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FLAVONE C-GLYCOSIDE AND A NEW TANNIN ISOLATED FROM THE LEAVES OF NEOCARYA MACROPHYLLA (SABINE) PRANCE (CHRYSOBALANACEAE) USING CENTRIFUGAL PARTITION CHROMATOGRAPHY

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ABSTRACT

Neocarya macrophylla (sabine) Prance is a plant of the traditional senegalese pharmacopoeia. Its leaves are used in the treatment of various diseases. The phytochemical investigation of its leaves resulted in the isolation of the new tannin (1), 1-O-galloyl-6-O-lutéoyl R-glucose (2) and Isoorientine (3). Their structures were isolated and characterized by using a combination of centrifugal partition chromatography (CPC), NMR (1D and ^{2}D) spectroscopic and mass spectrometry (ESI-MS) analyses, as well as by its comparison with literature values. This study constitutes the first phytochemical examination of the leaves of *N. macrophylla.*

KEYWORDS: *Neocarya macrophylla,* Leaves, Centrifugal Partition Chromatography, Preparative Chromatography, Tannin, flavone C-glycoside, NMR (1D and ^{2}D).

1. INTRODUCTION

Neocarya macrophylla, commonly known as ginger bread plum or "neou" oil tree, belongs to the *Chrysobalanaceae* family. *N. macrophylla*, famous for its numerous therapeutic virtues, is a plant of the traditional senegalese pharmacopoeia. The leaves, bark and roots of this plant are commonly used in Senegal and other African countries. Previous studies had already proved the physiological and therapeutic importance of the plant (leaves, barks, and roots).[1-5] Traditional medicine is still the mainstay of about 80 % of the population, especially in the developing countries. This is solely due to its acceptability, affordability and lesser side effects.[6] Medicinal plants have been a major source of most therapeutic agents.[7] *N. macrophylla* has been used in traditional medicine for the treatment of various diseases such as asthma, skin infections, treatment of wounds, dysentery, inflammations, pulmonary troubles, ear and eye infections.[8-10] In Senegal, a cigarette prepared from the stem bark of *N. macrophylla* is used as remedy for snake bite.[11] The previous phytochemical studies had demonstrated the presence of Stigmasterol, Bis-(5,7-diacetyl-catechin-4'-α-rhamnopyranoside), Epicatechin in the stem bark and quercetin in the leaves.[12- 16, 17] Methanolic extracts of the stem bark and leaves had shown that the plant possesses analgesic, antimicrobial, anti-inflammatory, Antibacterial and anti-biofilm activities.[18-22] This study presents for the first time the isolation by a one-step CPC system and elucidation of the structure of one flavone c-glycoside and two tannins including a new one from the aerial parts of *N. macrophylla*. In addition, the CPC system also offers many technological advantages such as versatile products, faster and inexpensive product development, retention of bioactivity integrity, higher throughput, higher yields, and reduced operating costs.[23-25]

2. MATERIALS AND METHODES

2.1 Plant material

The leaves of *N. macrophylla* were collected in January 2018 from region of Sedhiou in Senegal. The plant was authenticated at the Botanical Laboratory of the Faculty of Medicine, Pharmacy and Odontology (FMPO) of Cheikh Anta Diop University (CADU) and then dried at the Laboratory of Pharmacology of the same Faculty. The leaves (100 g) were shade dried, pulverized to powder, labelled and stored at room temperature for use.

2.2 Experimental procedures

2.2.1 Maceration followed liquid-liquid extraction

The powdered leaves (100 g) of *N. macrophylla* had been macerated for 24 h and extracted with 700 mL of ethanol. The crude obtained after filtration on Buchner and evaporation to dryness was weighed. A liquid-liquid extraction was carried out successively using a separatory funnel with 3 times 100 mL of heptane (0.599g), chloroform (CH_2Cl_2) (0.753 g), Ethyl-acetate (AcOEt) (1.072 g), butanol (BuOH) (2.87 g) and water (H_2O) (0.775 g).

2.2.2 Apparatus

2.2.2.1 CPC apparatus

The CPC instrument employed was an Armen SCPC-250 coupled with a Spot prep II system by Armen Instrument (Saint-Avé, France). It was fitted with 250 ml rotor containing 1800 twin-cells, UV dual detector 200-600 nm through loop of 10 ml. Rotation speed was adjustable in a range from 0 to 3000 and 1600 rpm was used in this study.

