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SHORT COMMUNICATION

Isolation and evaluation of antiglycation potential of polyalthic acid (furano-terpene) from *Daniella oliveri*

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Abstract A furano-diterpene (polyalthic acid) was isolated as a major stable compound for the first time from the oleoresin of the *Daniella oliveri* of the family Caesalpiniacea through column chromatography fractionation. Polyalthic acid was characterized using data obtained from EIMS, HREIMS, ESI-MS, MALDI-MS as well as 1D and 2D NMR and it was evaluated for its potential to inhibit the formation of advanced glycation end-products (AGEs) using a standard *in vitro* antiglycation procedure. Polyalthic acid indicated a negative antiglycation potential compared to standard inhibitor that has 85% inhibition, which is an indication that polyalthic acid may not contribute to the antiglycation activity of the plant as acclaimed in folkloric medicine. The negative antiglycation observed could indicate that the polyalthic acid could trigger glycation, thereby subjecting users to various degrees of complications. The bioactivity evaluation on molinspiration evaluator indicated that polyalthic acid could be a potential drug candidate. The biological and chemical insights gained on polyalthic acid provide a good basis for future research.

1. Introduction

Oxygenated heterocyclic diterpenes are of great medicinal value well known to possess various degrees of important pharmacological activities. Carnosic acid and carnosol (oxygenated diterpenes) from ethanolic extracts of dried leaves of sage (*Salvia officinalis*) have shown inhibition of (35S)tert-butylbicyclophosphorothionate(35S-TBPS) binding to rat brain membranes in an *in vitro* model [1]. The compounds were later observed to be strong antioxidants [2], activators of the human peroxisome

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proliferator-activated receptor gamma [3] and predicted to possess anti-angiogenic activity which possibly contributes to the chemo-preventive, antitumoral and antimetastatic activities of rosemary extracts [4]. Recently, eight new cassane-type heterocyclic diterpenes were isolated from the ethyl acetate extract of *Caesalpinia minax* and they were observed to possess significant antiproliferative activity against various cell lines [5]. Our interest was drawn to polyalthic acid, a bicyclic diterpene which is present in the exudates of *Daniella oliveri* as indicated in our preliminary findings. Literature search revealed that heterocyclic diterpenes have been isolated from this plant previously. Specifically, compounds isolated from *D. oliveri* wood include a diterpene lactone [6], daniellic acid and daniellic alcohol [7]. The biological significance of these diterpenes has not been established.

D. oliveri, a deciduous wood plant belonging to the family Caesalpiniacea, is known for the production of aromatic resins which are used as a mosquito repellent in various African countries [8]. The bark and wood are also burnt to act as mosquito repellants in some countries [9,10]. The bark extract is used traditionally for control of gastro-intestinal parasites [11], while the decoction of the root extract with other plant extracts is used in some parts of Southern Nigeria as remedy for hyperglycemia [12]. The leaf extract has been reported for its *in vitro* antioxidant potential [13], antimicrobial activities [14,15], *in vivo* anti-typhoid potential [16] and effect on skeletal muscles of experimental animals [17]. The resinous exudate obtained from the bark of the plant is used for wound healing purposes in Nigeria [18]. The exudate also possesses anti-wrinkle potential [19]. The gum and bark are taken in various preparations, internally and externally, and sometimes with other plant parts, to treat venereal diseases, ulcers and sores, circumcision wounds, leprosy, dysentery, colic, menstrual problems, cough, colds, angina, bronchitis, tuberculosis, kidney problems, appendicitis, headache, back-ache, rheumatism, fever pains, hernia, tooth-ache and snakebites [20]. In this paper we report for the first time the isolation, characterization and antiglycation activity of polyalthic acid from *D. oliveri* exudate.

2. Materials and methods

Plant materials were collected from mature *D. oliveri* tree at Ilorin, Nigeria. Botanical authentication was performed by a plant taxonomist at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria and a voucher specimen number UIH 964 was obtained. The resin was stored in a cool place until further use.

2.1. Isolation of polyalthic acid

Approximately 2 g of the plant exudates were dissolved in dichloromethane (DCM) and fractionated on a silica gel column chromatography eluting with hexane: DCM in an increasing order of polarity. Fractions were obtained and profiled on thin layer chromatography (TLC). Most fractions were too complex to be separable as they contained numerous compounds in minute quantities and were therefore not further processed. The fraction containing a major compound (semi pure) was re-chromatographed on a silica gel column chromatography using hexane: DCM in an increasing order of polarity to furnish a compound, which was later identified as polyalthic acid.

