Scientific evidence supporting the concept of garlic extract as an inhalation therapy in the treatment of Covid-19 infections:

An overview of the literature.

Allicin, the principal active ingredient in garlic and garlic extracts is a volatile compound with marked antimicrobial and antifungal properties. In published studies it has also been shown to possess antiviral activity against a wide range of viruses including Coronavirus (CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV).

Because of its volatile nature, the possibility exists to administer allicin *via* a nebuliser for the treatment of pulmonary infections, and results of published laboratory studies using a lung model and clinical isolates of microorganisms strongly suggest that the extract is likely to be active when administered by this route. To date there have no confirmatory clinical studies have appeared in the scientific literature, although there have been some anecdotal reports on the beneficial use of nebulised allicin.

Allicin liquid appears to be safe, relatively cheap and is freely available. It is therefore suggested that for a patient with a serious pulmonary infection associated with SARS-CoV-2 (Covid 19), and for whom no clearly defined effective form of antiviral therapy is available, the administration of a short course of nebulised allicin must surely be worthy of serious consideration.

Dr S Thomas, Medetec, December 2020

1. Summary

Garlic has been used empirically for millennia in the treatment or prevention of human diseases and metabolic disorders. However there is now a body of scientific evidence from a variety of sources which suggests it may also have value as an inhalation-therapy in the treatment for Coronavirus 2 (i.e., SARS-CoV-2).

Garlic extracts, particularly allicin, the principal active ingredient of the bulb, are active against a broad spectrum of bacteria and fungi, including multi-resistant strains. They can also prevent the formation of bacterial biofilms, a major obstacle to successful conventional antimicrobial therapy.

Garlic has been shown in *in vitro* studies to be active against many different viruses including Coronavirus (CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). Results of molecular binding studies predict that allicin would interact with Covid-19 active protease sites and thus help to prevent virus replication.

Because allicin rapidly passes through cell membranes and reacts with circulating glutathione, there is some debate concerning its ability to develop sufficient concentrations in tissue to exert the desired clinical effect. Fortunately, allicin is effective in the vapour phase, the only 'antibiotic' to function in this way. This has been demonstrated in laboratory studies, including one involving the use of a lung model. It is therefore theoretically possible to deliver allicin to the entire inner surface of the lung using an aqueous solution in a nebuliser, with obvious implications for the treatment of Covid-19 and other serious pulmonary infections.

Garlic is considered to be non-toxic when administered *via* the oral route as around 30 million tons are consumed annually. Few side effects have been reported when garlic has been used medicinally although consumption of large quantities of raw garlic may cause stomach pain. Isolated instances of tissue damage have been reported if raw garlic is applied to the skin for extended periods. The possible toxicity of allicin administered *via* the pulmonary route has been discussed in the literature. Although cytotoxic to lung cells *in vitro*, this effect is largely eliminated in the presence of glutathione, a substance known to be present in large quantities in alveolar fluid.

There are many different formulations of garlic, the composition of which is determined by the method of manufacture. The selection of an appropriate solution containing high levels of allicin would, however, probably be critical to the successful use of the material by the pulmonary route.

Administration of allicin by nebuliser is not a novel concept as it has formed the subject of anecdotal reports in the literature. However the evidence suggests is an idea worth revisiting in the present pandemic because of the wealth of scientific data now available.

Allicin liquid is appears to be safe, relatively cheap and is freely available. At least two liquid preparations are available in the UK; Allimed Liquid (Allicin International) and Allitech (Dulwich Health).

After reviewing the available evidence, for a patient with a serious pulmonary infection associated with SARS-CoV-2, a risk-benefit analysis would surely suggest that a course of treatment should be worth serious consideration.

2. History of garlic use

Garlic (*Allium sativum* L.; Family: Amaryllidaceae) is an aromatic herbaceous annual spice widely used throughout the world. In 2016, 26.5 million metric tonnes of garlic were produced, 80% of which was grown in China.

Garlic has been used to flavour food, treat disease and promote health for millennia. Sculptures of garlic bulbs were found in ancient Egyptian structures dating back to 3700 BC, while the Ebers papyrus, from around 1500 BC, describes 32 medical applications for garlic. It has been suggested that it was given to Egyptian slaves to increase their strength and ability to work. Well preserved garlic bulbs were discovered in the tomb of Tutankhamen (1320 BC). Similarly, early Greek military leaders are thought to have provided their soldiers with garlic before major battles. During excavations in the Knossos Palace on the Greek island of Crete, garlic bulbs were discovered dating from 1850–1400 B.C. Garlic has also been used medicinally in India and China for at least 5000 years.[1]

Aged garlic extract (AGE), is a folk herbal remedy that is believed to enhance the immune system and thus prevent cancer and heart disorders. Teas and tinctures prepared from fresh garlic, often with the addition of other agents such as honey, have been used for centuries to treat gastric infections, fight colds and fever, and help to treat or prevent serious, highly infectious diseases. In 1720 a thousand inhabitants of Marseille were reportedly saved from the plague by the use of garlic, which was also employed during a cholera outbreak in 1913, a typhoid fever and diphtheria outbreak in Beirut in 1918, and Spanish flu - the great influenza pandemic of 1918. Albert Schweitzer initially used garlic in Africa as the only available remedy against dysentery, typhus, diphtheria, and tuberculosis and even cholera.[1]

Garlic was used in Ayurvedic and Greek systems of medicine, and early Russian physicians also used it for the treatment of respiratory tract diseases. The ability of garlic to kill *Mycobacterium tuberculosis* was first demonstrated in a laboratory study published by Rao *et al.,* in Nature in 1946.,[2] and it was around this time that serious scientific interest in the properties of the plant appears to have begun. The potential medicinal benefits of garlic and its constituents have since been studied extensively *in vitro* and *in vivo*.

3. Chemistry of garlic compounds

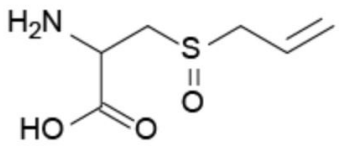
Bulbs of *A. sativum* contain two principal classes of organosulfur compounds, L-cysteine sulfoxides and γ-glutamylcysteine peptides. Detailed information on the chemistry of garlic can be found elsewhere.[3-7]

3.1. L-Cysteine sulfoxides

An intact garlic bulb has a slight, imperceptible odour, but if sliced or crushed it immediately develops a powerful characteristic smell. An enzyme, alliinase, released by the damage caused to the bulb, converts a family of cysteine sulfoxides to sulfenic acids which spontaneously react together to form allyl-thiosulphinates.

In the case of alliin (S-allyl-L-cysteine sulfoxide), which accounts for approximately 80% of the cysteine sulfoxides in garlic, this process results in the production of allicin (S-(2-propenyl)-2-propene-1-sulfinothioate) more commonly referred to as diallyl thiosulphinate, the most studied of the allyl thiosulphinates.

Allicin is an oily, colourless, unstable substance, the most biologically active constituent of garlic and the agent responsible for the familiar pungent odour.



S-allyl-L-cysteine sulfoxide

(alliin)

The conversion process is very rapid and is completed within 10-60 seconds of damaging the bulb. The half-life of allicin produced in this way is 2.5 days at 23°C.

Allicin was first isolated from garlic by Cavallito and Bailey in 1944,[8] and in 1947 it was shown to be responsible for many of the medicinal properties of garlic. It is assumed that the production of allicin by the bulb is a natural defence mechanism which prevents it from becoming spoiled by microorganisms if physically damaged.

