

IN VITRO ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF SEEDPOD AND QUERCETIN OF *NELUMBO NUCIFERA* GAERTN

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ABSTRACT

Objective: The objective of the study was to evaluate whether ethanolic extracts of *Nelumbo nucifera* (EENN) seedpod and quercetin (active component of NN) possess antibacterial properties against Gram (-) bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* and Gram (+) bacteria such as *Staphylococcus aureus*.

Methods: Antibacterial activities of EENN seedpod and quercetin were investigated using disc diffusion method, minimum inhibitory concentration against *E. coli* and *P. aeruginosa* and Gram (+) bacteria such as *S. aureus*.

Results: The antibacterial activity of both EENN seedpod and quercetin was found to be increased in dose-dependent manner. The maximum zone of inhibition was exhibited by both EENN seedpod and quercetin against *E. coli* (14 mm and 15 mm) and *P. aeruginosa* (13 mm and 15 mm). Gram-negative bacteria were more susceptible to the EENN seedpod extract and quercetin than Gram-positive bacteria.

Conclusion: The results of the present study suggested that the effect of EENN seedpod and quercetin against the tested bacteria *in vitro* may contribute to the *in vivo* activities of the EENN seedpod and quercetin.

Keywords: *Nelumbo nucifera*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

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INTRODUCTION

In ancient time, plant extracts have been used in traditional medical systems for the treatment of microbial diseases [1]. Drugs used in modern medical system have become less efficient because of the development of resistance in pathogenic microorganisms, and hence, new antimicrobial drug discoveries have become essential [2]. Antimicrobial activities of phytochemicals in various plant extracts were investigated and provide the source for the natural antimicrobial drugs [3]. In recent times, search for compounds with antimicrobial activity has gained increasing importance, due to alarming increase in the rate of infection by antibiotic-resistance microorganisms [4]. In earlier studies, it has been shown that therapeutic potential of plant origin is not associated with side effects contrary to the synthetic drugs [5,6]. Therefore, researches on drugs against microbial infections are increasingly turning their attention to folk medicine [7].

Nelumbo nucifera (NN) is a perennial, aquatic crop that is used throughout Asia and cultivated for its edible rhizomes, stems, seeds, and leaves. All parts of NN are shown to contain number of secondary metabolites such as alkaloids, flavonoids, steroids, triterpenoids, glycosides, and polyphenols [8]. Some of the plants have different constitutions and concentrations, thus have an activating or inhibiting effect on microbial growth [9,10]. Phenolics are widely distributed in plants, are a class of plant secondary metabolites that contain one or more hydroxyl derivatives of benzene rings, and may affect the growth and metabolism of bacteria and used for defensive functions in many plants [11,12]. In many parts of the world, lotus is utilized as herbal medicine for the treatment of many diseases including heart problems, hypertension, diarrhea, insomnia, and cancer [13,14]. Various parts of lotus plant such as buds, flowers, anthers, stamens, fruits, leaves, stalks, rhizomes, and roots were used in traditional medicine [15,16]. Lotus was studied for its pharmacological properties such as antipyretic, antiviral, antidiarrheal, anti-inflammatory

immunomodulatory, hypoglycemic, antioxidant, psychopharmacological, lipolytic, anticancer, and hepatoprotective activities [17]. In literature review, it was found that the seedpod of NN (white lotus) had not been screened for antibacterial properties so far. From this viewpoint, this study was carried out to evaluate the antibacterial activity of EENN white seedpod and its active component quercetin.

METHODS

Procedure

Collection, identification, and extraction of plant material

NN seedpod was collected from a pond near Avadi, Chennai, Tamil Nadu, India. The plant material was authenticated and a voucher specimen was deposited in the herbarium in National Institute of Siddha, Tambaram, Chennai (No: NISMB1442014). The plant materials were cleaned, dried in shade, and grinded to fine powder with the help of a mixer grinder, and ethanolic extract was prepared using 90% ethanol by Soxhlet apparatus and extract was stored at 4°C until use.

Identification of bacterial strains

Staphylococcus aureus, *Escherichia coli*, and *Pseudomonas aeruginosa* pure isolates used in this study were obtained from Hi-Media Laboratories. All samples were cultured and subcultured again for purity on Blood Agar plates. Colony morphology and Gram staining were done to confirm the identity of working strains.

Preparation of extract dilutions

A stock solution of the plant extract was made by dissolving 100 mg of extract in 10% dimethyl sulfoxide and then diluted using sterile Mueller-Hinton broth. Therefore, the initial concentration of the plant extract was (100 mg/mL). The above process was repeated several times to obtain other dilutions: 100, 50, 25, and 10 µg/mL.

