Antimicrobial activity of Lotus (*Nelumbo nucifera*) fresh seed epricarp against some bacterail species and *Candida albicans.*

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Abstract

Three fractions of phenolic compounds had been obtained during the extraction of *Nelumbo nucifera* seed epicarp that performed in Huazhong Agricultural University in China. Antimicrobial activity of these fractions in 100 mg/ml concetration has been expermented with certain bacteria and yeast. The result show sensitivity of two types of tested bacteria, *E. coli* and *Enterococcus faecalis*, to fraction 2, and sensitivity of *Enterococcus faecalis* to fraction 3 while the result show the resistance of other tested bacteria and *Candida albicans* to these compounds. In comparison of this study with others conclude that our bacterial species have high resistance not to antibiotics and also to medicinal plants too.

الخلاصة

تم الحصول على ثلاثة اجزاء من مركبات فينولية اثناء عملية الاستخلاص لقشرة بذور نبات ***Nelumbo nucifera*** التي تمت في مختبرات جامعة هيزهونغ للزراعة في جمهورية الصين. اختبرت الفعالية ضد المايكروبية لهذه المركبات الفينولية بتركيز 100 ملغم/مل ضد مجموعة من البكتريا والخميرة البيضاء. اظهرت النتائج حساسية نوعين من البكتريا المستخدمة في هذه التجربة وهي بكتريا ***E. coli*** و ***Enterococcus faecalis*** للجزء الثاني، وحساسية بكتريا ***Enterococcus faecalis*** للجزء الثالث. بينما اظهرت بقية انواع البكتريا والخميرة البيضاء مقاومة واضحة لهذه المركبات . عند مقارنة هذه الدراسة مع الدراسات الاخرى نجد ان انواعنا البكتيرية لها مقاومة شديدة ليس فقط للمضادات الحيوية وانما للنباتات الطبية ايضا.

Introduction

Lotus plants are common in Australia, China, India, Iran and Japan (Anonymous, 1966). Lotus was introduced from China to Japan and cultivated for more than 1000 years (Komatsu *et al*., 1975). *Nelumbo nucifera* belongs to the family of Nelumbonaceae, which has several common names (e.g. Indian lotus, Chinese water lilly and sacred lotus) and synonyms (*Nelumbium nelumbo, N. speciosa, N. speciosum* and *Nymphaea nelumbo*) (Sridhar & Bhat, 2007). All parts of *N. Nucifera* have been used for various medical purposes in oriental medicine (Kashiwada *et al*., 2005). The leaves, roots and emberyonic stage of the plant have reported to contain alkaloids such as roemerine, nuciferine, nouronucefirine, nelumboside and asimilobine (Kulkarni & Juvekar, 2008). It has been reported to have anti-sterss (Kulkarni and Juvekar, 2008), anti-oxidant (Rai *et al*., 2006), anti-inflammatory (Mukherjee *et al*., 1997) and anti-pyretic (Mukherjee *et al*., 1996) activities.

Phenolic compounds are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom (Crozier *et al*,. 2006) Total phenolics content (mg gallic acid equivalents (GAE)/g fraction) of fractions are shown that Fr3 had the highest phenolic content, followed by Fr2 and Fr1. The amount of total phenolic content varied between fractions of Epicarp of *Nelumbo Nucifera* Gaertn, ranging from 138.75, 768.11 and 872.87 mg GAE/g of dry material (fraction).

The high antioxidation properties of fractions F1, F2 and F3 of extract (60% MeOH) from Epicarp of *Nelumbo Nucifera* Gaertn were evaluated antioxidant activities by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene bleaching method. Fr3 had the highest in DPPH scavenging activity, and β-carotene bleaching method followed by Fr2 and Fr1 (Hussam, 2010)

The World Health Organisation (WHO) along with other various national authorities, now recognizes antimicrobial resistance in both medicine and agriculture as a major emerging problem of public health importance (Hearst *et al*., 2010).

Throughout history, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease. Almost as soon as antibacterial drugs were deployed, bacteria responded by manifesting various forms of resistance. As antimicrobial usage increased, so did the level and complexity of the resistance mechanisms exhibited by bacterial pathogens. The struggle to gain the upper hand against infections continues to this day, although the number of scientists who are developing new antibacterial agents is beginning to dwindle, even as bacteria evolve ever more clever mechanisms of resistance (Tenover, 2006).

