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ISOLATION AND STRUCTURAL DETERMINATION OF *MYO*-INOSITOL FROM *COELOCARYON PREUSSII* FRUIT SHELLS: ANTIOXIDANT ACTIVITY

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ABSTRACT
The methanolic extract of the fresh fruit shells of *Coelocaryon preussii* was studied to highlight its bioactive components. The investigation led to the isolation and identification of an inositol derivative named *myo*-inositol. The structure of this compound which was isolated for the first time from this species was established on the basis of its IR, NMR and Mass analyses. The evaluation of the antioxidant activity of this compound using the DPPH method gave a relatively interesting activity (IC_{50} = 34.8 µg/mL) compared to the reference compounds, vitamin C (IC_{50} = 5.3 µg/mL) and quercetin (IC_{50} = 3.1 µg/mL).

KEYWORDS: *Coelocaryon preussii*, fruit shells, *myo*-inositol, NMR, DPPH.

1. INTRODUCTION

Coelocaryon preussii is a tree of about 30 m in height, from the Myristicaceae family. Of all the plants of the genus *Coelocaryon*, it is the most known and used (**Moutsinga et al., 2024**; **Ouayogode et al., 2017**). Different parts of this plant are traditionally used to treat various ailments. Indeed, the seeds are used in the treatment of diabetes and hypertension, and the stem barks are used to treat diarrhea, cough, hemorrhoids, dysentery, venereal diseases, and rheumatism (**Moutsinga et al., 2024**; **Ouayogode et al., 2017**; **Onanga et al., 1999**). Phytochemical screening of the aqueous extracts of barks and leaves of this species revealed the presence of polyphenols, tannins, flavonoids, coumarins, and saponosides. In addition, sterols and sugars have been detected in the aqueous extract of barks (**Tchouya et al., 2015**). Furthermore, *β*-sitosterol and stigmasterol were isolated from the fruit barks of this plant (**Ouayogode et al., 2017**). Biological evaluation of aqueous extracts of the plant's bark and leaves at a concentration of 10 mg/mL was performed on various microbial strains. This study showed inhibition diameters of 12 mm for *Salmonella typhimurium* and *Streptococcus B* in the bark, and 13, 8, and 22 mm for *Salmonella typhimurium*, *Candida albicans* (ATCC P37037), and *C. albicans* (ATCC P37039), respectively, in the leaves (**Tchouya et al., 2015**). Despite the numerous uses in traditional medicine of *C. preussii*, very little chemical data exists on this plant, hence the importance of this study. Indeed, to the best of our knowledge, only three chemical studies on this species are available in the literature (**Moutsinga et al., 2024**;

Ouayogode et al., 2017; **Tchouya et al., 2015**). This study which is the first on the shells of *C. preussii* fruits, aimed to determine the phytochemical constituents and evaluate the *in vitro* antioxidant activity of the methanol extract, and an isolated compound.

2. MATERIAL AND METHODS

2.1. General experimental procedures

Melting points expressed in degrees Celsius (°C) were measured on a Wagner & Munz Kofler heating bench (Heizbank System Kofler WME). Infrared (IR) absorption spectrum was recorded on a Bruker Vector 22 Fourier Transform Spectrometer (Champs-sur-Marne, France). The absorption bands (ν) were expressed in cm^{-1}. The NMR spectra were carried out on a Bruker AM-400 spectrometer (400 MHz) using D_2O as solvent. The solvent signals were used as references. ESIMS spectrum was registered with an Agilent 6530 Q-TOF spectrometer (Les Ulis, France) equipped with an ESI source operating with a positive polarity. The antioxidant activity was assessed using a UV/Vis spectrophotometer (JENWAY Spectrophotometer).

2.2. Plant material

The fruits of *Coelocaryon preussii* were harvested in May 2024 in Lakota, southwest of Côte d'Ivoire. The botanical identification was confirmed by the botanist TEHE Henry from Centre Suisse de Recherche Scientifique (CSRS, Ivory Coast) where a voucher specimen has been deposited under the reference YC-CP 01-Lakota. The shells were separated from the almonds using a hammer.

2.3. Extraction and isolation

100g of fresh *Coelocaryon preussii* fruit shells were first diced and then macerated in 300 mL of methanol for 24 hours. A macerate was obtained after filtration. These operations were repeated three times in succession. The different macerates obtained were grouped together. Part (approximately 100 mL) of this macerate was concentrated under reduced pressure to give the crude extract CFMeOH and the remainder was left to rest at room temperature. After 24 hours, a precipitate formed within the liquid phase was collected by filtration. The solid obtained after drying the precipitate was air-dried and then recrystallized in methanol to give 70 mg of compound (**1**).

