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Original Research Article

Antimicrobial and Antioxidant Xanthone Derivative from *Piliostigma thonningii* leaf

Ogbiko Cyril[1*], Eboka C. Jonathan[2], Okoye F.B. Chiedu[3]

[1]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State Nigeria*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State Nigeria*
[3]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

ABSRTACT

Antimicrobial resistance and oxidative stress-related pathologies have guided the scientific community into the pursuit of natural product based research. The study aim to isolate and characterize antimicrobial and antioxidant compound(s) from the leaves of *Piliostigma thonningii*. Bioactivity-guided fractionation was carried out with the structure of the isolated compound elucidated using ^{1}H and ^{13}C NMR as well as liquid chromatography coupled mass spectrometry (LC-MS). The isolated compound was screened against standard bacteria strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as fungal strains of *Candida albicans* and *Aspergillus niger* using agar well diffusion method. The inhibitory zone diameter (IZD) were determined and compared with reference antimicrobial ciprofloxacin and miconazole. The free radical scavenging activity in comparison with the ascorbic acid standard was performed using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. The compound (8-hydroxyl-2,4,5-trimethoxyxanthone) displayed a lower antimicrobial and antioxidant activities in comparison to the positive standard employed. However, despite lower effect of the compound, this work opens the perspective to use this molecule as 'leads' for the design of novel and selective drug candidates of pharmaceutical relevance.

Keywords:8-hydroxyl-2,4,5-trimethoxyxanthone, antimicrobial, antioxidant, ascorbic acid, ciprofloxacin, miconazole

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Introduction

For thousands of years, people and animals have used plant-based treatments to alleviate illnesses.[1] Plants have long been used as a reliable source of disease prevention and therapy in many cultures.[2] Several countries of the developing globe still rely heavily on medicinal plants for healthcare, and what is even more interesting is that plants are the source of approximately 25% of modern drugs.[3,4,5]

Antibiotic resistance is now widely acknowledged as a serious worldwide healthcare issue since it has been linked to clinical treatment failure, extra mortality, and rising healthcare costs.[6] Many diseases, including inflammation, cancer, coronary heart disease, atherosclerosis, and Alzheimer's disease, have been linked to oxidative stress and its role in their onset and progression.[7,8] Antioxidants' ability to control oxidative stress is what makes them valuable in the treatment and management of human diseases.[9] Many adverse effects linked to the usage of synthetic antibacterial and antioxidative agents have prompted more research into alternative sources such as natural compounds.[10] Natural products are typically thought to be safer than synthetic ones, despite the fact that there is not enough scientific evidence to support this.[11,12]

*Corresponding author. Email: :ogbikoc@dufuhs.edu.ng;cyrilogbiko@gmail.com
Tel: +234-808-039-8933

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The leaves of *Piliostigma thonningii* Milne-RedH Schum (Fabaceae) have been employed in ethnomedicine to treat a number of conditions, including inflammatory disease states and diseases linked to microbial infections. Several pharmacological studies have been conducted to support some of the plant material's ethnomedicinal applications, including its usage as an antipyretic,[13,14,15] antimicrobial,[16,17] anthelmintic,[18] and anti-inflammatory agent.[16] In the current study, we isolated and analyzed a xanthone derivative from the plant's genus with a view of establishing its antioxidant and antimicrobial properties.

Materials and Methods

General experimental procedures

UV spectra were obtained in CH_2Cl_2 solution on a HEWLETT PACKARD 8452-A spectrophotometer. Low-resolution ESI-MS data were acquired in positive and negative ion mode, using a MICROMASS QUATTRO-LC instrument. ^{1}H and ^{13}C NMR experiments were recorded on a JEOL JNMECA 400 MHz spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard. Column chromatography was performed with silica gel 60 (200 - 400 mesh), while thin layer chromatography was performed with aluminum backed plates coated with silica gel F_{254}, and the plates were visualized by spraying with vanillin sulphuric acid, followed by warming.

Plant material

The leaves of *Piliostigma thonningii* were collected in April 2018 from Tambuwal, Sokoto State, Nigeria. The plant material was authenticated, voucher specimen deposited and herbarium number UDUH/ANS/0137 issued. The leaves were shade dried for 14 days (2 weeks) and pulverized using an electric blender.

