Jurnal Medical Aradean (Arad Medical Journal)
Vol. XIII, issue 3, 2010, pp. 19-25

PHYTONCIDES AND PHYTOALEXINS – VEGETAL ANTIBIOTICS

Robert DUKA[1], Dorina ARDELEAN

"Vasile Goldis" Western University Arad, Romania

ABSTRACT. Phytoncides and phytoalexins are antibiotic substances that have been isolated from a large number of plants .Tropical plants in particular possess many antibacterial compounds, such as sophoraflavanone G (*Sophora*), calozeylaxanthone (*Calophyllum*), α-mangostin, and the stilbene olygomers of gnemonal B and gnetin E. Other plants like garlic (*Allium sativum*) and onion (*Allium cepa*), mustard (*Sinapis alba*), horseradish (*Armoracia rusticana*), radish (*Raphanus sativus*) and lichens (like *Centraria islandica* and *Usnea barbata*) also manifest antibiotic properties. The antibacterial activities of sophoraflavanone G, calozeylaxanthine, α-mangostine are observed as strong against methicillin-resistant Staphylococcus aureus (MRSA) and against vancomicyn-resistant enterococci (VRE). This was evaluated by measuring minimum inhibitory concentration (MIC) values determined by the the agar dilution method of the Japanese Society of Chemotherapy. Allicin in garlic and dihydroallinine in onion are found to kill species of pathogens like methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Esterechia coli* and *Bacillus subtilis*. Interaction between phytoncides and phytoalexins and commercially available antibiotics, such as ampicillin, gentamicin, minocycline, fosfomycin, and vancomycin hydrochloride, evaluated by using fraction inhibitory concentrations (FIC) indices measured by the MIC values of the compounds, alone or in combination with the antibiotic, have shown that there is a level of synergism between them. Because of the strong anti-VRE and anti-MRSA activities and because some of them show synergistic interactions, these compounds could be used in the medical field to decrease infectious bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomicyn-resistant enterococci (VRE).

Keywords: Phytoncides, phytoalexins, vegetal antiobiotic, bacteria, antibacterial effects

INTRODUCTION

The word, phytoncide, which means "exterminated by the plant", was coined in 1937 by Dr. Boris P. Tokin ((1900-1984), a Russian biochemist from Leningrad University. He found that some plants give off very active substances which prevent them from rotting or being eaten by some insects and animals.

Phytoalexins are low molecular weight compounds (molecular weights are mainly 100 500) produced defensively following infection of plants by pathogenic microorganisms. They are natural antimicrobial compounds which are produced by plants as a defense against the attack of harmful insects and microorganisms. The production of phytoalexins can be induced by nonbiological stress, such as ultraviolet irradiation and by treatment with heavy metals. The detailed production mechanisms of phytoalexins are not clearly understood. The participation of active oxygen is thought to be one of the main reasons for the killing mechanism of the phytoalexins.

The main components of phytoncides are easily volatile terpen compounds that act on autonomic nerves, contributing to the stability of mind and concentration.

***Correspondence:** Robert Duka, "Vasile Goldiş" Western University Arad, Faculty of Medicine, Str. Feleacului nr. 1 Arad 310396 Romania, Phone 0040-257-256931, e-mail: medicina@uvvg.ro*
Article received: May 2010; published: August 2010

No toxicity reports of phytoalexins and phytoncides against humans have been found.

Enterococci and *Staphyloccocus aureus* are two of the leading causes of nosocomial infections in long-term health care facilities, and reports on vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphyplococcus aureus* (MRSA) infections in hospitals have increased worldwide. In recent years, there have been a number of reports on useful trials carried out to control the infections caused by VRE and MRSA. However, further trials are needed to find more reliable methods to control VRE and MRSA infections adequately. In this context the use of natural products as anti-VRE and anti-MRSE agents are promising candidates for study towards the prevention and treatment of VRE and MRSA infections. Furthermore, it is very important to investigate the interactions of the active natural products with commercially available antibiotics, with the hope of enhancing their activity.