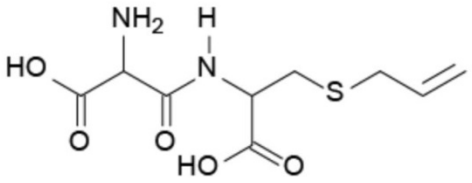
Alliinase is easily inactivated by heating or boiling and for this reason cooked unpeeled or uncrushed garlic has reduced biological or therapeutic effects, as the alliin cannot be converted to allicin.[9]

This has been demonstrated in animal studies. Platelet aggregation was shown to be lost by heating, although crushed garlic retained more anti-aggregatory activity compared to uncrushed garlic.[10] Similarly the ability garlic fed to rats to protect their DNA against damage caused by a chemical carcinogen was eliminated by first heating the uncrushed garlic cloves.[11] Physically damaging a clove and allowing it to stand for 10 minutes prior to heating preserved some activity. It is recommended therefore that if cooked garlic is consumed as part of the diet for medicinal reasons, it should be allowed to stand briefly after crushing prior to cooking.[9]

Allicin itself breaks down *in vitro* to form a variety of fat-soluble organosulfur compounds including diallyl trisulfide (DATS), diallyl disulfide (DADS), and diallyl sulphide. (DAS) In the presence of oil or organic solvents it produces ajoene and vinyldithiins.

Within the body allicin can react with glutathione and L-cysteine to produce S-allylmercaptoglutathione (SAMG) and S-allylmercaptocysteine (SAMC), respectively.

3.2. γ-Glutamyl-L-cysteine peptides



γ-glutamyl-S-allyl-L-cysteine

The γ-Glutamylcysteine peptides found in garlic comprise a number of water-soluble dipeptides, including γ-glutamylmethylcysteine, and γ-glutamylpropylcysteine. These are not affected by crushing the garlic bulb. The γ-glutamyl-S-allylcysteine (GSAC) is thought to be absorbed intact and hydrolysed to S-allylcysteine (SAC) and trans-S-1-propenylcysteine since metabolites of these compounds have been measured in human urine after garlic consumption.

Crushed garlic subjected to long-term storage in aqueous solution produces water-soluble organosulfur compounds, such as S-allylcysteine and SAMC. This is the process which occurs in the manufacture of 'Aged Garlic'.

The consumption of a commercial preparation of aged garlic extract that contains SAC, has been found to increase plasma SAC concentrations in humans. Water-soluble organosulfur compounds like SAC and its metabolite, N-acetyl-S-allylcysteine, may be used as reliable markers of compliance in clinical trials involving garlic intake.[3]

Both allicin and γ-glutamyl-*S*-allylcysteine (GSAC), as the source of *S*-allylcysteine (SAC) appear to be responsible for the hypotensive effects of garlic. The majority of the clinical trials on the possible cardiovascular effects of garlic have used supplements that are standardized on alliin or allicin potential.[5]

4. Overview of garlic preparations

Numerous garlic preparations are produced commercially but the different techniques employed in their manufacture markedly influences the chemical makeup and potentially the biological activity of the final product.[3]

4.1. Powdered (dehydrated) garlic

Powdered or dehydrated garlic is made from garlic cloves which are processed at a low temperature to prevent alliinase activity or inactivation. The resulting dried garlic is then pulverized and encapsulated or made into tablets. The United States Pharmacopeia specifies that powdered garlic supplements must contain no less than 0.1% γ-glutamyl-S-allylcysteine and no less than 0.3% alliin.

4.2. Garlic fluid extracts (aged garlic extract)

Aged garlic is produced by macerating cloves in a solution of water or ethanol and water for up to 20 months. During this time allicin is mainly converted to allyl sulfides, which are lost by evaporation or converted to other compounds. The resulting extract contains primarily water-soluble organosulfur compounds, such as SAC and SAMC. Garlic fluid extracts, including aged garlic extracts, are standardized to their S-allylcysteine content.

In controlled clinical trials, daily intakes of aged garlic extract between 1.2 -2.4 grams (containing 1.2 to 2.4 mg of S-allylcysteine) consistently resulted in reductions in systolic blood pressure of 9 -10 mm Hg and reductions in diastolic blood of 4-8 mm Hg in a majority of patients with uncontrolled hypertension. Additionally, aged garlic extract at doses of 2.4 to 7.2 g/day resulted in short-term reductions in *ex vivo* platelet aggregation and reductions in serum cholesterol concentrations up to 12 weeks.

4.3. Steam-distilled garlic oil

Steam distillation of crushed garlic cloves results in a product that contains mainly allyl sulfides, including DATS, DADS, and DAS, [12] which are usually subsequently dissolved in vegetable oil.

4.4. Garlic oil macerates

Incubation of crushed garlic cloves in oil at room temperature results in the formation of vinyldithiins and ajoene from allicin, in addition to allyl sulfides, such as DADS and DATS . Ether extracts are similar in composition to garlic oil macerates but more concentrated.

4.5. Pure allicin preparations

Preparations containing stabilised pure allicin are also available. In one manufacturing process is garlic subjected to controlled temperature and pressure extraction and a flood reaction process designed to produce stabilized liquid allicin dissolved in water. No chemical solvents are used. The resultant allicin liquid is added to a reaction vessel along with non GM maltodextrin and gum acacia before being passed through a spray dryer. The resultant powder, which is stable on storage, is then filled into capsules. Purity and potency of this preparation is confirmed by testing microbiologically against an MRSA bacteria and analysis by HPLC. [3]

Table 1: Principal constituent in different types of garlic extracts

Product	Principal Organosulfur Compounds	Delivers allicin - derived compounds?
Fresh garlic cloves	Cysteine sulfoxides (Alliin),γ-Glutamyl-L-cysteine peptides	Yes, when chopped, crushed, or chewed raw. Minimal, when garlic cloves are cooked before crushing or chopping.
Garlic powder (tablets)	Cysteine sulfoxides (Alliin), γ-Glutamyl-L-cysteine peptides	Varies greatly among commercial products. Enteric-coated tablets that pass the USP allicin release test are likely to provide the most.
Steam-distilled garlic oil (capsules)	Diallyl disulfide (DADS) Diallyl trisulfide (DATS)Allyl methyl trisulfide	Yes, but there is only 1% of oil-soluble sulfur compounds in 99% of vegetable oil.
Garlic oil macerate (capsules)	Vinyldithiins (E/Z)-ajoene Diallyl trisulfide	Yes
Aged garlic extract™ (tablets or capsules)	S-Allyl-L-cysteine (SAC) S-Allylmercaptocysteine (SAMC) trans-S-1-Propenyl-L-cysteine	Minimal
Pure allicin (capsules and liquid	Allicin only	Yes

Data abstracted from Higdon 2016 [3]

5. Pharmacokinetics of garlic compounds

When water is added to dried garlic powder, complete transformation of alliin to allicin takes place in less than a minute; however *in vivo* garlic powder garlic supplements produce very variable results. This is because at the pH of the stomach, typically pH 3.5 or below, alliinase is inactivated, although a moderate to high-protein meal can briefly raise the stomach pH to 4.4 or higher, a range in which alliinase is active. Some garlic supplements are therefore enteric-coated to allow the tablets to pass through the stomach to the small intestine which has a more favourable pH environment.

