroform. The chloroform extract was then concentrated at a reduced pressure to give the aglycone, CNAGL (3.25 g).

The CNAGL (0.5 g) was subjected to column chromatography on silica gel column eluted with mixtures of petroleum ether and ethyl acetate of increasing polarity [from petroleum ether to petroleum ether: ethyl acetate (1:4)]. Fractions were collected in 10 cm^3 aliquots. The elution was monitored by thin layer chromatography. Similar fractions were pooled together, washed, dried over anhydrous Na_2SO_4, and evaporated on a rotary evaporator. The major fraction was further purified on preparative thin layer chromatography.

RESULTS

The major fractions from the column, fractions 10 - 18, eluted with petroleum ether – ethyl acetate (1:1) yielded 0.085 g, and were further purified by preparative thin layer chromatography (silica gel) using petroleum ether : ethyl acetate (1:2) as mobile phase. This afforded two components, $CNAGL_1$ and $CNAGL_2$. The $CNAGL_1$ was obtained as orange powder and $CNAGL_2$ as yellowish-white solid.

The $CNAGL_1$ and $CNAGL_2$ were characterised by spectroscopy. The IR and GC/MS data for $CNAGL_1$ recorded were as follows:

IR υ_{max} (cm^{-1}): 605, 721, 772, 910, 1033, 1115
1204, 1264, 1378, 1463, 1641
1711, 2360 2853, 3281
GC/MS: m/z 397 (100), 280 (10), 191 (5), 73 (10)

The GC/MS spectrum of $CNAGL_2$ showed it to be a mixture of four compounds indicated in Table 1.

DISCUSSION

The $CNAGL_1$ was characterised as 4-hydroxyanthraquinone-2-carboxylic acid. The IR spectrum of the $CNAGL_1$ showed the absorption of carbonyls at 1641, 1711 cm^{-1}. It also indicated the presence of hydroxyl (-OH), stretching and bending at 3281 and 1463 cm^{-1}. The GC/MS spectrum of $CNAGL_1$ did not show the M^+ of the 4-hydroxyanthraquinone-2-carboxylic acid peak, but indicated a peak at m/z 397 for 4-hydroxyanthraquinone-2-carboxylic, diTMS ($C_{21}H_{24}O_5Si_2$). The GC/MS spectrum of $CNAGL_2$ showed it to be a mixture of four compounds, identified based on their retention time and fragmentation patterns. This is the first report of the presence of these compounds in *C. nigricans*, although β-sitosterol acetate, bis(2-ethylhexyl) phthalate, fatty acids and esters have been previously isolated from other *Cassia* species (Nageswara Rao et al., 2000; Jalalpure et al., 2004; Bahorun et al., 2005; Kumaran and Karunakaran, 2007). The mass spectra shown above for β-sitosterol acetate and bis (2-ethylhexyl) phthalate were comparable with spectral data previously reported (Nageswara Rao et al., 2000; Jalalpure et al., 2004).

Although the bioactivities of the compounds were not investigated in the present study, β-sitosterol acetate, heptadecanoic acid, 14-methyl-, methyl ester, β-cholest-3-ene and 4-hydroxyanthraquinone-2-carboxylic acid have potential therapeutic properties, while bis (2-ethylhexyl) phthalate is toxic to humans. β-sitosterol acetate has been shown to be highly effective in reducing enlarged prostates and in lowering blood cholesterol level (Amundsen et al., 2002; Wikipedia, 2009). It has been demonstrated to counteract the proliferative changes associated with colon carcinogenesis (Nair et al., 1984). Deepak et al. (2002) isolated β-sitosterol from methanol extract of the whole plant of *Tribulus terestris* and demonstrated that the compound possesses anthelminthic

Table 1. Components of methanol extract identified by gas chromatography/mass spectrometric analysis from *C. nigricans* leaves ($CNAGL_2$).

Component	Retention time (min)	Mass m/z (M^+)	Characteristic ions (relative abundances)
Heptadecanoic acid, 14-methyl-,methyl ester	17.626	298	298(100), 267(5), 255(55), 223(10), 199(50), 143(40), 129(20), 87(75), 74(85), 55(45), 43(70)
Bis(ethyl hexyl) phthalate	21.414	390	391(100), 279(35), 261(21), 167(40), 149(100), 113(5), 71(45), 57(45)
β-cholest-3-ene	21.733	370	370(100), 350(50), 257(20), 230(20), 215(55), 135(25), 108(50), 61(50), 55(45), 40(35)
β-sitosterol acetate	23.388	396	396(100), 275(50), 255(60), 213(25), 147(85), 81(100), 43(85)

activity. Fatty acid methyl esters promote the proliferation of the mesenchymal stem cells (Zhang et al., 2008). β-Cholest-3-ene has been found to be involved in the biosynthesis and metabolism of steroids (Schroepfer, 2000). The hydroxyanthraquinones have been demonstrated to possess good analgesic, anti-inflammatory and anti-plasmodial activities (Chidume et al., 2001; Yang et al., 2003; Obodozie et al., 2004). Georges et al. (2008) showed that anthraquinones are of value in the management of some agricultural pests. Phthalate esters have been shown to induce reproductive and developmental toxicity, resulting in reproductive malformations (Foster et al., 2001; Foster, 2006; Howdeshell et al., 2008; Lyche et al., 2009) via an antiandrogenic mechanism (Foster et al., 2001; Rider et al., 2009). Bis (2-ethylhexyl) phthalate is used as plasticizer for the manufacturing of intravenous and transfusion tubing and blood bags. It has been shown to reduce markedly sperm counts (Lyche et al., 2009; Wikipedia, 2009). Efforts are being made to further isolate and characterise the compounds, and also to test for their bioactivities.

In conclusion, the results show that the leaves of *C. nigricans* Vahl., which is one of the herbal plants used extensively in traditional medicine in Nigeria, contain potentially therapeutic and toxic principles.

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