ANTIMICROBIAL ACTIVITY OF NUTMEG EXTRACTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* [+]

الفعالية الضد مايكروبية لمستخلصات جوزة الطيب ضد المكورات العنقودية الذهبية والأيشيريشيا القولونية

Sabah J. Ameen [*]

المستخلص:

تم تقييم الفعالية الضد مايكروبية للمستخلصات المائية والكحولية لجوزة الطيب ضد الأيشيريشيا القولونية والمكورات العنقودية الذهبية. استخدمت أربع تراكيز لكل مستخلص (١٠٠%، ٧٥%، ٥٠%، ٢٥%).عند تركيز ١٠٠% كان المستخلص المائي والكحولي لجوزة الطيب فعالاً ضد الأيشيريشيا القولونية وبمنطقة تثبيط قطرها ١٦ ملم و١٩ ملم على التوالي، بينما عند تركيز ٧٥% كان قطر منطقة التثبيط هو ١٤ ملم و ١٩ ملم على التوالي.أظهرت المكورات العنقودية الذهبية مقاومة للتراكيز ٢٥%، ٥٠%، ٧٥% لكل من المستخلصات المائية والكحولية، بينما في تركيز ١٠٠% أظهرت منطقة تثبيط بقطر ١٢ ملم و ١٣ ملم على التوالي.

Abstract:

The antimicrobial activity of aqueous and ethanolic extracts of *Myristica fragrans* (nutmeg) has been evaluated against *E. coli* and *Staph. aureus*. Four concentrations were used for each type of extract,(100%, 75%, 50%, 25%).
In the 100% concentration, the aqueous and ethanolic extracts of nutmeg were effective against *E. coli* with inhibition zones of 16 mm and 19 mm respectively, while in the 75% concentration, the inhibition zones were 14 mm and 19 mm, respectively.
***Staphylococcus aureus* showed a resistant to the 25%, 50%, 75% concentrations of aqueous and ethanolic extracts, while the 100% concentration showed inhibition zones of 12 mm and 13 mm, respectively.**

Introduction:

Bacterial infections are a world wide problem. In the last decade antibiotics resistant infections occurred demanding new therapeutic strategies. For people living in developing countries, mainly medicinal plants and natural substances are available for the treatment of infectious diseases [1, 2].
Food borne pathogens such as *E. coli* which is widely distributed in nature. It's implicated in large numbers of food borne outbreaks in many parts of the world, including the developed countries [3].

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[*]**Assistant Lecturer/Institute of Medical Technology\ Baghdad**

Early humans recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive men and women treated illness by using plants [4].
Enormous advances have been made in medical care, but many people are still using herbal or alternative remedies [5].
One of these plants is nutmeg, the shelled seed, and mace, its aril, are two valuable products derived from the tree *Myristica fragrans* Houtt. (Myristicaceae) that have been highly coveted for their aromatic and medicinal properties since at least the beginning of the common era [6].
Nutmeg seeds contain 20-40% of a fixed oil commonly called nutmeg butter. This oil contains myristic acid, trymirisin and glycerides of lauric, tridecanoic, stearic and palmitic acids [7,8].
Other isolated compounds include the resorcinols malabaricone B and C [9] as well as lignans and neolignans [10].

In Iraq many studies used different kinds of herbs against different types of pathogens, [11] found that alcoholic and aqueous extracts of *Myrtus communis* had a bactericidal effect against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeroginosa*.
However [12] found that *Myrtus communis* oil had an antibacterial activity against *Streptococcus pyogens, Staph,aureus, Pseudomonas aeroginosa* and *E. coli*.
The aim of this study is to determine the antimicrobial effect of aqueous and ethanolic extracts of nutmeg (*Myristica fragrans*) against *E. coli* and *Staphylococcus aureus*.

Materials and Methods:

Source of organisms:
The cultures were obtained from the Microbiology Laboratory of Baghdad Teaching Hospital and were preserved in agar slants.

Plant materials:
The dry plant Myristica fragrans was obtained from the local spice markets.