5.1. Release characteristics of garlic tablets

Although garlic supplements prepared from dry powder do not actually contain allicin, the manufacturer may provide a theoretical value for the 'allicin potential' or 'allicin yield' of a supplement on the product label. These values represent the maximum achievable allicin

yield if the powdered garlic is dispersed in water at room temperature and the allicin content measured after 30 minutes.[3]

Because of the pH sensitivity of alliinase, it was proposed that the standard technique for assessing drug release from enteric-coated tablets under conditions that mimic those of the stomach and intestine should be adopted for assay purpose as the results obtained in this way would more accurately reflect clinically derived values.[13]

When samples of enteric coated tablets were tested using the U.S. Pharmacopeial method (USP 724A), 21/24 samples yielded less than 20% of the allicin obtained by the activation of alliin by alliinase in water. This was considered to be due to premature dissolution of the tablets with consequent loss of alliinase activity.[13]

The relevance of these findings in relation to published data on the clinical performance of garlic extracts was investigated by Lawson *et al*.[14] Some 30 clinical studies carried out in the early 1990s appeared to support the proposition that the use of garlic had a serum cholesterol lowering effects. The majority of these studies employed garlic powder tablets Kwai (Lichtwer Pharma, Berlin), standardized to yield 3600±5400 µg of allicin per daily trial dose. Later diet-controlled clinical trials failed to demonstrate any clinical effects thus casting doubt on the previous work. In an attempt to explain these apparently anomalous results, Lawson analysed multiple batches of garlic extract tablets that were manufactured during the relevant time periods. Ten batches of tablets were examined to determine disintegration times and the simulated allicin release (as a percentage of allicin potential) using the standard USP method.

Substantial differences were found between tablets manufactured before 1993 (the years when all but one of the positive trials were conducted) and those manufactured after that date (the years when all of the negative trials were conducted). They found that the older lots were more resistant to acid-disintegration (2.5 *vs* 1.3 hours, $P < 0.001$) and that they released three times as much of their potential allicin (44 *vs* 15% $P < 0.001$) as the newer lots. A second brand of tablets employed in a further trial which also gave negative results released no detectable amount of allicin; while a third set of tablets with high allicin release was used in a trial that gave positive effects. They suggested that, based upon these finding, those subjects involved in the later studies probably received considerably less allicin than did those in the older positive studies, which might account for the discrepancy in treatment outcomes.

They concluded that clinical trials using garlic powder tablets to assess any effect of garlic that might be related to allicin, cannot be considered valid when the trial shows no effect, unless the expected allicin release from the tablets has at least been determined under standardized drug release conditions

This evidence was, and still appears, persuasive. However their conclusions, which have since been widely quoted in the literature, were subsequently criticised and disavowed by the authors themselves after they devised an *in vitro* technique for measuring the bioavailability of garlic products based upon breath analysis which appear to contradict their earlier findings (see 5.3). [15]

5.2. Results of animal studies

The pharmacokinetics of three important garlic constituents (alliin, allicin and vinyldithiines) were investigated by Lachmann *et al*.[16] in an animal model using the radionuclide S-35. Maximum blood levels for the 35S-alliin were achieved within the first 10 minutes and virtually eliminated after 6 hours. Maximum blood levels were not achieved by the other constituents until 30 - 60 minutes (35S-allicin) or 120 minutes (vinyldithiines) and these were

still measurable at after 72 hours. The mean total urinary and faecal excretion after 72 h was 85.5% (35S-allicin) or 92.3% (labelled vinyldithiines) of the dose administered. Excretion levels in the urine suggested a minimum absorption rate of 65% (35S-allicin) or 73% (vinyldithiines). No unchanged 35S-allicin vinyldithiines were detected in the urine and no conjugates with sulfuric or glucuronic acid were detected. The results showed no differences in pharmacokinetic behaviour between 35S-allicin and the labelled vinyldithiines.

Although a number of biological activities have been attributed to various allicin-derived compounds, it is not yet clear which of these compounds or metabolites actually reaches target tissues, but considerable evidence suggests that the allyl thiosulfinates, or their transformation compounds (allyl polysulfides), or metabolite (allyl methyl sulfide, AMS), are responsible for most of the reported lipid-lowering, antioxidant, anti-atherosclerotic, and anticancer effects of whole garlic in both animals and humans.[5]

5.3. Results of human studies

Cconducting meaningful bioavailability studies on garlic and its derivatives has proved challenging. It is known that allicin is able to permeate freely through cell membranes and interact with the SH groups within the cell,[17] and when added to whole blood it is rapidly converted to allyl mercaptan (allyl thiol), a process which can take less than one minute.

Attempts to isolate allyl mercaptan in the blood, urine, or stool of volunteers after consuming a large amount (25 g) of chopped raw garlic were unsuccessful, but the metabolite allyl methyl sulphide (AMS) is known to reach maximum levels in four hours and to persist for over 30 hours, indicating that it is a product of systemic metabolism and thus a potentially indicator of allicin bioavailability.[5]

AMS is present in the breath, and Lawson and Wang[15] showed that the area under the 32-hour breath 'AMS concentration curve' (AUC) is linearly proportional to the amount of allicin consumed and thereby established a validated method for determining the bioavailability of allicin from any garlic product. In this context they employed the term 'allicin bioavailability' to represent the sum of three processes, specifically the formation of allyl thiosulfinates (mainly allicin) from alliin by enzymatic activity (garlic alliinase), followed by their absorption and metabolism to the quantifiable metabolite AMS.

They also proposed the term 'allicin bioequivalence' which refers to the metabolic formation of the allicin metabolite, AMS, from any S-allyl compound, without the assistance of garlic alliinase, including allyl polysulfides, alliin and possibly other S-allyl compounds, such as GSAC and SAC. The term is used particularly for products in which garlic alliinase is inactive. They combined these terms together and referred to them as 'allicin bioavailability or bioequivalence' (ABB).

In their seminal study Lawson *et al.*[5] used the AMS breath technique to determine the ABB of allicin from raw and cooked garlic together with a variety of commonly consumed supplements. They showed that for alliinase-inhibited garlic foods, 5.9 g of roasted garlic and 11 g boiled garlic must be consumed to obtain the same equivalence as 2 g raw garlic. For the three acidified commercial garlic foods (pickled, acid-minced, oil-chopped), 5.3 to 19 g provide the same equivalence as 2 g raw garlic.

An additional unexpected finding of their study was the finding of unpredictably high allicin bioequivalence for alliinase-inhibited garlic foods and for garlic extracts from which alliin and alliin-derived compounds were selectively removed leaving γ-glutamyl-S-allylcysteine (GSAC) as the only source for AMS production. They concluded that the body has metabolic

pathways that lead to partial transformation of alliin and GSAC to AMS without garlic alliinase.

The authors used their findings to retrospectively 'validate' results of previously published clinical studies involving specific garlic preparations. In so doing, they acknowledged that their previous report on the dissolution of the main garlic supplement (Kwai) used in early clinical trials (previously referred to) in which they criticised the low allicin release of some batches of garlic tablets was probably incorrect. When the same batches of the tablets used during the study were examined using the new bioavailability technique, they produced values for allicin bioavailability that were not significantly less than that of fresh crushed raw garlic some 19 years later. They therefore considered that results of the many clinical trials that have been conducted with this consistently standardized garlic supplement can be considered as valid for representing crushed raw garlic.

For non-enteric tablets, allicin bioavailability was typically above 65% for all brands tested, and not significantly affected by meal type but allicin bioavailability from enteric-coated tablets was found to be much more variable (36–104%) and to be strongly reduced (22–57%) when consumed with a high-protein meal, due to delayed gastric emptying. They concluded that the *in vitro* USP dissolution allicin release test was unreliable for predicting allicin bioavailability from enteric tablets, and only 20% accurate for predicting allicin bioavailability from non-enteric tablets and capsules, confirming the importance of determining allicin bioavailability *in vivo*.

Based upon their work, to avoid unnecessary product-related criticisms of future clinical research they recommended that allicin bioavailability of garlic supplements used in such studies should be determined prior to commencement of any study and shown to be at least 65%, based on these *in vivo* tests. During the study attention should be given to the influence of diet on allicin release. If an enteric tablet is to used, its allicin bioavailability should be validated and limitations on meal contents described.

