

Evaluation of Antibacterial effect of extracted fatty acids of Nutmeg *Myristica fragrans* (Houtt.) against locally isolated bacteria

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Abstract

This study has been done in order to evaluate the antibacterial activity of six different concentrations of fatty acids were extracted from *Myristica fragrans* (FAN) against five different G-ve bacterial isolates from pathological cases was studied, agar well diffusion method was used to determine this activity and the zone of inhibition in millimeters was the criterion, results showed inactivity of concentrations (10, 20, 40, and 60%) in comparison to partial activity in using of (80 and 100)% of FAN against all bacterial isolates except *P. aeruginosa*. which their results showed inactivity of 10% FAN against its' growth, in comparison to 20%, 40% and 60% whose appeared to inhibit the growth of *P. aeruginosa* actively, this impact was gradually progressed to be very active in using of 80% and 100% of FAN against the same bacteria.

تقييم الفعالية المضادة للبكتيريا للأحماض الدهنية المستخلصة من جوزة الطيب ضد البكتيريا

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الخلاصة

أجريت هذه الدراسة لغرض تقييم الفعالية المضادة للبكتيريا لستة تراكيز مختلفة من الأحماض الدهنية المستخلصة من جوزة الطيب ضد خمسة عزلات بكتيرية مختلفة معزولة من حالات مرضية واستعملت طريقة الانتشار حول الحفر لتحديد هذه الفعالية وكان المعيار هو منطقة التثبيط مقاسة بالمليميتر، أظهرت النتائج عدم فعالية التراكيز (10، 20، 40، 60)% بالمقارنة مع فعالية جزئية لكل من التراكيز (80% و 100%) من الأحماض الدهنية لجوزة الطيب ضد جميع العزلات البكتيرية عدا الزوائف الزنجارية والتي أظهرت نتائج عدم فعالية تركيز 10% من الأحماض الدهنية لجوزة الطيب ضد نموها، بالمقارنة مع التراكيز (20، 40، 60)% والتي أظهرت تثبيط نمو الزوائف الزنجارية بصورة فعالة، هذا التأثير ازداد تدريجيا ليصبح فعالا جدا باستعمال التراكيز 80% و 100% من الأحماض الدهنية لجوزة الطيب ضد نفس البكتيريا.

Introduction

Nutmeg (*Myristica fragrans* Houtt.), a tropical evergreen dioecious tree with a narrow range of distribution is the source of the high value medicinal spices, it consists from two parts, mace (the reddish aril) and nutmeg (endosperm or seed) (1). About 30-55% of the seed consists of oils and 45-60% consists of solid matter including cellulose materials. (2) The oil of nutmeg has been used for medicinal purposes and it is the fraction of the nutmeg which contains the pharmacologically active components (3). There are two types of oils can be extracted from nutmeg: Essential oil (volatile oil) which accounts for 5-15% of the nutmeg seed. (2) And fixed oil, who sometimes called the butter of nutmeg and it accounts for 22.3 to 46.9 wt% of fatty material. Many studies have been achieved to evaluate the antibacterial of nutmeg volatile oil components (4) in contrast to nutmeg fixed oil components. The aim of this study is to evaluate the antibacterial effect of fatty acids of Nutmeg against locally isolated pathogenic bacteria.

Material and Methods

This study has been achieved at Department of physiology and pharmacology/ veterinary medical College/ Diyala University from 7 April to 11 July 2011.

- **Plant material preparation:** Plant material of Nutmeg were purchased from the local markets of Diyala province, nutmeg fruits have been selected carefully then cleaned from the outer husk that shells the seeds. Seeds were grinded with electrical grinder (Emerald corp. china) then, extracted within n-hexane of analar grade (BDH, England) in Soxhulate apparatus (fine tech, South Korea) boiling range 60-80 °C (5) The oil was then poured in to a beaker and left for 5 days for the remaining solvent to evaporate. The resultant oil was heated with sodium hydroxide (BDH, England) then acidified with Hydrochloric acid (Merck, Germany) to obtain the crude fatty acids mixture (6).
- **Preparation of drug solution:** The extracted Fatty acids of Nutmeg (FAN) were dissolved in Di-methyl sulfoxide DMSO (Merck, Germany) to prepare different concentrations (10, 20, 40, 60, 80 and 100)% of (FAN).
- **Microorganisms' isolation and identification:** A total of 25 samples collected from animals (None had been received treatment.) with different pathological cases attending the Microbiology lab in the college, the identity of the cultures was confirmed by standard bacteriological methods (7). The cultures were plated on nutrient agar medium (Hi- Media, Mumbai, India) and incubated for 24 h at 37°C.

