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Cytotoxic Xanthone from the Stem-Bark of *Anthocleista vogelii* (Planch)

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Abstract

In this study, the crude, defatted extracts and the pure isolated xanthone (1,8-dihydroxy-2,5-dimethoxyxanthone) were screened *in vitro* for cytotoxicity activities, the xanthone was isolated from the defatted fraction of the aqueous methanolic stem-bark extract. The cytotoxic activity was evaluated using the brine shrimp lethality test of stem bark extract and the isolated compound, 1,8-dihydroxy-2,5-dimethoxyxanthone gave LC_{50} values less than 200 µg/mL, indicating a high pharmacological potential and could serve as anticancer agent.

Keywords: Cytotoxicity; Xanthone; *In vitro*; Cancer; Phytochemical; Crude extract; Brine shrimp

Introduction

Plant secondary metabolites have been reported to exhibit various biological activities ranging from antimicrobial, anti-inflammation, antioxidants, anticancer etc., that make some of them drug candidate. They have actually provided a significant number of lead/novel compounds to the pharmaceutical industry. Cancer is the leading cause of death worldwide, accounting for 8.2 million death in 2012 [1]; one out of every four deaths is from cancer [2]. It has also been predicted that as lifestyles changes in developing countries, there would likely be a significant rise in the incidence of cancers that have previously been associated with developed countries, (such as breast, colon, prostate and lung cancers), [3] in addition to those that are already common. According to WHO, it is reported that in Nigeria, about 100,000 cancer deaths are recorded annually while 250,000 new cases are recorded yearly. Some of the most effective anti-cancer agents to date are natural products or compounds derived from natural sources.

Therefore, the objectives of this study were to evaluate the cytotoxic effects of the extracts of the plants and isolated compound using the brine shrimp test method as a broad measure of anti-tumour activity [4].

Current estimates indicate that about 60% of anti-cancer and anti-infective agents available on the market are of natural product origin. It is reported that, 25% of these pharmaceutical agents are of plant origin [5].

Materials and Methods

General

Silica gel and Sephadex LH-20 were used as stationary phases for column chromatography. All analyses of samples (fractions and isolated compounds) were done using thin layer chromatography (TLC) technique. It was performed at room temperature using pre-coated plates (MERCK, silica gel 60 F254 0.2 mm). Detection of spots was by ultraviolet lamp (254 and 366 nm). Nuclear magnetic resonance (NMR) spectra data were obtained from a Brucker Spectrometer (600 MHz). Chemical shifts are expressed in parts per million (ppm).

Plant Material

The stem bark of *Anthocleista vogelii* was collected in the premises of Obafemi Awolowo University Campus, Ile-Ife. Collected plant was authenticated by Mr. Ibheneshbor of the University Herbarium, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife and voucher specimens (IFE 17320) were submitted for future reference. The stem bark was air-dried and pulverized.

Preparation of extract

1.5 kg of the pulverized stem-bark of *A. vogelii* was extracted with 95% aqueous methanol for 72 hours. The extract was filtered using cotton wool and Whatmann No. 1 filter paper. This procedure was repeated until it was observed that a considerable constituent of the plant material has been extracted. The extract was subsequently concentrated in vacuo at 40ºC using rotary evaporator. The crude extract (130 g) was reconstituted in methanol and there after partitioned with n-hexane to give n-hexane and defatted fractions. The fractions were separately concentrated on rotary evaporator to give 54 g and 60 g for n-hexane and defatted fraction respectively. The defatted extract was subsequently subjected to repeated column chromatography using silica gel and sephadex LH-20.

Phytochemical screening of the crude extract

Phytochemical tests [6] were carried out for the presence of alkaloids (Dragendorff's, Meyer's tests), anthraquinones (Borntrager's test), cardiac glycosides (Keller-illiani test), flavonoids (Schinoda's test), steroids and terpenes (Liebermann-Burchard test), saponins (froth test) and tannins (ferric chloride test).

Isolation from defatted fraction

30 g of defatted fraction of the stem-bark of *A. vogelii* was suspended in methanol and pre-adsorbed on equal weight of silica gel after which it was allowed to dry. The pre-adsorbed sample was loaded on (82 g) of silica gel and subjected to gradient elution using n-hexane, dichloromethane, ethyl acetate and methanol. A total of 180 fractions

were obtained. Based on TLC profile, the fractions were pooled into 5 sub-fractions (MK1 – MK5). The sub-fraction MK5 (0.6 g) was subjected to further column chromatography using silica gel (60-120 mesh) as stationary phase. Elution was carried out using DCM and methanol in increasing order of polarity to give 20 fractions which were bulked into 4 sub-fractions (KS1 – KS4) based on their TLC profile. On standing, fraction KS3 yielded a yellowish solid which gave a single spot on TLC ($CHCl_3$: MeOH, 8:2) when viewed under UV lamp (254 nm), this yielded a compound 1 (5 mg) which was subjected to ^1H and ^{13}C NMR and IR spectroscopic analysis.

Cytotoxicity evaluation

The cytotoxicity evaluation of the stem-bark of *A. vogelii* was carried out using brine shrimp lethality test according to the procedure described by Meyer [7]. The brine shrimp lethality assay was used for the evaluation of cytotoxicity effects of the crude extract, defatted fraction and the isolated compound. Little quantity of the *Artemia salina* L. (Artemiidae) brine shrimp eggs was sprinkled into a crucible containing seawater and crucible was left half covered to allow illumination. After a period of about 48 hours, the eggs hatched into matured nauplii.