Although allicin bioavailability from capsules was unaffected by meal type, only one of three brands gave higher than 65% and this brand was uniquely made with a coarse garlic powder, which coarseness was probably responsible for its high value. Hence, capsules should not be used unless high allicin bioavailability has been demonstrated.

The well-known garlic odour on the breath and skin after fresh garlic consumption is a qualitative indicator of good absorption.[15]

6. Antimicrobial properties of garlic

Although preparations of garlic have been used empirically for centuries in the treatment of disease caused by various types of microbes, Louis Pasteur is credited as the first to demonstrate its antimicrobial properties in the laboratory, writing in 1858 that garlic killed bacteria including *Helicobacter pylori* and other strains found to be resistant to other agents.

Garlic extracts have since been shown to possess antibacterial and antifungal properties and the thiosulfinates, particularly allicin, are thought to be responsible for this activity. Allicin-derived compounds, including DATS and ajoene, also have some antimicrobial activity *in vitro*.

In a comprehensive review of the chemistry and pharmacology of garlic and its constituents Gaber El-Saber Batiha *et al.* [6] reported that it is active against a wide range of microorganisms including antibiotic-resistant, Gram-positive and Gram-negative bacteria

such as *Shigella*, *Escherichia coli,* *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus faecalis*, *Staphylococcus pyogenes*, *Salmonella enterica*, *Klebsiella aerogenes*, *Vibrio*, *Mycobacteria*, *Proteus vulgaris*, and *Enterococcus faecalis*.

The method of production or extraction (aqueous, chloroform, methanolic, and ethanolic) was said to have a marked effect upon activity. Ethanolic garlic was more active against *E. coli* and *Sal. typhi* than the aqueous extract. Aqueous garlic extract showed antibacterial activity toward Gram-negative (*Klebsiella pneumoniae* and *E. coli*) as well as Gram-positive (e.g., *Bacillus subtilis* and *S. aureus*) strains, but methanolic garlic extract showed antimicrobial activity against all tested strains except *S. aureus*. However, hexane, ethyl acetate, and chloroform extracts did not show any antibacterial effect. Moreover, garlic extracts prevented the growth of enterotoxigenic *E. coli* strains and other pathogenic intestinal bacteria, which are the main cause of diarrhoea in humans and animals. Garlic extracts also showed a broad spectrum fungicidal effect against a wide range of fungi including *Candida, Torulopsis*, *Trichophyton*, *Cryptococcus*, *Aspergillus*, *Trichosporon*, and *Rhodotorula* species. Anti-protozoal activity has also been reported,[6] and numerous reports suggest it may be active against *Borrelia burgdorferi* the causative organism of Lyme disease.

In laboratory studies using clinical isolates, fresh garlic extract exhibited marked inhibitory activity against *C. albicans* and MRSA but was less against *P. aeruginosa*. When garlic extract was tested in combination with the antibiotics fluconazole and itraconazole the antifungal activity was greater than that produced by either agent individually. Similarly garlic extract was shown to act synergistically with cefotaxime and ceftriaxone against *P. aeruginosa.*[18]

A systematic review and meta-analysis on the use of Allicin as add-on therapy for *Helicobacter pylori* infection suggested that if facilitates eradication of the organisms and improves healing and reduces remission of symptoms although the authors suggested that the results be treated with caution due to the limited sample size.[19]

6.1. Biofilm and quorum sensing

Under appropriate conditions many bacteria have the ability to clump together and attach themselves to a hard surface to form a 'biofilm' within which the bacteria organize themselves into a coordinated functional community, a process facilitated by the production of a slimy extracellular matrix comprising a polymeric conglomeration of extracellular polysaccharides substances (EPS), proteins, lipids and DNA which forms a protective coating to the cells. The cells within the biofilm matrix are physiologically distinct from planktonic cells of the same organism. Biofilms may form on living or non-living surfaces and are ubiquitous. The most familiar is probably that which forms on the teeth as dental plaque, a major cause tooth decay and gum disease. A biofilm may contain of a single species or a diverse group of microorganisms and its formation may be initiated by many different factors including exposure of planktonic cells to sub-inhibitory concentrations of antibiotics.

Biofilm production is influenced by a process termed 'quorum sensing' (QS) a process by which bacteria interact or 'communicate 'with each other by means of small diffusible signalling molecules which pass between cells. These activate the expression of genes which, in addition to biofilm formation, can control functions like bioluminescence and virulence. It follows that quorum sensing inhibitors (QSIs), chemicals which interfere with QS, may offer

therapeutic benefits when used alone or in conjunction with antibiotic therapy in the treatment of different pathogens.[20]

Garlic extract and some of its constituents have been shown in numerous studies involving different bacterial species to be effective QS inhibitors, active against numerous species including *P. aeruginosa* both *in vitro* and *in vivo*. This is important because *P. aeruginosa*, and the biofilms it produces, cause many serious clinical problems.

The activity has been shown to be due at least in part to the presence of a sulfur-containing compound ajoene. Ajoene treatment of biofilms *in vitro* demonstrated a clear synergistic, antimicrobial effect with tobramycin, killing or removing biofilms and preventing lytic necrosis of polymorphonuclear leukocytes. Notably, in a mouse model of pulmonary infection, a significant clearing of infecting *P. aeruginosa* was detected in ajoene-treated mice compared to a non-treated control group.[21]

Lihua *et al*.[22] investigated the effects of allicin on *P aeruginosa* biofilm formation and the production of quorum-sensing controlled virulence factors such as exotoxin A, elastase, pyoverdin and rhamnolipid. They found that allicin inhibited early bacterial adhesion, reduced EPS secretion, and down-regulated the production of virulence factors leading them to conclude that allicin has potential as a therapeutic agent for controlling *P. aeruginosa* biofilm.

P. aeruginosa is the predominant micro-organism of chronic lung infections in cystic fibrosis patients, forming biofilm microcolonies throughout the lung which are highly tolerant to otherwise lethal doses of antibiotics. The biofilm also protects against the bactericidal activity of polymorphonuclear leukocytes (PMNs). *In vitro* and *in vivo* studies indicate that a QS-inhibitory extract of garlic renders *P. aeruginosa* sensitive to tobramycin, as well as respiratory burst and phagocytosis by PMNs.[23]

P. aeruginosa also colonizes urinary catheters, and is responsible for causing recurrent catheter-associated urinary tract infections (UTIs). Using a mouse UTI model, garlic extract was evaluated as a prophylactic agent to prevent such infections. It was reported that oral treatment significantly lowered renal bacterial counts and protected mouse kidney from tissue destruction. It was proposed that this decreased virulence could be attributed the QSI properties of garlic.[24]

Garlic is also active against biofilms produced by other bacterial species including *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Serratia marscens* and MRSA. It also exhibited potent activity against systemic and deep tissue infections induced in mice caused by *P. aeruginosa* and MRSA. No adverse haematological or histological changes were seen in these animals but there was evidence of some tissue protective effective.[25]

In a further study the antibacterial and anti-biofilm activity of garlic extract was evaluated against eight Shiga toxin producing *E.coli* (STEC) isolates, seven of which strains were multidrug resistant. Shiga toxin producing *E. coli* (STEC) is an entero-haemorrhagic strain of *E. coli* which can result in bloody diarrhoea and potentially lead to deadly haemolytic uremic syndrome (HUS). All the strains examined exhibited dose dependent sensitivity towards garlic. Compositional and content changes in the biofilm were also noted.[26]

Prevention of biofilm formation by *Listeria monocytogenes*, an important and potentially dangerous contaminant of processed food has also been reported.[27]