Bacteria were diagnosed depending upon standard morphological and physiological characteristics (Gram -ve bacilli microscopically, alpha or beta hemolysis on blood agar). Biochemical tests (Catalase, Coagulase, Oxidase, IMViC and TSI), and culture on manitol salt agar (selective and differential medium).

- **Antibacterial assay:** The agar diffusion assay described by (8) was adopted. Briefly, *Escherichia coli*, *Salmonella typhimurium*, *proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were incubated at 37°C for 24 h, and diluted to final concentration of approximately 10^6 CFU/ml; 0.5 MacFarland. Each bacterial suspension was spread over the surface of Mueller-Hinton agar containing seven wells of 6 mm diameter. These wells were filled with 100 µl of 10%, 20%, 40%, 60%, 80% and 100% (FAN) in addition to sterile DMSO whom served as a negative control. The plates were allowed to stand for at least 1 h for diffusion to take place and then incubated at 37°C for 24 h. The zone of inhibition (mean of three assays) was recorded to the nearest size in millimeters (9). the results were expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9 - 12 mm, partially active; 13 - 18 mm, active; >18 mm, very active (10).

Results

The antibacterial activity of different concentrations of the fatty acids of Nutmeg (FAN) has been evaluated against locally isolated gram-negative pathogenic bacteria. (*Escherichia coli*, *Salmonella typhimurium*, *proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). Agar well diffusion method that utilizes the zone of inhibition in milliliters as a criterion were adopted, table 1 showed the antibacterial activity of different concentrations of (FAN) against bacterial isolates, the method of (6) was used to recognize which one of these dilutions is inactive, relatively active, active or very active against each bacterial isolate, the proportionality relationship between FAN concentrations and zone of inhibition was derived by dose response relationship and figured graphically figures 1-5. Results showed the inactivity of all concentrations of (FAN) against *K. pneumoniae* except the 100% (FAN) whom showed partial activity (Table 1 and Fig. 1), on the other hand, 100% (FAN) inhibits *E. coli* growth actively in comparison to the other (FAN) concentrations. (Table 1 and Fig. 2), *P. vulgaris* resist

the antibacterial effect of 10%, 20%, 40% and 60% of (FAN), while the concentration of 80% (FAN) was partially active against *P.vulgaris* and 100% (FAN) inhibits the growth of *P.vulgaris* actively. (Table 1 and Fig. 3), All concentrations of (FAN) showed partial activity against *S. typhimurium* except the 10% (FAN) whom inactive against the same bacteria. (Table 1 and Fig. 4), *P. aeruginosa* are insensitive to 10% of (FAN), while all of 20%, 40% and 60% of (FAN) appear to be active against *P. aeruginosa*, both of 80% and 100% are very active against *P. aeruginosa*. (Table 1 and Fig. 5).

Table (1) Diameter of mean (triplicate) zone of inhibition (mm)

Bacterial isolates	Concentrations of (FAN).						
	%10	20%	40%	60%	80%	100%	DMSO
<i>K. pneumoniae</i>	0	0	0	4	8	9	0
<i>E. coli</i>	0	4	7	10	10	14	0
<i>P. vulgaris</i>	0	0	0	7	9	13	0
<i>P. aeruginosa</i>	3	13	13	17	20	22	0
<i>S. typhimurium</i>	0	9	10	10	11	12	0