2.2. Instruments

Electron-impact mass spectra (EI-MS) were obtained on a variant MAT 311 mass spectrometer connected to a computer system. High-resolution electron impact mass (HREI-MS) spectra were recorded on a JEOL-JMS HX 110 mass spectrometer. ESI-MS data were obtained on QqTOF-MS/MS instrument (QSTAR XL mass spectrometer Applied Biosystem/MDS Sciex, Darmstadt, Germany) at room temperature. Samples were dissolved in appropriate polar solvent, and working dilution was prepared in acetonitrile-water containing 0.1% trifluoroacetic acid. Analysis was carried out by electrospray ionization (ESI) and collision-induced dissociation (CID), positive ion mode on the instrument at room temperature. Matrix assisted laser desorption/ionization (MALDI) mass spectrum data were obtained on a Ultraflex TOF (Bruker Daltonics, Bremen, Germany) mass spectrometer, equipped with a Smart beam laser (Nd: YAG, 355 nm) and an electrostatic reflector. Infrared spectra were determined on a JASCO A-302 spectrophotometer Fourier Transform-Infrared (FTIR) spectrophotometer using KBr pellet. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-400 spectrometer. Multiplicity of the carbon signals was determined by using DEPT 90° and 135° experiments. Homonuclear ^{1}H–^{1}H connectivities were determined by using COSY 45° experiment. One bond ^{1}H–^{13}C bond connectivities were determined by using HMQC experiment. Two- and three-bond ^{1}H–^{13}C connectivities were determined by HMBC experiment. Tetramethylsilane (TMS) was used as the internal reference. All NMR spectra were measured in $CDCl_3$.

2.3. In vitro antiglycation assay

The *in vitro* antiglycation potential of polyalthic acid isolated from *D. oliveri* was evaluated following standard procedure [21], by dissolving 10 mg/mL bovine serum albumin (BSA) in 67 mM phosphate buffer (pH 7.4). Glucose (50 mg/mL) was dissolved in 67 mM phosphate buffer (pH 7.4). 3 mM sodium azide was added to inhibit bacterial growth. 1 mg/mL concentration of the extract was used to calculate antiglycation activity along with the standard inhibitor. The dissolved samples (60 mL in each well of 96-well plate) were incubated for a week at 37 °C. After a week, the samples were allowed to cool to room temperature. Then 6 μL of 100% trichloroacetic acid (TCA) was added to each of the well and the supernatants containing unbounded glucose, inhibitor and interfering substances were removed after centrifugation at 14,000 rpm for 4 min. Pellets were obtained at the bottom of the wells. Solvent was removed from each well and 60 mL of phosphate buffer saline (PBS) pH 10 was added to dissolve the pellets and the comparison of fluorescence intensity at 370 nm excitation and emission at 440 nm was made using a spectrofluorimeter, RF-1500 Shimadzu, Japan. Rutin was used as the standard inhibitor. Inhibition percentage was calculated as

$$\% \text{ inhibition} = 100 - [(OD_{sample}/OD_{blank}) \times 100],$$

where OD_{sample} and OD_{blank} are the optical densities of sample and blank, respectively.

2.4. Data analysis

The 50% cytotoxic (IC_{50}) was calculated from dose-response-inhibition curves after nonlinear regression analysis. The results

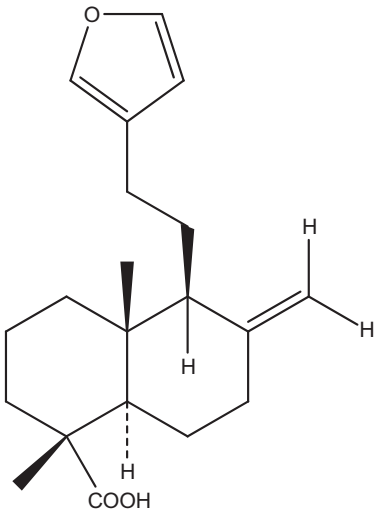

Fig. 1 Structure of polyalthic acid.

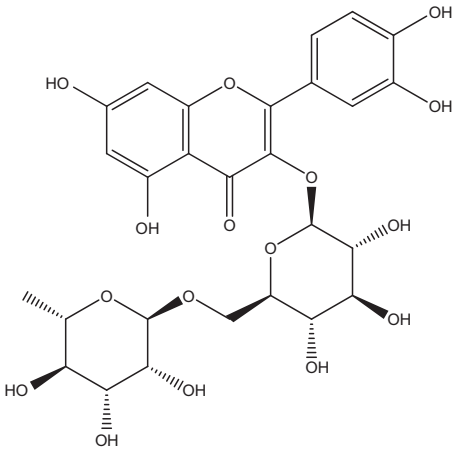

Fig. 2 Structure of rutin.

were presented as the mean ± standard error of the mean values of three different experiments.

2.5. *Spectroscopic data of polyalthic acid*

Polyalthic acid, $C_{20}H_{28}O_3$ (19 mg); EI-MS m/e: (M^+) 316 (34%), 189 (34%), 121 (89%), 81 (100%); UV λ_{max} 248 nm; IR (KBr) cm^{-1}: 3415 (O–H stretch), 2959 and 2871 (C–H stretch), 1712 (C═O stretch of carboxylic acid), 1665 (C═C stretch), 1453 (O–H bend), 1214 (C–O–C ether) and 900 (C–H bend) and 889 (furan ring): HREIMS; 316.2028 amu; calc. 316.20 indicating $C_{20}H_{28}O_3$. MALDI-TOF; $[M+H]^+$ 317: ESI-TOF-MS; $[M+H]^+$ 317. NMR ($CDCl_3$ ppm): 0.78 (3H, CH_3), 1.21(3H, s, CH_3) 1.3–2.57 (16H, M, $–CH_2–$ and –CH–), 4.56 and 4.87(1H each, s and –CH), 6.23 (1H, s, proton on furan ring), 7.17 and 7.32 (1H each s, protons on furan ring), 9.40 (lH, brs, COOH). Other NMR data which include COSY, HMQC, HMBC and NOESY were examined and compared with the data in literature [22,23].

