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Full Length Research Paper

Pharmacological activity of 2,3,8-tri-O-methyl ellagic acid isolated from the stem bark of *Irvingia gabonensis*

George, I. Ndukwe [1*] and Yimin Zhao[2]

[1]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.
[2]Beijing Institute of Pharmacology and Toxicology, Beijing, PR China.

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2,3,8-Tri-O-methyl ellagic acid was isolated from the stem bark of *Irvingia gabonensis.* The acid showed significant antimicrobial activity on some pathogens.

Key words: Ellagic acid, *Irvingia gabonensis*, clinical isolates, *in vitro* studies, pharmacological activity.

INTRODUCTION

Irvingia gabonensis (Irvingiaceae) is widely distributed in tropical West Africa and represented by two varieties: (i) fruits with sweet edible scanty fibrous pulp, fluted or cylindrical hole and (ii) fruits with bitter inedible very fibrous pulp and buttressed hole (Watt et al., 1962). The stem bark is claimed to be very useful in ethnomedicinal treatments in some parts of West Africa. Irvine (1935) reported that the shavings of the stem bark are eaten to stop diarrhea or dysentery in French Equatorial Africa while Dalziel (1948) reported that aqueous extract of the bark is rubbed for pain relief by the Mendes of Sierra Leone. The powdered kernels is applied to burns and also used as an astringent (Irvine, 1935). Nyakandiyi (1999) reported that the methanol extract from the stem bark has excitatory effect on the phrenic nerve of rats, while Raji et al. (2001) reported that the methanol extract of its stem bark has antidiarrhea and antiulcer properties in rats. Folklore has it that the aqueous/alcoholic extract of the stem bark finds use in the treatment of dysentery, typhoid fever and fungal (skin) infections. There is no literature available on the chemical compounds isolated from this plant.

MATERIALS AND METHODS

Plant material

The plant material was collected from Ihiala, Anambra State, Nigeria (6°N and 7°E) and identified by Mr. Abdullahi Musa of the Herbarium, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen, number 103947, was deposited there. The collected sample was air-dried, pulverized using a mill hammer and stored in polythene bags for use.

Extraction procedure

Air-dried and pulverized plant material (325.60 g) was defatted with redistilled petroleum spirit (60 – 80°C) by the use of soxhlet extractor to afford 6.00 g (1.85%) of fatty acids and their derivatives. The defatted pulverized plant material was then successively and exhaustively extracted with redistilled chloroform and methanol. The various extracts were concentrated *in vacuo* at 40°C using a rotavapor. This gave 0.80 g (0.24%) of dry crude chloroform extract and 22.28 g (6.00%) of dry crude methanol extract. The various extracts were stored in a refrigerator and later subjected to bioassay studies (Ndukwe et al., 2005). The most active fraction (methanol) was column chromatographed on silica gel. Elution with chloroform gave pale yellow crystals which were further purified using silica gel preparative thin layer chromatography with melting point of 296 – 297°C. The crystals were characterized purely by spectral techniques as 2,3,8-tri-O-methyl ellagic acid. The antimicrobial activity of this acid was determined *in vitro* on clinical isolates of *Streptococcus pneumponiae, Vibro cholera, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli* and *Salmonella typhymurium* using the disc diffusion method of Baeur et al. (1966) and Barry and Thornsberry, 1985).

Antimicrobial screening test

Pure clinical isolates of *S. pneumoniae, V. cholerae, S. aureus, K. pneumoniae, P. aeruginosa, B. cereus, E. coli* and *S. typhi* obtained from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were grown on a nutrient agar slant in bijou bottles in an incubator at 37°C for 24 h. Stock solutions of the acid were prepared by initially dissolving 0.5 g of the extract in 0.5 ml of DMSO to obtain a stock solution of concentration 1000 mg/ml. From this stock solution, concentrations of 200, 160, 120, 100, 80, 60, 20

*Corresponding author. E-mail: ndukweilogeorge@yahoo.com.

and 10 mg/ml were prepared by serial dilution. The cork and bore diffusion method of Bauer et al. (1966) and Barry and Thornsberry (1985) were used in the antimicrobial screening. Inoculation of the prepared plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. A control was set up alongside using pure DMS0 for each strain of organism. The plates were incubated at 37°C for 24 h after which they were examined for zones of inhibition of growth.

Determination of the minimum inhibitory concentration (MIC)

Double strength nutrient agar was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 42 sets of universal bottles and sterilized in an autoclave at 121°C for 15 min. The agar was allowed to cool to 45°C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allow to solidify for one hour. Extracts' concentrations of 100, 80, 60, 50, 40, 30, 10 and 0.5 mg/ml respectively were prepared by serial dilution. Each plate was divided into six equal sections and labeled accordingly to correspond to six test organisms. Two 5 mm diameter paper discs (Whatman No.1) were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 ml of each bacterial suspension was taken and transferred aseptically and carefully into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 h at 37°C after which they were observed for growths or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

Determination of the minimum Bactericidal concentration (MBC)

This was carried out to know if the organisms could be killed completely or their growths could only be inhibited. Another 42 sets of plates of nutrient agar were prepared according to the manufacturers' standard and sterilized in an autoclave as earlier described. The paper discs in all the plates from the MIC tests were re-activated. Emphasis was mostly paid to the MIC plates and the preceding plates. The re-activation was done in a mixture of 0.5% egg lecithin and 3% Tween 80 solution in a test tube. The reactivated organisms were sub-cultured into appropriately labeled quadrants of the sterilized nutrient agar plates using wire loop into each test tube and streaking uniformly on the labeled quadrants. This was then incubated for 24 h at 37°C after which they were observed for growths. The MBC was the quadrant with the lowest concentration of the extract without growth.