Extraction and fractionation

About 2000 g of pulverized dried leaves of *Piliostigma thonningii* were extracted with 10 L of absolute methanol (MeOH) under continuous stirring for 72 h at room temperature (25℃) and the extract concentrated *in vacuo* with a rotary evaporator to obtain a dark green semisolid. The dried extract (200 g, 73.4% w/w) was reconstituted in 100 mL of MeOH and the dispersion made up to 200 mL with water. The crude extract was then partitioned successively with n-hexane (625 mL x 4), ethyl acetate (500 mL x 4) and n-butanol (500 mL x 3). All fractions were concentrated using rotary evaporator to obtain approximately 3.6 g (1.8% w/w) of n-hexane fraction (NHF), 62.8 g (31.4% w/w) of ethyl acetate fraction (EAF), 33.8 g (16.9% w/w) of n-butanol fraction (NBF) and 94.6 g (47.3% w/w) of aqueous residual fraction (ARF) respectively.

Isolation and purification of EVLC

About 5 g of ethyl acetate fraction (EAF) was subjected to VLC (silica gel 500 g, sintered funnel 5 L) eluting with 500 mL each of n-hexane: ethyl acetate (100:0, 90:10, 80:20, 70:30, 60;40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) and dichloromethane: MeOH (90:10, 70:30, 50:50, 30:70, 0:100) resulting in 16 fractions coded LC_1 to LC_{16}. Based on similar TLC profile, LC_1 and LC_2; LC_3 and LC_4; LC_7 and LC_8; LC_9 and LC_{10} were bulked together. This resulted in 12 fractions coded VLC_1 - VLC_{12}. VLC_6 fraction which showed better antioxidant and antimicrobial activities was chromatographed by gradient elution technique using n-hexane (NH): ethyl acetate (EA) (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) and EA: MeOH (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) before been separated on Sephadex LH-20 column (3 x 60 cm), eluted with EA: MeOH (1:1) which afforded a pure compound coded EVLC (33.56 mg). The purity of the isolated compound was confirmed by TLC (solvent system: EA: MeOH (8:2); one spot; R_f 0.63).

Antimicrobial assay

Antimicrobial activity of the compounds was screened on laboratory strains of bacteria and fungi using previously reported protocols.[19]

Antioxidant assay

The antioxidant activity of the compounds was screened as previously reported.[20]

Results and Discussion

Structural and spectra information of EVLC

EVLC: Yield 33.56 mg: yellowish crystals; UV: λmax 261.5 nm; m/z= 304.16 [M^+ + 2]. Spectra data are shown in Tables 1 and 2 as well as in Figures 1 - 4. The proposed structure of the compound is shown in Figure 5.The ^{1}H - NMR spectrum of the compound revealed three methoxy protons at δ 3.87 (3H, singlet), δ 3.93 (3H, singlet) and δ 4.00 (3H, singlet) attached to the xanthone skeleton at carbons 2, 4 and 5 respectively. The presence of four aromatic protons at carbons 1, 3, 6 and 7 with δ 6.33 (1H, doublet, *J* = 2.5 Hz, ArH), δ 6.31 (1H, doublet, *J* = 2.5 Hz, ArH), δ 7.33 (1H, doublet, *J* = 9.3 Hz, ArH) and δ 7.16 (1H, doublet, *J* = 9.2 Hz, ArH) respectively. The meta (2J) coupling between protons on carbon 1 and 3 and the ortho (1J) between protons on carbon 6 and 7 unequivocally confirms their position. The signal at about 13.26 ppm is attributed to the phenolic proton attached to carbon 8; its highly deshielded position confirms its involvement in a hydrogen bond with the carbonyl group (carbon 9). The ^{13}C NMR spectrum of the compound gave resonances for three methoxy carbons at carbons 2, 4 and 5 with δ 55.9, 61.9, and 157.3 respectively; four protonated aromatic and olefinic carbons 1, 3, 6 and 7 with δ 92.2, 97.0, 120.5, and 112.9 respectively; one carbon bearing the hydroxyl group (carbon 8) and a carbonyl carbon 9 at δ 151.0 and 181.3 respectively. M^+ + 2 of 304.16 correspond to the molecular formula $C_{16}H_{14}O_6$. The compound 8-hydroxyl-2,4,5-trimethoxyxanthone was elucidated in substantial agreement with 1- hydroxyl-3,7,8-trimethoxyxanthone previously reported in the literature.[21]

Antimicrobial activity

The result of the antimicrobial determination of the isolated compound and positive control ciprofloxacin and miconazole against the four strains of microbes tested is shown in Table 1.