Various spices, onion, garlic, oak and pine trees (and bark) and many other plants give off phytoncides.

Garlic is found almost everywhere in the world and has been used in traditional medicine for over 4000 years to treat disorders of arthritis, common cold, diabetes, malaria, and tuberculosis. The microbiologist Louis Pasteur studied the bactericidal properties of garlic, and during the Second World War garlic was called "Russian penicillin" because the Russian government turned to this ancient treatment for its soldiers when supplies of antibiotics had been exhausted.

It has been shown experimentally that garlic possesses therapeutic and preventive activities against bacterial infection, atherosclerosis, high total cholesterol, and hypertension; it also aids in prolongation of blood coagulation time (Iqbal Ahmad et al.).

PHYTOALEXINS AND PHYTONCIDES

The structures of phytoalexins and phytoncides sophoraflavonone G, calozeyloxanthine, α-mangostin, and stilbene oligomers, as used in the anti-VRE and anti MRSA tests (Figs. 1, 2, 3, 4a and 4b).

Sophoraflavanone G is a flavanone derivative, calozeyloxanthine and α-mangostin are the xanthone derivatives, and stilbene oligomers (gnemonol B and gnetin E) belong to the polyphenol group, respectively.

Chemically, allicin is known as 2-propene-1-sulfinothioc acid S-2-propenyl ester; thio-2-propene-1-sulfinic acid S-allyl ester. Allicin is produced by an enzymatic reaction when raw garlic is either crushed or somehow injured. The enzyme, allicinase, stored in a separate compartment in garlic, combines with a compound called allicin in raw garlic and produces allicin.

ISOLATION OF PHYTOALEXINS AND PHYTONCIDES

Sophoraflavanone G isolated from *Sophora* spp. (Leguminosae) was used to destroy MRSA (Sakagami et al., 1998).

Calozeyloxanthone was isolated from the root bark of *Callophyllum moonii* as a yellow crystalline compound. It was identified from the plant material collected from the Kanneliya forest in the southern province of Sri Lanka (Sakagami et al., 2002).

Alpha-mangostin is isolated as follows; Stem bark of *G. mangostana* L. is dried, powdered, and extracted with hexane, methylene chloride and methanol, respectively. Silica gel column chromatography of the hexane extract and melthylen chloride extract give two major compounds: alpha-mangostin and beta-mangostin as yellow needles (Sakagami et al., 2005).

The stilbene oligomers gnemonol B and gnetin E were isolated from gnetaceous plants.

It is known that the processing of garlic and onion into extracts, essence, and dehydrated foods leads to the formation of products with significantly different physicochemical and biological characteristics.

When garlic is extracted with ethanol and water at room temperature, it yields the oxide of diallyl disulfide, allicin, which is the source

Jurnal Medical Aradean (Arad Medical Journal)
Vol. XIII, issue 3, 2010, pp. 19-25

of the garlic odor. Under the influence of allinase the precursor allicin decomposes to 2-propenesulfenic acid. Allicin possesses hypolipidemic, antimicrobial, and hypoglycemic activities, and heat-unstable allicin is considered to be a principal antibacterial constituent.

However, heat treatment at 100˚C for 20 minutes could not eliminate the bactericidal potency and its activity remained in the garlic powder.

Thus it seems that the garlic contains two types of antibacterial ingredients: the heat-labile allicin and heat-stable sulfur compounds, both of which work together against bacteria.

SYNERGISM OF ANTIBACTERIAL COMPOUNDS WITH COMMERCIALLY AVAILABLE ANTIBIOTICS

Antimicroabial compounds were prepared in 50% dimethylsulfoxide solution. A solution of phytoalexin (or phytoncide) in combination with respective antibiotics was prepared by the doubling dilution method with sterilized water, and each solution was poured into

Synergistic effects between the test compounds and the commercial antibiotics against VRE and MRSA are summarized in Table 1.

