World Journal of Advanced Pharmaceutical and Medical Research

Journal homepage: https://zealjournals.com/wjapmr/

ISSN: 2799-0656 (Online)

(RESEARCH ARTICLE)

Nypa fruticans endophytic fungus Cichorin A displays a weak cytotoxic activity against mouse lymphoma

Gideon Oyewole Alade * and Kola' Kayode Ajibesin

Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria.

World Journal of Advanced Pharmaceutical and Medical Research, 2023, 04(02), 008–011

Publication history: Received on 26 February 2023; revised on 09 April 2023; accepted on 12 April 2023

Article DOI: https://doi.org/10.53346/wjapmr.2023.4.2.0021

Abstract

A keen interest and attention have been given to studies on Endophytic fungus as a substitute or an alternative to the traditional source of drugs which is principally plant as a result of its interesting bioactive products. This may be an additional means of increasing the yields of those already obtained from plants or a source of entirely new ones. In essence, it is a treasure trove of therapeutically active metabolites. The aim of the study was to challenge Cichorin A isolated from *Pestalotiopsis sp*. against mouse lymphoma cell line. The result showed that it has a weak activity with a growth inhibitory effect of 31.2%

Keywords Cichorin A; Fungus; Endophytic; Cytotoxic; *Nypa fruticans*

1. Introduction

"Endophytic fungus" is a terminology referring to specific fungus housed in the tissues of plants. It may be accommodated in its host completely or partly as long as it lives, yet without a single negative effect on the host [1,2]. Among several ways in which it supports the plant is in the production of hormones, nutrients essential for plants' development and metabolites that are capable of protecting them from biotic and abiotic invaders [3]. Of utmost importance to drug development is the production of a wide variety of biologically active metabolites which can sometimes be identical to those from medicinal plants [4,5]. A lot of new and novel compounds are being increasingly isolated from endophytic fungi [6,7] and this technology has been shown to be an alternative way to sourcing biologically active metabolites in a sustainable way unlike the use of plants that involves the use of large quantities of sample especially leaf [8,9]. As at 2022, only approximately one-twentieth (5%) of fungal species worldwide has been identified and characterised and it has been shown that this is a good resource for potential development of drugs that can solve a lot of challenges of failed therapy [10] and sometimes with proven high therapeutic features [11]. Folkloric uses of various parts of *Nypa fruticans* Wurmb of the family Arecaceae include toothache, asthma, leprosy, tuberculosis and sore throat among several others [12,13,14,15]. Cichorin E and F, previously isolated from the plant; *Cichorium intybus* L. (Compositae) were reported to show weak cytotoxic activity on breast and Ewing's sarcoma cells, respectively [16]. The present work aimed to report Cichorin A for its cytoxicity activity.

2. Material and method

2.1. Plant collection

Identification and plant authentication of *Nypa fruticans* were carried out at the herbarium of the Department of Pharmacognosy & Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria with a voucher specimen (NDUP 140) banked. A sample of five leaves of *Nypa fruticans* was collected from the mangrocve within the premise of Onne Naval Base, Rivers State, Nigeria.

* Corresponding author: ALADE G. O

2.2. General experimental procedures

Bruker DRX600 NMR spectrometer was used to record ^{1}H NMR spectra. HPLC was run using a Dionex UltiMate 3000 HPLC system with a photodiode array detector (DAD-3000RS) attached. Commercially C18 pre- filled column obtained from Knauer, Germany, and of 125mm high and an internal diameter was utilized. Separation on semi-preparative HPLC system from Lachrom-Merck Hitachi having a pump and UV specifications of L7100 and UV detector L7400, respectively was carried out. Running of column chromatography was done on Sephadex LH20 (GE HealthCare).

2.3. Endophytic fungus isolation and its molecular characterisation

The strain of the isolated fungus was gotten from a diseased –free leaf of *P. reclinata*. The leaf sample was cleansed in sterilized clean water, 70% (v/v) ethyl alcohol for 2-3 minutes and finally sterilized water. The cleansed sample was gently placed on a malt agar contained in a petri dish for a minute to be sure of complete sterilization. The leaf was thereafter cut into sizes of approximately 10 x 10mm and cultured in an antibacterial containing malt agar. The composition of the culture medium: 0.015 kgL^{-1} malt extract, 0.015 kgL^{-1} agar, 0.0001 kgL^{-1} streptomycin and 0.0002 kgL^{-1} chloramphenicol dissolved in distilled water (pH: 7.4-7.8). Culture of plates was done for three weeks at 21°C till detection of the growth of hyphae, transferring to fresh culture of the same composition and constantly looking out for purity of culture.

The resulting isolated fungus was subjected to DNA extraction, amplication, gel electrophoresis, sequencing (ITS region) using standard method [17] to arrive at its character as *Pestalotiopsis sp.* [18]. The voucher strain; LNF-L-1 was then banked in Prof. Peter Proksch laboratory at the Institute of Pharmaceutical Biologie and Biotechnologie, Heinrich-Heine University, Duesseldorf, Germany.

