Antibacterial Activity of Nutmeg (*Myristica fragrans*) Seed Extracts Against Some Pathogenic Bacteria

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Abstract

This study was designed to evaluate the antibacterial activity of water, ethanol and acetone *Myristica fragrans* seed extract tested against four bacteria species; two Gram-positive bacteria (*Bacillus subtilis,* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The susceptibility of these different bacterial species toward the extracts of this plants seed was compared with each other and between crust and bulb and with selected antibiotic (ciprofloxacin) used as positive control. Results showed that ethanol and acetone extracts exhibited antibacterial activity against gram positive bacterial species only, the diameter of inhibition zone reached 25mm against *Staph. aureus* by crust extract. Crust extract showed antibacterial activity more than the pulp extract. It is concluded that this plant can be indispensible source for secondary metabolites.

Keywords: *Mysritica frangrans*, plant extract, antibacterial activity.

Introduction

Several plants were reported for their many therapeutic and pharmaceutical virtues, especially antioxidant, anti-tumor, and anti-infectious activities. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses [1]. These benefits are due to their big content of bioactive compounds [2]. Since the introduction of antibiotics there has been tremendous increase in the resistance of diverse bacterial pathogens [3, 4]. This shift in susceptibility greatly affects the ability to successfully treat patients empirically. Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments [5]. They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also. Nutmeg tree is one of several tree species in the genus Myristica. The most important commercial species is *Myristica fragrans*, an evergreen tree indigenous to the Banda Islands in the Moluccas (or Spice Islands) of Indonesia. The nutmeg tree is important for two spices derived from the fruit: nutmeg and mace. Nutmeg is the seed of the tree, roughly egg-shaped and about 20 to 30 mm (0.8 to 1 in) long and 15 to 18 mm (0.6 to 0.7 in) wide, and weighing between 5 and 10 g dried, while mace is the dried "lacy" reddish covering of the seed. The first harvest of nutmeg trees takes place 7–9 years after planting, and the trees reach full production after 20 years. Nutmeg is usually used in powdered form. This is the only tropical fruit that is the source of two different spices. Several other commercial products are also produced from the trees, including essential oils, extracted oleoresins, and nutmeg butter [6].

Like other herbs and spices, the assumed health benefits of nutmeg have been many and varied, but substantially unproven. Historically, nutmeg has been used for stomach cramps to cure plague. Studies have shown that it lowers blood pressure and sooth a stomach ache as well as stop diarrhea and (at low doses) help to detoxify toxins in the body. The essential oil of the nutmeg is considered the most efficacious part of the plant. Nutmeg is known to have anti-inflammatory properties and can be used to treat joint and muscle pain. In holistic medicine it is considered an excellent liver tonic which removes toxins. Nutmeg oil is also a good herb for the kidney, helping in dissolving kidney stones as well as

relieves infections; many believe that heart problems may also be alleviated by nutmeg, as it increases blood circulation and stimulate the cardio-vascular system. It is also good for digestion, getting rid of both gas and stomach aches and relieving vomiting, diarrhea, and flatulence as well as encourages appetite. Nutmeg can also help with respiratory problems such as a cough from the common cold. It is often found as an ingredient in cough syrups. It is also said to be able to help with asthma [7].

Nutmeg extracts have a potential use as antifungal and antibacterial. This later antibacterial effect is interesting, as it appears to single out pathogenic bacteria while leaving normal flora unharmed. For example, the *157 E coli* strain is sensitive to nutmeg extract while the non-pathogenic strains of *E. coli* are not. A similar phenomenon happens in the mouth. *Streptococcus mutans*, the bacteria that cause cavities, are killed by nutmeg extract but the harmless bacteria are unaffected. From nutmeg, one can make a suitable insecticide against cockroaches. Nutmeg also provides protection to healthy cells against damage from radiation but, it is unclear what effect it has on cancer cells [8].Thus, it is aimed to investigate the antibacterial effect of seed pulp and crust extracts.

Material and methods

Plant Material

Nutmeg (*M. fragrans)* seeds were bought from local market, Baghdad. The collected seeds were separated to pulp and crust, and both coarsely powdered and then extracted separately.

Microbial Organisms

Two Gram-positive bacteria (*B. subtilis*, and *Staph. aureus*) and two Gram-negative bacteria (*E. coli*, and *P. aeruginosa*) were used throughout this study which kindly supplied by Dept. of Biotechnology, Al-Nahrain University. Gram-negative strains were cultured using nutrient broth while brain-heart infusion broth for Gram-positive strains.

Preparation of Crude Extracts

Dried seeds of *M. fragrans* (pulp and crust) were powdered separately and passed through sieve #10. 30 g. the sieved powder was weighed accurately and subjected to extraction using a soxhlet apparatus at room temperature using ethanol (99%), acetone or water successively. Before extraction with the next solvent, the powder was air dried to remove the adhering solvent. Extracts were then filtered, concentrated in a rotary flash evaporator and dried using a vacuum oven. Percentage yield of each extract was calculated and the dried extract was stored in air tight containers for further studies.