2.2.2.2 CPC separation procedure

CPC is a preparative liquid-liquid chromatographic technique, without any support solid, based on partition of solutes between two immiscible liquid phases. The first phase is chosen to be the stationary phase held in the rotor by the action of a centrifugal force. The second, the mobile phase, flows continuously through the stationary phase. Compounds are separated according to their partition coefficient Kd expressed as the ratio of their concentration in the stationary phase and their concentration in the mobile phase.[26] The biphasic solvent systems used is the ternary mixture AcOEt/ MeOH/ Water (v/v/v) (4.1 /1.9 / 4) in isocratic mode.

2.2.3 Purification of butanol extract by CPC

CPC Characteristics

A part of the crude of BuOH (500 mg) extracts was purified by CPC. The system used for this purification corresponds to the ternary mixture AcOEt, MeOH, and H2O (11.74/10/78.26 v/v/v). The ascending mode is chosen to achieve the purification, the stationary phase is the lower phase and the mobile phase is the upper phase. The purification chromatogram is shown in Figure 1. From [0 to 120 min]. We worked in ascending mode then in descending mode from [130 to 180 min] followed by extrusion.

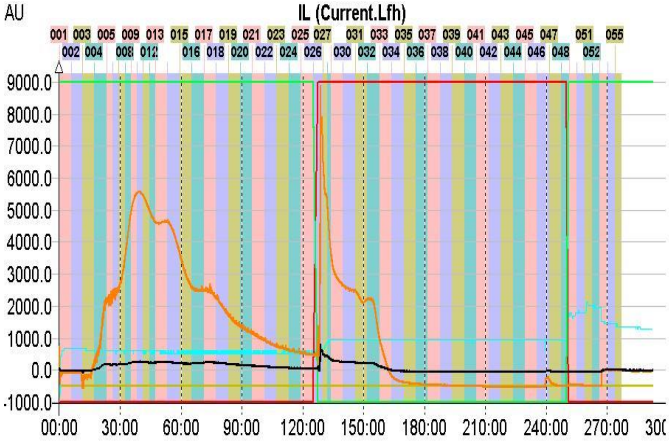

Figure 1: Chromatogram purification of *Neocarya macrophylla* butanol leaf extract by CPC: From [0 to 120 min] elution with 100 % of the upper phase then from [130 to 180 min] elution with 100 % of the lower phase followed by extrusion.

3. RESULTS AND DISCUSSION

Phytochemical screening carried out on the BuOH extract of *Neocarya macrophylla* leaves revealed the presence of different secondary metabolites families. Thus, the study showed the presence of polyphenols, flavonoids, tannins and saponins.

The yield of the purification by CPC is 62%. Three majority molecules were isolated (**1**) (61 g); (**2**) (62 g); (**3**) (7 g) and their purities have been confirmed by analytical HPLC (analytical conditions CH_3CN/H_2O (70/ 30)) and LC/MS.

3.1 Identification of compounds

The known compounds **2** and **3** were readily identified by their spectral data and by comparison with reported corresponding compounds in the literature as 1-O-galloyl-6-O-lutéoyl R-glucose (**2)** and Isoorientine (**3)** (Figure 2).[27,28]

