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Influence of methanol fruit and leaf extracts of *Myristica fragrans* (Myristicaceae) on the activity of some antibiotics

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The emergence of bacterial resistance to antibiotics is a serious draw back in the management of infections. In this study, the antibacterial activity of the methanol fruit and leaf extracts of *Myrisctica fragrans* Houtt. (Myristicaceae) against typed strains of *Staphylococcu saureus*, *Bacillus suibtilis*, *Enterococcus faecalis*, *Proteus vulgaris*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* were determined using a modified Kirby-Bauer agar well diffusion method. Their influence on the minimum inhibitory concentration of ciprofloxacin, tetracycline, erythromycin and amoxicillin against the test organisms were also determined using themicro-dilution method. The extracts exhibited antibacterial activity against all the test organisms. In the presence of sub-inhibitory concentrations of the leaf and fruit extracts (1.0 and 2.0 mg/mL respectively), the antibacterial activity of amoxicillin against all the organisms was nullified. The antibacterial activity of ciprofloxacin against all the organisms was also cancelled by the fruit extract while the leaf extract acted similarly against all the test organisms except *E. faecalis* and *S. typhi*both of which saw four-fold reduction in susceptibility. The antibacterial activity of erythromycin against all the test organisms were nullified by both extracts except *S. aureus* and *B. subtilis* where the leaf extract caused an 8-fold reduction in activity. In the presence of the leaf extract, tetracycline lost activity against *S. aureus*, *P. aeruginosa* and *P. vulgaris*, its activity was reduced 16-fold against *B. subtilis* and *E. faecalis* and 32-fold against *E. coli*. The fruit extract caused a complete loss of activity of tetracycline against *S. aureus*, *P. aeruginosa*, *B. subtilis* and *E. faecalis*; there was 16-fold, 8-fold and 32-fold reduction in activity against *P. vulgaris*, *S. typhi* and *E. coli* respectively. The extracts of *M. fragrans* enhanced the resistance of these organisms to all the antibiotics used.

Key words: Antibiotic resistance, antibacterial, resistance enhancing, minimum inhibitory concentration, *Myristica fragrans*.

INTRODUCTION

The discovery of penicillin by Alexander Fleming paved the way for major research into the discovery and

synthesis of various kinds of antibiotics (Bennett and Chung, 2001). However, infectious diseases that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapy (Levy and Marshall, 2004).

Many mechanisms have been established for microbial resistance to antimicrobial agents and include drug inactivation or modification, alteration of target site, alteration of metabolic pathway, reduced drug accumulation in the organisms and alteration of membrane permeability (Nelson, 2002).

Some of the factors that promote bacterial resistance to antimicrobial agents include mis-diagnosis, drug counterfeiting, misuse and abuse of antibiotics and disposal of antibiotics in food production and animal rearing, indiscriminate prescribing, noncompliance and under dosing (Spratt, 1994).

Efforts are being made to curb the problem of microbial resistance to antibiotics including improving infection control, developing new antibiotics, using antibiotics more appropriately and modulating microbial resistance to already existing antibiotics (Adu et al., 2009; Gbedema et al., 2010).

The problem of bacterial resistance to antibiotics, has limited the use of cheap and routine antibiotics, and this has necessitated the need for a continued search for new antimicrobials (Sibanda and Okoh, 2007). The search for new antibiotics is usually difficult considering the number and nature of mechanisms and factors associated with resistance (Levy and Marshall, 2004, Stewart and Costerton, 2001).

Nutmeg is the dried kernel of the seeds of *Myristica fragrans* Houtt. which belongs to the family Myristicaceae. It is a popular food additive used in many formulations. *M. fragrans* is also used in the perfumery and pharmaceutical industries, in toothpaste and as a major ingredient in some cough syrups. In traditional medicine, nutmeg and its oil are used for disorders related to the nervous and digestive systems such as infantile diarrhoea. Nutmegs, maces and their oils are largely used for flavouring and as carminative (Evans, 2002). The aim of the study is to determine the antibacterial activity of methanol fruit and leaf extracts of *M. fragrans* and their influence on activity of some antibiotics including amoxicillin, ciprofloxacin, tetracycline and erythromycin.