2.4. Identification of compound (1)

*Myo-inositol (**1**)*: white amorphous solid; mp 226°C; IR ν_{max} 3360, 3159, 2922, 1417, 1145, 1045, 999 cm^{-1}; ESIMS (*m/z*) $[M+Na]^+$ 203.1184 (Molecular formula $C_6H_{12}O_6$); ^1H and ^{13}C NMR data (See **Table 1**).

2.5. Phytochemical screening

Phytochemical screening was carried out by color and precipitation reactions in tubes according to the methods described by **Kaboré et al.** (**2021**) and **Harborne** (**1998**) with some modifications.

2.5.1. Alkaloids test

The alkaloids were characterized using Nessler's reagent. Two to three drops of Nessler's reagent were added to 2 mL of extract. The appearance of a yellowish precipitate indicates the presence of alkaloids.

2.5.2. Polyphenols

Polyphenols were detected by ferric chloride. One drop of 2% ferric chloride alcoholic solution was added to 2 mL of extract. The appearance of a blue-black or green color, more or less dark, indicates the presence of phenolic compounds.

2.5.3. Anthraquinones test

Potassium hydroxide (KOH) was used to characterize anthraquinones. To a few milliliters (3-5 mL) of extract, an equivalent volume of 10% aqueous potassium hydroxide was added. After stirring, the presence of anthraquinones is confirmed by a red color change in the aqueous phase.

2.5.4. Indoles test

Indoles were characterized by Salkowski's reaction. Two to three drops of Salkowski's reagent were added to 2 mL of extract. The appearance of a yellowish precipitate indicates the presence of indoles.

2.5.5. Amino acids test

Amino acids were detected by the ninhydrin test. 1 mL of freshly prepared ninhydrin solution was added to 1 mL of the extract. The appearance of a blue-violet color indicates the presence of amino acids. The ninhydrin solution was prepared by mixing 0.2 g of ninhydrin, 95 mL of butan-1-ol, and 10 mL of acetic acid.

2.6. Antioxidant activity

The antioxidant activity of the extracts was evaluated according to the method described by **Kouadio et al.** (**2020**) with some modifications. Indeed, 3.94 mg of DPPH powder were dissolved in 100 mL of an ethanol-water mixture (50:50) to give a 100 µM solution. The preparation was protected from light using aluminum foil. A range of eight (8) concentrations of standard solutions (Quercetin and Vitamin C), extract and the compound to be tested, ranging from 400 to 3.125 µg/mL (400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) were prepared in ethanol-water mixture (50:50). 2.5 mL of plant extract and 2.5 mL of DPPH 100 µM solution were introduced into dry and sterile tubes. After shaking, the tubes were protected from light for 30 minutes. The absorbance of the mixture was then measured at 517 nm using a blank consisting of 2.5 mL of ethanol-water (50:50) and 2.5 mL of DPPH 100 µM solution. Vitamin C and Quercetin served as positive controls. The percentage of DPPH inhibition was calculated using the following formula:

$$I(\%) = \frac{A_0 - A_1}{A_0} * 100$$

A_0: Absorbance of the blank

A_1: Absorbance of the sample
The IC_{50}, representing the concentration of extract needed to trap 50% of DPPH radicals, is determined graphically.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Phytochemical screening of the methanol extract from fresh *Coelocaryon preussii* fruit shells revealed the presence of alkaloids, polyphenols, anthraquinones, amino acids, and indoles. Furthermore, polyphenols, tannins, flavonoids, coumarins, and saponosides have already been detected in the aqueous extracts of barks and leaves of this species, while sterols and sugars were present only in the same extract of barks (**Tchouya et al., 2015**). This is the first report of chemical groups from the fruit shells of *C. preussii*.

3.2. Structural elucidation of compound (1)

Purification by recrystallization of the methanolic extract of fresh *Coelocaryon preussii* fruit shells led to compound (**1**).

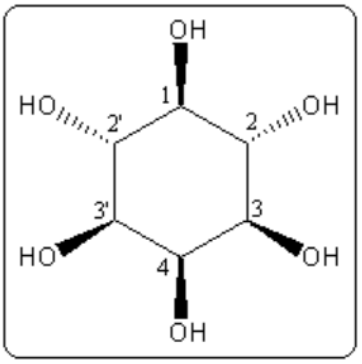

Figure 1: Structure of *myo*-inositol isolated from the fresh fruit shells of *Coelocaryon preussii.*

Compound (**1**) was obtained as a white, amorphous solid (**Fig. 1**). ESIMS (**Fig. 2**) indicated a $[M+Na]^+$ ion peak at *m/z* 203.1184, consistent with the molecular formula $C_6H_{12}O_6$. The IR absorption broadband at 3159 cm^{-1} (**Fig. 3**) indicated the presence of hydroxyl groups and bands at 1417, 1145, 1045, and 999 cm^{-1} were attributed to C-OH stretching.

