ALLICIN LIQUID

Summary of Product Characteristics (SmPC)

1. NAME OF THE ACTIVE MATERIAL

Allicin liquid with a 100% allicin yield at concentrations of 1000 or 5000ppm

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Allicin liquid is the only commercially available extract of allicin from fresh garlic produced via a cool flood reaction system.

Allicin liquid contains traces of nitrogen, phosphorus, potassium, copper, iron, manganese, selenium, zinc, calcium and magnesium.

Allicin liquid can be manufactured and supplied at nominal concentrations of 1000ppm and 5000ppm. This can be readily diluted down to low concentrations for a series of practical applications.

3. CHEMICAL STRUCTURE

Allicin
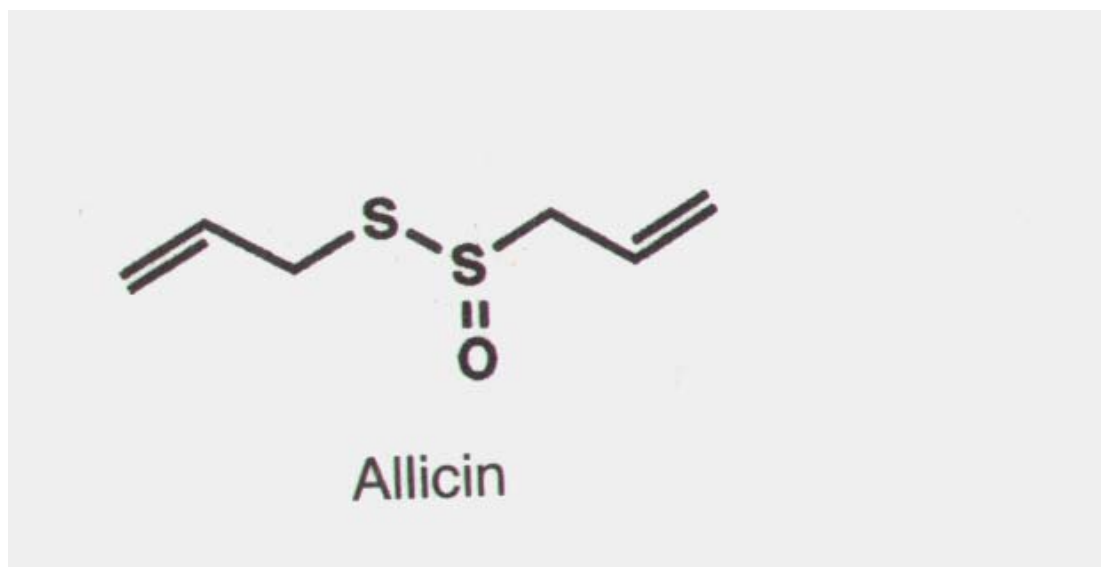
The sulphur-sulphur and sulphur-oxygen bonds are responsible for many of the beneficial properties associated with allicin. Although similar to the penicillin structure these bonds are very reactive and in fresh garlic they break down very quickly into a series of thiosulphinate components.

4. MANUFACTURING PROCESS

Allicin liquid is the result of a patented process, which produces pure allicin. It is the first time that this material has been produced on a commercial scale and has a wide range of potential applications across many markets.

Allicin liquid is made from fresh, raw garlic. Heads of garlic are specifically selected to ensure that they contain a significant enzyme activity (allinase enzyme). Garlic heads are split into cloves, which are left unpeeled and then subjected to filtration and a temperature controlled extraction process designed to produce pure liquid allicin dissolved in water. No chemical solvents are used. The alliin amino acid in fresh garlic is subjected to complete conversion by the allinase enzyme and to ensure a large volume of active agent is harvested. The volume of active agent produced is directly related to the enzymatic concentration and activity.

5. ACTIVITY

Allicin liquid has demonstrated significant antibacterial, antifungal, larvicidal and antiviral properties.

5.1 Antibacterial, antifungal, antiviral and larvicidal properties

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ABSTRACT — Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit:

i) antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains *of Escherichia coli*
ii) antifungal activity, particularly against *Candida albicans*
iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia* and
iv) antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica.*

1. Introduction

Garlic is one of the edible plants, which has generated a lot of interest throughout human history as a medicinal panacea. Wide ranges of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Moreover, garlic has been reported to reduce blood lipids and to have anticancer effects. Chemical analyses of garlic cloves have revealed an unusual concentration of sulfur-containing compounds (1—3%) [1,2].

Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl sulfides. However, it was not until 1944 that Cavallito and his colleague's [3] isolated and identified the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur compound, which they termed allicin, from the Latin name of the garlic plant, *Allium sativum.* Pure allicin is a volatile molecule that is poorly miscible in aqueous solutions and which has the typical odor of freshly crushed garlic [4]. Final proof of the chemical structure of allicin (figure 1*)* came in 1947, when it was shown that allicin could be synthesized by mild oxidation of diallvl disulfide [2]. The debate on the presence of allicin in crushed cloves versus its absence in odorless intact cloves was resolved after Stoll and Seebeck [5] isolated, identified, and synthesized an oxygenated sulfur amino acid that is present in large quantities in

garlic cloves and which they named alliin *(figure 1)*. Alliin was found to be the stable precursor that is converted to allicin by the action of an enzyme termed allinase, which is also present in the cloves [6]. Only one isomer of alliin ((+)-S-allyl-L-cysteine-sulfoxide) was found to be present, which in itself had no antimicrobial activity. Numerous investigators studied the amounts of alliin and allicin present in different strains of garlic. Considerable variations have been reported, ranging from 2.8 to 7.7 mg/gram found in Romanian red [2].
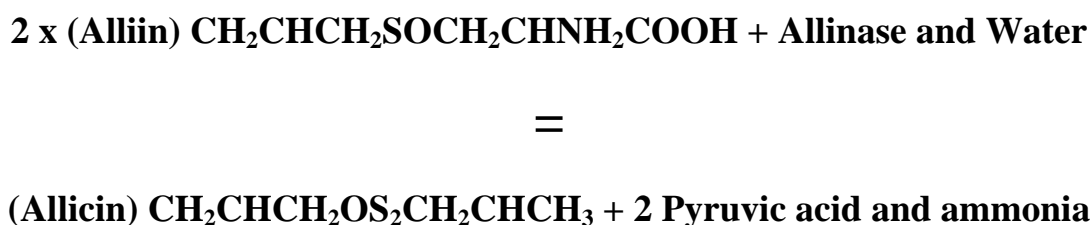
2 x (Alliin) $CH_2CHCH_2SOCH_2CHNH_2COOH$ + Allinase and Water

=

(Allicin) $CH_2CHCH_2OS_2CH_2CHCH_3$ + 2 Pyruvic acid and ammonia

Figure 1. Generation of allicin from crushing a garlic clove.

The transformation of alliin into the biologically active allicin molecule upon crushing of a garlic clove is extremely rapid, being complete in seconds. The enzyme responsible for the lysis is allinase, or alliin-lyase (E.C.4.4.1 4), a pyridoxal 3-phosphate-dependent glycoprotein consisting of two subunits 17, 81. Allinase is present in unusually high amounts in garlic cloves: at least 10% of the total protein content (10 mg/g fresh weight).

The gene coding for the enzyme has been cloned, and upon translation, found to consist of 448 amino acids with a protein molecular mass of 51.45 kDa and together with a carbohydrate content of 5.5-6%, gives 55000 kDa [*7,* 8]. Allinase has 10 cysteine residues, all of them in S-S bridges, and their reduction, or the removal of the pyridoxal coenzyme factor, renders the enzyme inactive. Expression of a recombinant allinase has been achieved in the baculovirus system, and although protein yields were impressive, the enzymatic activity was very poor due to difficulties with folding of the protein (Mirelman *et al.*, unpublished results). Moreover, in the clove, allinase is found closely associated with a lectin [9]. The site of linkage of the carbohydrate moieties of allinase has been identified at Asp 146 [9]. Significant homology has been reported between the garlic and onion allinases, although alliin was not detected in the latter species.

Garlic cloves are odour-free until crushed. Cross-section studies have indicated that the substrate alliin and the enzyme allinase are located in different compartments [2, 6]. This unique organization suggests that it is designed as a potential defense mechanism against microbial pathogens of the soil. Invasion of the cloves by fungi and other soil pathogens begins by destroying the membrane, which encloses the compartments that contain the enzyme and the substrate. This causes the interaction between alliin and allinase that rapidly produces allicin and which in turn inactivates the invader. The reactive allicin molecules produced have a very short half-life, as they react with many of the surrounding proteins, including the allinase enzyme, and making it into a quasi-suicidal enzyme. This very efficient organization ensures that the clove defense mechanism is only activated in a very small location and for a short period of time, whereas the rest of the alliin and allinase remain preserved in their respective compartments and are available for interaction in case of subsequent microbial attacks. Moreover, since massive generation of allicin could also be toxic for the plant tissues and enzymes, its very limited production and short-lived reactivity, which is confined to the area where the microbial attack takes place, minimizes any potential self-damage to the plant.

