

# Significance of Garlic Extract in CanXida RMV

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*Garlic extract contains bioactive compounds with broad-acting antimicrobial properties. It has a unique effect on the gut where it acts against pathogenic fungi and bacteria but supports normal gut microbiome at the same time.*

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## **Executive Summary**

Garlic extract is a broad-acting antimicrobial which can counter many pathogenic bacteria, fungi, and protozoans in the oral cavity, stomach, and intestine. Its bioactive compounds manifest an excellent pharmacokinetics profile and biosafety. Organosulfur compounds in the extract are effective against drug-resistant bacteria and fungi, support the normal microbiome, assist the immune system in eliminating pathogens, and possess antioxidant and anti-inflammatory properties. It is well established through clinical and research outcomes that garlic extract not only eliminates pathogenic strains from the digestive tract, but it also fosters the growth of beneficial microbiota and stimulates the healing of the internal lining of the gut for long-lasting results. Bioactive compounds in whole garlic extract or in purified form have demonstrated significant health benefits in pre-clinical research and clinical trials. CanXida RMV formula is developed with aged garlic extract supplemented with 2% pure allicin for wholesome results against a wide variety of bacteria, fungi, and gut ailments. Garlic extract contributes significantly to CanXida RMV antimicrobial activity due to its potent bioactive compounds.

## 1. Introduction

Garlic (*Allium sativum*) is cultivated all over the world and is a universal folk spice as well as a food source. Hippocrates the “Father of Medicine” perhaps the greatest prehistorian healer, wrote 2500 years ago “Let your food be your medicine, let your medicine be your food.” Garlic fits into the description of an ideal food, which is both super nutritious and a medicine.

Garlic contains bioactive compounds with proven antimicrobial activity, antioxidant, and anti-inflammatory properties. Although Garlic has been employed as a general aseptic for wound healing among ancient Chinese, Romans, and Greeks, the first scientific study on the antimicrobial properties of garlic was done by Louis Pasteur in 1858. Since then, garlic has proven its mettle against a range of pathogenic bacteria, protozoa, and fungi.

## 2. Bioactive Compounds of Garlic

Garlic consists of several 33 sulfur-containing compounds which are effective as antioxidants, antibacterial, antifungal, antiprotozoal, as well as have immune modulatory properties. The chief components of garlic extract are alliin, allicin, ajoene, and their derivatives called allyl sulfides. These compounds possess potent therapeutic potential and have been used in the treatment of various diseases as crude extracts or purified substances. Besides, garlic extract also contains polyphenols which are well-established antioxidants. A general overview of the bioactive compounds in garlic extract is given in Table 1.

### 2.1. Allicin

In 2006, Professor Virginia Lanzotti from University degli Studi di Napoli Federico, Naples, Italy, reported that intact garlic bulbs don't contain allicin but rather it is produced during the cutting and crushing process when an enzyme converts alliin into allicin. Allicin gives garlic its characteristic odor and flavor. It

is slightly soluble in water and often acts as a precursor during the synthesis of other bioactive compounds.

*Table 1: Major bioactive compounds of garlic with antimicrobial activities.*

| Activities    | Bioactive Compound | Mechanism of Action                                                                 |
|---------------|--------------------|-------------------------------------------------------------------------------------|
| Antibacterial | Allicin            | Inhibits bacterial enzymes                                                          |
|               | DADS               | Cell wall disruption                                                                |
| Antifungal    | DADS               | Changes cell structure<br>Inhibits growth and germination                           |
|               | DATS               | Changes cell structure<br>Inhibits growth and germination                           |
| Antiviral     | Allicin            | Inhibits human enzymes used by viruses                                              |
|               | DATS               | Enhancing Natural killer-cell (NK-cell) activity that destroys virus-infected cells |
| Antiprotozoal | Allicin            | Preventing the parasite's RNA, DNA, and protein synthesis.                          |
|               | DATS               | Preventing the parasite's RNA, DNA, and protein synthesis.                          |
|               | Ajoene             | Inhibits human enzymes necessary for the activity of the parasite                   |

### 2.2. Organosulfur Compounds

Organosulfur compounds originate from allicin and are classified as oil-soluble and water-soluble. Oil-soluble compounds include diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and diallyl tetra-sulfides. Garlic extract consists of 66.7% DADS, 14.6% DATS, 13.3% DAS, and 5.4% diallyl tetra-sulfide.

Water soluble compounds involve S-allyl cysteine (SAC), S-methyl cysteine (SMC), and S-allyl mercapto-cysteine (SAMC). These compounds are often odorless and have bioactive properties.