Table 1: Comparison of the ^{1}H - NMR data of 8-hydroxyl-2,4,5-trimethoxyxanthone

Carbon Number	δ H[a] (*J* in Hertz)	δ H[b] (*J* in Hertz)
1	6.22	6.33 (d, *J* = 2.5, 1H)
2 – OCH_3	3.81	3.87 (s, 3H)
3	6.20	6.31 (d, *J* = 2.5, 1H)
4 – OCH_3	3.88	3.93 (s, 3H)
4a	-	-
5 – OCH_3	3.76	4.00 (s, 3H)
5a	-	-
6	7.22	7.33 (d, *J* = 9.3, 1H)
7	7.05	7.16 (d, *J* = 9.2, 1H)
8 - OH	13.10	13.26 (s, 1H)
8a	-	-
9	-	-
9a	-	-

a. at 400 MHz in $CDCl_3$ (Reference Compound)[21]
b. Derived from ^{1}H spectrum at 400 MHz in $CDCl_3$ from this study (Isolated Compound).

Table 2: Comparison of the ^{13}C NMR data of 8-hydroxyl-2,4,5-trimethoxyxanthone

Carbon Number	δ 13C[a] (*J* in Hertz)	δ 13C[b] (*J* in Hertz)
1	92.0	92.1
2	164.0	164.0
2 – OCH_3	-	55.9
3	96.8	97.0
4	166.5	166.5
4 – OCH_3	-	61.9
4a	148.9	149.3
5	157.1	157.3
5a	149.3	149.3
5 – OCH_3	-	57.3
6	120.5	120.5
7	112.8	112.9
8	151.0	151.1
8a	115.8	112.9
9	181.1	181.3
9a	104.1	104.1

a. at 400 MHz in $CDCl_3$ (Reference Compound)[21]
b. Derived from ^{1}H spectrum at 400 MHz in $CDCl_3$ from this study (Isolated Compound).

8-hydroxyl-2,4,5-trimethoxyxanthone showed promising antibacterial and antifungal activity against the microbial strains at 50 µg/mL. The reported activity of 8-hydroxyl-2,4,5-trimethoxyxanthone could be attributed to the fact that xanthones have long being established to possess antimicrobial activity.[22] The compound and the fraction (VLC_6) from which it was isolated (Table 3) had activity against the same set of microorganisms with the compound exhibiting a better antifungal than antibacterial activity. This could suggest that while the compound may act in synergism to exhibit its antibacterial action, it may be solely or largely responsible for the observed antifungal activity.

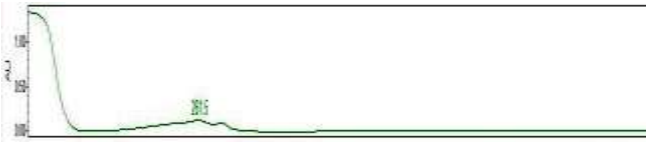

Figure 1: UV – Vis spectrum of 8-hydroxyl-2,4,5-trimethoxyxanthone

Antioxidant activity

The isolated compound when screened for antioxidant activity using the DPPH free radical scavenging assay exhibited promising DPPH free radical scavenging activity with an IC_{50} value of 12.92 µg/Ml comparable to the standard drug, ascorbic acid which exhibited a better free radical scavenging activity with an IC_{50} value of 5.80 µg/mL (Figure 6, Table 4).A lower IC_{50} value depicts a higher antioxidant activity hence the lower antioxidant activity observed for the compound when compared to that of the ascorbic acid standard could be attributed to the hydrogen bonding among the hydroxyl group at carbon-8 and the carbonyl functional group at carbon-9 (Figure 4) as previously reported.[23,24] The unmasked hydroxyl group attached to carbon-8 could be the primary target for DPPH free radicals resulting in the observed antioxidant activity.