3. Results and discussion

Polyalthic acid (Fig. 1) has two C-Me groups [23] and the NMR spectrum of polyalthic acid in $CDCl_3$ distinctly revealed two tertiary methyl groups at δ 0.78 and 1.21 ppm, one being more deshielded than the other due to the proximity of a carboxyl group on ring A, 14 cyclic methine and methylene protons, an exocyclic methylene group and two α and one β-furan protons. The two exocyclic vinyl protons were well discernable at δ 4.56 and 4.87 ppm and the furan ring protons at δ 7.17 and 7.32 ppm. Thus, the compound was identified as polyalthic acid. The formation of advanced glycation end-products (AGEs) otherwise known as antiglycation indicated that the compound has a negative antiglycation potential when compared with the standard inhibitor drug, rutin (Fig. 2) which had 85% inhibition at 1 mM concentration and an IC_{50} of 269.04 ± 3.79 mg/mL. Consequently, polyalthic acid may not be a primary component responsible for the antidiabetic activity reported for the plant extract in folkloric medicine and *in vivo* studies [12]. The negative antiglycation observed could be an indicator that polyalthic acid could trigger glycation, thereby subjecting users to various degrees of complications.

In previous research, polyalthic acid has been isolated from *Polyalthia fragrans* [24] and reported to suppress the mutagenicity of Trp-P-1 [25]. It has also been shown that kaurenoic and polyalthic acids are capable of promoting the inhibition of rhodamine 6G efflux in *Saccharomyces cerevisiae* with Pdr5p enzyme, the protein that confers multiple drug resistance [26].

Polyalthic acid isolated from *Croton reflexifolius* was identified as the main active relaxing agent when assessed in isolated guinea pig tracheal rings [27] and was reported to be a gastro-protector agent in animal studies. Polyalthic acid used to treat rats orally showed a gastro-protective effect similar to that elicited by carbenoxolone [28].

The online chemoinformatics software Molinspiration (version 2011.06) was used to establish the molecular properties (Table 1) and drug-likeness/bioactivity score of polyalthic acid compared with the standard, rutin (Table 2). Drug-likeness is based on Lipinski's "Rule of 5" [29], which considers whether various molecular properties and structure features of a particular molecule are similar to those of known drugs. These properties, such as hydrophobicity, electron distribution, hydrogen bonding characteristics, molecule size and flexibility, influence the absorption, bioavailability, transport properties, reactivity, toxicity, metabolic stability of the molecule and thus potential for use as a pharmaceutical drug [29,30].

The rule was formulated by Lipinski et al. [29]. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). The rule is important for drug development where a pharmacologically active lead structure is optimized stepwise for increased activity and selectivity, as well as drug-like properties [31]. Polyalthic acid and rutin were checked for the bioactivity by calculating the activity score for GPCR ligand, ion channel modulator, kinase inhibitor, and nuclear receptor ligand [29].

Rutin violated three of the Lipinski's rules while polyalthic acid violated none (Table 1). The positive bioactivity score of polyalthic acid, especially its ability to act as nuclear receptor ligand and enzyme inhibitor, is a clear indication that the compound is a potential drug candidate. It can be a viable lead compound in the development of drug through structure activity relationship studies.

Table 1 Molecular properties of polyalthic acid and rutin.

Compound	MW/formula	Log P[a]	Molecular polar surface area/volume	No. non-H atoms	No. H bond acceptors[b]	No. H bond donors[c]	Rotatable bonds	No. of rule of five violations[d]
Polyalthic acid	316.441 $C_{20}H_{28}O_3$	4.689	50.439/316.057	23	3	1	4	0
Rutin	610.521 $C_{27}H_{30}O_{16}$	−1.063	269.427/496.068	43	16	10	6	3

MW: molecular weight.
[a]Log P is based on octanol–water partition coefficient.
[b]H bond acceptors include O and N atoms.
[c]H bond donors include OH and NH groups.
[d]Rule of 5 violations are based on the molecular descriptors used by Lipinski et al. [29] for "drug-like" molecules (log $p \leq 5$, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5).

Table 2 Bioactivity score of polyalthic acid and rutin.

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Polyalthic acid	0.38	0.28	−0.42	0.80	0.13	0.60
Rutin	−0.05	−0.52	−0.14	−0.23	−0.07	0.12

4. Conclusion

Polyalthic acid, a diterpenoid isolated for the first time from exudates oleoresin of *D. oliveri*, indicated no antiglycation potential. Other biological assays would be substantive to amply validate the pharmacological significances of polyalthic acid obtained in the plant. The biological and chemical insights on polyalthic acid provide a good basis for future research.

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