The ability of garlic products to prevent biofilm formation on hard surfaces within the body has also been investigated. A major challenge in orthopaedic surgery is the formation of prosthetic joint infections (PJIs), largely due to the formation of biofilms. Aggressive surgical

treatment and the use of antibiotics are of limited value in these situations. The potential value of allicin for this serious problem was investigated in a rabbit PJI model. After the insertion of a sterile stainless-steel screw and polyethylene washer into the lateral femoral condyle, a suspension of *Staphylococcus epidermidis* was introduced into the knee joint. Fourteen days later, rabbits randomly received continuous lavages of normal saline, vancomycin allicin or allicin plus vancomycin. The lowest number of viable bacterial counts of *S. epidermidis* recovered from the site was in the rabbits treated with allicin plus vancomycin. Biofilm formation was significantly reduced or undetectable by SEM in these animals leading the authors to conclude that intra-articular allicin inhibits biofilm formation and enhances the bactericidal effect of vancomycin on implant surface *in vivo*. [28]

Formation of a biofilm is also a major challenge in dental practice leading to tooth decay and gum disease. In a search for a new root canal irrigant, to prevent the formation of biofilm produced by *E. faecalis*, garlic extract (GE) was compared with a 5.25% solution of NaOCl, the standard treatment. All the concentrations of GE employed displayed considerable anti-microbial efficacy but a 70% concentration was most effective and exhibited similar anti-microbial efficacy to the current therapy.[29]

The antimicrobial activities of allicin, s-allyl cysteine (SAC), diallyl disulfide (DADS), and s-allyl mercaptocysteine (SAMC) were investigated on standard strains of microorganisms responsible for chronic suppurative otitis media and otitis externa. These microorganisms were *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Acinetobacter baumannii*, *S. aureus*, *Enterococcus faecium*, *C. albicans*, and *Candida tropicalis*. Allicin and SAC showed significant antimicrobial activity against all the tested microorganisms, even at low concentrations. These two derivatives may be used to treat infections in the future but SAMC was found to possess limited antimicrobial activity.[30]

Furner-Pardoe *et al*.[31] reproduced a 1,000-year-old medicinal recipe containing onion, garlic, wine, and bile salts, known as 'Bald's eyesalve', which they found to be active against a range of Gram-negative and Gram-positive wound pathogens both in planktonic culture and in a soft-tissue wound biofilm model. The authors suggested that while the presence of garlic in the mixture could explain the activity against planktonic cultures, they asserted that garlic has no activity against biofilms and it was the combination of ingredients that was responsible for the overall beneficial effect of the formula.

However this conclusion is not entirely consistent with the findings of others, including Wu *et al*.,[32] who compared the activity of garlic extract with that of the principal antimicrobial component, allicin. These were compared using both planktonic bacteria and bacterial biofilms formed by two strains of *S. epidermidis*. They found that the minimal inhibitory concentration (MIC) and the minimum biofilm inhibitory concentration (MBIC) for pure allicin were identical at a concentration of 12.5 micrograms/mL. MBICs for standardized garlic extracts were significantly lower, with 1.56 and 0.78 micrograms/mL allicin for garlic water and ethanol extract, respectively. However, viability staining followed by confocal laser scanning microscopy showed 100% bactericidal effect on biofilm-embedded bacteria at a concentration of 3.13 micrograms/mL allicin. The authors concluded that although extracts of fresh garlic are more potent inhibitors of *S. epidermidis* biofilms than pure allicin, allicin exerts a unique bactericidal effect on biofilm-embedded organisms.[32]

6.1.1 Garlic and wound healing

The effect of allicin on wound healing was invested in an experimental diabetes model involving 50 Wistar albino rats. The diabetic animals were divided into three groups and treated with (allicin, physiological serum and control, where no dressing was used, and a

group of nondiabetic rats were divided into two groups (allicin and control, where no dressing was used). The area of an experimental wound was monitored and biopsied at intervals. On days 7 and 14, wound surface areas were smaller in the allicin group than in other groups but there were no statistically significant differences between the groups on day 21.[33]

A novel preparation comprising nanoparticles containing garlic extract which provided slow sustained release of garlic components was shown to produce a significant enhancement of antimicrobial and anti-biofilm activity relative to free garlic. The preparation was also able to penetrate and disrupt well-established biofilms formed by methicillin-resistant *S. aureus* (MRSA) biofilm, suggesting a possible future role in soft tissue infections. [34]

7. The antiviral activity of garlic

Although garlic has been used empirically for aeons as a treatment for conditions we now know to be viral in origin, a review of the literature suggest that serious scientific consideration of the use of garlic as an antiviral agent appears to have begun in the early 1980s.[35]

In 1992 Weber *et al.*[36] attempted to isolate and identify the individual constituents responsible for garlic's antiviral activity. They examined the *in vitro* virucidal effects of fresh garlic extract, its polar fraction, and specific compounds including: diallyl thiosulfinate (allicin), allyl methyl thiosulfinate, methyl allyl thiosulfinate, ajoene, alliin, deoxyalliin, diallyl disulfide, and diallyl trisulfide.

The relative potency of the individual agents was found to be ajoene > allicin > allyl methyl thiosulfinate > methyl allyl thiosulfinate. Ajoene was found in oil-macerates of garlic but not in fresh garlic extracts. No activity was recorded for the garlic polar fraction, alliin, deoxyalliin, diallyl disulfide, or diallyl trisulfide. Fresh garlic extract, in which thiosulfinates appeared to be the active components, was virucidal to each virus tested. The predominant thiosulfinate in fresh garlic extract was allicin.

A list of viruses with their family and common infection syndrome on which garlic extract and its organosulfur compounds reported to have antiviral activity is shown in Table 1 (after Rouf *et al.* 2020[7])

Table 2: Viruses against which garlic extracts have demonstrated activity.

Name of virus	Family	Common infection/Syndrome	References
Adenovirus-3 (AdV-3); Adenovirus-41(AdV-41)	Adenoviridae	Cold and respiratory tract illness	Khanal et al. (2018)
Porcine reproductive and respiratory syndrome virus (PRRSV)	Arteriviridae	Reproductive and respiratory tract illness in animal	Dietze et al. (2011)
Coronavirus (CoV); Severe acute respiratory syndrome coronavirus (SARS-CoV)	Coronaviridae	Cold and respiratory tract infection (both in human and animal)	Mehrbod et al. (2013)
Dengue virus (DENV)	Flaviviridae	Hemorrhagic fever	Alejandria (2015)
Herpes simplex virus-1 (HSV-1); Herpes simplex virus-2 (HSV-2);	Human cytomegalovirus (HCMV)	Herpesviridae Genital herpes, cold sores and other sexual infection	(Straface et al., 2012; Taylor, 2003)
Influenza A virus subtype H9N2 (IAV-H9N2); Influenza B virus (IBV); Influenza A virus-H1N1 (IAV-H1N1)	Orthomyxoviridae	Flu in both human and animal	Klenk et al. (2008)
Coxsackie B 3 (CBV-3), Echovirus-11 (ECHO); Enterovirus-71 (EV-71)	Picornaviridae	Minor febrile illness, aseptic meningitis, encephalitis and paralysis	(Dalldorf & Gifford, 1951; Khetsuriani et al., 2006; Lin et al., 2002)
Human rhinovirus-2 (HRV-2)		Cold and respiratory tract infection in human	Rossmann et al. (1985)
Hepatitis A virus (HAV)		Infectious disease of the liver in human	Matheny & Kingery (2012)
Measles virus (MeV); Newcastle disease virus (NDV);Parainfluenza virus-3 (PIV- 3)	Paramyxoviridae	Cold and respiratory tract illness in human	Enders (1996)
Vaccinia virus (VV)	Poxvirus	Skin infection, fever and common cold	Silva et al. (2010)
Vesicular stomatitis virus (VSV)	Rhabdoviridae	Foot and mouth disease in animals and flulike illness in human	Ludwig & Hengel (2009)
Human immunodeficiency virus-1 (HIV-1); Reticuloendotheliosis virus (REV)	Retroviridae	Causes immunosuppression in human (HIV) and poultry (REV)	(Wang et al., 2017; Palefsky & Holly, 2003;)
Porcine Rotavirus (PRV); Rotavirus SA-11 (RV-SA-11)	Reoviridae	Gastrointestinal infection and diarrhoea in human and animal	(Crawford et al., 2017; Vlasova et al., 2017)
Potato Virus Y (PVY)	Potyviridae	Virus infecting potato	Kreuze et al. (2020)
Spotted wilt virus (SWV)	Bunyaviridae	Virus infecting Tomato	Abad et al. (2005)
Grapevine leafroll-associated virus 2 (GLRaV-2)	Closteroviridae	Virus infecting grapevine	Alkowni et al. (2011)