MATERIALS AND METHODS

Collection and preparation of plant materials

The fresh leaves of *M. fragrans* were obtained from the Physique Garden at the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The fruits were obtained from the Kumasi central market. They were authenticated by Dr. G. H. Sam, Department of Herbal Medicine where a voucher specimen of the leaves (KNUST/HM1/2013/L-014) has been deposited. The leaves were washed, dried at 28 to 30°C for 5 days and milled into coarse powder using a laboratory mill machine (Type 8, Christy & Norris, UK). The fruits were also similarly processed. All the chemicals and culture media were purchased from Sigma-Aldrich, St Louis, MO, USA unless otherwise stated.

The powdered leaves and fruits materials (200 g each) were soxhlet extracted using methanol and concentrated under reduced pressure using a rotary vapor (Buchi, Germany). The concentrates were lyophilized. A brown semi-solid extract was obtained for the leaves giving a yield of 12.25% w/w; whiles fruits had yield of 7.76% w/w (related to the dried material).

Test organisms

Enterococcus faecalis ATCC 29212, *Salmonellatyphi* ATCC 19430, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* 25922, *Bacillus subtilis* NCTC 10073 and *Proteus vulgaris* NCTC 4175.

Preliminary phytochemical screening

The methanol leaf and fruit extracts were screened for secondary metabolites using methods described by Sofowora (1993) and Harborne (1998).

Determination of antimicrobial activity

The antimicrobial activity of the extracts was determined using both the Kirby-Bauer agar disc diffusion method and the broth dilution method using micro-dilution method (Eloff, 1998).

Petri dishes containing 20 mL of Muller-Hinton agar were poured and allowed to set. Overnight cultures of the test organisms grown at 37°C in Muller-Hinton broth were diluted to 0.5 McFarland standards with saline. Ten microliters of the bacterial culture was spread over the surface of the agar and allowed to dry for 10 min. Filter paper discs (6 mm in diameter) soaked in the various concentrations (20.0, 10.0, 5.0 and 2.5% w/w) of the extracts were placed on the inoculated agar. Discs containing tetracycline (10µg/disc) were placed as positive control.

These were incubated at 37°C for 24 h. The antibacterial activity against each test organism was quantified by determining mean zone of growth inhibition. The procedure was done in triplicate and the mean zones of inhibition recorded.

The minimum inhibitory concentrations (MIC) of the extracts and the test antibiotics (ciprofloxacin, tetracycline, erythromycin and amoxicillin) against the various organisms were determined using the micro-dilution method (Eloff, 1998). The 96-well plates were prepared by dispensing 150 µL of inoculated broth and 50 µL of plant extract or antibiotics constituted in broth or 50µL broth (MHB) in the case of negative control in each well. The plates were incubated at 37°C for 24 h. Presence of bacterial growth was determined by the addition of 20 µL (0.2 mg/mL) of 3-(4,5-

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Table 1. Antibacterial activity of methanol leaf and fruit extracts of *M. fragrans* against test organisms. Mean zones of inhibition are mean of zone of growth inhibition plus or minus standard deviation of triplicate experiments.

Concentration (%w/w)/organisms	Mean zones of inhibition (mm) ±SEM							
	40.0		20.0		10.0		5.0	
	LE	FE	LE	FE	LE	FE	LE	FE
S. aureus	13.4±0.3	16.5±0.1	12.5±0.0	14.0±1.0	11.0±0.2	13.0±0.0	9.8±0.2	11.5±0.1
P. vulgaris	13.6±0.1	22.0±1.0	12.2±0.2	20.5±0.1	10.8±0.3	17±2.0	9.1±0.2	15.0±1.0
P. areuginosa	15.0±0.0	12.5±0.1	13.8±0.2	10.5±0.2	12.0±0.3	10.0±1.0	9.7±0.4	8.5±0.1
B. subtilis	12.5±0.1	15.5±0.0	11.4±0.3	13.5±0.2	10.8±0.2	12.0±1.0	9.3±0.3	9.5±0.1
S. typhi	13±0.3	16.0±1.0	11.5±0.2	13.5±0.1	9.5±0.1	10.2±0.2	0.0	0.0
E. faecalis	13.8±0.4	16.4±0.2	11.6±0.1	13.8±0.1	10.4±0.2	11.3±0.2	8.4±0.1	9.4±0.3
E. coli	12.3±0.2	13.5±0.2	10.2±.2	11.5±0.0	8.5±0.1	9.5±0.1	8.0±0.1	8.5±0.1

