IJBPAS, May, 2022, 11(5): 2561-2570

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN-VITRO ANTIBACTERIAL ACTIVITY OF NIRANTHIN

SOMADE PM[1*] AND SOMADE PP[2]

1: Dept. of Physiology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India

2: Intern, Krishna Institute of Medical Sciences, Karad, Maharashtra, India

***Corresponding Author: Dr. Prakash M. Somade: E Mail: saishaprakash@gmail.com**

Received 20th June 2021; Revised 19th July 2021; Accepted 29th Aug. 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6116

ABSTRACT

Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobials from plants. This study was carried out to determine the antibacterial activity of Niranthin.

The antibacterial tests used were the agar well diffusion assays at concentration 1gm/ml. Minimum Inhibition Concentration (MIC) was determined in Niranthin that showed some efficacy against the tested microorganisms. Gentamicin (10µg) was used as a positive control. Niranthin showed better antibacterial activity against the study organisms compared to Gentamicin. Niranthin exhibited a significant bactericidal activity against *S. aureus* as compared to its efficacy against *P. aeruginosa* and *E. coli.*

This in-vitro study corroborated the antimicrobial activity of Niranthin indicating that it could be potential source of new antimicrobial agent.

Key words: Niranthin, antibacterial activity, MIC

INTRODUCTION

Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant **[1]**. Moreover, antibacterial pharmaceuticals are not accessible to the majority of the communities in the developing countries **[2]**. Increase in resistance calls for new antibacterial drugs, one source of which are medicinal traditional plants. Plants may provide

natural source of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally **[3]**.

Niranthin, a lignan isolated from the aerial parts of the plant *Phyllanthus amarus*, exhibits a wide spectrum of pharmacological activities **[4]**. Niranthin also exhibits anti-inflammatory and anti-allodynic properties **[4]** and has been shown to possess anti-viral activity against human hepatitis B virus in vitro **[5]**. The lignan-rich fraction containing niranthin from aerial parts of *Phyllanthus amarus* exhibits cytotoxic effects in the K-562 cell line **[6]**. Chowdhury *et al* have shown niranthin capability as a potent anti-leishmanial agent **[7]**. Recently Conrado *et al* have reaffirmed Antileishmanial and Antitrypanosomal Activity activity of niranthin and other lignans from Niranthin **[8]**. Recently study by Chopade *et al* indicates that the Niranthin may have potential clinical applications in the management of anxiety **[9]**.

This study looks into the in vitro antibacterial activity of Niranthin against three pathogenic microorganisms (*Staphylococus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) that cause the most common cases of infectious diseases.

MATERIALS AND METHOD

Plant marker- The Niranthin [6-[(2*R*,3*R*)-3-[(3,4-dimethoxyphenyl)methyl]-4-methoxy-2-(methoxymethyl)butyl]-4-methoxy-1,3-benzodioxole] purchased (Product code : N006, Lot. no. : T18C277) from Natural Remedies Pvt. Ltd., Bangalore. Purity of Niranthin was determined by the manufacturer by HPLC area normalization and was certified above 95.0%.

Preparation of the test micro-organisms

This process followed the previously established procedures for testing antimicrobial agents. Standard cultures of bacteria from the Y. C. College of Science Karad. A Gram positive bacterium; *Staphylococcus aureus*, was used as a wound/skin pathogen and Gram negative bacteria; *Escherichia coli*, was used to represent pathogens that cause gastro enteritis while *Pseudomonas aeruginosa*, was used as an environmental pathogen. Standardized bacterial suspension was prepared by picking a colony of respective bacteria using sterile wire loop and suspending it in 5 ml infusion liquid media. The dilutions formed the bacterial stock solutions for use in the agar-well diffusion assays.

Preparation of culture media

Mueller Hinton agar (Hi media lab) was used for direct sensitivity testing. The media was prepared and treated according to manufacturer's guidelines. Thirty five

(35) g medium was mixed with one litre of distilled water, enclosed in a screw cap container and autoclaved at 121 °C for 15 minutes. The medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37 °C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile.