Table 3: Antimicrobial activity of the 8-hydroxyl-2,4,5-trimethoxyxanthone in comparison with ciprofloxacin and miconazole standards

Compound Code	Test Organisms / IZD (mm) at 50.00 µg/mL			
	SA	*PA*	*AN*	*CA*
VLC_6	12.00 ± 0.25**	9.00 ± 0.02**	4.33 ± 0.78**	2.00 ± 0.08**
8-hydroxyl-2,4,5-trimethoxyxanthone	6.50 ± 0.7***	9.60 ± 0.37**	6.10 ± 0.56**	4.00 ± 0.00**
CIP (5.00 µg/mL)	17.50 ± 0.11*	20.47 ± 0.17*	NT	NT
MICO (50.00 µg/mL)	NT	NT	12.00 ± 0.00*	18.70 ± 0.07*
DMSO	-	-	-	-

SA = Staphylococcus aureus, PA = Pseudomonas aeruginosa, AN = Aspergillus niger, CA = Candida albicans, CIP = ciprofloxacin, MICO = miconazole, DMSO = dimethylsulphoxide, IZD = inhibitory zone diameter, VLC_6 = the VLC fraction from which 8-hydroxyl-2,4,5-trimethoxyxanthone was isolated, NT = not tested; Values with different asterisk indicate statistical significance ($P < 0.05$).

Table 4: DPPH free radical scavenging assay of the isolated compound

Test compounds	IC_{50} (µg/mL)
VLC_6	9.43
8-hydroxyl-2,4,5-trimethoxyxanthone	12.92
AA	5.80

AA = ascorbic acid; VLC_6 = the VLC fraction from which the compound was isolated