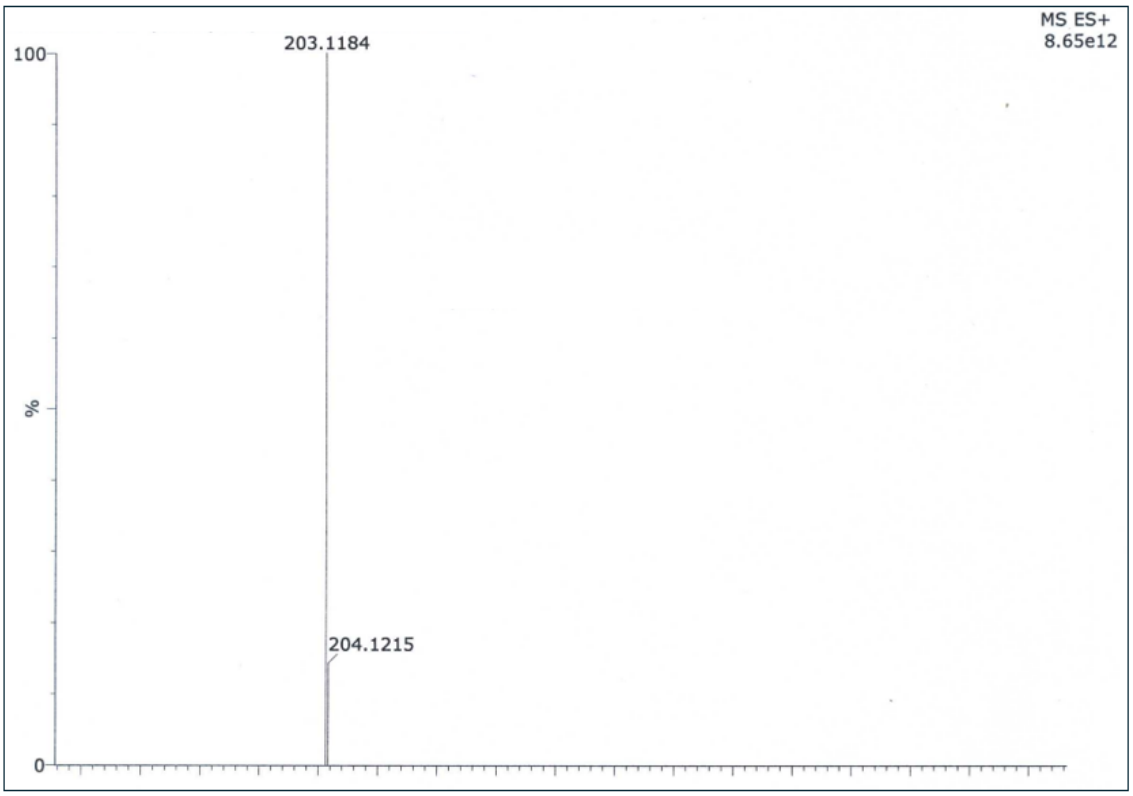

Figure 2: ESI-MS (+) spectrum of compound (1).

The ^1H and ^{13}C spectra (**Fig. 4** and **5**) were characteristic of a symetric sugar (**Obendorf et al., 2000**). Indeed, ^1H NMR spectrum (**Fig. 4**) showed six (6) protons resonances (**Table 1**) at δ_H 3.34 (1H; t; *J* = 9.3 Hz; H-1), 3.68 (2H; t; *J* = 9.9 Hz; H-2/2'), 3.58 (2H; dd; *J* = 9.9, 2.9 Hz; H-3/3'), and 4.11 (1H; t; *J* = 2.9 Hz; H-4).