Preparation of aqueous and ethanolic extract:
Nutmeg was cleaned and washed in sterile distilled water and then crushed using an electric blender. 20 g of nutmeg were soaked in 100 ml of hot sterile water and allowed to stand for 72 hrs. The extract was considered as 100% in concentration. The crude extracts were filtered. The concentrations, 75%, 50%, 25% were made by diluting the concentrated extract with the required volume of sterile distilled water [14].
Similarly, 20 g of nutmeg were soaked in 100 ml of the 90% ethanol in a conical flask that sealed with foil and allowed to stand for 72 hrs. then filtered to obtained ethanolic extract, [15]. The concentrations, 75%, 50%, 25% were made by diluting the concentrated extract with the required volume of ethanol.

Antimicrobial activity:
The selected bacteria are inoculated into 10 ml of sterile nutrient broth, and incubated at 37˚C for 18-24 hrs. The nutrient broth cultures were swabbed on the surface of sterile Mueller Hinton agar plates.
By using sterilized cork borer, agar wells were prepared with (10 mm diameter) [16].

By using micropipette, 100 micro liters of different concentrations of nutmeg extracts (aqueous and ethanolic extracts) (100%, 75%, 50% 25%) were added to different wells in the plate, plates incubated at 37˚C for 24 hrs. the diameter of inhibition zones measured in mm and the results were recorded.

Results and Discussion:

Results of antibacterial activity of aqueous and ethanolic extracts of nutmeg against *E. coli* revealed that this bacteria were sensitive to the concentrations of 100% and 75% in both aqueous and ethanolic extracts which were 16 mm and 14 mm, respectively in aqueous concentration as showed in table (1) and (19, 16 mm), respectively in ethanolic extract as shown in table (2), however at concentration of 50%, both aqueous and ethanolic extracts, showed a decrease in inhibition zone to 12 and 14 mm respectively the bacteria was resistant to both extracts of nutmeg in 25% concentration.

Table (1): The in vitro activity of aqueous extract of nutmeg against *Escherichia coli* and *Staphylococcus aureus*.

Bacterial Types	Concentration of aqueous extract %	Mean inhibition zone of diameter ± SE.
E. coli	100 75 50 25	16 14 12 0
Staph. aureus	100 75 50 25	13 12 0 0

Table (2): The in vitro activity of alcoholic extract of nutmeg against *Escherichia coli* and *Staphylococcus aureus*.

Bacterial Types	Concentration of alcoholic extract %	Mean inhibition zone of diameter ± SE.
E. coli	100 75 50 25	19 16 14 11

Staph. aureus	**100** **75** **50** **25**	**14** **12** **0** **0**

Also, the results of nutmeg extracts (both aqueous and ethanolic) against *Staphylococcus aureus* showed inhibition zone in concentration 100% (13mm in aqueous extract and 14mm in ethanolic extract) and in 75% concentration (12mm in both extracts), however it has no effect in other concentrations.

In Iraq, many studies used different kinds of herbs (except nutmeg) against different types of pathogens [11, 12, 13, 17].

However, our results are in agreement with the results obtained by Indu, *et al* who found that nutmeg aqueous extract had an antibacterial activity against different serotypes of *E. coli, Salmonella spp., Listeria*, and *Aeromonas hydrophila*. [14]

Another study revealed that ethyl alcohol extract of nutmeg had an antibacterial activity against the enteropathogenic *E. coli.* [18]

Other study in Morocco showed that *Myristica fragrans* has antimicrobial activity against both gram positive and gram negative bacterial species. [19]

While others found that mace which is a layer that surrounds the nutmeg seeds have two antimicrobial agents the first one is malabaricone B and the second one is malabaricone C and these agents exhibited a significant level of antimicrobial activity against many microorganisms including *Candida albicans* and *Staphylococcus aureus*. [9,20]

Other study concluded that ethanolic extracts and essential oils of 14 spices (including nutmeg) had antimicrobial activity against *Salmonella* and other enterobacteria such as *E. coli*. [21]

Another researches support the information on the antibacterial activity of *Myristica fragrans*, particularly against *Streptococcus mutans* [22], and against oral microorganisms [23] and also against oral colonizer bacteria [24].

In conclusion; the aqueous and ethanolic extracts of nutmeg were effective against both *E. coli* and *Staph. aureus* and further study required to reveal which part of their constituents has the antibacterial activity.

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