Garlic has also been shown to be active in vivo, against infectious bronchitis virus, a coronavirus which is a highly infectious avian pathogen.[37]

8. Therapeutic uses of garlic

Despite the widespread use of garlic products, only limited evidence supporting their use has been provided from controlled clinical trials. Some studies were not well blinded; others are only of short duration; some have only small numbers of patients; and many are not well controlled. More importantly, many different garlic preparations have been used in these investigations and not all are standardized, and even some that have been, are reported to show unacceptable variability with respect to the nature and amount of the organosulfur compounds they contain and release in vivo. [38] [5]

8.1. Cardiovascular effects

Garlic supplements have been evaluated in numerous controlled clinical trials since the mid-1980s, focusing primarily on serum cholesterol and blood pressure. Whilst the effects on blood pressure have been moderately consistent for hypertensive subjects, their value in the treatment of serum lipids remains the subject of some debate.

Xiong *et al* [39] found garlic to be an effective and safe approach for hypertension but recommended more rigorously designed randomized controlled trials focusing on primary endpoints with long-term follow-up are still warranted before garlic can be recommended to treat hypertensive patients.

Ried *et al*.[40] in their review concluded that garlic supplements have the potential to lower blood pressure in hypertensive individuals but also to regulate slightly elevated cholesterol concentrations, and to stimulate the immune system.

A particularly important study was described by Gardner *et al*.,[41] in which 192 adults with low-density lipoprotein cholesterol (LDL-C) levels of 130 to 190 mg/dL (3.36-4.91 mmol/L) were randomly assigned to receive either raw garlic, powdered garlic supplement (Garlicin), aged garlic extract supplement (Kyolic tablets) or placebo using active ingredients that were fully characterised prior to the commencement of the trial using the method previously described by Lawson *et al*.[5] In each case garlic doses equivalent to an average-sized, 4 gram, clove were consumed six day each week for six months. No statistically significant effects on high-density lipoprotein cholesterol, triglyceride levels, or total cholesterol /high-density lipoprotein cholesterol ratio were found during the six month study.

Garlicin is a dried garlic preparation which releases high amounts of allicin *in vivo* and under simulated gastrointestinal conditions, approximating to the levels produced by a similar weight of raw garlic. The dose used was twice the label-recommended dose and the allicin potential at this dose was considerably greater than used in previous clinical trials including those reporting significant effects on serum lipid concentrations. Hence, this trial was better positioned than previous studies to identify a cholesterol-lowering effect from a garlic powder supplement.

Kyolic aged garlic extract powder has previously been used in two controlled clinical trials for effects on serum lipid concentrations with positive outcomes. In these earlier studies it was employed at a dose of 7.2 g daily for 6 months, four times larger than the Gardner study which still used up to three times the recommended dose.

The strengths of this investigation include the extensive chemical characterization of the products, the inclusion of raw garlic, the extended treatment period and the patient population which was substantially larger than almost all previous studies.

The authors cautioned that the results of this trial should not be generalized to other populations or health effects. Garlic might lower LDL in specific subpopulations, such as those with higher LDL concentrations, or may have other beneficial health effects. Also only one dosage level was used and effects might emerge at higher doses, if tolerated. However, they concluded that based on current evidence garlic supplements or dietary garlic in reasonable doses are unlikely to produce beneficial effects on lipid levels.

A meta-analysis of six studies which investigated the effect of garlic on plasma High Lipoprotein(a) levels also did not find a significant alteration in plasma levels after garlic consumption. When the studies were categorized according to the duration of therapy, there was no effect in the subgroup of trials lasting 12 weeks or less, a significant elevation of plasma Lp(a) concentrations was found in trials lasting more than 12 weeks.[42]

An umbrella review of the strength of evidence on the impact of garlic and garlic supplement intake on biomarkers of cardiovascular disease was undertaken by Schwingshackl *et al.* [43] They examined eight systematic reviews on the effects on blood pressure parameters and nine systematic reviews which examined the effects of garlic on lipid parameters. They concluded that the effect of garlic on systolic blood pressure showed consistent results across publications, with 7 out of 8 meta-analyses demonstrating a substantial decrease in systolic blood pressure and 6 out of 8 meta-analyses reporting significant reductions in diastolic blood pressure levels following interventions with garlic.

The results on blood lipids were more complex. Eight of nine meta-analyses synthesizing the effect of garlic on blood lipids reported significantly decreased total cholesterol levels. But inconsistent results were achieved for HDL-cholestrol, LDL-cholesterol, and triglycerides. The authors concluded that garlic products have some positive effects on indicators and biomarkers of cardiovascular disease, typically without causing any serious side effects but given the substantial heterogeneities between the different trials a conservative interpretation of the outcome seems to be appropriate.

Garlic may also reduce he thrombotic risk by producing a modest but significant decrease in platelet aggregation, although mixed results on fibrinolytic activity and plasma viscosity have been published. Atherosclerotic plaque volume reduction in humans also have also been recorded.[38]

The ability of garlic to prevent the development of hypertension was investigated in a cross-sectional study involving 22,812 adults in China published in 2020 the results of which indicated that frequency of garlic consumption is inversely related to development of prehypertension.[44]

8.2. Vulvovaginitis

Vulvovaginitis is a common inflammatory condition that can have a significant effect upon quality of life.[45] The term actually encompasses a variety of inflammatory lower genital tract disorders that may be secondary to infection, irritation, allergy or systemic disease. Infection may be bacterial or fungal in origin, or caused by *Trichomonas vaginalis* a common sexually transmitted parasite. After bacterial vaginosis, vulvovaginal candidiasis is estimated to be the second most common cause of vaginitis and *C. albicans* accounts for 85-90% of cases.[46] Approximately 75% of U.S. women experience vulvovaginitis candidiasis during their reproductive years. Between 40% and 50% of these women have recurrent episodes, and 5% to 8% experience chronic candida infections and approximately 3 million women have recurrent infections.[45]

Although effective pharmaceutical treatments are available, many women choose to self-medicate with a variety of products. According to Van Kessel *et al*.,[47] *Lactobacillus* recolonization (via yogurt or capsules) may be of value for treatment of both yeast vaginitis and bacterial vaginosis with little potential for harm.

In a controlled trial patients diagnosed with Candida vaginitis were randomly divided into two groups. One received daily doses of garlic in the form of Garcin tablets, the other fluconazole tablets 150 mg, over a period of seven days. The symptoms of the disease improved by about 60% in the Garcin group and 71.2% in the fluconazole group ($p > 0.05$) and laboratory results also showed significant differences before and after intervention in both groups ($p < 0.05$). The authors concluded that Garcin tablets could be a suitable alternative to fluconazole for the treatment of Candida vaginitis. [48]

Garlic has also been topically. In a trial involving 64 patients, a vaginal cream containing garlic and thyme was found to be as effective as clotrimazole vaginal cream for Candida vaginitis.[49] The use of raw garlic to treat long-term symptomatic group B Streptococcal vulvovaginitis in eight cases was reported by Cohain.[50] However considerable caution should be used when applying garlic to mucus membranes in this way as it has the potential to cause serious tissue damage.