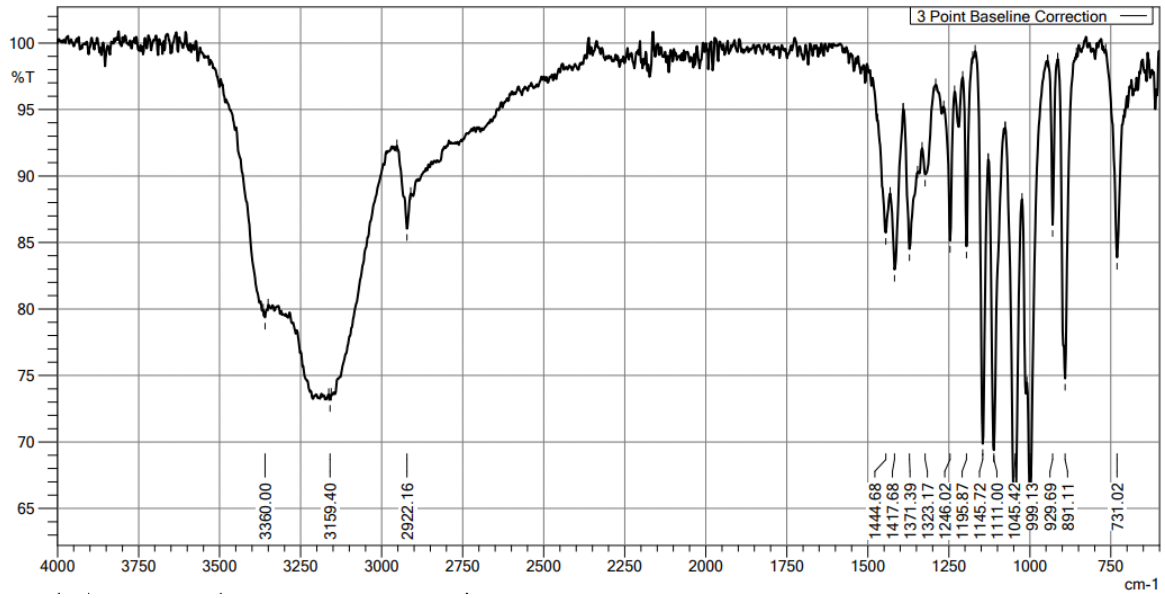

Figure 3: IR spectrum of compound (1).

Table 1: NMR spectroscopic data (400 MHz, D_2O) for compound (1).

Position	δ_H, m (*J*, Hz)	δ_C (ppm)	COSY	HMBC
1	3.34, t (1H, 9.3)	74.4	H-2/H-2’	C-2/C-2’
2/2’	3.68, t, (2H, 9.9)	72.4	H-1; H-3/H-3’	C-1; C-3/C-3’
3/3’	3.58, dd (2H, 9.9, 2.9)	71.2	H-4; H-2/H-2’	C-1; C-2/C-2’
4	4.11, t (1H, 2.9)	72.2	H-3/H-3’	C-2/C-2’; C-3/C-3’

The ^{13}C NMR spectrum (**Fig. 5**) revealed chemical shifts for four carbons at δ_C 71.2 (C-3/3’), 72.2 (C-4), 72.4 (C-2/2’), and 74.4 (C-1).