	Inactive		Partially active		Active		Very active
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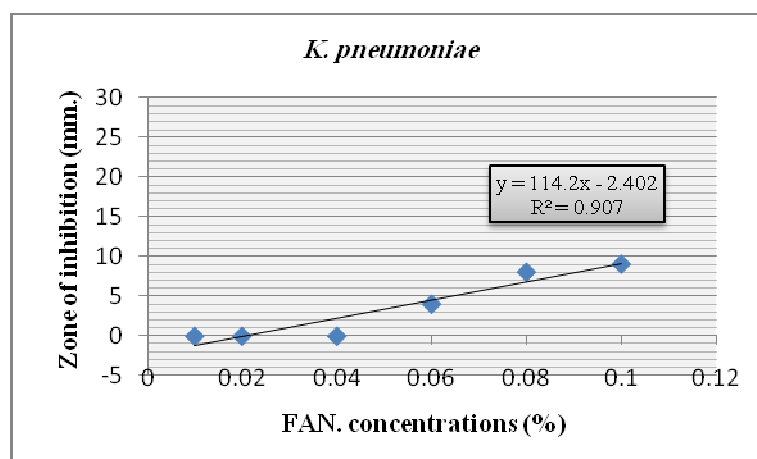


Figure (1) Relationship between (FAN). concentrations (%) and zone of inhibition (mm) for *K. pneumoniae*

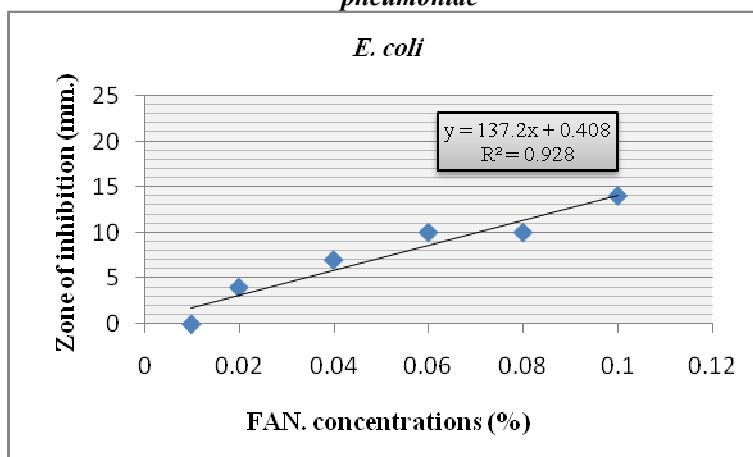


Fig. (2) Relationship between (FAN). concentrations (%) and zone of inhibition (mm) for *E. coli*

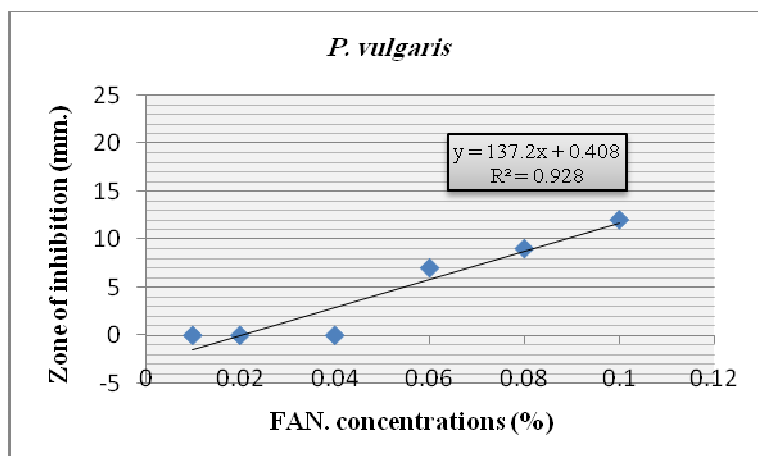


Fig. (3) Relationship between (FAN). concentrations (%) and zone of inhibition (mm) for *P. vulgaris*