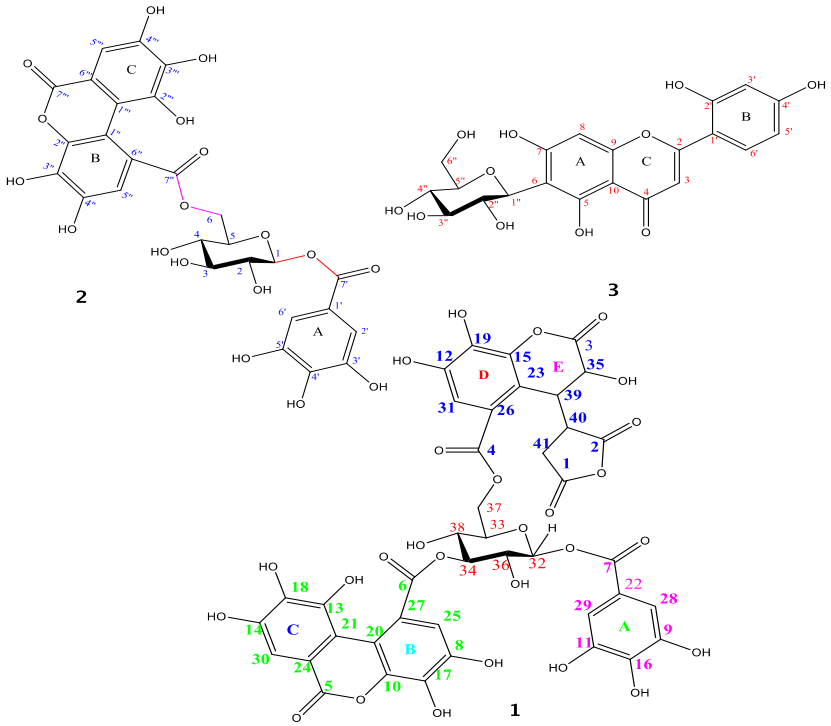

Figure 2: 1-O-galloyl-6-O-lutéoyl R-glucose (2), Isoorientine (3) and tannin 1.

The figure 1 shows the procedure adopted to characterize and identify the new tannin **1**. The numbering used is based on previous work of the same nature in maize to make the interpretation more readable. The ppm chemical shifts of the NMR (^{1}H and ^{13}C), COSY, HSQC, and HMBC spectra are respectively shown in Table 1. In the HMBC correlations, the coordinates of each peak observed in the contest trace are a distinct ^{1}H and ^{13}C from 2, 3, see 4 links (Figure 3). This is extremely useful for making assignments and the cartography to the covalent structure.

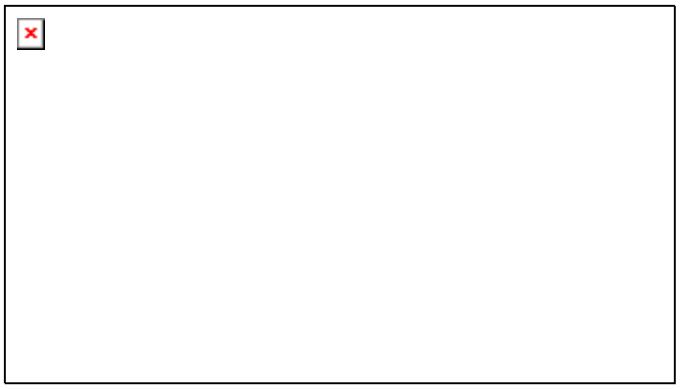

Figure 3: HMBC spectrum of compound 1.

Tableau 1: Chemical shifts in ^{1}H NMR and ^{13}C NMR of compound 1 in MeOD, δ in ppm.

Carbone N°	^{13}C, □ppm	^{1}H, mult. (J en Hz), □ppm	Correlations COSY	Correlations HSQC	Correlations HMBC
1	173,64				H41
2	173,015				H39 ; H40
3	169,36				H35
4	168,73				H37
5	166,09				H30
6	165				H25
7	164,85				H28 ; H29
8	145,97				H25
9	145,17				H28
10	145,17				

11	144,99				H29
12	144,73				H31
13	144,18				
14	144,14				H30
15	139,97				H39
16	139,45				H28 ; H29
17	138,96				H25
18	137,26				H30
19	136,14				H31
20	124				
21	123				
22	118,65				H28 ; H29
23	117				H39
24	116,2				H30
25	116,2,	7,49 s		H25	
26	114,79				H31
27	114,53				H25
28	109,48	7,1 s		H28	
29	109,48	7,1 s		H29	
30	108,9	6,80 s		H30	
31	106,76	6,65 s		H31	
32	91,1	6,53 d	H32 ; H36	H32	
33	72,82	4,84	H33 ; H37	H33	
34	69,66	5,4	H34 ; H38	H34	
35	65,5	4,82	H35 ; H39	H35	
36	65,36	5,25	H36 ; H34	H36	
37	63,31	4,89 ; 4,38	H37 ; H37' ; H33	H37 ; H37'	
38	60,97	5,89	H38 ; H33	H38	
39	40,29	5,06	H39 ; H40	H39	
40	38,58	3,8	H40 ; H41	H40	
41	29,08	2,23	H41 ; H40	H41	

Compound **1** was obtained as a dark brown amorphous powder. Compound **1** exhibited in the negative ion-mode ESI-MS (Fig. 4), a quasi-molecular ion peak at m/z 953.3 $[M-H]^-$ (calcd for 953.3) compatible with the molecular formula $C_{41}H_{30}O_{27}$.