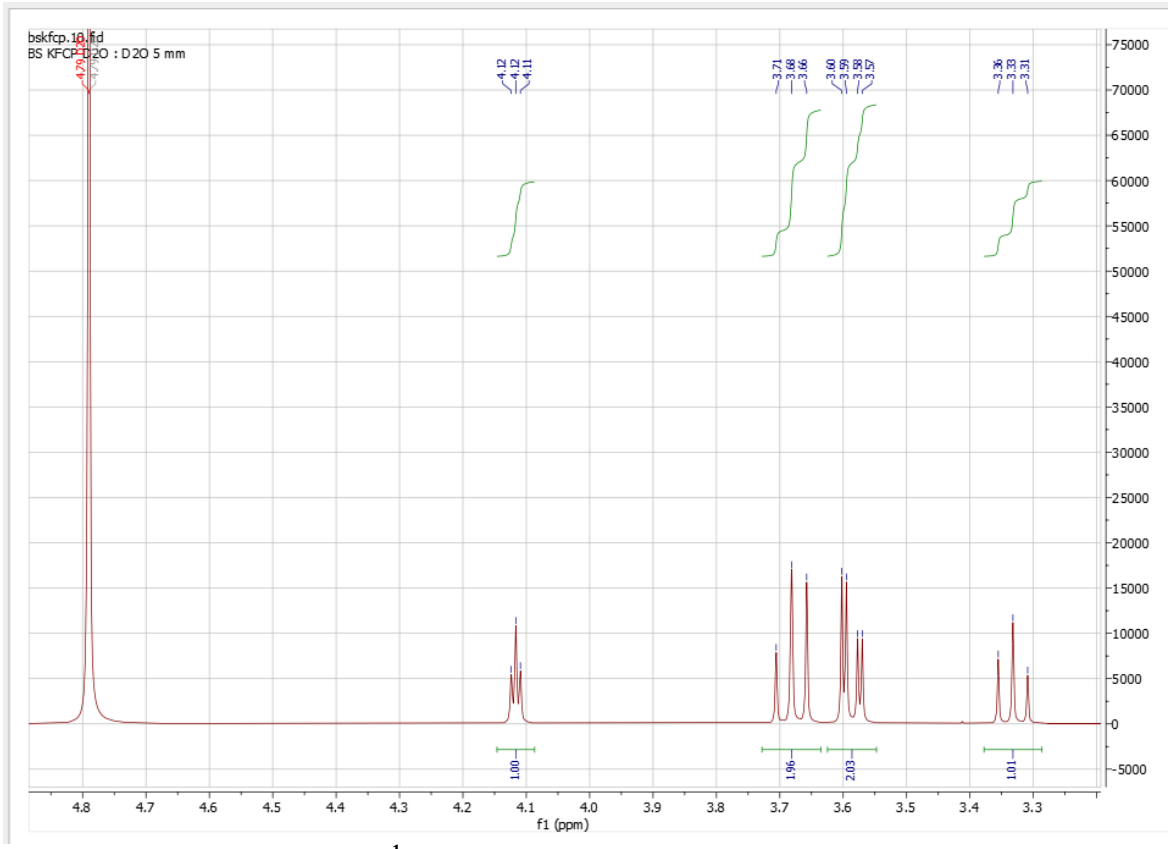

Figure 4: ^1H NMR spectrum in D_2O of compound (1).

The different assignments were made using HSQC spectrum analysis (**Fig. 6**), which showed $^{2}J_{C\text{-}H}$ correlations between protons at δ_H 3.34 (1H; t; J = 9.3 Hz), 3.68 (2H; t; J = 9.9 Hz), 3.58 (2H; dd; J = 9.9, 2.9 Hz), and 4.11 (1H; t; J = 2.9 Hz), and carbons at δ_C 74.4, 72.4, 71.2, and 72.2 respectively.

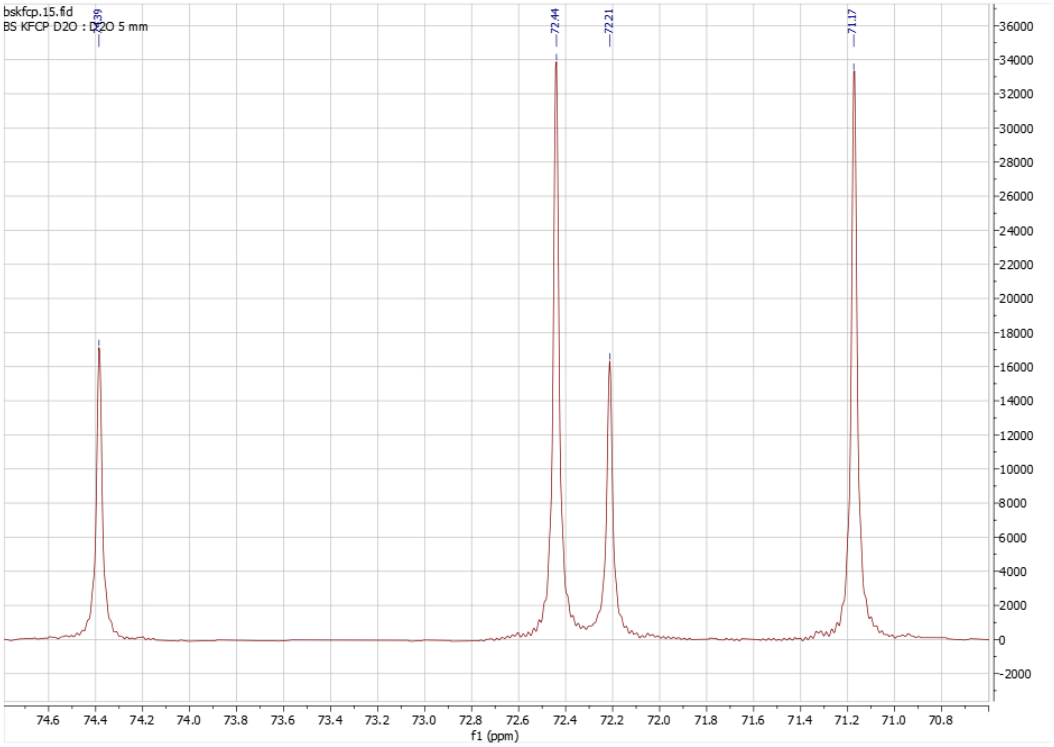

Figure 5: ^{13}C NMR spectrum in D_2O of compound (1).

In the HMBC spectrum (**Fig. 7**), correlations were observed between the signal of the proton H-1 and that of carbons C-2/2', these latter correlated with the proton C-4. In addition, correlations were observed between the protons H-3/3' and carbons C-1, and C-2/2'.

