Phytochemical Investigation and Antimicrobial Screening of *Cardiospermun grandiflorum* (Sweet) [*Sapindaceae*]

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ABSTRACT

Methanol extract of the aerial parts of *Cardiospermum grandiflorum* was investigated for the phytochemical constituents and antimicrobial activities.

The extract was screened for the presence of saponins, tannins, steroids, alkaloids, reducing sugars, glucosides and flavonoids using standard methods. Antimicrobial assay was carried out on the extract against two gram positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*; three gram negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiellac pneumonas* and fungi: *Candida albicans*, *Candida krusei*, *Aspergillus niger*, *Rhizopus stolonifer* and *Pencilillum notatum* by the agar diffusion method between concentrations 6.25 – 200 mg/ml.

Preliminary screening of methanol extract of *C. grandiflorum* revealed the presence tannins, steroids and reducing sugars. The extract showed average activity against the organisms used and was most active on *C. albicans* between concentrations 50-200 mg/ml with zones of inhibition between 12-16 mm.

Phytochemical investigation of the methanol extract yielded L-pinitol. The structure and relative configuration of the compound was elucidated on the basis of the spectroscopic data, especially MS and NMR techniques.

Keywords: Phytochemical, antimicrobial, *Cardiospermum grandiflorum*, *Candida albicans*

1. Introduction:

The *Sapindaceae* family is a tropical and subtropical woody family represented by 150 genera and about 2000 species [1]. The species of *Cardiospermum* includes *grandiflorum, corindum* and *halicacabum.* Plants of this family occur as trees, herbs and lianas [2-3]. Previous phytochemical investigations of these plants revealed the presence of fatty acids, cyanolipids, triterpenoid saponins, polyprenols, flavonoids, sphingolipids, alkaloids, coumarins, and ellagic acid derivatives [2-4]. Their secondary metabolites exhibited interesting biological activities such as antiplasmodial, anti-inflammatory, antiulcer, cytotoxic, antioxidant and antibacterial [4] .

Cardiospermum grandiflorum Sweet commonly called balloon vine (climber) originated from America, Africa and West Indies. It is called Ako-ejirin in South-west Nigeria.

The leaves are taken as vegetable in Ghana. Moreover, the leaves have application for dermatological troubles, chest problems and fever while the fruits are used traditionally for abortion [5-6]. There is little or no report on the chemical constituents of the aerial parts of *C. grandiflorum*, therefore, this paper reports phytochemical investigation and antimicrobial screening of *C. grandiflorum*.

2. Materials and Methods

2.1 Plant collection and identification

The aerial parts of *C. grandiflorum* were collected along Arulogun road, Ojoo, Ibadan in October 2010. The plant sample was authenticated at the Herbarium of the Forestry Research Institute, Ibadan and voucher specimen (FHI 109489) of the plant was deposited at the herbarium for further reference.

2.2 Plant preparation and extraction

The air-dried, ground plant samples (738.1 g) of *C. grandiflorum* were soaked in methanol for 72 h. The extract was concentrated under reduced pressure at 40 ^{o}C. The crude methanol extract was partitioned in hexane to yield a defatted methanol extract of *C. grandiflorum* (21.7 g).

2.3 Phytochemical analysis

The standard procedures were used to test for the presence of secondary metabolites in the plant extract [7].

2.4 Antimicrobial screening of the extract

The extract was assayed for antibacterial activity against two gram positive *Staphylococcus aureus* and *Bacillus subtilis*; three gram negative bacteria *Pseudomonas aeruginosa, Escherichia coli*, *Klebsiellac pneumonas* and

five fungi namely *Candida albicans*, *Candida krusei*, *Aspergillus niger*, *Rhizopus stolon* and *Penicillum notanun* using agar diffusion.

2.4a Antibacterial assay - pour plate method

An overnight culture of each organism was prepared by taking two loopful of the organism from the stock and inoculating each into the sterile nutrient broth of 5 ml each for 18-24 h at 37 ^{o}C. From overnight culture, 0.1 ml of each organism was added into 9.9 ml of sterile distilled water to get 1:100 (10^{-2}) dilution of each organism. From the diluted organism, 0.2 ml was taken into the prepared sterile nutrient agar which was at 45 ^{o}C. This was aseptically poured into the sterile plates and allowed to set on the bench for about 45 min. A sterile cork-borer of 8 mm diameter was used to make the wells according to the number of the concentrations of the extract and the controls. Extract of different concentrations were introduced into the wells accordingly. These plates were allowed to stay on the bench for about 2 h to allow the extract to diffuse properly into the nutrient agar. The plates were incubated in the incubator for 18-24 h at 37 ^{o}C.