FE = Fruit extract, LE = leaf extract; SEM: standard error mean.

Table 2. Minimum inhibitory concentration (mg/mL) of methanol leaf and fruit extracts of *M. fragrans* against test organisms.

Extract	Minimum inhibitory concentration (mg/mL) of extract against the test organisms						
	S. aureus	*P. aeruginosa*	*B. subtilis*	*P. vulgaris*	*S. typhi*	*E. faecalis*	*E. coli*
Leaf extract	4.0	8.0	2.0	4.0	2.0	2.0	4.0
Fruit extract	16.0	16.0	8.0	16.0	8.0	16.0	16.0

dimethylthiazole-2- yl)-2,5-diphenyltetrazolium bromide (MTT).

The minimum inhibitory concentrations (MIC) of the antibiotics against the various organisms were re-determined as above in the presence of sub-inhibitory concentrations of the extracts (1.0 and 2 mg/ml for the methanol leaf and fruit extracts, respectively).

RESULTS

Phytochemical tests

The preliminary phytochemical analysis indicated the presence of alkaloids, glycosides (including saponin glycosides) and both condensed and true tannins in both extracts.

DISCUSSION

The antimicrobial activity of *M. fragrans* has already been established by other workers (Singh et al., 2005). In this study, the methanol extracts of both the leaves and fruits of *M. fragrans* were found to have antibacterial activity against all the test organisms used and the activity increased with increasing concentration. Though, the leaf extract exhibited a more potent activity against all the test organisms than the fruit extract, as indicated by lower MIC values (Table 2), the fruits extract consistently showed larger zones of growth inhibition against all the test organisms except *P. aeruginosa* where the reverse was true (Table 1). This may be attributed to factors associated with the diffusion of the active constituents into the agar. The minimum inhibitory concentrations of the leaf extracts ranged from 2.0 mg/mL against *B. subtilis, S. typhi* and *E. faecalis* to 8.0 mg/mL against *P. aeruginosa;* while that of the fruit extract ranged from 8.0 mg/mL for *B. subtilis* and *S. typhi* to 16.0 mg/mL for all the other test organisms.

Resistance of microorganisms to antibiotics has usually been blamed on factors associated with the organisms though other factors may be responsible. Thus, under certain circumstances, it may be more appropriate to describe the situation as reduced activity of the antibiotic rather than increased resistance of the organism. This is because it may be possible that the lack of activity of the antimicrobial agent against the organism may be due to factors outside the organism.

In this study the reference antibiotics (amoxicillin, ciprofloxacin, erythromycin and tetracycline) showed activity against all the test organisms with MIC ranging from 1 to 128 µg/ml (Table 3). In the presence of sub-inhibitory concentrations of the methanol extract of *M. fragrans* leaves (1.0mg/ml) and fruits (2.0mg/ml) the MIC of the antibiotics ranged from 8 to 512 µg/ml (Table 4). The extracts thus caused drastic reduction in the antibacterial activities of all the antibiotics.

Amoxicillin lost its activity completely against all test organisms at all the concentrations used in the presence of both extracts (Table 4). Ciprofloxacin also lost its activity completely against all the test organisms used except *P. vulgaris* and *E. faecalis* in the presence of the

Table 3. Minimum inhibitory concentration of selected reference antibiotics against the test organisms.