FIC index values were judged as follows:

- FIC index ≤0.5 results in synergetic effect.
- FIC index 0.5-1.0: partially synergetic effect.
- FIC index 1.0>: no synergetic effect.
- FIC index ≥2.0: antagonistic effect. (Iqbal Ahmad et al.)

ANTIBACTERIAL EFFECTS OF GARLIC AGAINST ENTERO-HEMORRHAGIC ESCHERICHIA COLI (O157:H7)

The symptoms caused by entero-hemorrhagic Escherichia coli (O157:H7) are characterized by the sudden onset of severe abdominal cramps and bloody diarrhea with no fever or low-grade fever. The illness

sterilized plastic Petri dishes separately. Sterilized MH agar was poured into the above Petri dishes and mixed. After cooling, the MIC values of phytoalexins or phytoncide alone, the antibiotics alone, and their combinations, were examined. The Fraction inhibitory concentration (FIC) indices were calculated by the method of Didry et al., and the interactive effects of the phytoalexin or phytoncide and the commercial antibiotics were examined.

Synergism between sophoraflavanone G and vancomycin hydrochloride or fosfomycin against MRSA was observed. Synergism between calozeyloxanthine and vancomycinhydrochloride against VRE was observed. Synergism between alpha-mangostin and gentamicin against VRE, and alpha-mangostine and vancomycin hydrochloride against MRSA was also observed. The other test compounds possessed partial synergism except for the case of gnetin E (ampicillin against MRSA, and ampicillin or fosfomycin against VRE).

ANTIBIOTICS

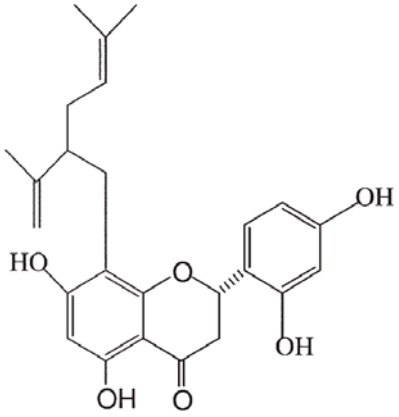
develops a hemolytic uremic syndrome (HUS), which differentiates it from other types of food poisoning and can often be fatal to the patient, especially in infants.

The bacterium O157:H7 is a remarkably resistant organism that can survive for over three years just in water without any nutrients. It can also change certain biochemical characters, often leading to microbiological misdiagnosis.

Fresh garlic powder prepared from garlic harvested on year previously was tested with the test-tube method, combined with the nutrient agar plate method and the results were that garlic powder easily killed O157:H7 as shown in Table 2 (Sasaki, J et al., 1999).

Using the nutrient agar plate test, it was additionally found that garlic powder killed other species of pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*.

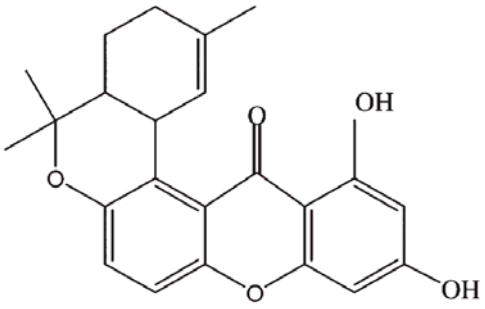
Some studies suggest that odor modulates mental activity to reduce stress and aids recovery from distress (Sasaky, J et al., 2003)



Sophoraflavanone G

[2 (S)-5,7,2',4'-tetrahydroxy-8-lavandulylflavanone]