2.4b Antifungal assay - surface plate method

A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates and allowed to set. Then 0.2 ml of the 10^{-2} of each organism was spread on the surface of the set agar using a sterile spreader to cover all the surface of the agar. The wells were made using a cork borer of 8 mm diameter. Various concentrations of the extract and the controls were added into the well. All the plates were left on the bench for 2 h to allow the sample to diffuse properly into the agar. The plates were incubated in the incubator for 48 h at 26-28 ^{o}C.

The assays were done in duplicates and the activity was reported by measuring the diameter (in mm) of the inhibition zone around each disk [8].

2.5 General experimental procedure

Optical rotation was measured using a Rudolph research Autopo III automatic polarimeter. Melting point obtained on a Fisher-Johns micro melting point apparatus and was uncorrected. IR spectrum data recorded on a FT-IR spectrometer, Perkin Elmer instrument. High-Resolution ESI mass spectrum was recorded using the Micromass Autospec instrument. NMR spectra (^{1}H, ^{13}C) were determined on DRX-400 spectrometer data (400 MHz for ^{1}H and 100 MHz for ^{13}C) in deuterowater. Silica gel Thin Layer Chromatographic (TLC) separations were carried out using precoated plastic sheet (Alltech, 0.25mm thickness silica gel with fluorescent indicator, 40×80 mm). TLC spots were viewed by exposure to UV light at 254 nm or phosphomolybolic acid with heating. Glass column of varying sizes (depending on weight of extract) and silica gel (70-230 mesh) were used for open column chromatography. Polyamide adsorbent (for tannins removal) was Discovery DPA-6S adsorbent, Supleco.

2.5a Removal of tannins

The defatted methanol extract (21.7 g) of *C. grandiflorum* was dissolved in water/ethanol (1:1). The extract was partially soluble and filtered. The residue recovered was sugar-like while the filtrate solution was made up with equal volume of acetonitrile and air-dried to give 9 g of methanol extract. This was dissolved in water/ethanol (1:1) and introduced into column (length = 82 cm, internal diameter = 3.0 cm) packed with 150 g of polyamide adsorbent. The column was preconditioned and eluted with water/ethanol (1:1) solvent mixture [9].

The eluted fractions were collected and made up with equal volume of acetonitrile and left to dry under streams of air in the fume cupboard. The tannins-free methanol extract (4 g) was subjected to open column chromatography.

2.5b Fractionation

The methanol extract (4 g) was pre-adsorded on silica gel and loaded on column (length = 82 cm, id = 3.0 cm) packed with 120 g of silica gel. The column was first eluted with hexane and increasing concentration of ethyl acetate and methanol. A total of thirty - four 100 ml fractions were collected. Fractions were spotted on analytical pre-coated TLC plates and similar fractions were pooled resulting in nine combined fractions. Fractions 23- 24 eluted with 60% ethyl acetate in methanol gave **1**.

L-pinitol (1):

White solid (93 mg),

R_f =0.56 (4:1 EtOAc: MeOH); mp 171-173 ^{o}C;

$[\alpha]_D^{23}$ = -56.1 (*c* 1.9 × 10^{-1}, MeOH)

IR (MeOH) 3328, 2939 cm^{-1}

^{1}H NMR: Table 2

^{13}C NMR: Table 2

HRESIMS m/z $[M+Na]^+$ calcd for $C_7H_{14}O_6Na$, 217.074; found 217.0679.