Antibiotic	Minimum inhibitory concentration (µg/mL) of antibiotic against the test organisms						
	S. aureus	*P. areuginosa*	*B. subtilis*	*P. vulgaris*	*S. typhi*	*E. faecalis*	*E. coli*
Amoxicillin	8.0	-	16.0	32.0	32.0	64.0	64.0
Ciprofloxacin	1.0	1.0	2.0	2.0	1.0	2.0	1.0
Erythromycin	64.0	128.0	64.0	64.0	128.0	128.0	128.0
Tetracycline	16	32.0	8.0	8.0	16.0	8.0	4.0

- = No activity.

Table 4. Minimum inhibitory concentration (MIC) of reference antibiotics in the presence of sub-inhibitory concentrations of *M. fragrans* extracts.

Antibiotic	MIC (µg/mL) of antibiotic against the test organisms in the presence of *M. fragrans* extract													
	S. aureus		*P. areuginosa*		*B. subtilis*		*P. vulgaris*		*S. typhi*		*E. faecalis*		*E. coli*	
	LE	FE	LE	FE	LE	FE	LE	FE	LE	FE	LE	FE	LE	FE
Amoxicillin	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	-	-	-	-	-	-	8.0	-	-	-	8.0	-	-	-
Erythromycin	512.0	-	-	-	512.0	-	-	-	-	-	-	-	-	-
Tetracycline	-	-	-	-	128.0	-	-	128.0	64.0	128.0	128.0	-	128.0	128.0

-= No activity, FE = fruit extract, LE = leaf extract.

leaf extract both of which had a four-fold reduction in activity. Similarly, erythromycin lost activity against all the test organisms except *S. aureus* and *B. subtilis*, both of which also, in the presence of the leafextract, had an eight-fold reduction in activity.

Tetracycline was more resilient to the action of the extracts. Both extracts nullified its activity against *S. aureus* and *P. aeruginosa*. The methanol leaf extract nullified its activity against *P. vulgaris* while the fruits extract nullified its activity against *B. subtilis* and *E. faecalis*. There were four-fold and eight-fold reduction in activity against *S. typhi* by the leaf and fruit extracts, respectively. There was a sixteen-fold reduction in activity against *E. faecalis* and *B. subtilis* in the presence of the leaf extract and against *P. vulgaris* in the presence of the fruit extracts. *E.coli* showed a thirty-two-fold reduction in activity in the presence of both the leaf and fruit extracts.

The reduction in activities of the antibiotics seen in this study can be due to several factors that may be associated with the interaction between the antibiotics and the organism, the antibiotic and the phytoconstituents of the extracts or the organism and the phytoconstituents.

In the first instance, the phytoconstituents may act to enhance the various resistance mechanisms the organisms employ to evade the antibiotic action. The phytoconstituents may be protein activators, or co-enzymes, binding to and activating enzymes that are involved in the resistance mechanisms of the organisms (Lambert, 2002). The mechanisms may be genetic and naturally associated with the organisms or may be introduced as a result of the environment in which the organism finds itself. Thus, an organism very sensitive to a particular antimicrobial agent in one environment may be resistant to the same antimicrobial agent in another environment. In the second instance, the phytoconstituents may interact with the antibiotics by binding to the active moieties or react chemically with them resulting in loss of activity (Adu et al., 2009). In the third situation, the phytoconstituents may interact with the surface structures of the organism reducing the permeability of the cell to the antibiotics. It is known that certain substances protect organisms from the lethal effects of certain agents (Keweloh et al., 1989) and it may be possible that some phytoconstituents of *M. fragrans* exhibited this effect.

The effects of all these are that the extracts may prevent the antibiotics from reaching the target sites by inhibiting the penetration of the antibiotic into the organism or produce conformations that make the antibiotics unable to fit its receptor. It may also bind to or modify the functional groups that are responsible for the activity (Barza et al., 1976).

Minimum inhibitory concentration (MIC) is an important laboratory diagnostic tool used to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). MIC is generally regarded as a basic laboratory measurement of the activity of an antimicrobial agent against an organism (Budak and Ubeyli, 2011).