Agar-Well Diffusion Assay [10]

A concentration of 1 g/ml of the Niranthin was designed from the stock solution for agar well diffusion assay. Cultures of *S. aureus, E.coli* and *P. aeruginosa* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. The sensitivity testing of the plant extracts was done using the agar well diffusion method17 whereby, wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. About 50 µl of the Niranthin, of the concentration 1 g/ml, was dispensed into respective wells and 10 µg Gentamycin was used as a positive control since it is a broad spectrum antibiotic. Physiological saline/Dimethyl sulfoxide (DMSO) was used as negative control. All the tests were run in triplicates for quality results. The set up was incubated for 24 hours at 37 °C. Twenty four (24) hours later, the zones of inhibition were measured using a ruler and a pair of divider then results reported in millimeters (mm).

Minimum Inhibition Concentration (MIC) Evaluation [11]

The MIC was evaluated on Niranthin showed antibacterial activity in the agar well diffusion assay on any organism. This test was performed at five concentration of Niranthin (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml) employing doubling dilutions of Niranthin in Brain heart infusion broth up to the fifth dilution. One (1) ml of the resultant broth was put in test tube and equal amounts of the Niranthin (1 ml) were added to the first test tube and serial dilution done with the last 1 ml being discarded. To complete the test, each organism was separately suspended in 5 ml of Brain heart infusion broth and incubated overnight, after which 0.1 ml was added to all the test tubes and preparation incubated at 37 °C for 18 hours. After incubation, a loop full from each tube was sub cultured on nutrient agar to see if bacteria growth was inhibited (Minimum Bactericidal Activity). Growth of bacteria on solid media indicated that particular concentration of the extract was unable to inhibit the bacteria. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation.

Data Analysis

Microsoft Excel® was used to enter and capture data. Various graphs and tables were extracted from this data. Data was then exported to SPSS for further analysis. The MIC for each microorganism was analyzed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant

RESULTS AND DISCUSSION

The results showed that Niranthin demonstrated antibacterial activity against the *S. aureus, E.coli* and *P.aeruginosa*. It can be noted from **Figure 1 & 2**, that *Staphylococcus aureus* was the most susceptible of the three organisms and *E.coli* the least. Niranthin showed antibacterial activity against studied microbes in following order *S. aureus* >> *P.aeruginosa* >> *E.coli*. The highest zone of inhibition to Niranthin was against S. aureus, while lowest against *E.coli,* signifying higher activity against *S. aureus*. The zones of inhibition produced by Niranthin against the test organisms indicated its susceptibility to the microbes. It was observed that the zones of inhibition varied from one organism to another.

According to statistical analysis results of one way ANOVA of *Staphylococcus aureus* the P-value was 0.241, F- calculated was 1.3 and F-critical was 15.7, one way ANOVA for Pseudomonas aeruginosa; P-value was 0.808, F-calculated was 0.225 and F-critical was 6.94. Lastly one way ANOVA for *Escherichia coli* P-value was 0.530, F-calculated 0.455 and F-critical 6.61.

These observations of present study are likely to be the result of the differences in the cell wall structure between gram-negatives and gram-positive bacteria, with gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics.

In-silico studies were performed for predictions of Molecular Properties and Drug-likeness of Niranthin by Web Molecular Editor v1.5.1 (http://www. http://www.molsoft.com/mprop/). It predicts an overall drug-likeness score using and Molsoft's chemical fingerprints. The training set for this mode consisted of: 5K of marketed drugs from WDI (positives), 10K of carefully selected non-drug compounds. Chemical structure of Niranthin is shown in **Figure 3**. H^1NMR for Niranthin is depicted in **Figure 4**. While **Figure 5** shows C^{13} NMR for Niranthin. The summarized details of in-silico predictions of Molecular Properties and Drug-likeness of Niranthin are given in **Table 1**.

In conclusion Niranthin showed antibacterial activity against disease-causing organisms and this suggests that Niranthin could be useful in chemotherapy. From the findings of the current study, the

following recommendations could be made; Firstly, there is a need to further study antibacterial activity of Niranthin in detail and secondly, it is necessary to determine Niranthin's toxicity, side effects and pharmacokinetics effects.