Preparation of Tested Microorganisms

The average number of viable *B. subtilis, Staph. aureus, E. coli, and P. aeruginosa* microorganisms per ml of the stock suspensions was determined by means of the surface viable counting method [9]. Inoculums (10^8-10^9) colony-forming units per ml were used. A fresh stock suspension was prepared; the experimental conditions were maintained constant so that bacterial suspensions with closed viable counts would be obtained.

In Vitro Examination of Anti-Microbial Activity of Extracts

The cup-plate agar diffusion method was adopted according to Kavanagh *et al* [10]. In order to assess the antibacterial activity of the prepared extracts, aliquot of 0.6ml of standardized bacterial stock suspensions (10^8-10^9) colony- forming units per ml was mixed with 60ml of sterile nutrient agar. Aliquot of 20ml of the inoculated nutrient agar was distributed into sterile Petri dishes. The agar was left to set in each of these plates, 1 cup (10 mm in diameter) was cut using a sterile cork borer No. 4. The agar discs were removed and filled with 0.1ml of the extract and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated in the upright position at 37ºC for 18 hrs. Replicates were carried out for the extract against each of the tested organism. Simultaneous addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of growth inhibition zones were measured.

Results and Discussion

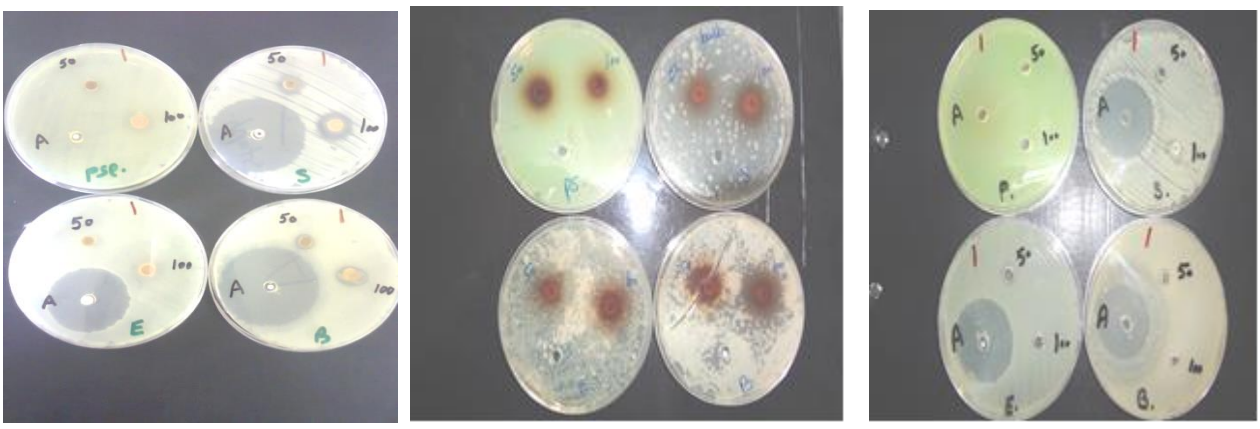
The three types of extracts (ethanol, acetone, aqueous) were subjected to preliminary screening for their antibacterial activity against four standard bacterial species namely*: E, coli, P. aeruginosa, Staph. aureus,*

and B. subtilis. Ethanol and acetone extracts for pulps and crusts showed high activity against gram positive bacteria while gram negative were resistant, the diameter of inhibition zone reached 25 mm against *S . aureus* and to 20 mm in diameter against *B. subtilis* at conc.100mg/ml, but the aqueous extract showed no antibacterial activity against those bacterial species(Fig.(1, 2)). This antibacterial activity was compared with that of antibiotic ciprofloxacin which is considered popular in treatment of diseases caused by the four types of bacterial species. The diameter of inhibition zone caused by ethanol and acetone extracts approached to that of antibiotic at conc.100 mg/ml. Crust extracts displayed greater activity than extracts of the pulp.

Table (1)
Antibacterial activity of M. fragrans extracted seed pulps examined by disc diffusion method using different solvent compared with ciprofloxacin.

Bacterial isolate	Mean Diameter of Growth Inhibition Zone (mm)							
	Ethanol 50 (mg/ml)	100	Acetone 50 (mg/ml)	100	Aqueous 50 (mg/ml)	100	Ciprofloxacin 100 (mg/ml)
Staph .aureus	15	20	22	23	-	-	30
B. subtilis	10	15	15	20	-	-	30
E. coli	-	-	-	-	-	-	25
P. aeruginosa	-	-	-	-	-	-	-