2. Antibacterial activity of allicin
The antibacterial properties of crushed garlic have been known for a long time. (see table 1). Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including

species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus,* and *Clostridium.* Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic [10]. Garlic extracts are also effective against *Helicobacter pylon,* the cause of gastric ulcers [11]. Garlic extracts can also prevent the formation of *Staphylococcus* enterotoxins A, B, and C1 and also thermonuclease [12]. On the other hand, it seems that garlic is not effective against toxin formation of *Clostridium botulinum* [13].

Cavallito and Bailey [4] were the first to demonstrate that the antibacterial action of garlic is mainly due to allicin [3]. The sensitivity of various bacterial and clinical isolates to pure preparations of allicin [14] is very significant. As shown in *table I* Mirelman *et al.*, unpublished results) the antibacterial effect of allicin is of a broad spectrum. In most cases the 50% lethal dose concentrations were somewhat higher than those required for some of the newer antibiotics. Interestingly, various bacterial strains resistant to antibiotics such as methicillin resistant *Staphylococcus aureus* as well as other multidrug-resistant enterotoxicogenic strains of *Escherichia coli, Enterococcus, Shigella dysenteriae, S. flexneni,* and *S. sonnei* cells were all found to be sensitive to allicin. Allicin also had an in vivo antibacterial activity against *S. flexneri* Y when tested in the rabbit model of experimental shigellosis [15].

On the other hand, other bacterial strains such as the mucoid strains of *Pseudomonas aeruginosa, Streptococcus β hemolyticus* and *Enterococcus faecium* were found to be resistant to the action of allicin. The reasons for this resistance are unclear but may be because these bacteria tend to have a mucoid coat, making penetration difficult. So it is assumed that hydrophilic capsular or mucoid layers prevent the penetration of the allicin into the bacteria, but this has to be studied more in depth.

Table 1. Sensitivity of various bacterial species to allicin.

Bacterial Strain	Allicin Concentration (LD$_{50}$ μg/ml)	Comments
Escherichia coli	15	Sensitive to antibiotics
Escherichia coli	15	Multidrug resistant MDR
Staphylococcus aureus	12	Sensitive
Staphylococcus aureus	12	Methicillin resistant
Streptococcus progenies	3	Sensitive
Streptococcus β hemolyticus	>100	Clinical MDR strain
Proteus marbles	15	Sensitive
Proteus mirabilis	>30	Clinical MDR strain
Pseudomonas aeruginosa	15	Sensitive to cefprozil
Pseudomonas aeruginosa	>100	MDR mucoid strain
Acinetobacter baumanii	15	Clinical isolate
Klebsiella pneumoniae	8	Clinical isolate
Enterococcus faecium	>100	Clinical MDR strain

3. Antifungal activity of allicin

Garlic extracts also have a strong antifungal effect and inhibit the formation of mycotoxins like the aflatoxin of *Aspergillus parasiticus* [17]. Allicin was assumed to be the main component responsible for the inhibition of fungal growth. A concentrated garlic extract containing 34% allicin, 44% total thiosulfinates, and 20% vinyldithiins possessed potent in vitro fungistatic and fungicidal activity against three different isolates of *Cryptococcus neoformans.* The minimum inhibitory concentration of the concentrated garlic extract against 1×10^5 organisms of *C.neoformans* ranged from 6 to 12 μg/mL. In addition, in vitro synergistic fungistatic activity with amphotericin B was demonstrated against all isolates of *C. neoformans* [18]. Pure allicin was found to have a high anticandidal activity with a minimum inhibitory concentration of 7 μg/mL [19]. Yamada and Azuma [20] report that pure allicin was effective in vitro against species of *Candida, Cryptococcus, Trichophyton, Epidermphyton,* and *Microsporum* at low concentration (minimal inhibitory concentrations of allicin was between 1.57 and 6.25 μg/mL). Allicin inhibits both germination of spores and growth of hyphae [20]. The sensitivities of various clinically important yeasts to a pure preparation of allicin were determined and found to be very significant *(table II)* (Mirelman *et al.*, unpublished results). The mode of action of allicin on the fungal cell has not yet been elucidated but it is assumed to function on thiol enzymes as in other microorganisms (see below).