5 mg of the test Sample was dissolved in 5 mL of sea water to give a concentration of 1000 µg/mL which was taken as stock solution. Stock solution, concentrations of 100 and 10 mg/mL were prepared using serial dilution methods. To each test solution 10 matured nauplii were added. The negative control solution was prepared using distilled water in place of the test samples. All experiments were carried out in triplicate. The number of survivors was noted after 24 hours. The LC_{50} was computed using Finney programme.

Results

Phytochemical screening of the crude extract

The phytochemical constituents of the crude extracts of the stem-bark of *A. vogelii* investigated revealed the presence of some bioactive compounds such as alkaloids, flavonoids, cardiac glycosides, Saponnin and tannins (Table 1). However, anthraquinones, phlobatannins, steroids and terpenes were absent in the extract.

Secondary Metabolite	Level of Detection
Alkaloids	+
Tannins	+
Cardiac glycosides	+
Flavonoids	+
Steroids/terpenes	-
Saponins	+
Phlobotannins	-
Anthraquinones	-

Table 1: Phytochemical Screening result of *Anthocleista vogelii* extract.

Evaluation of cytotoxic activity

The brine shrimp test was carried out in triplicate on the crude extract, defatted fraction and on the compound 1 at concentrations 1000, 100, and 10 mg/mL (Table 2). The LC_{50} as calculated by Finney program at confidential limit of 95% for each test sample were 54.3, 149.8, and 62.18 for crude extract, defatted, and compound 1 respectively.

Sample	Concentration (ppm)	No. of shrimp loaded	LC_{50}(µg/mL)
Crude	1000	10	54.30
	100	10	
	10	10	
Defatted fraction	1000	10	149.80
	100	10	
	10	10	
Compound 1	1000	10	62.30
	100	10	
	10	10	

Table 2: Showing the result of LC_{50} values of test samples.

Isolation and structural elucidation

Structural elucidation of isolated compound: Compound 1 (5 mg) appeared as a yellowish crystalline solid. The IR spectrum showed diagnostic absorption bands at 3200 cm^{-1} (O-H), 2371.42 cm^{-1} (C-H), 1609 cm^{-1}(C=C) conjugated, 1655 cm^{-1}(C=O) conjugated ketone. The ^1H NMR spectrum of compound 1 is a typical xanthone. The presence of two intrinsic singlets at 3.89 and 3.87 ppm indicated the presence of two methoxyl groups at position 2 and 5. Also, the presence of two singlets at 13.51 and 13.49 ppm indicated the presence of two chelated hydroxyl groups at positions 1 and 8. Chemical shifts at 7.96 (d, J = 8.3Hz) and 6.91 (d, J = 8.3Hz) are ortho related protons at positions 3 and 4 respectively while protons at positions 6 and 7 with chemical shift 6.84 (1H, d, J = 7.26Hz) and 6.85 respectively.

Similar spectroscopic data has been reported for swertiaperennin isolated from *A. vogelii* [8,9]. However, the methoxy groups in swertiaperennin (1,8-dihyroxy-4,7-dimethoxyxanthone) are at positions 4 and 7 while the protons at 5 were meta coupled compared to compound 1 which has the two methoxy group at positions 4 and 7 with ortho related protons at positions 2 and 3. Hence, compound 1 was elucidated as 1,8-dihydroxy-2,5-dimethoxyxanthone. The proposed structure is as shown in Figure 1.

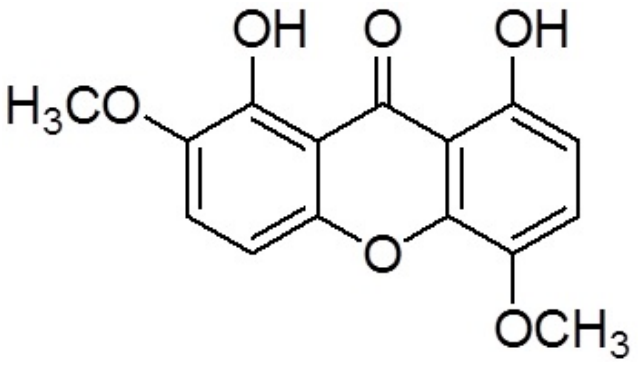

Figure 1: Proposed structure of 1,8-dihydroxy-2,5-dimethoxyxanthone.

Discussion and Conclusion

The brine shrimp test has been reported to describe a rapid, economical and simple bioassay for testing plant extracts, synthetic and isolated compounds lethality which in most cases correlates reasonably well with cytotoxic, anti-tumour [10] and insecticidal activities [11].

Plants extract having LC_{50} values >1000 ppm (extract) are considered inactive. However, plants having LC_{50} <200 ppm (extract) and <5 ppm (isolated compound 1) are considered highly active [12]. Thus, both the extracts and the pure compound 1 are considered active. However, the extracts are highly active compared to the pure compound, this could be as a result of several compounds present in the extract. The results of these screening supports a further investigation of the active compound identified. These could be confirmed as hit molecules especially when the cytotoxicity of the compound is low; showing selectivity which is vital for drug molecules.

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