- : No inhibition zone



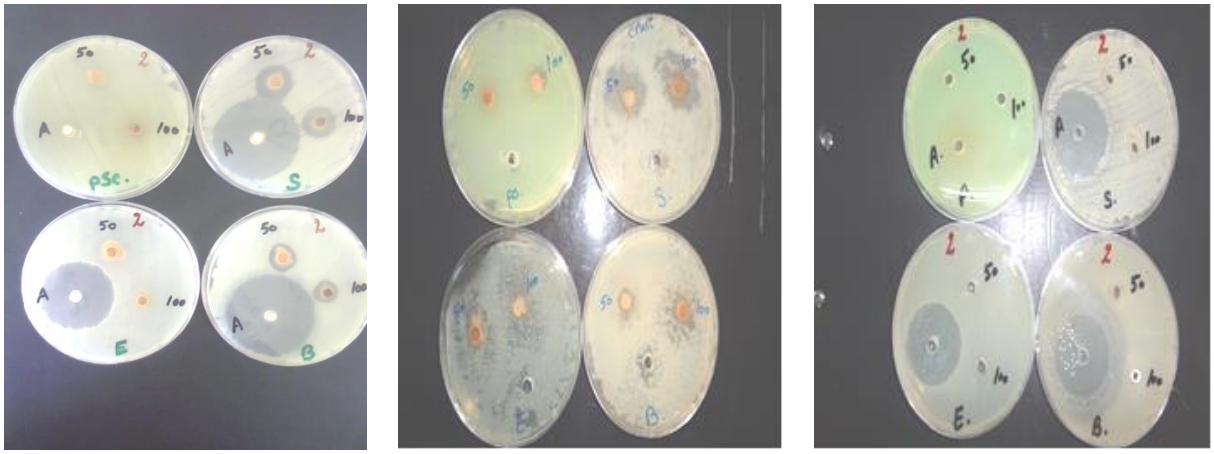
Ethanol extracts ***Acetone extract*** ***Aqueous extract***

Fig. (1) Inhibition zones caused by seed pulp extract against E. coli, P. aeruginosa, Staph. aureus, and B. subtilis after 18hrs of incubation period.

Table (2)
Antibacterial activity of M. fragrans extracted seed crust examined by disc diffusion method using different solvents compared with ciprofloxacin.

Bacterial isolate	Mean Diameter of growth Inhibition Zone (mm)						
	Ethanol		Acetone		Aqueous		Ciprofloxacin
	50 (mg/ml)	100	50 (mg/ml)	100	50 (mg/ml)	100	100 (mg/ml)
Staph .aureus	20	18	25	25	-	-	30
B. subtilis	14	12	10	15	-	-	30
E. coli	-	-	-	-	-	-	25
P. aeruginosa	-	-	-	-	-	-	-

- : No inhibition zone



Ethanol extracts *Acetone extract* *Aqueous extract*

Fig. (2) Inhibition zones caused by seed crust extract against E. coli, P. aeruginosa, Staph. aureus, and B. subtilis after 18hrs of incubation period.

Nutmeg oil contains monoterpenes such as -pinene, camphene, -pinene, sabinene, myrcene, a-phellandrene, a-terpinene, limonene, 1, 8-cineole, g-terpinene, linalool, terpinen-4-ol, safrole, methyl eugenol and myristicin,as their active principles. Their mode of antimicrobial action is related to their ability to inactivate microbial adhesion, enzymes and cell envelope proteins [11].

The cell wall structural nature of Gram-negative enteric bacteria may be responsible for the observed resistance. For instance, the cell wall of Gram-negative bacteria contains 15-20% polysaccharides and 10-20% lipid, whereas that of Gram-positive bacteria contains 35-60% polysaccharides and only 0-2% lipid [12]. The cell membrane of *E. coli* contains 20% lipid [13]. The polysaccharides and the lipid contents of the cell wall affect the permeability of active components, and thus the observed resistance to *M. fragrans* extract by the diarrhea microorganisms [14, 15].

The Gram-positive bacteria were more sensitive to the antimicrobial compounds in spices than Gram-negative bacteria. The extent of sensitivity varied with the isolates and environmental conditions imposed. Certain spices have a direct effect on the rate of fermentation by stimulating acid production in starter cultures. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in spices [16].

It is concluded that Nutmeg *(Myristica fragrans*) has antibacterial activity against gram positive bacteria in contrast to gram negative bacteria which are resistant to all studied extracts. The antibacterial activity of crust extract is more effective than bulb extracts.

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الخلاصة

صممت الدراسه الحاليه لتقييم التاثير ضد البكتيري للمستخلص المائي والكحولي والاسيتوني لقشر ولب بذور نبات جوز الطيب على بعض البكتيريا المرضيه . حضرت المستخلصات ودرس تأثيرها في نمو اربع انواع بكتيرية مرضيه مختلفة اثنان منها موجبه لصبغه كرام *Bacillus* (*subtilis*, and *Staphylococcus aureus*) واثنان سالبه لصبغه كرام (*Escherichia coli*, and *Pseudomonas aeruginosa*). باستخدام طريقه الانتشار عبر الوسط ألزرعي. قورنت النتائج بين الأنواع الاربعه وبين تاثير القشر واللب وبين تأثير المضاد الحيوي الواسع الانتشار (سيبروفلوكسيلين) على هذه الأنواع من البكتيرية المرضية.اظهرت النتائج بان المستخلص الكحولي والاسيتوني يمتلك فعاليه ضد بكتيريه ضد عزلات البكتريا الموجبه فقط وقد وصل قطر التثبيط الى ٢٥ ملم ضد المكورات العنقوديه. وان تاثير القشرة كان اكثر من تاثير اللب على البكتريا.