4. Antiparasitic properties of allicin

The antiparasitic effects of freshly crushed garlic were known by many ancient cultures. Albert Schweizer used to treat people suffering from dysentery or intestinal worms with freshly crushed garlic. One of the traditional Chinese medical treatments for intestinal diseases is an alcoholic extract of crushed garlic cloves. Several years ago we found out that *Entamoeba histolytica,* the human intestinal protozoan parasite, is very sensitive to allicin, as only 30 μg/mL of allicin totally inhibits the growth of amoeba cultures [21]. More recently we have found that at lower concentrations (5 μg/mL), allicin inhibited by 90% the virulence of trophozoites of *E. histolytica* as determined by their inability to destroy monolayers of tissue-cultured mammalian cells in vitro [22].

Allicin (30 μg/mL) also very efficiently inhibited the growth of other protozoan parasites such as *Giardia lamblia, Leishmania major, Leptomonas colosoma,* and *Crithidia fasciculata (*Mirelman *et al*, unpublished results). Some allicin toxicity towards tissue-cultured mammalian cells was observed at concentrations above 100 μM [22]. Interestingly however, at these high allicin concentrations no damage to the mammalian cells was seen if the incubations were done in the presence of amoebic trophozoites, suggesting that the affinity of the allicin molecules is towards the parasite targets. The reason for microbial cells' higher sensitivity to allicin than that of mammalian cells is that most of the microbial cells do not have, or have very small amounts of, glutathione (or its equivalent thiol molecules such as trypanothione) and thus lack the ability to reactivate the essential SH-enzymes that are thiolated by allicin (see below section 6).

Table II Effect of allicin on various fungal pathogens

Fungal strain	Allicin concentration MIC (μg/mL)	Comments
Candida albicans	0.3	
Candida albicans	0.8	Clinical isolates
Candida neoformans	0.3	
Candida parapsilosis	0.15	

Candida tropicalis	0.3	
Candida krusei	0.3	
Torulopsis glabrata	0.3	
Torulopsis glabrata	1.9	Clinical isolates

5. Antiviral activity of allicin

Fresh garlic extracts in which allicin is known to be the main active component have been shown to have *in vitro* and *in vivo* antiviral activity. Among the viruses, which are sensitive to garlic extracts are the human cytomegalovirus, influenza B, herpes simplex virus type 1, herpes simplex virus type 2, parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human rhinovirus type 2 [23]. The allicin condensation product, ajoene, seems to have in general more antiviral activity than allicin. Ajoene was found to block the integrin-dependent processes in a human immunodeficiency virus-infected cell system [24]. Interestingly, there are some viruses like the garlic plant virus X which are resistant to the antiviral effects of garlic extracts [25].

Most recently a double blind placebo controlled study has shown significant protection from the common cold virus. Conducted by The Garlic Centre and published in *Advances in Therapy* this is the first serious work to show both prevention, treatment and reduction of re-infection benefits from taking Allimax Powder capsules once daily [16].

6. Mechanism of action of allicin

Inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism involved in the antibiotic effect [3]. Recently, we have studied the mechanism of action of pure allicin molecules with thiol groups in more detail [14]. This study confirmed the ability of allicin to react with a model thiol compound (L-cysteine) to form the S-thiolation product S-allylmercaptocysteine. The identification of the thiolation product was proven by nuclear magnetic resonance as well as by mass spectroscopy.

The main antimicrobial effect of allicin is due to its interaction with important thiol-containing enzymes. In the amoeba parasite, allicin was found to strongly inhibit the cysteine proteinases, alcohol dehydrogenases [22], as well as the thioredoxin reductases (Ankri *et al.*, unpublished results) which are critical for maintaining the correct redox state within the parasite. Inhibition of these enzymes was observed at rather low concentrations (<10 μg/mL). Allicin also irreversibly inhibited the well known thiol-protease papain, the $NADP^+$-dependent alcohol dehydrogenase from *Thermoanaerobium brockii,* and the NAD^+-dependent alcohol dehydrogenase from horse liver. Interestingly, all three enzymes could be reactivated with thiol-containing compounds such as DTT, mercaptoethanol and glutathione [14] At concentrations that are at least a log higher (> 100 μg/mL), allicin was also found to be toxic to tissue-cultured mammalian cells [22]. As mentioned above, the significant difference in sensitivity between the microbial and mammalian cells may be explained by the much higher concentrations of glutathione that the mammalian cells possess.