Metronidazole, the gold standard for treatment of trichomoniasis was compared with garlic on multiplication and motility of trophozoites in an *in vitro* study. The treatments were found to be broadly comparable, suggesting that commercially available garlic could be a promising phytotherapeutic agent for trichomoniasis.[51]

8.3. Oral Health

Garlic may have a useful role to play in oral health.[52] Jiang *et al*.[53] suggested that the anti-inflammatory, antimicrobial, anti-oxidation and immunomodulation properties of allicin may make it an effective way to control the pain, promote ulcer healing and prevent the recurrence of recurrent aphthous mouth ulcers. This they subsequently confirmed in a randomized, double-blinded, placebo-controlled clinical trial involving 96 subjects half of whom were treated with 'adhesive oral tablets' containing allicin. The remainder received the vehicle only. Subjects who received the active agent experienced significantly reduced ulcer size and pain on days 2, 4, and 6, compared with the controls.[54]

Garlic extracts were also shown to be active against two periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and possess antiproteolytic activity against the protease of *P. gingivalis*, suggesting that it may have therapeutic value in the treatment of periodontitis and other oral infections.[55]

Raw garlic treatment should be used with care for this indication, as a patient with toothache who placed crushed garlic cloves in the buccal vestibule overnight developed a serious garlic burn injury in the area manifesting as slough and ulceration.[56]

A further major area of interest for the use of garlic is the prevention and treatment of various types of cancers. The literature contains a wealth of publications (over 1100) on this topic including 35 reviews. This application falls outside the remit of this current review.

Garlic has also been shown to protect rats against 'frailty' due to senile osteoporosis.[57]

8.4. Garlic in the treatment of pulmonary disease

Because allicin is excreted partly by the respiratory organs garlic and perhaps for this reason it has long been used to treat respiratory tract diseases.

8.4.1 The common cold

Garlic is widely believed by many to be of value in treating or preventing the common cold. On average, children have six to eight colds per year and adults have two to four and consequently large quantities of complementary products and alternative therapies (CAMs) are purchased annually to treat the condition. A survey off 2440 outpatients and elective inpatients in a UK hospital over a 3-month period revealed that of the 73% who responded, (1789 subjects) 60% had used complementary and alternative medicine CAM. Most commonly employed were preparations of cod liver oil (n = 481), and garlic (n = 255) to improve immunity and prevent or treat illnesses like the common cold.[58]

Despite the ubiquitous nature of the condition limited serious research has been conducted on the role of garlic in this area. In one study 146 volunteers were randomized to receive a placebo or an allicin-containing garlic supplement over a 12-week period between November and February. It was recorded that the active-treatment group had significantly fewer colds than the placebo group (24 vs 65, $P < .001$), significantly fewer days challenged virally (111 *vs* 365, $P < 0.05$) and a significantly shorter duration of symptoms (1.52 *vs* 5.01 days, $P < .001$). [59] In a subsequent systematic review Lissman *et al*.[60] concluded that although this single trial suggested that garlic may prevent occurrences of the common cold more studies were needed to validate this finding.

Additional evidence was provided by the results a second randomized, double-blind, placebo-controlled parallel intervention study in which 120 healthy subjects (60 per group) were enrolled to determine the effect of aged garlic extract supplementation (2.56 g/d) on immune cell proliferation and cold and flu symptoms. After 45 days, gammadelta-T cells and natural killer NK cells were shown to be significantly raised compared to placebo. After 90 days illness diary entries showed that although the incidence of colds and flu, a secondary outcome, were not statistically different, the group consuming the aged garlic extract appeared to have reduced severity in the number of symptoms reported, the number of days affected, fewer incidences where the subjects functioned sub-optimally and the number of work/school days missed due to illness,[61]

Taken together, these findings suggest that the evidence that garlic offers real potential for ameliorating the effects of the common cold is persuasive although it should be noted that different garlic preparations were used in these two studies, neither of which might have been optimal.

8.4.2 Cystic fibrosis

Cystic fibrosis (CF) is a genetic condition which leads to a build-up of thick sticky mucus in the lungs, digestive system and other organs. Colonisation or infection of this mucus can be life threatening. *P. aeruginosa* is the predominant cause of chronic lung infections in cystic fibrosis patients, leading to the formation of biofilm micro colonies throughout the lung. Quorum sensing (QS) renders the biofilm bacteria highly tolerant to otherwise lethal doses of antibiotics, and protects against the bactericidal activity of polymorphonuclear leukocytes (PMNs). In one study the synergistic effects of garlic and tobramycin and the activity of PMNs, was evaluated. In the laboratory, garlic-treated biofilms were susceptible to both

tobramycin and PMN grazing. In the second part of the study garlic extract was administered as treatment for a mouse pulmonary infection model. Mice were treated with garlic extract or placebo for 2 days then *P. aeruginosa* was instilled in the left lung of the mice. Bacteriology, mortality, histopathology and cytokine production were used as indicators. The garlic treatment initially provoked a higher degree of inflammation, and significantly improved clearing of the infecting bacteria indicating that a QS-inhibitory extract of garlic renders *P. aeruginosa* sensitive to tobramycin, respiratory burst and phagocytosis by PMNs, as well as leading to an improved outcome of pulmonary infections.[23]

These encouraging results have so far not been replicated *in vivo*. Only one study was found in which garlic was evaluated in a randomised controlled trial. Thirty four 34 patients were randomised to garlic or olive oil capsules (both 656 mg daily). Clinical outcomes and safety bloods were measured at baseline and after 8 weeks treatment. Eight patients withdrew, leaving 26 for analysis (13 garlic). Although there was no statistically significant effect of garlic compared to placebo in this pilot study, there was a suggestion of improvement with garlic which should the authors suggested be investigated in a larger trial.[62]

Note that the choice of a less than optimal garlic preparation used in this study may once again have influenced treatment outcomes.

8.4.3 Tuberculosis

The emergence of multi-drug resistant *Mycobacterium tuberculosis* (MDR) and extensively drug resistant (XDR) strains represents a serious clinical problem. The potential value of garlic and its extracts as an effective alternative or complementary therapy therefore cannot be ignored.

It has long been known from laboratory studies that garlic has inhibitory effects on *M. tuberculosis* including drug resistant strains,[63-65] but in a more recent study garlic has been shown to be effective against the organism in an animal model. Allicin solution given intraperitoneally not only reduced the bacterial burden in the lungs of mice infected with *M. tuberculosis*, but also induced strong anti-tubercular immunity *via* pro-inflammatory cytokines in macrophages. Furthermore, garlic extract reversed the immune dampening effects of frontline anti-TB drugs. The authors concluded that allicin/garlic extract alone or as an adjunct to classical antibiotics holds great promise for treatment of drug-sensitive as well as drug-resistant TB. [66]

8.5. Allicin vapour for pulmonary disease

Although laboratory studies suggest that allicin is active against a broad spectrum of bacteria and fungi. Reiter *et al.*[4] has suggested that when ingested orally, allicin which enters the circulatory system reacts with glutathione in the cells and blood making effective therapeutic concentrations difficult to achieve at the site of an infection arguing it is therefore not suitable for the treatment of pulmonary infections. This conclusion was based in part on the results of clinical research which suggested that although clinical trials on the effect of consuming garlic or garlic oil capsules on cystic fibrosis patients were encouraging, the treatment did not produce statistically significant results significant improvements.[62]

They proposed that for pulmonary infections allicin vapour might be an alternative route of administration. They examined the inhibitory effects of allicin vapour on a range of clinical isolates of different strains of bacteria and showed that with the exception of one very resistant strain of *P. aeruginosa* it was inhibitory to the growth of all organisms examined,

and that multi-drug resistant strains (MDRs) of *S. pneumoniae* were equally as susceptible to allicin as the non-MDR strains.

