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Antimicrobial activity of Narciclasine against *Escherichia coli* and *Candida albicans*

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Abstract
Microbial infections are a major cause of several health complications. This is partly due to the fact that available anti-microbials have become ineffective against these pathogens. Despite the advancement of science and technology, plant derived natural products are still a major source of therapeutic agents. Plants belonging to the Amarylldiaceae family have shown to have several biologically significant compounds. Narciclasine is found to be one of the useful bioactive compounds of this family as it predominantly consists of pharmacologically active alkaloids. The need for newer effective therapeutic agents against microbial infections has led us to study the anti-microbial properties of narciclasine. Here we have tested various concentration of narciclasine against two major microbial pathogens – *Escherichia coli* and *Candida albicans.* We used the microdilution method and optical density measurements to decipher the potential of narciclasine to inhibit bacterial and fungal growth. The minimum inhibitory concentration of narciclasine against *E coli* and *C albicans* was found to be 22.72µg/ml and 11.36µg/ml respectively. This study results show that narciclasine has the potential to inhibit the bacterial and fungal growth.

Keywords: narciclasine, anti-microbial activity, microdilution, natural products, *Escherichia coli*, *Candida albicans*

1. Introduction
Despite the advancement in science, infectious diseases are a major cause of morbidity and mortality worldwide. At present antibiotics is the main mode of therapy for microbial infections. However, repeated use of antibiotics over a period of time has led to the development of multidrug resistant strains [1]. There is severe threat to human health and hence the need to discover newer antimicrobials. Natural products have been important for discovery of new drugs [2]. Medicinal plants and herbs have been an important source of these natural products, and have been used in traditional medicine [3]. Many active compounds from plant extracts have been the starting point of useful drugs for various ailments. Narciclasine belongs to the Amarylldiaceae family. The pharmacologically active components of this plant are the alkaloids. These alkaloids have shown to exhibit several useful properties like anti-proliferative, anti-inflammatory and antitumor [4]. Narciclasine has shown to exhibit cytotoxic effects in human cancer cells but not in normal cells [5]. *Escherichia coli* are one of the most predominant bacterial pathogen found in community acquired infections in various hospitals [6]. Fungal infections are an important cause of morbidity and mortality of hospitalized patients [7]. *Candida albicans* is the common yeast species to cause nosocomial infection and accounts for 50-70% of invasive candidiasis [8]. Here we have attempted to study the effect of various concentrations of narciclasine in inhibiting the growth of *E coli* and *C albicans*.

2. Material and Methods
Narciclasine was purchased from Tocris Bioscience (R&D Systems, USA). Narciclasine was dissolved in the appropriate amount of Dimethyl sulfoxide (DMSO) as per the manufacturer's instructions. The stock concentration of narciclasine was 1 mg/ml. *Escherichia coli* and *Candida albicans* culture were received from Department of Microbiology, JIPMER. It was subcultured and incubated at 37'C for 24 hours. The optical density readings were taken using microplate reader at 600nm (Mindray, China).

2.1 Determination of antimicrobial activity of narciclasine against *E coli* and *C albicans*
The antimicrobial activity of narciclasine against *E coli* and *C albicans* was determined using microdilution and optical density measurements. In order to determine the minimum concentration of narciclasine needed to inhibit the *E coli* and *C albicans* growth, different concentrations of narciclasine were prepared from stock of 1 mg/ml. (NC(A) =0.5 mg/ml, NC(B)= 0.25 mg/ml, NC(C)= 0.125 mg/ml)
The constituents were added in the 96 well plate according to the following template. All the measurements were done in triplicates.

Table 1

Group	LB/YPD medium (µL)	*E coli or C albicans* culture in medium (µL)	Narciclasine (µL)	Resulting NC Concentration (µg/ml)
Medium only	200	0	0	0
Medium+ *E coli or C albicans*	200	20	0	0
Medium+ *E coli or C albicans* +NC(A)	200	20	20	45.45
Medium+ *E coli or C albicans* +NC(B)	200	20	20	22.72
Medium+ *E coli or C albicans* +NC(C)	200	20	20	11.36

Abbreviations: LB- Luria Bertani; YPD- Yeast extract peptone dextrose; NC-Narciclasine

Optical density readings were taken every 10th minute till the 540th minute at 600nm wavelength. The plate was kept in sterile condition and maintained at 37'C throughout the experiment. The plates were subjected to mild and gentle shaking throughout the experiment.

3. Results

3.1 Antimicrobial activity of narciclasine against *Escherichia coli*

The effect of different concentrations of narciclasine on the growth of *E coli* is depicted in Fig 1.