Inoculation procedure

The bacterial inoculum was prepared with Mueller-Hinton broth which was similar to the disc, then incubated at 37°C for 18–24 h, and the bacterial concentration was adjusted to a $0.5 (1.5 \times 10^8 \text{ CFU/mL})$. Sterile broth and the suspension were diluted in the ratio of 1:100 to obtain a cell number of approximately 10^6 CFU/mL . To the tubes containing 0.5 mL of diluted extracts, 0.5 mL of the standardized bacterial suspension was added to produce a final cell count of about $5 \times 10^5 \text{ CFU/mL}$. A tube containing broth, extract solvent either distilled water or 10% (v/v) Tween-20, and the inoculums were known as positive growth controls [18]. The negative control was a tube containing broth without inoculum, and extract solvent which was incubated overnight at 37°C.

Antimicrobial susceptibility testing

Disc diffusion test

Disc diffusion method for antimicrobial susceptibility testing was carried out based on recommendations given by the Clinical Laboratory Standards Institute, 2007 [18].

Preparation of impregnated discs

To 100 mg of extract, 1 mL of their respective solvents was added to prepare a stock solution of plant extract and impregnated in sterilized 6 mm blank discs. Distilled water and dimethyl sulfoxide-loaded discs were used as negative controls for methanolic extract respectively. All impregnated discs were fully dried in 45°C incubator for 18–24 h before the application on bacteria. The standard antibiotic disc used as positive controls was vancomycin (30 µg) for all strains.

Application of impregnated discs

The discs which were impregnated with plant extracts using sterile forceps were applied on the inoculated Mueller-Hinton agar once it has completely dried [19]. The discs were gently pressed to obtain uniform contact with agar surface. In each one of the test plates, three discs were placed in equidistance to each other to avoid the overlapping of inhibition zone. Three treated discs, one is treated with distilled water, one negative control, and the last one was treated with extract. The plates were then inverted and incubated for 24 h at 37°C. The antibacterial activity assessment was measured with the diameter of inhibition zone around the treated discs and the control discs. If activity is present, their diameters were measured to the nearest whole millimeter with a ruler. All tests were done for three times to obtain reliability, and the average of the three replicates for each extract, and antibiotic was calculated.

Determination of minimum inhibitory concentration (MIC) values

The lowest concentration of extract dilution with no visible growth was taken as MIC value. The tubes were then further incubated another 24 h and plated again to observe for presence or absence of growth after an incubation period of 48 h and at the same period of MIC value was recorded too.

Statistical analysis

Results for all the parameters analyzed were expressed as the mean \pm standard error of the mean. The statistical analysis of data was conducted with Statistical Package for the Social Sciences (SPSS) software (version 13.0; SPSS Inc., IL, USA) for Windows. Comparisons among groups that were more than two were performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test.

RESULTS

The antibacterial activity of the EENN seedpod extracts was evaluated at different concentrations (10, 25, 50, and 100 µg/mL) against three bacterial strains by the disk diffusion method and the results are summarized in Table 1. The antibacterial activity of both EENN seedpod extract and quercetin (Table 2) was found to be increased in dose-dependent manner. The maximum zone of inhibition was exhibited by both EENN seedpod and quercetin

against *E. coli* (14 mm and 15 mm) (Fig. 1) and *P. aeruginosa* (13 mm and 15 mm). The moderate zone of inhibition was found in both EENN seedpod and quercetin against *S. aureus* (12 mm and 15 mm). However, the results revealed that the EENN seedpod showed slightly lower antibacterial activity when compared to quercetin which shows the major activity of any drug is contributed by its quercetin which is a flavonoid, and these results were compared with the standard antibiotic vancomycin (30 µg/mL). It was observed from the result that antibacterial activity of the EENN increased with the increase in the concentration used. Statistical significance of the data demonstrated that concentration of extract at 10 µg exhibited least significant ($p < 0.05$), 25 and 50 µg exhibited moderate significant ($p < 0.01$), and 100 µg and standard showed highly significant ($p < 0.001$) maximum zone of inhibition.

DISCUSSION

Recently, many medicinal plants are being studied for the presence of phytochemicals which include various classes of bioactive compounds such as alkaloids, flavonoids, and tannins, which has many pharmacological activities. Different parts of many plants such as root, stem, leaves, seeds, and flowers were reported for various biopharmacological activities such as antiviral, antibacterial, antifungal, antioxidant, and anti-inflammatory properties. Bacterial infection has become one of the most serious global health issues nowadays and requires natural remedy since drug-resistant microbes have become a serious issue. Lotus has been used both as food and medicine in Asia, particularly in India [20]. Gram-negative bacteria were more susceptible to the EENN seedpod and quercetin than Gram-positive bacteria which correlate with the previous reports done in NN flowers [21]. The results of this study revealed that the EENN seedpod showed slightly lower antibacterial activity