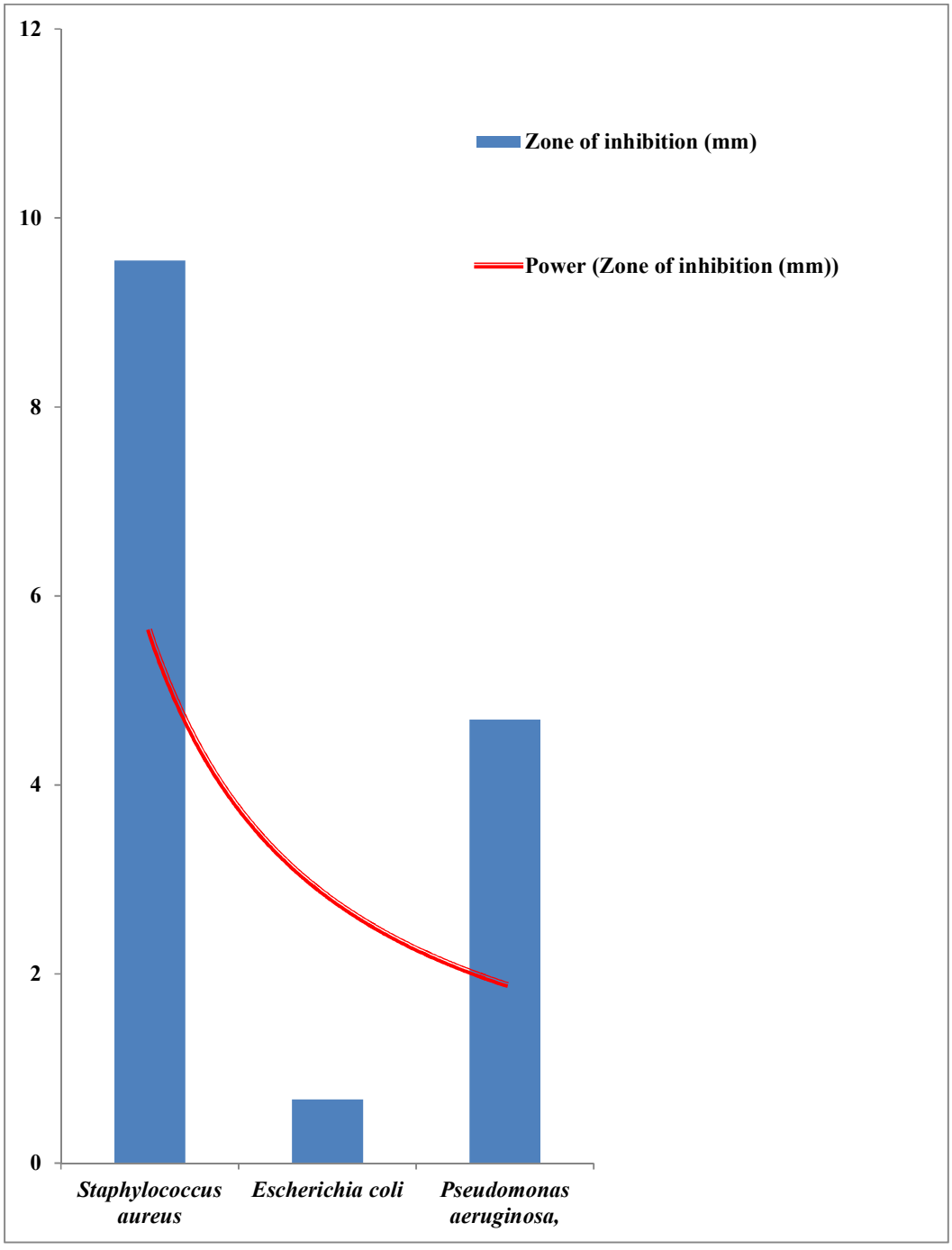

Figure 1: Chart showing the Zone of inhibition of Niranthin