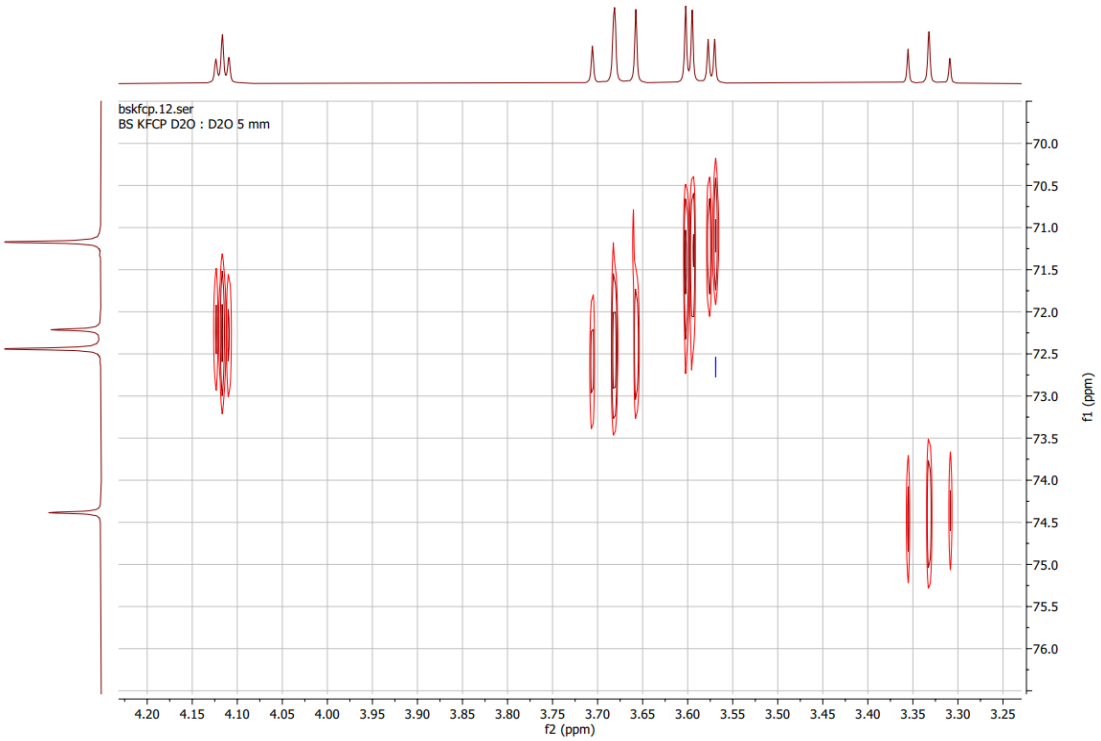

Figure 6: HSQC spectrum in D_2O of compound (1).

The homonuclear correlation spectrum ^{1}H-^{1}H COSY (**Fig. 8**) allowed unambiguous confirmation of the structure of compound (**1**). Indeed, this spectrum showed correlations between the proton H-1 and protons H-2/2', these latter correlating with the protons H-3/3', which in turn correlated with the proton H-4. The coupling constants (J = 9.9, 9.3, and 2.9 Hz), between the different proton systems made it possible to confirm the spatial arrangement of the hydroxyl groups of compound (**1**). The large coupling constant values (J = 9.9 and 9.3 Hz) indicate an arrangement of protons in different planes, while the weak coupling constant (J = 2.9 Hz) is attributable to an arrangement of these in the same plane.