RESULTS AND DISCUSSION

The crude extracts of the stem bark of *I. gabonensis* had various antimicrobial activities but it was highest for the crude methanol extract hence this fraction was further investigated. The acid- 2,3,8-tri-O-methylellagic acid (Table 1), mp 296 - 297°C, pale yellow crystals(DMSO) was isolated from this fraction and its structure esta-blished purely by spectral data shown below:

1H-NMR (400 MHz, DMSO-D_6):
ä: 7.55 (1H singlet), 7.65 (1H singlet), 4.01 (1H singlet), 4.04 (1H singlet), 4.06 (1H singlet).

Table 1. Properties of 2,3,8-Tri-O-methylellagic acid isolated from the stem bark of *Irvingia gabonensis*.

Parameter	^{13}C-NMR	^{1}H-NMR
1	111.21	-
2	140.96	-
3	140.20	-
4	152.63	-
5	111.66	7.55s
6	112.53	-
7	158.33	-
1'	111.96	-
2'	141.49	-
3'	140.84	-
4'	153.81	-
5'	107.47	7.65s
6'	113.38	-
7'	158.52	-
OMe(4')	56.73	4.01s
OMe(3')	61.31	4.04s
OMe(3)	61.02	4.06s
OH(4)	-	-

Molecular formula: C17H12O8. Percentage composition: C 59.3%, H 3.51%, O 37.18% .Physical description: Pale yellow needles (DMSO). Melting point: 296 - 2970C.

Table 2. Antimicrobial screening of the acid isolated from the stem bark of *Irvingia gabonensis*.

Test organism	Diameters of zones of inhibition (mm)
S. Pneumoniae	19
Vibrio cholerae	24
S. Aureus	25
K. Pneumoniae	20
P. Aeruginosa	19
B. cereus	21
E. coli	25
S. Typhi	22

^{13}C-NMR (400MHz, DMSO-D_6),
ä: 111.21 (C-1), 140.96 (C-2), 140.20 (C-3), 152.63 (C-4), 111.66 (C-5), 112.53 (C-6), 158.33 (C-7), 111.96 (C-1'), 141.49 (C-2'), 140.80 (C- 3'), 153.81 (C-4'), 107.47 (C-5'), 113.38 (C-6'), 158.52 (C-7'), 56.73 (OMe-4'), 61.31 (OMe-3') and 61.02 (OMe-3).
EI-MS, m/z: 74.0, 87.1, 103.1, 115.1, 131.1, 145.1, 159.1, 172.1. 187.1, 202.1, 215.1, 230.1, 241.1, 258.1, 273.2, 286.1, 301.2 314.1, 329.2, 334.2[M^+]

This acid was very active on all the microorganisms used in this study (Table 2). The report by Irvine (1935) that the bark is eaten to stop diarrhea or dysentery in French Equatorial Africa might be possible given that *B. cereus*

Table 3. MIC of the acid isolated from the stem bark of *Irvingia gabonensis* on some pathogens.

Test organisms	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
B. cereus	-	-	-	-	-*	+	++	+++	+++
S. aureus	-	-	-	-*	+	++	+++	+++	+++
E. coli	-	-	-	-*	+	++	+++	+++	+++
P. aeruginosa	-	-	-	-	-*	+	++	+++	+++
K. Pneumoniae	-	-	-*	+	++	++++	+++	+++	+++
S. Typhi	-	-	-	-	-*	+	++	+++	+++

* = MIC concentration; - = No growth, + + = growth.

Table 4. MBC of the acid isolated from the stem bark of *Irvingia gabonensis* on some pathogens..

Test organisms	Concentration (mg/ml) of extract								
	100	80	70	60	50	40	20	10	0.5
B. cereus	-	-	-*	+	++	++	+++	+++	+++
S. aureus	-	-	-*	+	+	++	+++	+++	+++
E. coli	-	-	-*	+	+	+++	+++	+++	+++
P. aeruginosa	-	-*	+	+	++	++	+++	+++	+++
K. Pneumoniae	-	-*	+	++	++	+++	+++	+++	+++
S. Typhi	-	-	-	-*	+	++	+++	+++	+++

main causative agent of diarrhea or dysentery could be exterminated by this acid obtained from the bark of this tree and at such a low concentration as 70 mg/ml (Table 3). Also the claim that the bark is found useful in the treatment of typhoid fever is equally justified by an MBC of 60 mg/ml determined for this acid on *S. typhi* which is the causative agent of typhoid (Table 4). The populace of the West African sub-region relies on plants' concoctions/decoctions for curing typhoid. If toxicological studies of this acid are conducted, it is expected that it could be formulated into a useful drug for the treatment of typhoid and some other diseases whose causative agents are involved in this studies. Although Nawwar et al. (1994), Yazaki and Hillis (1976) and Tchinda et al. (2003), had variously isolated this acid from some other plants, but the pharmacological activity had not been reported. It may be necessary to carry out further toxicological studies on it to ascertain if it could be put into pharmaceutical use.

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