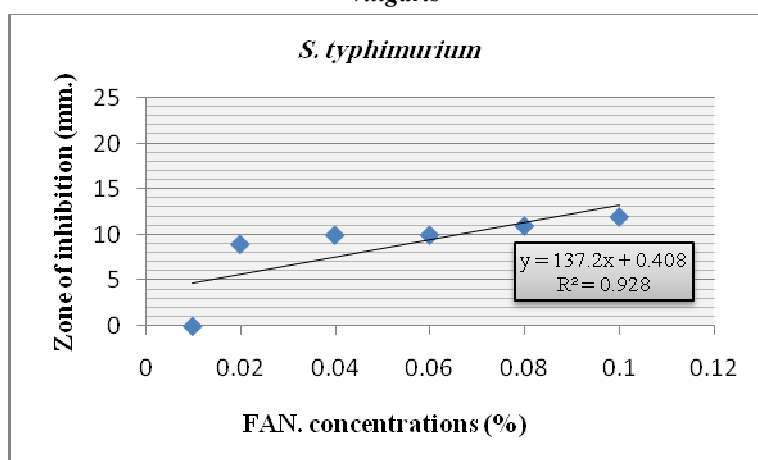


Fig. (4) Relationship between (FAN). concentrations (%) and zone of inhibition (mm) for *S. typhimurium*

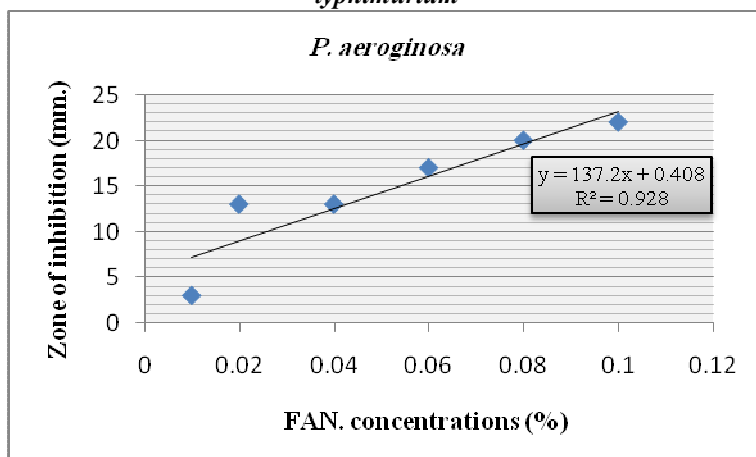


Fig. (5) Relationship between (FAN). concentrations (%) and zone of inhibition (mm) for *P. aeruginosa*

Discussion

The antibacterial activity of fatty acids of Nutmeg (FAN) were investigated in this study, agar well diffusion method were used to determine this activity and the zone of inhibition in millimeters was the criterion, the composition of fatty acids of Nutmeg was studied by (11) and (12) and the fatty acids were exist in nutmeg were., Lauric acid 0.4%, Myristic acid 71.8%, Palmatic acid 14.3%, Stearic acid 1.2%, Myristoleic acid 0.8%, Palmioleic acid 4.8%, Oleic acid 5.2% and Linoleic acid 1.5%. The chief antibacterial activity can be attributed to the inhibitory capability of these fatty acids on

the enoyl- acyl carrier protein reductase (FabI) whom responsible of bacterial fatty acids synthesis (13). Fatty acid synthesis in bacteria is essential to the production of a number of lipid-containing components, including the cell membranes (14). All results conducted to inactivity of 10%, 20%, 40% and some times 60% (FAN) against all tested bacterial isolates but except *P. aeruginosa*, while both of 80% and 100% (FAN) showed partial activity against all tested bacterial isolates but *P. aeruginosa* this can be explained by weak sensitivity of gram negative bacteria to antibacterial activity of fatty acids due to chemical composition of their cellular membrane(15). Results also showed inactivity of 10% (FAN) against *P. aeruginosa* in contrast to 20%, 40%, and 60% which appeared to be active against *P. aeruginosa* and this activity developed to be very active in using of 80% and 100% of (FAN) against the same bacteria, this activity might be attributed to presence of myristic and palmitic acids in (FAN) both of them thought to be chelating agents to Ca^{+2} and Mg^{+2} from the Lipopolysaccharide (LPS) of the outer membrane of *P. aeruginosa* causing release of LPS thereby destabilizing the membrane and making it permeable to the rest fatty acids that exist in (FAN) (16). In addition to the capacity of fatty acids to inhibit the growth and virulence factors in *P. aeruginosa* in comparison to other bacterial isolates that used in this study (17, 18).