Fig. 1 The structure of sophoraflavanone G



(1) Calozeyloxanthone

Fig. 2 The structure of calozeyloxanthine

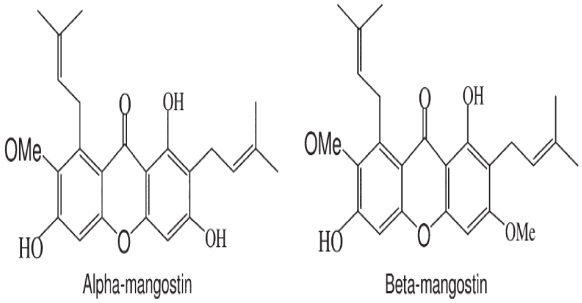
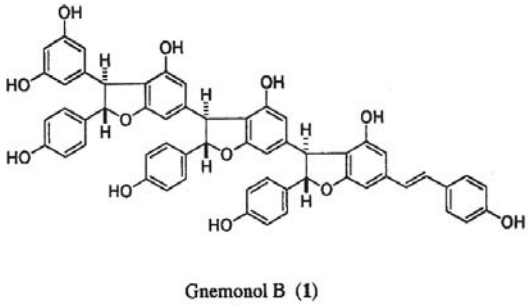
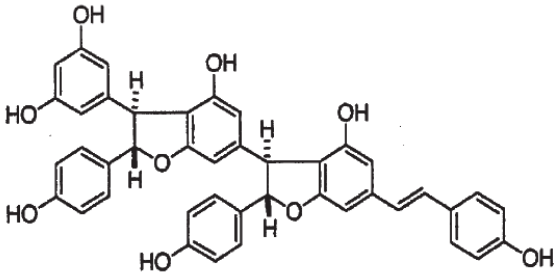


Fig. 3 The structure of alpha-mangostin and beta-mangostin



Gnemonol B (1)

Fig. 4.a The structure of stilbene oligomers (Gnemonol B)



Gnetin E (2)

Fig. 4.b The structure of stilbene oligomers (Gnetin E)