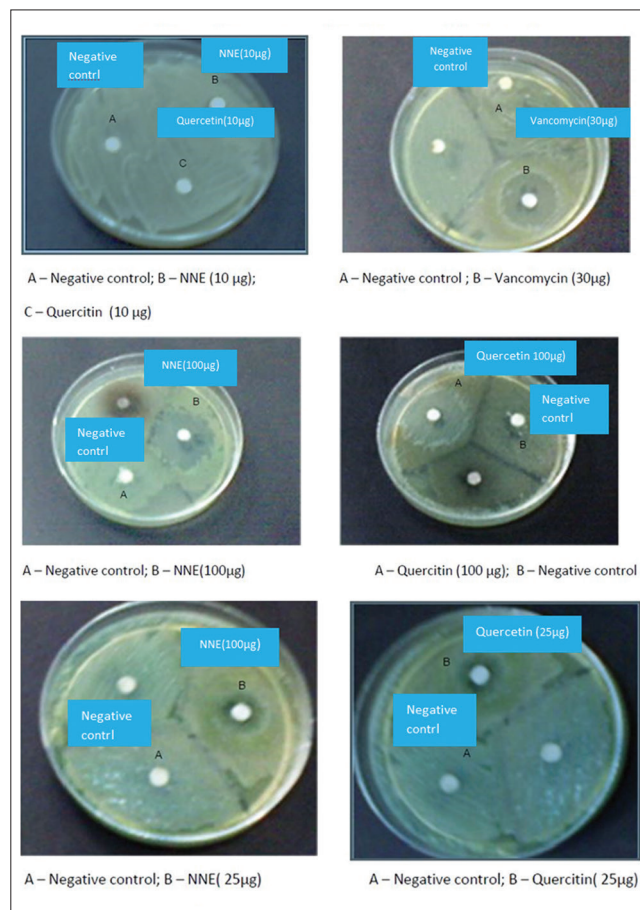


Fig. 1: Antibacterial activities shown by zones of inhibition by NNE and quercetin on *Escherichia coli*

Table 1: Antibacterial activity of NN extract

Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
10 (A)	1.05 \pm 0.01*	1.06 \pm 0.01*	1.02 \pm 0.01*
25 (B)	5.13 \pm 0.28**	4.01 \pm 0.18**	5.07 \pm 0.21**
50 (C)	7.89 \pm 0.51**	6.13 \pm 0.31**	8.14 \pm 0.24**
100 (D)	14.57 \pm 0.89***	12.42 \pm 0.95***	13.54 \pm 0.56***
Positive control	15.56 \pm 0.95***	13.69 \pm 0.84***	15.09 \pm 0.82***
Negative control	NI	NI	NI

NI means no inhibition zone. Each value is expressed as mean \pm standard error of mean (n=3). *p<0.05, **p<0.01, and ***p<0.001 as compared with negative control. *N. nucifera: Nelumbo nucifera*

Table 2: Antibacterial activity of quercetin

Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
10 (A)	1.04 \pm 0.56*	1.02 \pm 0.83*	1.06 \pm 0.95*
25 (B)	5.41 \pm 0.21**	4.23 \pm 0.17**	5.13 \pm 0.11**
50 (C)	7.91 \pm 0.21**	7.13 \pm 0.19**	7.05 \pm 0.23**
100 (D)	15.64 \pm 0.12***	15.72 \pm 0.13***	15.58 \pm 0.14***
Positive control	15.14 \pm 0.10***	13.61 \pm 0.17***	15.05 \pm 0.19***
Negative control	NI	NI	NI

NI means no inhibition zone. Each value is expressed mean \pm standard error of mean (n=3). *p<0.05, **p<0.01, and ***p<0.001 as compared with negative control

when compared to quercetin. It was suggested that the antimicrobial component in the plant that is bioactive compounds interacts with enzymes and proteins of the microbial cell membrane causing its disruption and causes the dispersal of flux of protons toward cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis [22,23]. The quercetin of NN seedpod is responsible for antibacterial activity. This supports usage of NN for treating fever and also in traditional medicine. In a previous study, it was shown that ethanolic extract of white NN flowers possessed strong antibacterial activity when compared to the pink flowers, this well correlated with our study similar strong antibacterial activity was evaluated in white NN seedpod also and more activity was attributed to quercetin which is a flavonoid the predominant component in many plant extracts [21].

CONCLUSION

The findings of this study confirmed the therapeutic potency of white lotus seedpod and quercetin used in traditional medicine. Hence, it can be concluded that the EENN possess a powerful antibacterial action against the organism which were tested which is due to quercetin. Herbal extracts have less toxicity compared to synthetic drugs and also reduce the side effects. Through this work, we have found that white lotus seedpod contains flavonoids quercetin which acts against microorganisms and forms a basis for natural source of antibacterial drug.

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AUTHORS' CONTRIBUTIONS

P.N. Ruvanthika contributed for carry out experiment and computation of data. S. Manikandan contributed for data analysis and preparation of manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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