2.4. Cultivation of the fungus

The cultivation medium was a sterilized 1 L Erlenmeyer flask containing 100 g of commercially purchased rice in 100 ml of distilled water, autoclaved at a temperature of 121 °C, at a pressure of 2 bar and a duration of a quarter of an hour. A total of 6 flasks were engaged. Three small slices of the mycelia were gently placed on the pre-sterilized rice in the flask under a sterile condition and allowed for growth which occurred within three weeks.

2.5. Extraction and Fractionation

After a satisfactory growth, an adequate quantity of ethylacetate that was sufficient enough to cover the rice in the flask (0.4L) was carefully added to each flask for 12 hours for fungal growth termination as well as extraction of the fungus. A fresh volume of the same amount of solvent was added to the residue to repeat the process after which both were added together. Solvent was removed from the extract *in vacuo* to yield 860 g and then partitioned between *n*-hexane and methanol (90%). Concentration of the resulting methanol fraction yielded 400 mg. Based on the HPLC fingerprint, the n-hexane fraction was discarded while the vacuum liquid chromatography of the methanol fraction was run on silica gel employing n-hexane : ethylacetate (100:0) gradiently at 10% interval to end at 100 ethylacetate with each being collected separately to grant 11 fractions. With use of thin layer and hplc chromatographic fingerprints, the fifth fraction with a weight of 75 mg with mobile phase hexane : ethyl acetate (3:2) was further subjected to more process of chromatographic separation with Sephadex LH-20 and (DCM:MeOH (50:50) as the stationary and mobile phase, respectively. The latter afforded 6 bulked sub-fractions according to the TLC features, (silica gel $F_{254,}$)/ EtOAc-MeOH-H_2O (8:1.5:5), out of which fraction 3 (19 mg) was finally run on semi-preparative HPLC (Merck, Hitachi L-7100) employing a Eurosphere C18 column (0.3m × 0.08m, length × internal diameter) and MeOH-H_2O), 10% MeOH, 10% MeOH and 100% MeOH at 0, 5, 35 and 45 min., respectively which eventually was isolated pure and coded LNF-L-1. It was thereafter elucidated on the basis of one dimensional NMR spectroscopy and comparism with literature.

2.6. Biological assay

Evaluation of the the cytotoxicity activity was done in consonant with the method of El Amrami et al. [17] utilizing lymphoma cell line L5178Y.

3. Result and discussion

This compound; Cichorin A (4 mg) (Figure 1), which is solid and white in colour, has previously been isolated from plants but now obtained from an endophytic fungus (Figure 1). It exhibits UV λ_{max} (MeOH) absorption of 250.0 and 255.9 nm in ultra violet spectrum. The proton NMR (500 MHZ, $CDCl_3$) is as presented in Table 1.

Table 1 1H -NMR spectra data of compound LNF-L-1

Compound LNF-L-1 (600 MHZ)	
Atom number	**δH [ppm]**
1	4.31 (2H, s)
2	
3	5.06 (1H)
4	4.90 (1H, s)
5	6.88 (1H, d, J = 9.0 Hz)
6	7.63 (1H, d, J = 9.0 Hz)
7	6.86 (1H, d, J = 2.0 Hz)
8	2.23 (3H, s Me-8)
9	6.42 (1H, d, J = 2.0 Hz)
10	3.96 (3H, s, oxygenated methyl protons,-OMe)
11	
12	4.86 (1H, s)
13	1.72 (3H, s)

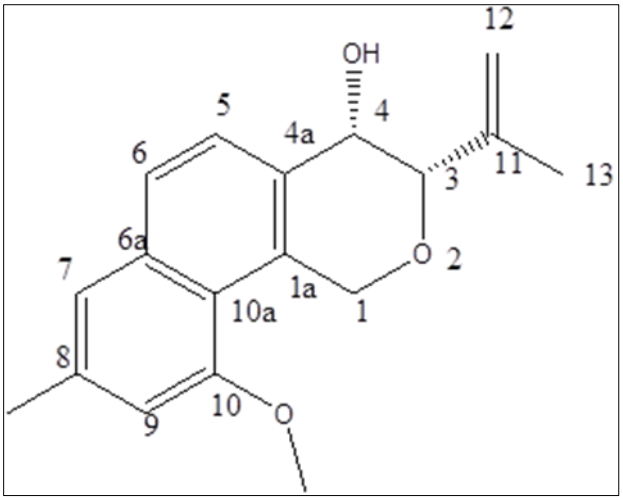

Figure 1 Cichorin A

By comparism with an earlier reported study, this compound was characterized as cichorin A [19].

A growth inhibitory activity of 31.2% was obtained against against mouse lymphoma cell line at 10 µgml-1.

4. Conclusion

The cytotoxicity activity of cichorin A was weak with a percentage growth inhibitory activity of 31.2 when challenged against lymphoma cell line L5178Y (mouse).

Compliance with ethical standards

Acknowledgments

The authors are grateful to Prof. A.T Oladele for helping in plant collection.

Disclosure of conflict of interest

No conflict of interest is declared by the authors.

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