Material and Methods

Microorganisms tested

Four bacterial species (*E. coli, Streptococcus faecalis, Psudomonas aeruginosa* and *Klebsiella pneumoniae*) in addition to *Candida albicans* were isolated from different pathogenic samples in pathogenic bacterial laboratory in Biology Departement of College of Science in Thi-Qar University and by using different biochemical tests and different culture media the diagnosis and the identification of bacteria and *Candida albicans* has been done. In order to prepare pure culture, every microorganismes had been tested cultured on Blood agar supplemented with 5% blood and incubated in 37° C for 24 hr.

Preparation of plant material

The powder of F1, F2 and F3 were isolated by the Natural Product Laboratory of Huazhong Agricultural University (China), PR China. The method of extraction is according the (Husam, 2010)

Antibacterial activity

One hundred milligram per milliliter concentrations were prepred from each fraction. Muller – Hinton agar and Sabouraud agar plates have been prepared then 200 µl of overnight bacterial and yeast broth cultures were plated as lawn form on the agar dishes and left to dry. Holes were made in the agar by sterile stainless steel cylinder (diameter = 6 mm) and 50 µl of 100mg/ml of each fraction were added in the holes, then incubated in 37℃ for 24 hr.

Chlomphinicol and Nystatin used as positive control while sterile normal saline was used as negative control in this experiment.

Inhibition zones were expressed in mm as diameter of clear zones around the holes.

Results and Discussion

The antimicrobial activity of Fraction 1, Fraction 2 and Fraction 3 of epicarb extract of *Nelumbo nucifera* against certain pathogens is shown in table 1. This study show no effect of epicarp extract fractions on the tested pathogens except some positive results with just two types of tested bacteria. The highest clear zone was obtained in fraction 2 with *Enterococcus faecalis* (14 mm) followed by the effect of same fraction on *E. coli* (12 mm) and finally there was a moderate effect of fraction 3 in 100 mg/ml concentration on *Enterococcus faecalis* with clear zone about (10 mm).

Another study show that *Nelumbo nucifera* pollen essential oil have inhibitory effect on growth of food born pathogen bacteria especially on Salmonella typhimurium and E. coli in low concentration which indicate the possibility of used as food preservation addidatives (Sitiiwet, 2009).

Other studies show that the stone lotus seed extracts in different solvents have significant in vitro antibacterial action which probably means that there are different compounds with antibacterial action (Ying *et al*., 2009). Also high antimicrobial activity of polyphenols that extract from another plant with high antioxidation effect has been show on certain bacteria. (Kosaleck *et al*., 2005) show high antimicrobial effect of polyphenolic compounds on many gram positive an gram negative bacteria except *Enterococcus faecalis* and *E. coli* and this is inversely effect with this study, and according to *Candida albicans* the (Kosaleck *et al*., 2005) study compounds have fungicidal effect while our fractions had no effect on this yeast.

On the other hand, there were studies show the mode of action of some phenolic compounds that extract from some plants, where there was study show that flavonoids compounds cause inhibition to DNA synthesis and RNA synthesis in different types of bacteria (Mori *et al*., 1987).

Table (1): Effect of extract fractions of *Nelumbo nucifera* epicarp against certain pathogens

Phenolic compound (100mg/ml)	Inhibition Zones (mm)				
	E. coli	***Enteroccous faecalis***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Candida albicans***
F1	**NIZ**	**NIZ**	**NIZ**	**NIZ**	**NIZ**
F2	**12**	**14**	**NIZ**	**NIZ**	**NIZ**
F3	**NIZ**	**10**	**NIZ**	**NIZ**	**NIZ**
Chloramphenicol (positive control)	**9**	**12**	**8**	**NIZ**	**NIZ**
Nystatin (Positive control)	**NIZ**	**NIZ**	**NIZ**	**NIZ**	**16**
Normal saline (Negative control)	**NIZ**	**NIZ**	**NIZ**	**NIZ**	**NIZ**

NIZ: No Inhibition Zone

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