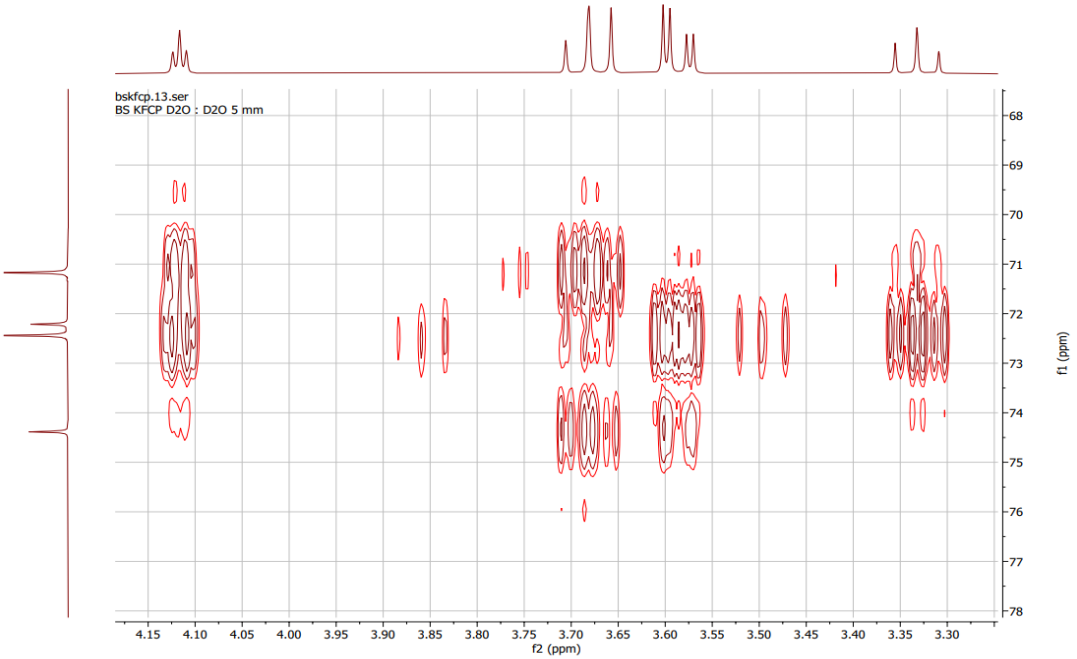

Figure 7: HMBC NMR spectrum in D_2O of compound (1).

From these spectral data, and in comparison, with the literature, compound (**1**) was identified as *myo*-inositol (**Fujisawa et al., 2017; Ow et al., 2012; Cerdán et al., 1985**).

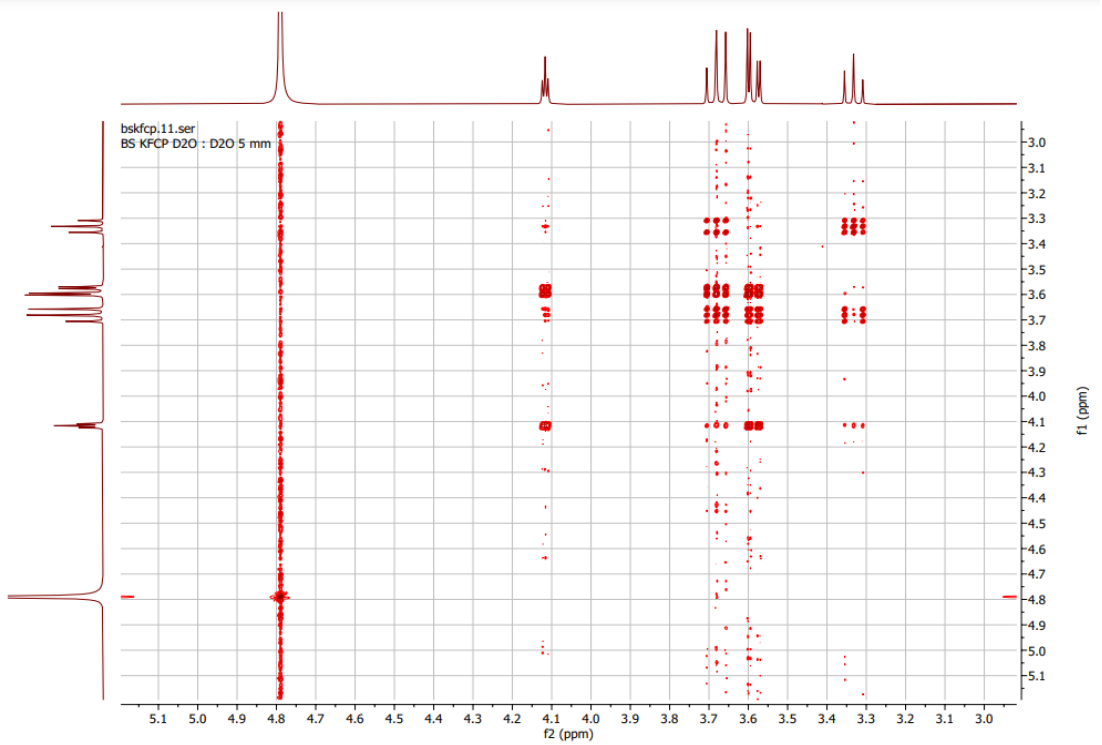

Figure 8: COSY NMR spectrum in D_2O of compound (1).

This molecule which was first discovered in 1850 by Sherer from muscle extract (**Loewus et Murthy, 2000**), is essential for the development of plants, animal and some organisms (**Naithani et al., 2026; Ow et al., 2012; Chhetri et al., 2006**). This compound is reported for the first time from *Coelocaryon preussii*. It is a precursor to biosynthesis of many compounds involved in phosphorus storage, signal transduction, stress, protection, hormonal homeostasis and cell wall biosynthesis in plants (**Iqbal et al., 2002**). *Myo*-inositol was reported to be commonly found in a wide variety of agricultural products such as citrus fruits, beans, grains and nuts (**Dipshika et Sukumar, 2025; Joardar et al., 2023; Clements et Darnell, 1980**). It has also been detected in the brains of male Wistar rats (**Cerdán et al., 1985**), and is considered as potential marker of prostate cancer (**Serkova et al., 2008**). This compound has been used as part of treatment for diabetes mellitus (**Clements et Reynertson, 1977**). In addition, **Giordano et al.** (**2011**) reported that *myo*-inositol may improve metabolic syndromes in postmenopausal women.