To test their hypothesis further they developed a lung test rig. This enabled them to expose simulated lung air-passage surfaces coated with a thin layer of growth medium seeded with clinical isolates of test bacteria (*Pseudomonas, Streptococcus, and Staphylococcus* sp.) to allicin and gentamicin in order to examine the feasibility of combating lung infections by direct inhalation.[67] Their results confirmed their earlier studies which suggested that allicin has an antibacterial effect when administered in this way. The model also facilitated differential sensitivity testing of the various bacteria to different antibiotics making it a useful device for pre-clinical trials of novel antibiotics.

They also examined the effects of allicin on mammalian cells, including those from lung tissue. They found the cell lines to be highly susceptible to allicin but toxicity was reduced by incorporating 1 mM GSH into the growth media. This effectively reduced the concentration of allicin *via* the formation of *S*-allylmercaptoglutathione.

At first sight these toxicity results would appear to challenge the wisdom of using allicin vapour for treating lung infections, however, the authors suggested that these *in vitro* findings must be considered in context. In the lungs epithelial cells are continually supplied with GSH which protects them against oxidative insults. The GSH concentration in alveolar fluid has been reported to be 140 times that of plasma. This creates an environment which is entirely different from that which exists in *in vitro* studies. It is also known that pathogenic bacteria mainly colonize the gel layer of the mucus within the lung, which is separated from the epithelial cells by an additional sol layer. The pathogen organisms would therefore be more exposed to the inhaled allicin, whereas the epithelial cells beneath would be more protected both chemically and physically. Furthermore, research has also shown that GSH levels in *E. coli* drop significantly during allicin exposure. This intrinsic protection against allicin due to endogenous GSH and other low molecular weight thiols in the bacteria themselves must be overcome before tissue cells will be inhibited by allicin.

They therefore concluded that toxicity of allicin to lung tissue *in vivo* is unlikely to be an issue.

As there are no volatile antibiotics available to treat pulmonary infections, using allicin vapour in conjunction with oral antibiotics could provide an effective treatment option for pulmonary disease as synergistic effects between garlic extracts and beta-lactams (cefazolin, oxacillin, and cefoperazone) and the antifungals amphotericin-B and polymixin-B has already been demonstrated.

From these findings it is clearly reasonable to assume that given the proven antibacterial, antiviral and anti-inflammatory properties of allicin, administration by nebuliser might offer an important new approach to the treatment of a range of serious respiratory infections including, potentially, Covid-19.

9. Garlic as a treatment for COVID-19

In late 2019 an outbreak of a new serious pulmonary disease (Coronavirus Disease COVID-19), caused by a new Coronavirus strain (i.e., SARS-CoV-2), was reported in China which quickly spread throughout the world, with over 70 million reported cases and in excess of 1.5 million deaths reported to date.

The symptoms and severity of COVID-19 vary from asymptomatic disease to severe acute respiratory infection. Fever, dry cough, dyspnea, myalgia, fatigue, loss of appetite, olfactory and gustatory dysfunctions are the most prevalent general symptoms.

According to Donma *et al.*,[68] the condition is characterised by decreased numbers of immune system cells such as suppressed regulatory T cells, cytotoxic and helper T cells, natural killer cells, monocytes/macrophages and increased pro-inflammatory cytokines. They suggested that garlic constituents have the potential to decrease the expression of the pro-inflammatory cytokines and to reverse the immunological abnormalities and immune system dysfunctions observed in patients with COVID-19 infection thereby relieving some symptoms detected during COVID-19 infection. Furthermore, they proposed that consumption of garlic may act as a preventive measure against infection with SARS-CoV-2 virus.

Similar claims had previously been made that the anti-inflammatory properties of garlic acting in this way could offer benefits in the treatment of Dengue virus infections, blocking the oxidative stress response thereby reducing inflammation and the progression of severe disease.[69]

As with most viral infections, the crucial event for the viral life cycle of COVID-19 is the entry of genetic material inside the host cell. This is facilitated by a large number of glycosylated spike (S) proteins which cover the surface of the SARS-CoV-2 virus which bind to the host cell receptor. A type 2 serine protease located on the host cell membrane then promotes virus entry into the cell by activating the S protein. Once in the cell the viral RNA is released and polyproteins are translated from the RNA genome. Viral RNA is then replicated and structural proteins are synthesized, assembled, and packaged in the host cell after which more new viral particles are released.[70] All of these stages are achieved by the action of different proteolytic enzymes either of the host or the virus acting in a concerted fashion to regulate and coordinate specific steps of the viral replication and assembly. It follows therefore that the proteases involved in these three steps are important potential therapeutic targets because molecules which interfere with their activity could help to prevent virus replication.[71]

Khubber *et al.*[72] predicted that constituents of garlic could act in this way by the formation of hydrogen bonds with the protease molecule. Using molecular screening and docking techniques. Sen *et al*. [73] examined 1040 compounds from selected spices and identified nine compounds found in onion, garlic, ginger, peppermint, chili and fenugreek which showed promise as potential home remedies against COVID-19.

Thuy *et al*.[74] also used a molecular docking technique to predict the ability of 17 organosulfur compounds found in garlic essential oil to produce an inhibitory effect on the host angiotensin-converting enzyme 2 (ACE2) protein. All the compounds they examined had strong interactions with the amino acids of the ACE2 protein and the main protease of SARS-CoV-2. The greatest activity was found in allyl disulfide and allyl trisulfide but the docking results also indicated that the various molecules had a synergistic effect suggesting that garlic essential oil could be a valuable natural antivirus source, which might help to prevent the invasion of the human body by the coronavirus virus.

Oso *et al*.[75] similarly concluded that the predicted pharmacokinetic properties, binding affinity, and binding free energy of three natural products, curcumin, allicin, and gingerol for the proteases cathepsin K, COVID-19 main protease, and SARS-CoV 3 C-like protease suggest that all three molecules could be developed into treatments for the prevention of coronavirus entry and replication into the human body. Similar opinions on the potential

value of garlic for the prophylaxis and treatment of COVID-19 infection have been expressed by others.[76] [77]

It is therefore possible that garlic, in common with some other phytopharmaceuticals, might have a useful role to play by addressing secondary respiratory tract infections as well as pulmonary fibrosis, diffuse alveolar damage, pneumonia, and acute respiratory distress syndrome, as well as associated septic shock, lung and kidney injury, all of which are symptoms associated with COVID-19 infection.[78]

The antiviral properties of garlic and its potential value for the treatment of human disease (including Covid-19) have been comprehensively described in a seminal review prepared by Rouf *et al*. [7] who concluded:

> *"Pre-clinical data demonstrated that garlic and its organo-sulphur components have potential antiviral activity against different human, animal and plant pathogenic viruses through blocking viral entry into host cells, inhibiting viral RNA polymerase, reverse transcriptase, DNA synthesis and immediate-early gene 1(IEG1) transcription*" and that
>
> "*Clinical studies demonstrated a prophylactic effect of garlic in the prevention of widespread viral infections in humans through enhancing the immune response. ..... Garlic possesses significant antiviral activity and can be used prophylactically in the prevention of viral infections*".

In the case of garlic specifically, it would be possible to administer allicin *via* the pulmonary route together with garlic extract in the form of a suitable oral preparation to exert its effects elsewhere within the body. Given the wealth of *in vitro* data and garlics apparent lack of toxicity, this surely must be an option worthy of serious consideration for infected patients for whom there are few realistic antiviral therapeutic options available

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