References

1. Latha, P. G.; Sindhu, P. G.; Suja, S. R.; Geetha, B. S.; Pushpangadan, P. & Rajasekharan, S. 2005. Pharmacology and chemistry of *Myristica fragrans* Houtt. - a review. *J. Spices & Aromatic Crops*. 14: 94-101.
2. Forrest, J. E. & Heacock, R. A. 1972. Nutmeg and Mace, the psychotropic spices from *myristica fragrans*. *Lloydia*, 35(4): 440-449.
3. Nadkarni, K. M. 1988. *Myristica fragrans*. In: *Indian Materia medica* 3rded., Bombay Popular Prakashan, Bombay, India, PP. 830-834.
4. Jaiswal, P.; Kumar, P.; Singh, V. K. & Singh, D. K. 2009. Biological Effects of *Myristica fragrans*. *ARBS Ann. Review of Biomedical Sci.*, 11:21-29.
5. Sonavane, G. S.; Sarveiya, V. P.; Kasture, V. S. & Kasture, S. B. 2002. Anxiogenic activity of *Myristica fragrans* seeds. *Pharmacol Biochem Behav.*, 71:247-252.
6. Johnson, W. R. & Fritz, E. 1989. *Fatty Acids in Industry: Processes, Properties, Derivatives and Applications*. Marcel Dekker, Inc New York, USA, P. 23.
7. Cheesbrough, M. 1984. *Medical Laboratory Manual for Tropical Countries*. Vol. II. Microbiology. Linacre House, Jordan Hill, Oxford. PP. 372-39.
8. Smânia, A.; Delle Monache, F.; Smânia, E. F. A.; Gil, M. L.; Benchetrit, L. C. & Cruz, F. S. 1995. Antibacterial activity of substance produced by the fungus *Pycnoporus sanguineus* (Fr.) merr. *J. Ethopharmacol*, 45: 177-181.
9. Norrel, S. A. & Messley, K. E. 1997. *Microbiology Laboratory Manual Principles and Applications*. Prentice Hall, Upper Saddle River. New Jersey.
10. Junior, A. & Zani, C. 2000. Biological Screening of Brazilian medicinal plants. *Brazilian J. of Sci.*, 95(3): 367-373.
11. Pathak, S. P. & Ojha, V. N. 1957. The component glycerides of nutmeg butter (*Myristica fragrans*). *J. Sci. Food Agric.*, 8: 537-540.
12. Spricigo, C. B; Pinto, L. T.; Bolzan, A. & Novais, A. F. 1999. Extraction of essential oil and lipids from nutmeg by liquid carbon dioxide. *J. Supercritical Fluids.*, 15: 253-259.
13. Zheng, C. J.; Yoo, J. S.; Lee, T. G.; Cho, H. Y.; Kim, Y. H. & Kim, W. G. 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters* 579: 5157-5162
14. Heath, R. J.; White, S. W. & Rock, C. O. 2001. Lipid biosynthesis as a target for antibacterial agents. *Prog. Lipid Res.* 40: 467-497.

15. Agoramoorthy, G.; Chandrasekaran, M.; Venkatesalu, V. & Hsu, M. J. 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Brazilian J. of Microbiol.*,38(4):739-742.
16. Krogfelt, A. K.; Utley, M.; Krivan, C. H.; Laux, C. D. & Cohen, S. P. 2000. Specific phospholipids enhance the activity of β -lactam antibiotics against *Pseudomonas aeruginosa*. *J. Antimicrobial Chemotherapy*. 46(3): 377-384.
17. Levison, M. E. 1973. Effect of colon flora and short-chain fatty acids on growth in vitro of *Pseudomonas aeruginosa* and Enterobacteriaceae. *Infection and Immunity*, 8 (1) 30-35.
18. Inoue, T.; Shingaki, R. & Fukui, K. 2008. Inhibition of swarming motility of *Pseudomonas aeruginosa* by branched-chain fatty acids. *FEMS Microbiol. Letters*, 281: 81-86.