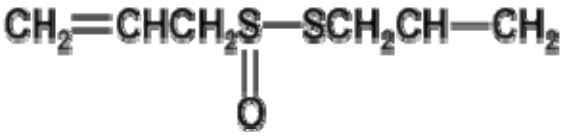


Fig. 5 Allicin

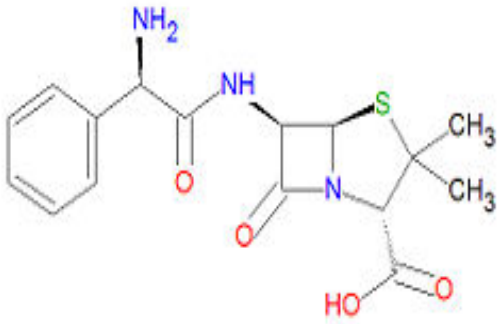


Fig. 6 Structure of Ampicillin

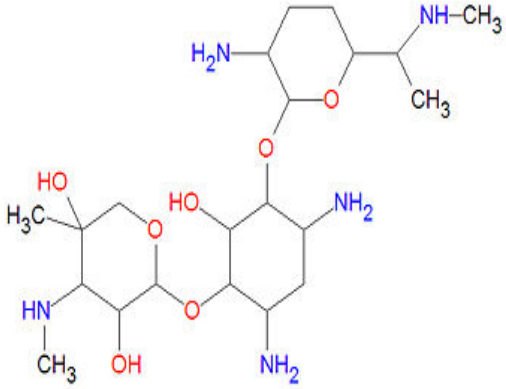


Fig. 7 Structure of Gentamicin

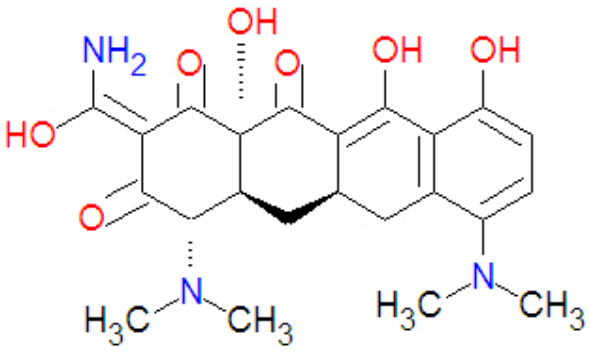


Fig. 8 Structure of Minocylcine

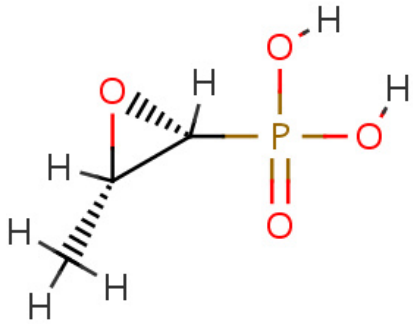


Fig. 9 Structure of Fosfomycin

Table 1

Synergistic effects between the test compounds and the commercial available antibiotics in vitro against VRE and MRSA

FIC index (average)	Ampicillin	Gentamicin	Minocycline	Fosfomycin	Vancomycin Hyrochloride
SFG (M)	0.73	0.6	Nt	0.48	0.16
CZXT (V)	0.750	0.625	0.563	Nt	0.453
Alpha-M (VRE)	0.606	0.451	0.969	0.826	0.508
Alpha-M (MRSA)	0.779	0.667	0.586	0.504	0.441
Gnemonol B (M)	0.708	0.501	0.833	0.750	0.750
Gnemonol B (V)	0.703	0.550	0.678	0.763	0.634
Gnetin E (M)	1.195	0.667	0.913	0.809	0.854
Gnetin E (V)	1.030	0.770	1.008	1.091	0.746

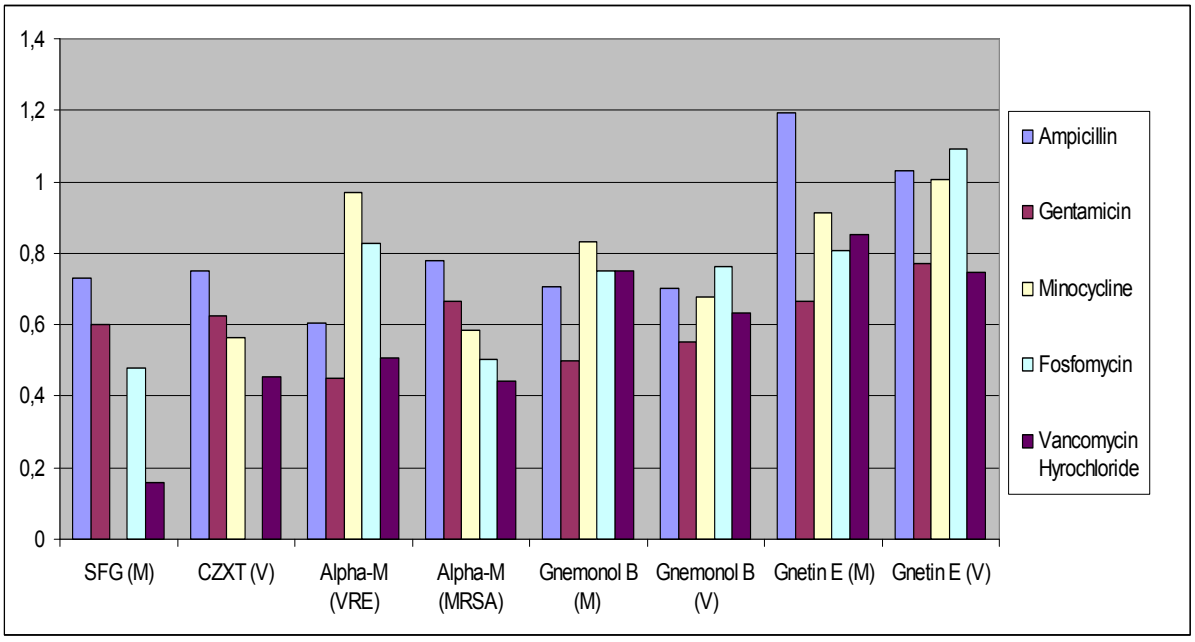


Fig. 10 Synergistic effects between the test compounds and the commercial available antibiotics in vitro against VRE and MRSA

Recent data showed that garlic's odour (flavour) had a bactericidal potency due to the volatiles released from grated garlic or its juice. It was observed trough experiments that by placing grated garlic or other samples in the lid of a Petri dish, which was then covered with the bacteria-streaked agar dish, tha bacteria were killed. Other types of foodstuff, such as onion (*Allium cepa*), horseradish (*Armoracia rusticana*), and *Houttuyna cordata*, produced similar results and their odor also killed bacteria.