The antibiotics used in this study act by different mechanisms and the mechanism of resistance employed by the test organisms also differ for each antibiotic and also for each organism. This presupposes that the resistance enhancement exhibited by the extract may not involve the known resistance mechanisms of the organisms to the antibiotics neither does it involve the mechanism at the site of action of these antibiotics. Probably, the extracts created a new environment or condition that does not allow the antibiotic to penetrate the organism or does not allow the antibiotics access to their sites of action.

The results of the study indicate that concurrent administration of certain substances with antibiotics may result in treatment failures due to interaction of these substances with the antibiotics outside the infecting organisms, a situation that can be best described as resistance enhancing. It further suggests that certain factors including concomitant administration of antibiotics and other substances including certain foods and food additives can alter bacteria susceptibility to antibiotic therapy.

Since *M. fragrans* is used as food additive, it can be said that certain foods containing fruit extracts of *M. fragrans* cannot be taken when a patient is on antibiotic therapy. The finding indicates that various materials used as foods or food additives should be investigated for possible bacterial resistance enhancing effects as found in this study. The study further indicates that some attention should be paid to the choice of foods for patients on antibiotic therapy less we risk treatment failures, the ultimate effects of bacterial resistance to antibiotics.

Conclusion

The study confirms that the methanol leaf and fruit extracts of *M. fragrans* have antibacterial activity. In the presence of sub-inhibitory concentrations of the extracts, there was profound loss of antibacterial activity of amoxicillin, erythromycin, tetracycline and ciprofloxacin against all the test organisms.

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Conflict of Interests

The authors declare no conflict of interests.

REFERENCES

Adu F, Gbedema SY, Annan K (2009). Antimicrobial and resistance modulatory activities of *Corynanthepachyceras.* Phcog. Res. 1(5):280-284.

Andrews JM (2001). Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48(1):5-16.

Barza M, Baum J, Kanne A (1976). Inhibition of antibiotic activity in vitro by synthetic melanin. Antimicrob. Agents Chemother.10(3): 569-570.

Bennett JW, Chung KT (2001). Alexander Fleming and the discovery of penicillin. Adv. Appl. Microbiol. 29:163-184.

Budak F, Ubeyli ED (2011). Detection of resistivity for antibiotics by probabilitistic neutral networks. J. Med. Syst. 35:87-91.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64(8): 711-713.

Evans WC (2002). Nutmeg and nutmeg oil:In"Trease and Evans Pharmacognosy", 15th Edition, ED Undersp. 269-270.

Gbedema SY, Adu F, Bayor MT, Annan K, Boateng JS (2010). Enhancement of antibacterial activity of amoxicillin by some Ghanaian medicinal plant extracts. Int. J. Pharm. Sci. Res.1(11):145-152.

Harborne JB (1998). Phytochemical methods.A guide to modern techniques of plant analysis. Chapman and Hall, London. pp. 182-190.

Keweloh H, Heipieper H, Rehm H (1989).Protection of bacteria against toxicity of phenol by immobilization in calcium alginate. Appl. Microbiol. Biotechnol. 31:383-389.

Lambert PA (2002). Mechanism of antibiotic resistance in *Pseudomonas aeruginosa*. J. R. Soc. Med. 95 (4): 22-26.

Levy SB, Marshall B (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10: S122-S129.

Nelson ML(2002). Modulation of antibiotic efflux inbacteria. Curr. Med. Chem. 1(1):35-54.

Sibanda T, Okoh AI (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr. J. Biotechnol. 6(25):2886-2896.

Singh G, Marimuthu P, Carola S de Heluani, Catalan C (2005). Antimicrobial and Antioxidant Potentials of Essential Oil and Acetone Extract of *Myristica fragrans* Houtt.(Aril Part). J. Food Sci. 70(2): M141–M148.

Sofowora A (1993). Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons, New York. pp. 97-145.

Spratt BG (1994). Resistance to antibiotics mediated by target alterations. Science 264:388-393.

Stewart PS, Costerton JW (2001).Antibiotic resistance in bacterial biofilms. Lancet 358(9276):135 - 138.