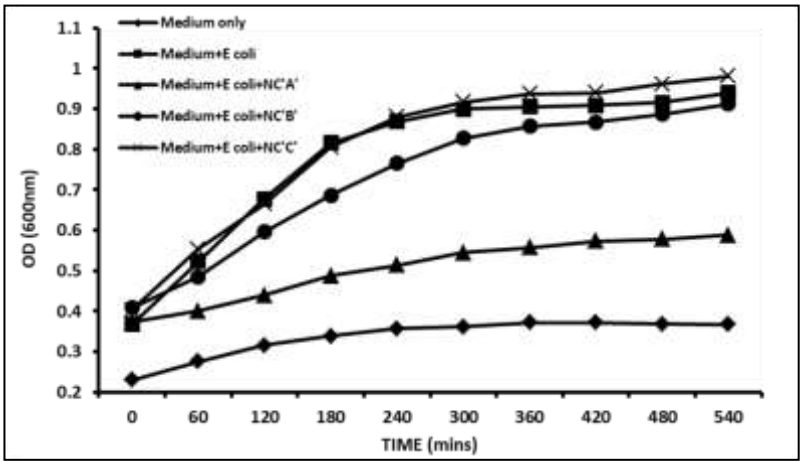

Fig 1: Graph showing the antibacterial activity of narciclasine

The pattern depicted clearly shows that narciclasine inhibits the bacterial growth. The control which consisted of only *E coli* culture showed exponential growth whereas the various concentrations of narciclasine significantly inhibited bacterial growth. The minimum concentration of narciclasine needed to inhibit the *E coli* growth is 22.72µg/ml (NC'B').

3.2 Antimicrobial activity of narciclasine against *Candida albicans*

The effect of different concentrations of narciclasine on the growth of *C albicans* is depicted in Fig 2.

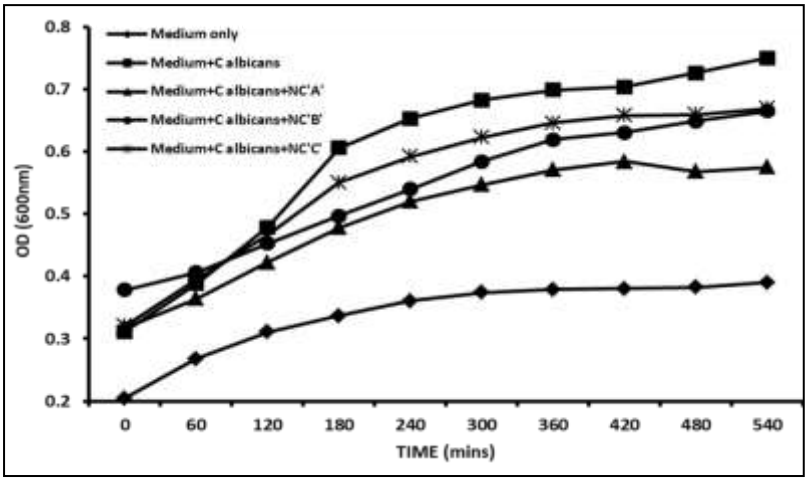

Fig 2: Graph showing the anti-fungal activity of narciclasine

The pattern depicted clearly shows that narciclasine inhibits the fungal growth. The control which consisted of only *C*

albicans culture showed exponential growth whereas the various concentrations of narciclasine significantly inhibited fungal growth. The minimum concentration of narciclasine needed to inhibit the *C albicans* growth is 11.36µg/ml (NC'C').

4. Discussion

Natural products and their derivatives have been serving as vital source of therapeutic agents. They have been a source of new drugs and have proven to improve the outcome in several health areas [9]. Antibiotic resistance is a big challenge to the efficient management of infections such as pneumonia and septicemia. There is need for newer antimicrobials as microbes are becoming increasingly resistant to the antimicrobials currently in use [10]. Due to the high cost of development of newer drugs and the chance for antimicrobial resistance the discovery of newer drugs have been making slow progress [11]. The increase in the incidence of antibiotic resistant bacterial infections in clinics and the community has led to the need for newer strategies to tackle bacterial infections.

Plants belonging to the Amarylldiaceae family are known for their useful and pharmacologically active alkaloids [12]. The extracts of several other plants in this family have shown to possess antibacterial and antifungal activities [13]. Narciclasine also known as lycoricidinol, is an isocarbostyril alkaloid of the *Narcissus* species and belongs to the Amarylldiaceae family. Narciclasine has shown to exhibit significant antitumor effects in cancer cells. It acts through apoptosis mediated cytotoxic effects in human cancer cells but does not seem to affect human fibroblasts [5]. Microbial infections are a major cause of health disorders. Gram negative pathogens are the major causative agents of newborn infection. *Escherichia coli* are one of the major pathogens which cause early onset infections in newborns especially in pre-terms [14].

Here we studied the bacterial and fungal growth inhibition by narciclasine using broth dilution and optical density measurements. The minimum concentration of narciclasine which was found to be most effective in inhibiting the growth of *Escherichia coli* was 22.72µg/ml. The minimum concentration of narciclasine found to be most effective in inhibiting the growth of *Candida albicans* was found to be 11.36µg/ml. This suggests a better anti-fungal potential of narciclasine than anti-bacterial. Further investigations could focus on the mechanism of action of narciclasine that underlies its anti-microbial potential.

5. Conclusion

The existence of increasingly resistant microbes and surge of microbial infections have resulted in the need for more potent and newer antimicrobials. Narciclasine has the potential to be an active anti-bacterial and anti-fungal compound especially against *E coli* and *Candida albicans*.

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8. Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

9. Ethical approval

This study has been approved by the JIPMER Scientific Advisory Committee (JSAC), Puducherry, India. (JSAC 19/16/2015)

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