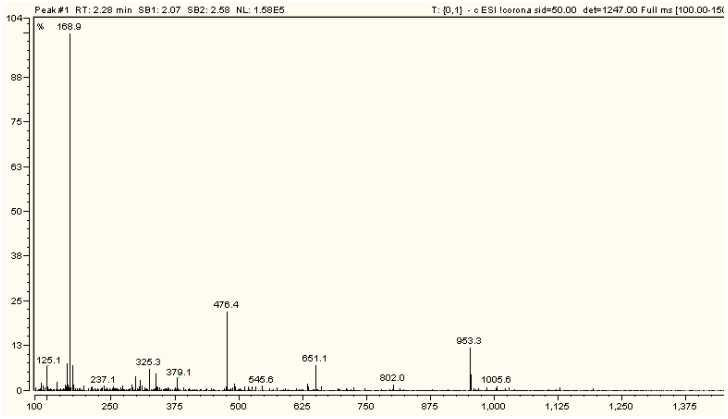

Figure 4: Spectrum masse of compound 1.

The ^{13}C NMR spectrum of compound **1** shows the presence of 41 carbons, among which the following characteristic carbons can be identified: Seven most unshielded carbonyls C-1, C-2, C-3, C-4, C-5, C-6 and C-7 which come out respectively at 173.64; 173.01; 169.73; 166.09; 165 and 164.85 ppm corresponding to the chemical shifts of the carbonyls of the esters. Twelve aromatic quaternary carbons C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18 and C -19 which come out respectively at 145.97; 145.17; 144.99; 144.73; 144.18; 144.14; 139.97; 139.45; 138.96; 137.26; 136.14 ppm. As in the case of compound 2[24], the carbons C-9

and C-10 at high intensity count as two carbons (due to the existing symmetry at the level of the aromatic ring **A**). The values of the chemical shifts of these very deshielded carbons in the aromatic zone show that the latter carry hydroxyl groups (-OH). Seven aromatic quaternary carbons C-20; C-21; C-22; C-23; C-24; C-26 and C-27 respectively at 124; 123; 118.65; 117; 114.79 and 114.53 ppm. Five aromatic CH CH-25; CH-28; CH-29; CH-30; and CH-31 which respectively come out at 116.2; 109.48 (very high intensity signals count as two CH); 108.9 and 106.76 ppm. Eight CH-32 aliphatic CH; CH-33; CH-34; CH-35; CH-36; CH-38; CH-39 and CH-40 which respectively come out at 91.1; 72.82; 69.66; 65.5; 65.36; 60.97; 40.29; and 38.58 ppm. The first six unarmored CH are at the foot of an oxygen tank. Two aliphatic CH_2 (C-37 and C-41) which come out at 63.31 and 29.08 ppm respectively. The armored C37 is at the foot of an oxygen.

The proton spectrum of **1** present: A singlet which resonates around 7.49 ppm corresponding to proton H-25. A singlet which counts for 2H due to the existing symmetry at the level of the aromatic nucleus **A** at 7.09 ppm which corresponds to the two aromatic protons H-28 and H-29. Two aromatic H-30 and H-31 singlet signals resonating respectively towards 6.86 and 6.65 ppm. A singlet signal of 1H intensity at 6.53 ppm corresponding to the anomeric proton H-32 of sugar. Seven H-33 protons; H-34; H-35; H-36; H-38; Aliphatic H-39 and H-40 which resonate respectively towards 4.84, 5.4, 4.82, 5.23, 5.85, 5.06 and 3.8 ppm suggest the presence of an electronegative element causing a deshielding of these protons. Two methylenes at 4.38 ppm and 4.96 ppm in the aliphatic zone deshielded by one oxygen corresponds to H-37. Two methylenes which resonate at 2.23 ppm corresponding to H-41.