3. Results and Discussion

The methanol extract of *C. grandiflorum* showed the presence of tannins, steroids and reducing sugars. The extract displayed broad spectrum activity against the test microorganisms. The activities of the extract against gram positive and gram negative organisms ranged between 10 - 18 mm and 12 - 16 mm respectively between the concentrations of 50 – 200 mg/ml (Table 1). Moderate inhibitory activities were observed against *S. aureus* and *E. coli* while low inhibitions were exhibited against *B. subtilis*, *P. aeruginosa* and *K. pneumonae*. The extract showed growth inhibition against the fungi used between diameter ranges of 10 - 16 mm at concentrations between 25 – 200 mg/ml. Higher activity was displayed against *C. albicans* while the effect of the extract was moderate on all other test fungi. Generally, the extract was observed to be more active against fungi than bacteria used. This result corroborates the traditional usage of the plant for skin infections especially those caused by *C. albicans* such as eczema and scabies [6].

Analysis of HRESIMS, ^{1}H NMR, ^{13}C NMR, and IR spectra were used to determine the structure of **1**. The HRESIMS showed a compound with base ion peak at m/z 217.0679 (M+Na) which produced a molecular formula of $C_7H_{14}O_6Na$.

All signals in the proton NMR spectrum were observed between 3.48 - 4.30 ppm that suggested the presence of sugar protons. The H-1 and H-6 appeared as a 2H multiplet centered at the δ 3.63, H-2 and H-3 as triplet at δ 4.08 and 4.30 (J = 3.6 Hz), H-4 and H-5 as multiplet at δ 3.75 and 3.43 respectively. In proton – proton decoupling experiment, irradiation of the signal at δ 4.30 (H-3) altered the multiplicity of protons H-2 (δ 4.08) and H-4 (δ 3.43) signals while the irradiation of H-4 changed the multiplicity of H-3 and H-5 at δ 3.75 which further confirmed the assignment of the protons. Protons of methoxy group were singlet at δ 3.48.

The ^{13}C NMR spectrum showed the presence of seven carbon atoms, one methyoxy and six oxygenated methines between 57.9 – 81.9 ppm. IR spectrum of **1** showed absorptions at 3328 cm^{-1} due to hydroxyl group, 2831 and 2939 cm^{-1} for C-H (sp^3) stretching.

The spectral data was suggestive of L-pinitol as reported in the literature thus, **1** is L-pinitol also known as 1-O-methyl-L-chiro-inositol [10].

Table 1: Antimicrobial activity of aerial parts of Cardiospermum grandiflorum methanol extract

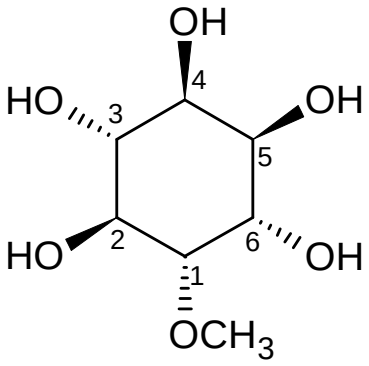
Conc. mg/mL	Microorganism/Zones of inhibition (mm)									
	S. a	E. c	B. s	P. a	K. p	C. a	C. k	A. n	R. s	P. n
200	18	16	14	12	12	16	14	12	14	12
100	14	14	12	10	10	14	12	10	12	10
50	12	12	10	-	-	12	10	-	10	-
25	10	-	-	-	-	10	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
MeOH	-	-	-	-	-	-	-	-	-	-
Gent.	38	36	34	36	36	NT	NT	NT	NT	NT
Tioc.	NT	NT	NT	NT	NT	24	26	26	24	22

S. a., - Staphylococcus aureus, B.s., - Bacillus subtilis, P.a., - Pseudomonas aeruginosa, E.c., - Escherichia coli, K.p., - Klebsiellae pneumonae C. a., - Candida albicans; C. k., - Candida krusei; A. n., - Aspergillus niger; R. s., - Rhizopus stolonifer; P. n., - Penicillum notatun. Gent., - Gentamycin (10 µg/mL), Tioc., - tioconazole (70 % w/v), MeOH – Methanol, NT – Not tested, (-) no activity, Size of well – 8 mm

TABLE 2: $^{1}H^{a}$ NMR and $^{13}C^{b}$ NMR Assignment of compound 1 in D_2O

Positions	δ $^{1}H^{a}$	δ $^{13}C^{b}$
1	3.63, m	81.2
2	3.43, m	73
3	4.30, t (3.6 Hz)	72.4
4	4.08, t (3.6 Hz)	68.2
5	3.75, m	73.9
6	3.63, m	71.4
1`	3.48, s	57.9

[a]400 MHz; [b]100 MHz



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