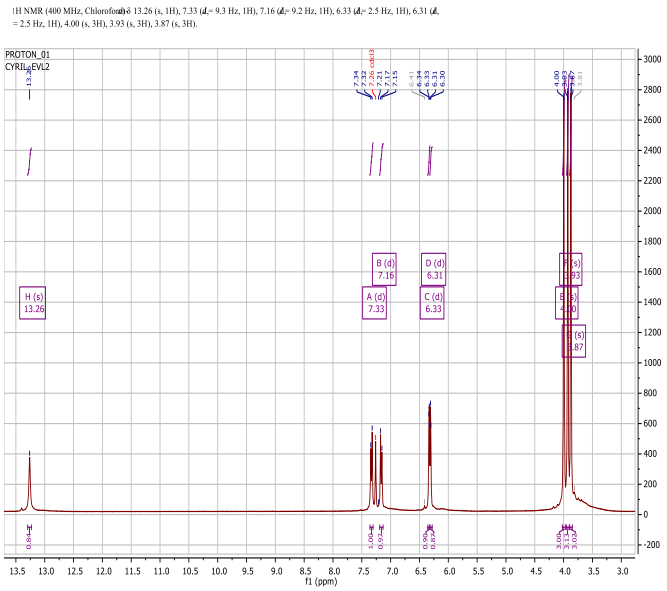

Figure 2: ^{1}H NMR spectrum of 8-hydroxyl-2,4,5-trimethoxyxanthone

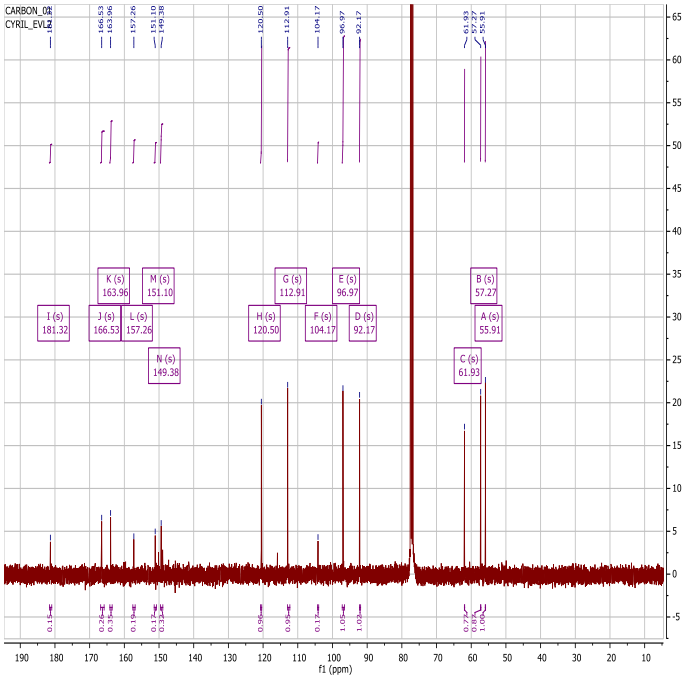

Figure 3: ^{13}C NMR spectrum of 8-hydroxyl-2,4,5-trimethoxyxanthone

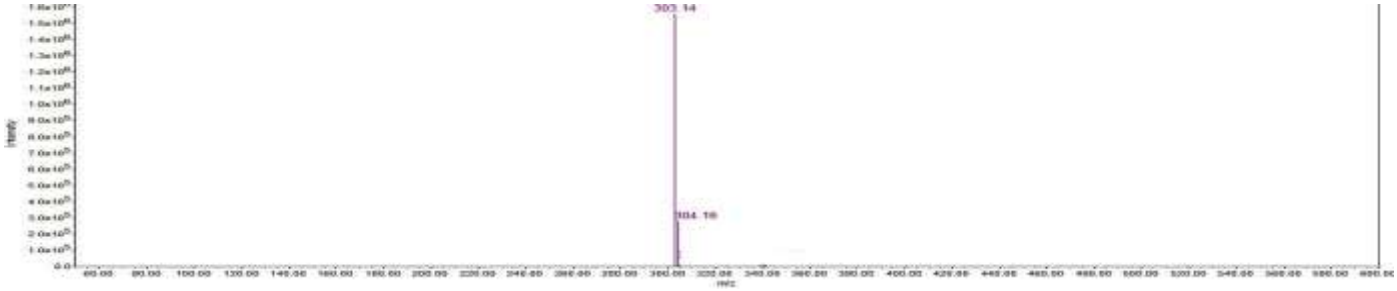

Figure 4: LC - MS spectrum of 8-hydroxyl-2,4,5-trimethoxyxanthone

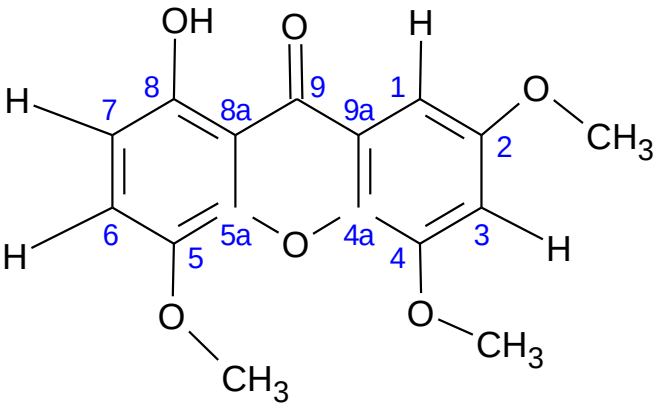

Figure 5: Chemical structure of 8-hydroxyl-2,4,5-trimethoxyxanthone

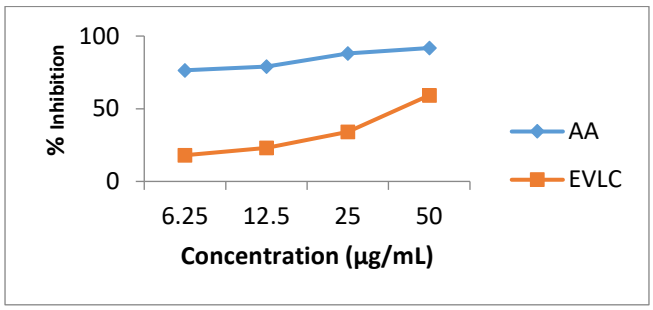

Figure 6: DPPH free radical scavenging activity of 8-hydroxyl-2,4,5-trimethoxyxanthone (EVLC) isolated from *P. thonningii* leaf compared to ascorbic acid positive control. Each value is the mean ± standard deviation.

Conclusion

Our present investigation resulted in the isolation of a xanthone derivative 8-hydroxyl-2,4,5-trimethoxyxanthone for the first time from the genus of the Piliostigma. The compound exhibited appreciable antimicrobial and antioxidant activity with possible synergistic activity. The findings from the study contribute significantly to the observed efficacy of the plant material in ethnomedicine but with further antioxidant and antimicrobial model testing recommended.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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