The analysis of the COSY spectrum has identified the following couplings: The H-39 proton couples with the H-35 and H-40 protons in 3J and H-40 which in turn couples with the H-41 protons. These couplings give us the following fragment (Figure 5):

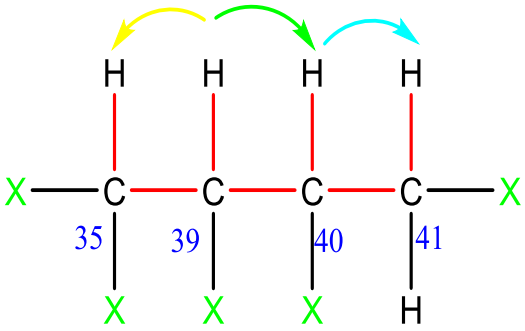

Figure 5: **Aliphatic fragment.**

On the COSY spectrum we distinguish the couplings of a spin system of six protons coupled together H-32/ H-36/ H-34/ H-38/ H-33/ H-37. These six couplings as well as the chemical shift of carbon CH-32 at 91.1 ppm corresponds to an anomeric carbon. The value of the chemical shifts of the six CH and CH_2 on the carbon and proton spectrum allows us to confirm the presence of a glucoside sugar.[29] (Figure 6).

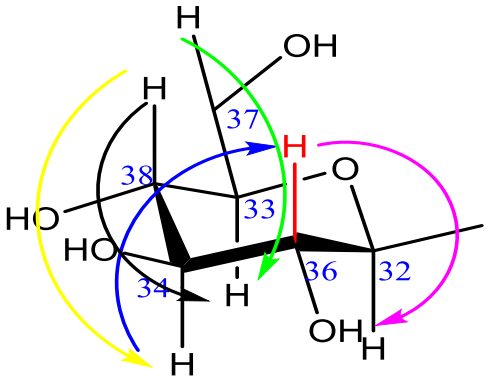

Figure 6: COSY correlations of the osidic fragment of compound 1.

As in the case of compound **2**, detailed analysis of the ^{1}H, ^{13}C, DEPT, HSQC and COSY, NMR spectra of **1** revealed the presence of signals for five substructures **A**, **B**, **C**, **D** and **E** in the presence of a sugar. Substructures **A**, **B** and **C** have characteristics similar to those of compound **2**. At this stage we have already been able to propose substructures for compound **1** while taking into account the degree of unsaturation necessary DI=26 corresponding to 7 double bonds carbonyl and 12 double bonds provided by NMR spectra, and therefore 7 cycles.

The aromatic singlets H-28 and H-29 located at 7.09 ppm are identified by six HMBC correlations, two of which with a methine C-28 (δC 109.48 ppm, its symmetry), C-7 (δC 165.85 ppm), C-9 (δC 145.17 ppm), C-11 (δC 144.99 ppm), C-22 (δC 118.65 ppm) and C-16 (δC 139.45 ppm) corresponding to the carbons of aromatic ring A which corresponds to a galloyl group (Figure 7). The correlation of the anomeric proton H-32 with the carbonyl C-7 allows the bonding of galloyl to sugar.

The aromatic proton H-25 at 7.5 ppm is identified by its correlations with the carbonyl C-6 (δC 164.96 ppm) and the quaternary carbons C-8 (δC 145.97 ppm), C-17 (δC 138.96 ppm), and C-27 (δC 114.53 ppm) of the aromatic ring B. This nucleus is linked to the sugar thanks to the correlation of the carbonyl C6 with the proton H-34 of glucose. The aromatic singlet proton H-30 at 6.80 ppm is identified by its correlations with the carbonyl C-5 (δC 166.09 ppm), C-14 (δC 144.14 ppm), C-18 (δC 137, 26 ppm), and C-24 (δC 116.2 ppm) corresponding to the aromatic ring **C**. The aromatic ring **D** is formed by the correlations of the aromatic singlet proton H31 at 6.65 ppm with carbons C-4, C-12, C-19, C-23 and C-26.

However, the ring resonances of compound **1** are easily distinguished from **2** by the other two carbonyl resonances of esters C-1 (δC at 173.64 ppm) and C-2 (δC 173.015 ppm). In addition, the CH-35, CH-39, CH-40 and CH-41 fragment is identified with the analysis of the spectrum of COSY as well as the presence of the proton CH-31.