The volatile allicin in garlic is primarily responsible for garlic's odor and sulfur compounds are produced when cells are ruptured, resulting in the formation of different thiosulfinates and related sulfonic acid-derived compounds by reaction taking place between the enzyme allinase and the volatile precursor allin. It can also blister the skin and kill bacteria, viruses, and jungi. The evidence suggests that garlic uses allicin for protection against bacteria and parasitic threats. This is a kind of defense system acquired over evolution to guard against attack (Bratman S., 2000).

Table 2

Anti-O157 avtivity of garlic powder prepared from old or fresh garlic bulbs

Sample	Number of O157 (cfu mL $^{-1}$)		
	0 h	6 h	24 h (treatment)
1% Old garlic powder	$4.0*10^7$	$8.0*10^6$	0
1% Fresh garlic powder	$4.0*10^7$	0	0
Control (water alone)	$4.0*10^7$	$8.0*10^7$	$8.0*10^8$

*cfu, colony forming unit

CONCLUSIONS

Synergism between phytoalexins or phytoncides and commercially available antibiotics could be used to decrease usage of antibiotics, contributing to the decrease of nosocomial infectious bacteria such as MRSA VRE. The use of phytoalexins or phytoncides isolated from natural products could also be valuable for the prevention of infectious bacteria such as VRE and MRSA etc. No reports of bacteria resistant to antibacterial compounds isolated from natural products were found. The use of antibiotics could also be decreased because of the partial synergism between the antibacterial compounds and the commercially antibiotics, which means that the detection ratio of the resistant bacteria would become lower.

Surprisingly, the odor of garlic and of other plants show antibacterial activity against pathogenic bacteria, These findings suggest that there are still undeveloped research fields that could contribute more to the medical area.

One of the important tasks that should be conducted urgently is a broad review of the analyses of the functional foods, including fruit, seaweed, fish, shellfish and other natural sources. The results of these experiments are essential if we are to create effective therapeutic strategies for disease treatments combining functional foods and herbs with Western medicines.

REFERENCES

Bratman, S., Herbs Prima Publishing Roseville, CA 2000

Iqbal Ahmad, Farrukh Aqil and Mohammad Owais "Modern Phytomedecine" Wiley-Vch: 142-153

Iqbal Ahmad, Farrukh Aqil and Mohammad Owais "Modern Phytomedecine" Wiley-Vch: 80, 82,138

Mauri, P., Simonetti, P., Gardana, C., Minoggio, M., Morazzoni, P., Bombardelli, E., Pietta, P., "Rapid Commun. Mass Spectrom." 2001, 15, 929-934

Sakagami, Y. Kajimura, K., Wijensinghe, W.M.N.M., Dharmaratne, H.R.W. "Planta Medicine" 2002, 66, 543-545

Sakagami Y., Iinuma M., Piyasena K.G.N.P., Dharmaratne, H.R.W. Phytomedicine 2005, 12, 203-208

Sakagami Y., Mimura M. Kajimura K. Yokoyama H., Inuma M., Tanaka T. Ohyama M. Lett. Appl. Microbiol. 1998, 27, 98-100

Sasaki, J., Kita, T., Ishita, K., Uchisawa, H., Matsue, H.J., "Nutri. Sci. Vitaminol" 1999, 45, 785-790.

Sasaky, J., Kita, J.J. "Nutr. Sci. Vitaminol." 2003 49, 297-299