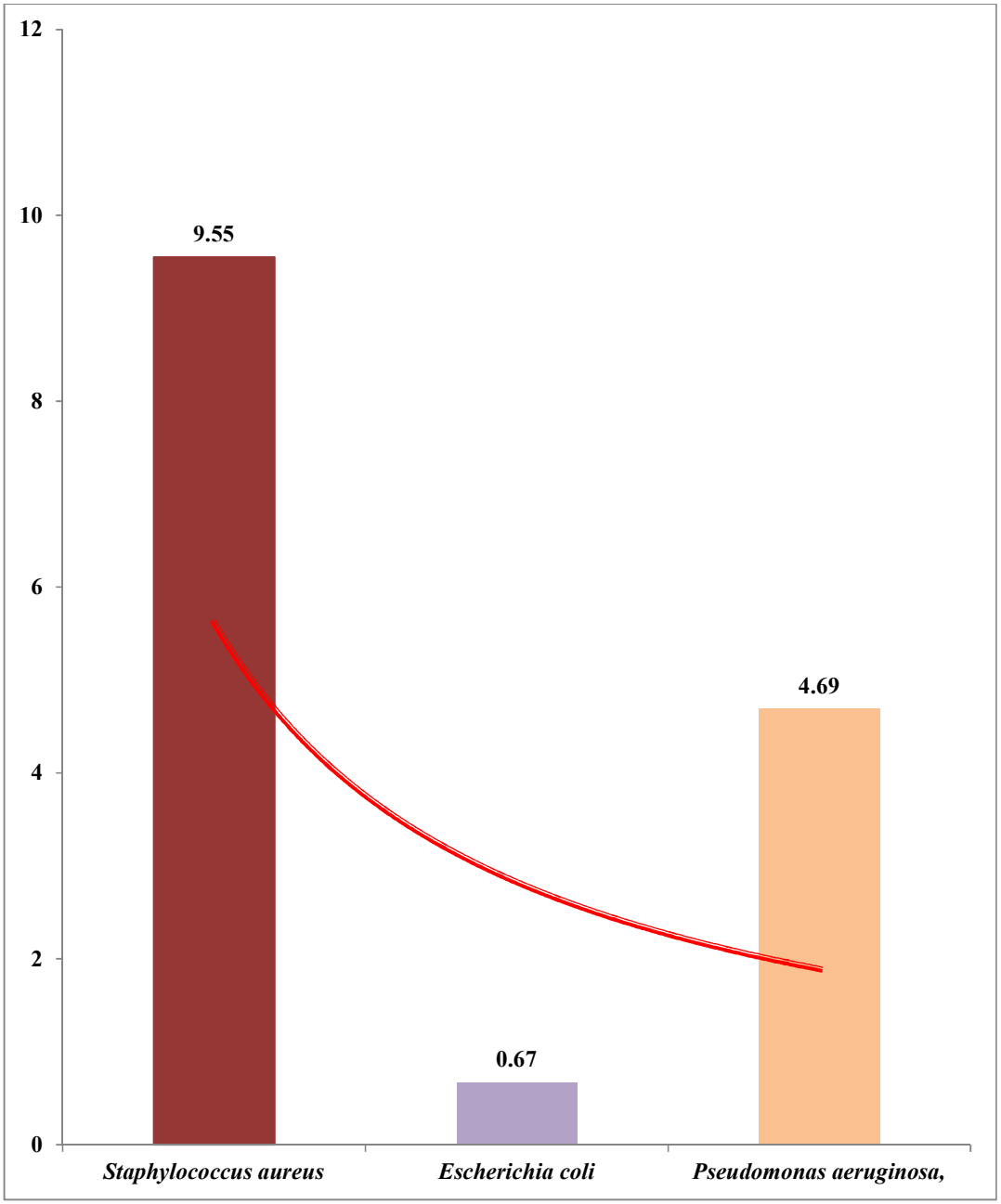

Figure 2: Chart showing the Minimum inhibition concentration of Niranthin in µg/ml