In the HMBC spectrum, we observe the correlation between the H-41 proton at 2.17 ppm with the C-1 carbonyl (δC 173.64 ppm) and the correlation between the H-39 and H-40 protons with the C-2 carbonyl (δC 173.015ppm). This shows that the C-1 and C-2 carbonyls are respectively bonded to C-40 and C-41. We also observe the correlations of the proton H-39 with the carbons C-35, C-40, C-41 and a carbonyl C-3. These correlations enable the justification of the fragment proposed during the interpretation of the spectrum of COSY. The **E** obtained substructure is linked to the **D** ring by the correlation of the proton H-39 with the aromatic quaternary carbon C-23 (Fig.7).

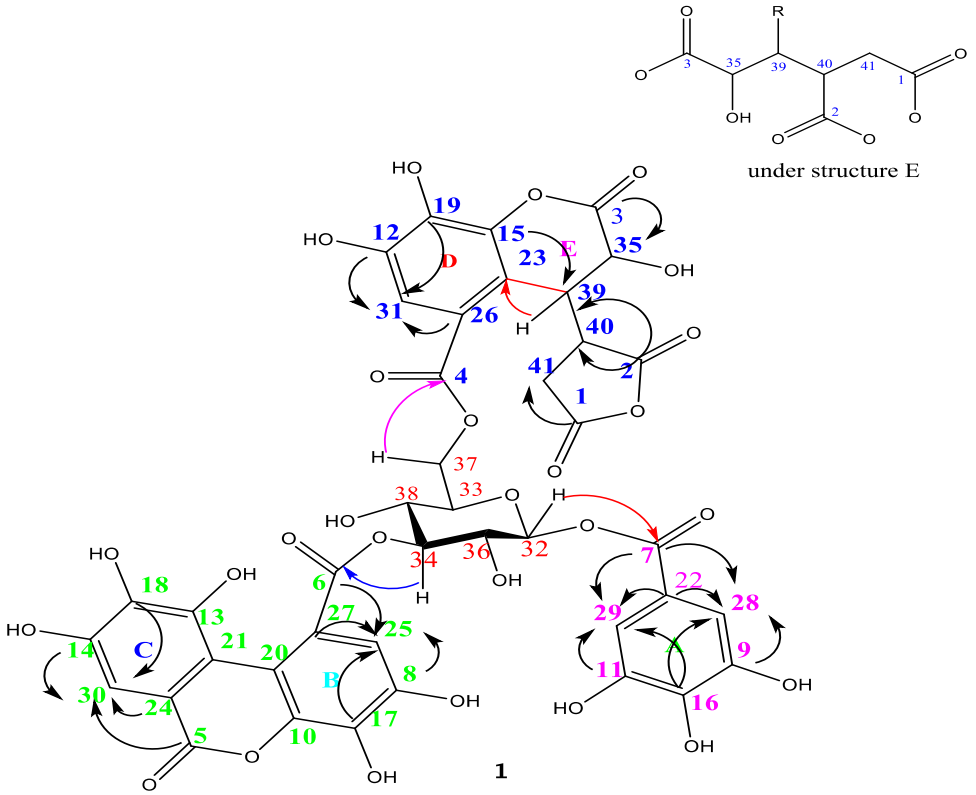

Figure 7: Selected key HMBC interactions for compound 1. (HMBC).

Based on these analyses, compound **1** is identified as being a gallic tannin which is not described in the literature.

4. CONCLUSION

The study of the butanol extract of *Neocarya macrphylla* enabled the isolation of three molecules and determine the structures corresponding by CPC and spectroscopic isolation methods (NMR and mass Ionization by Electrospray). There are two tannins, including a new one unknown in the databases [cf. SciFinder, Reaxys, Dictionary of natural products (DNP)] and a C-glycosyl flavonoid known from the literature. At this stage of characterization, we have not yet elucidated whether the

sugar is a *D*- or *L*-Glucose. However, we admitted that generally it is a *D*-glucose based on the results described in literature. The analysis and review of other excerpts (heptane, dichloromethane, ethyl acetate and water) are in progress. A inventory of all the metabolites of *N. macrophylla*, is in progress and each constituent will be subjected to biological activity tests.

Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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