Allicin also specifically inhibits other bacterial enzymes such as the acetyl-CoA-forming system, consisting of acetate kinase and phosphotransacetyl-CoA synthetase [26]. The inhibition is noncovalent and reversible. (^{14}C) acetate incorporation into fatty acids of isolated plastids was inhibited by allicin with a 50% inhibitory concentration (I_{50} value) lower than 10 mM. Furthermore, allicin at bacteriostatic concentrations (0.2 to 0.5 mM) was found to partially inhibit, in *Salmonella typhimurium,* the DNA and protein synthesis, but the effect on RNA synthesis was immediate, suggesting that this could be a primary target of allicin action [27]. *E. coli* RNA polymerase, in its alpha-subunit, contains a single sulfhydryl group which was shown to react with the monomercuric derivative of fluorescein, a specific reagent for

thiol groups (fluorescein monomercuracetate) [28]. This suggests that RNA polymerase could also be a target for allicin.

The condensation product of allicin, ajoene, which has a similar oxygenated sulfur group, has been shown to inhibit the proliferation of *Trypanosoma cruzi,* possibly by inhibition of phosphatidylcholine biosynthesis [29]. Ajoene was also recently shown to inhibit phosphatidylcholine biosynthesis in the human pathogenic fungus *Paracoccidioides brasiliensis* [30]. The inhibition capacities shown for ajoene clearly suggest that additional microbe-specific enzymes may also be targets for allicin.

It is reasonable to conclude, therefore, that the broad-spectrum antimicrobial effects of allicin (and ajoene) are due to the multiple inhibitory effects they may have on various thiol-dependent enzymatic systems. It is difficult at this stage to state, which are the more lethal targets. It could very well be that the effect of allicin may be at different levels. Some enzymes such as the thiol proteases, which cause severe damage to the host tissues, may be inhibited at the lowest concentrations.

At low concentrations the inhibition of these enzymes may not be lethal, but sufficient to block the microbe's virulence. At slightly higher concentrations other enzymes such as the dehydrogenases or thioredoxin reductases may be affected, and even partial inhibition of these enzymes could be lethal for the microorganism.

All the above descriptions on the wide range of biological activities that allicin has been found to have should have propelled this molecule into becoming a prime candidate for therapeutic use. Recently it has been possible to patent the manufacture of allicin in commercial grade quantities. This is not the first time that economic considerations will prevent a natural compound with superb medicinal properties to reach those patients that could most benefit from it. Allicin will therefore find a readily appreciative audience amongst those who purchase over the counter "medications" for a wide variety of conditions.

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7. AGRICULTURAL & HORTICULTURAL APPLICATIONS

Allicin liquid can be successfully used as a novel foliar and root feed. Since the component parts of allicin are essentially carbon, nitrogen, sulphur, hydrogen and oxygen together with significant quantities of phosphorus and many essential plant micronutrients these are all made bioavailable to the plant. Allicin liquid can be readily diluted to specific conditions. When sprayed onto plants it will act as a natural foliar feed and will also show significant antifungal activity. Since invading insects are less likely to attack crops sprayed with allicin liquid, healthier plants develop and flourish. Allicin liquid should be diluted 1 part concentrate to 50 parts water and prepared for spraying. Applications should be made during the growing season.

8. UNDESIRABLE EFFECTS

The incidence of side effects whilst using Allicin liquid is extremely low. Very few people report an odour whilst using the product. Sensitivity can occur very infrequently and a rash is the most obvious sign of continued use. However, when used topically the odour is not persistent to human use, but insects – with a highly developed sense of smell can detect allicin for many hours after use. Since Allicin liquid is made from fresh garlic it can be seen to have a safety record dating back thousands of years and is unlikely to cause any problems. Always follow the recommendations stated on the packaging for using Allicin liquid.

9. PHARMACOLOGICAL/PHARMACOKINETIC PROPERTIES

The allicin that makes up Allicin liquid is slightly acidic and it may also contain traces of alliin and allinase enzyme as the natural degradation reaction of garlic derived chemicals takes place. Thus, the breakdown thiosulphinate chemicals including diallyl disulphide, diallyltrisulphide and ajoene are readily produced from allicin liquid as it degrades. Therefore a genuine 100% yield is guaranteed from each application of Allicin liquid. All other garlic supplements are made by methods that destroy most, if not all, of the allicin.

Once absorbed by the plant or soil, Allicin liquid breaks down as predicted empirically to form a series of thiosulphinate compounds. None of these components can be easily measured or even detected without recourse to HPLC technology. One extremely beneficial component formed is ajoene and this has also demonstrated significant antiviral properties.