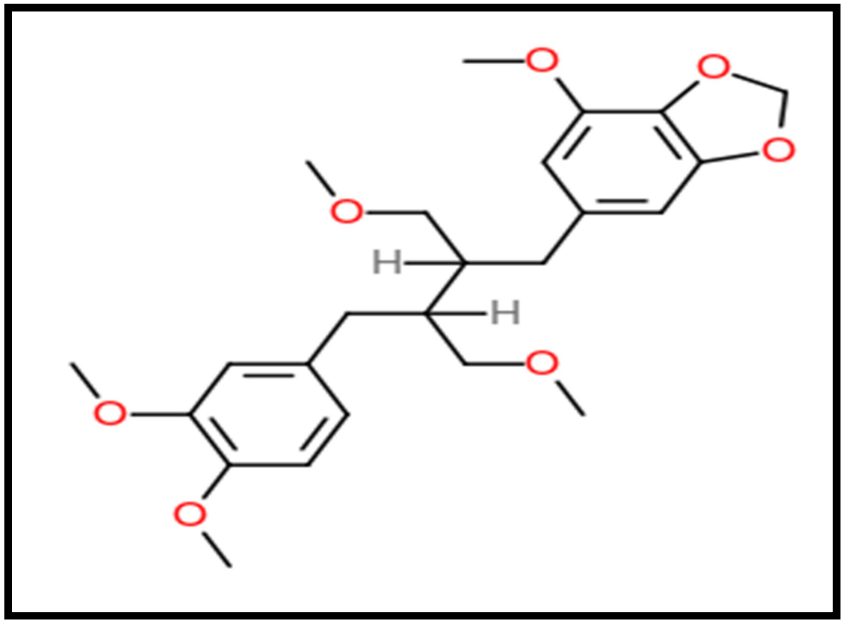

Figure 3: Molecular structure of Niranthin

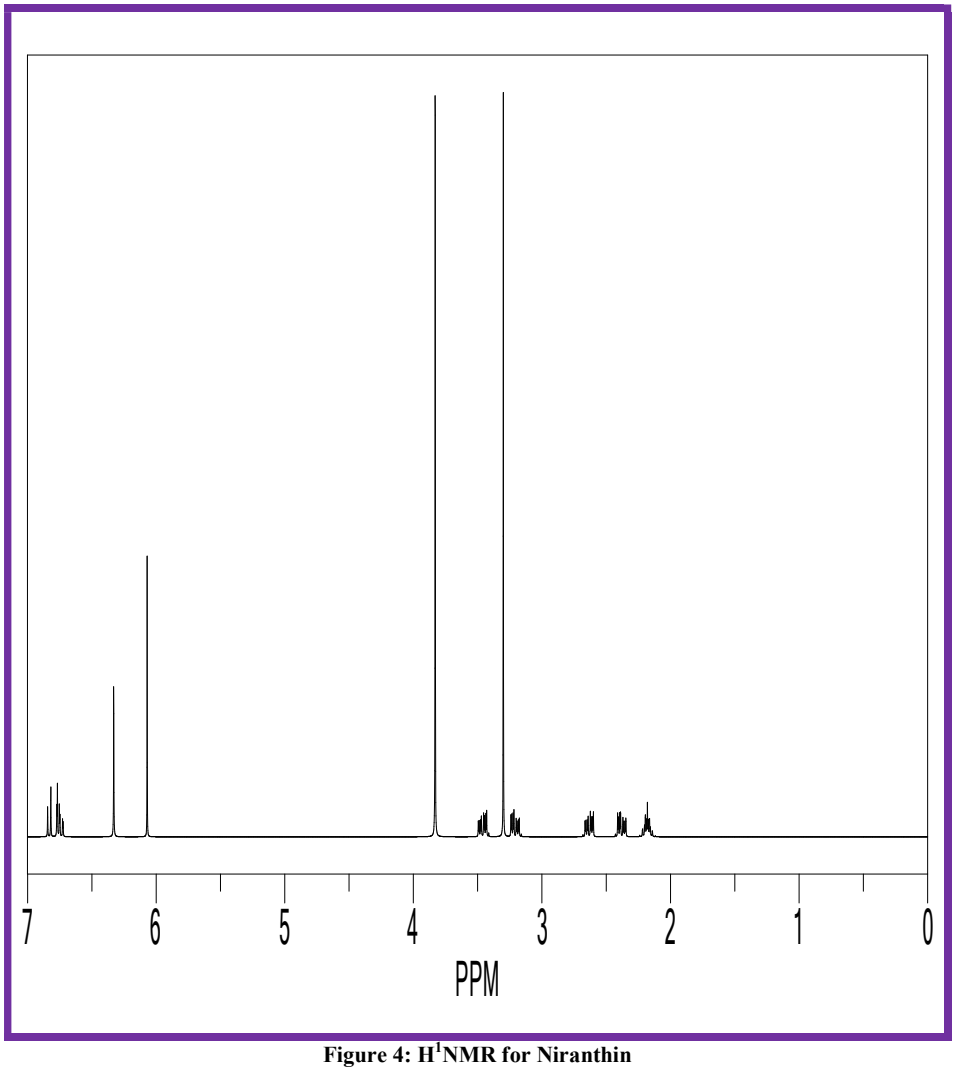

Figure 4: H^1NMR for Niranthin

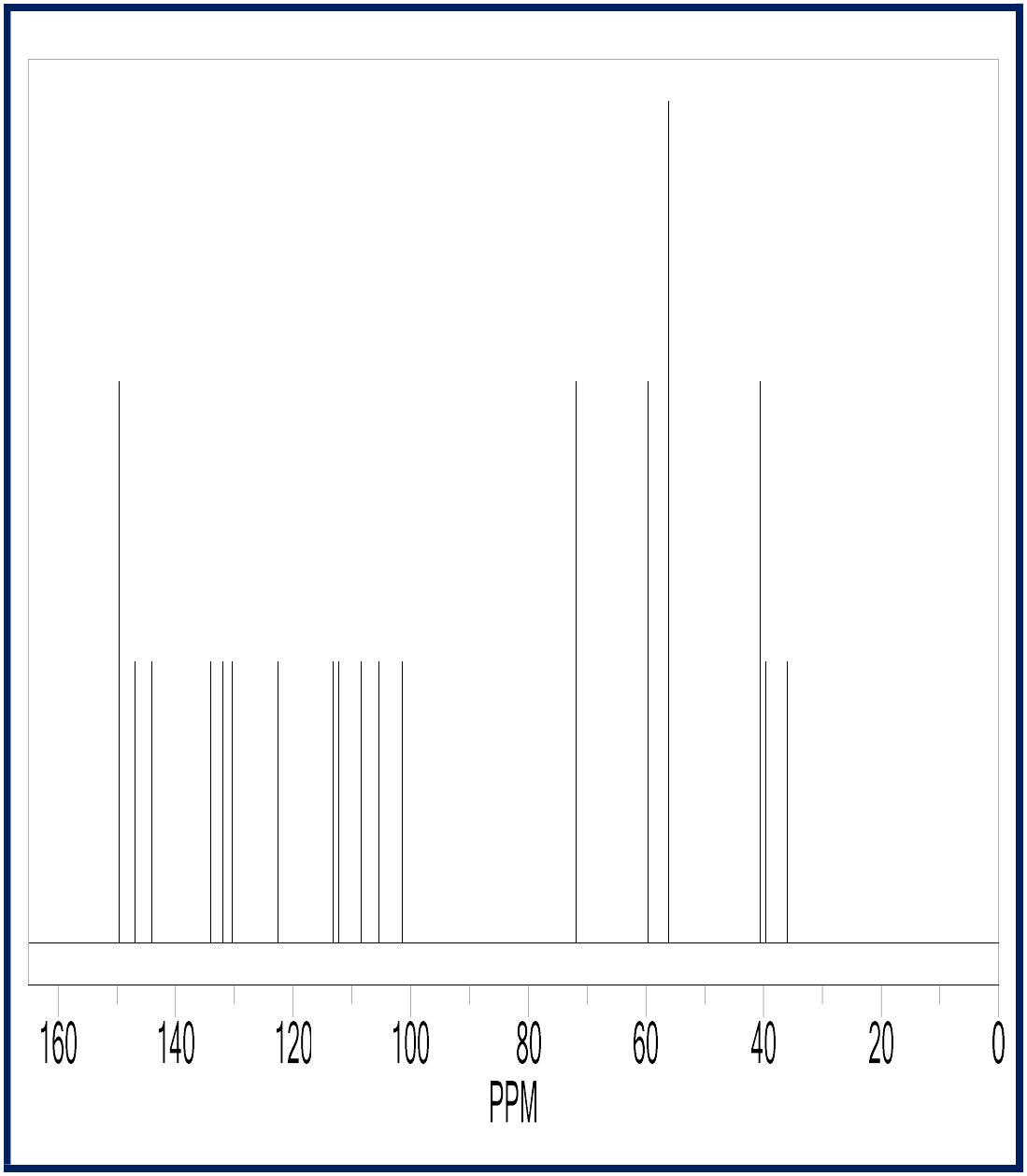

Figure 5: C^{13} NMR for Niranthin

Table 1: In Silico predictions of Molecular Properties and Drug-likeness of Niranthin

Properties	Molecular Properties of Niranthin
Molecular formula:	C24 H32 O7
Molecular weight:	432.21
Number of HBA (Hydrogen bond acceptor):	7
Number of HBD (Hydrogen bond donor):	0
MolLogP (octanol/water partition coefficient) :	3.68
MolLogS (water solubility Log(Mol/L)):	-3.61 (in Log(moles/L)) 105.94 (in mg/L)
MolPSA(Molecular Polar Surface Area (PSA) and Volume) :	56.51 A^2
Molecular Volume :	435.67 A^3
pKa of most Basic/Acidic group :	<0. / 18.11
Number of stereo centers:	2
BBB Score : The Blood-Brain Barrier (BBB) Score: 6-High,0-Low *(DOI: 10.1021/acs.jmedchem.9b01220)*	3.99

Funding source- None to report.

Conflicts of interest- We all the authors declare no conflict of interest

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