3.3. Antioxidant activity

The antioxidant activity of the crude methanolic extract and *myo*-inositol from the fruit shells of *Coelocaryon preussii* was evaluated by DPPH method. The choice of this method for measuring antioxidant activity lies in its simplicity, speed and the formation of a stable radical (**Bea et al., 2020**). Two reference compounds were used according to their mode of action: a phenolic compound (Quercetin) and a non-phenolic compound (Vitamin C). The results are presented in **Fig. 9**.

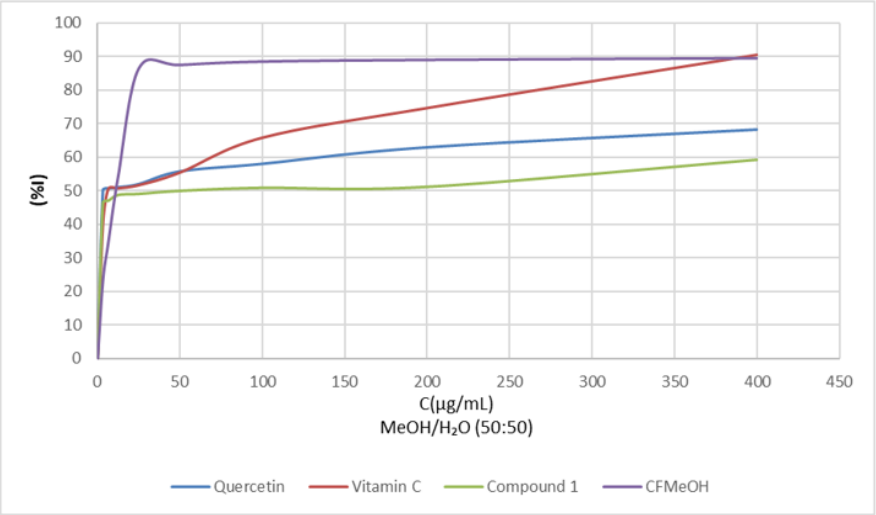

Figure 9: Antioxidant activity of *myo*-inositol and the methanolic extract of *Coelocaryon preussii* shells.

The results showed that all tested compounds possess antioxidant activity. The references used, Vitamin C (IC_{50} = 5.3 µg/mL) and Quercetin (IC_{50} = 3.1 µg/mL), are more active than the crude extract CFMeOH (IC_{50} = 5.5 µg/mL) and compound (**1**) (IC_{50} = 34.8 µg/mL). In addition, the crude extract CFMeOH exhibited anti-radical activity comparable to those of the reference compounds. The high activity observed in this extract suggested that it could be a good source of bioactive compounds. This finding led to purify the extract, resulting in the isolation of compound (**1**). The evaluation of the antiradical activity of compound (**1**) yielded a relatively modest activity compared to that of the extract from which it is derived and to the reference compounds. The crude extract's free radical scavenging potential could be due to a synergistic effect of the molecules it contains. To our knowledge, this study is the first to evaluate the antioxidant potential of the crude methanolic extract of *C. preussii* fruit shells. Furthermore, compound (**1**) (*Myo*-inositol) is already known in the literature as an antioxidant (**Rolnik et al., 2024; Osman et al., 2023**). It also possesses anticoagulant (**Rolnik et al., 2024**) and *in vitro* sperm quality improvement (**Osman et al., 2023**) properties.

4. CONCLUSION

The phytochemical investigation of the methanolic extract of fresh fruit shells of *Coelocaryon preussii* led the isolation and structural identification of a cyclitol named *myo*-inositol. This compound is isolated for the first time from this species. The evaluation of the antioxidant activity by DPPH method of this compound showed a relatively modest activity (IC_{50} = 34.8 µg/mL) compared to reference compounds, vitamin C (IC_{50} = 5.3 µg/mL) and Quercetin (IC_{50} = 3.1 µg/mL). This study is the first to highlight the presence of bioactive compounds in *C. Preussii*'s fruit shells. The presence of *myo*-inositol in the shells of *Coelocaryon preussii* could help to justify the different uses of this plant in traditional medicine especially in the treatment of diabetes.

5. ACKNOWLEDGEMENTS

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6. COMPETING INTERESTS

Authors declared that no competing interests exist.

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