### 2.3. Other Bioactive Compounds

Intact garlic cloves also contain saponins and organic selenium compounds which possess

antioxidant properties with the potential to cure chronic inflammation.

### 3. Therapeutic Potential of Garlic

There is a renewed interest in the various bioactive compounds of garlic or whole cell extracts for antimicrobial effects, antifungal, and wound healing properties (Table 2). Garlic extract has shown the potential to treat various ailments of the digestive system ranging from

*Table 2: List of selected clinical trials using garlic extract as an active ingredient in treatment or prevention. Source: clinicaltrials.gov*

| Clinical Trial ID | Health Condition                           | Status     |
|-------------------|--------------------------------------------|------------|
| NCT02347319       | Liver Health                               | Completed  |
| NCT03542721       | Chronic Fatigue                            | Unknown    |
| NCT00339768       | Gastric Lesions                            | Completed  |
| NCT02019368       | Antioxidant Effect                         | Completed  |
| NCT04830410       | Irritable Bowel Syndrome                   | Recruiting |
| NCT01393665       | Chronic Liver Disease                      | Completed  |
| NCT04449731       | Antiviral Activity                         | Completed  |
| NCT05841589       | Antimicrobial Activity                     | Completed  |
| NCT05396651       | Paediatric Irritable Bowel Syndrome        | Completed  |
| NCT00002033       | Antiviral Activity                         | Completed  |
| NCT04718519       | Antiviral Activity                         | Completed  |
| NCT03105960       | Oral Antimicrobial                         | Unknown    |
| NCT00954902       | Antioxidant Effect                         | Completed  |
| NCT05594329       | Anti-inflammatory                          | Completed  |
| NCT04716764       | Anti-inflammatory and Antioxidant activity | Unknown    |
| NCT04046653       | Antioxidant Effect                         | Completed  |
| NCT00455416       | Antioxidant Effect                         | Unknown    |
| NCT01390116       | Immune Modulation                          | Completed  |
| NCT02926508       | Prebiotic Activity                         | Unknown    |
| NCT04647071       | Antimicrobial                              | Completed  |
| NCT05082727       | Antiviral Activity                         | Recruiting |
| NCT05050071       | Oral Antimicrobial                         | Completed  |
| NCT04998617       | Oral Antimicrobial                         | Recruiting |
| NCT02691117       | Topical Antifungal                         | Terminated |
| NCT00275405       | Anti-inflammatory                          | Suspended  |
| NCT01198223       | Oral Antimicrobial                         | Completed  |
| NCT01959646       | Immune Modulation                          | Completed  |
| NCT05256784       | Probiotic Activity                         | Completed  |
| NCT03795636       | Oral Antimicrobial                         | Completed  |
| NCT03625635       | Antioxidant Effect                         | Unknown    |
| NCT00079170       | Antioxidant Effect                         | Completed  |
| NCT05016999       | Intestinal Microbiome Modulation           | Completed  |
| NCT03102411       | Gastric Health                             | Completed  |

oral antibacterial mouthwash to broad-spectrum antimicrobial activity in the stomach and intestine. It also improves liver function. Furthermore, it assists the immune system in the gut in fighting against pathogens, scavenging the reactive oxygen species (antioxidant activity), and mitigating

Among bioactive compounds of garlic, Allicin and Ajoene are **Drug Bank-**approved investigational medicine with accession IDs DB11780 and DB17735, respectively.

The garlic extract in **CanXida RMV** is intended to maintain the gut microbiome by eliminating pathogenic strains of bacteria and fungi and is supported by clinical evidence as well as repeated positive \*9research outcomes.

inflammation, as can be seen from the list of clinical trials in Table 2. A research study published in the Archives of Oral Biology journal showed that garlic extract inhibits viral, bacterial, and fungal growth in the oral cavity (Bakri et al 2005).

Another study published by Li and colleagues from Xi'an University of Health Sciences demonstrated that garlic extract provided a complementary effect to antibiotics in eliminating multi-drug-resistant bacteria. Similar results were also reported by Magryś and coworkers and were published in Springer Nature (Magryś et al 2021) as well as by others (Ashrat et al 2022 & Elmwolid et al 2019).

### 3.1. Antifungal Activity

Garlic inhibits fungal growth by fungistatic and fungicidal effects. The fungistatic effect prevents the growth of yeast by preventing the formation of new buds. As a result, the immune system gets enough time to eliminate the fungal infection. In the fungicidal effect, it directly kills the fungi by binding with the

enzymes of the fungi or damaging the cell wall of the pathogenic fungi.

Garlic has strong antifungal properties to counter drug-resistant fungal strains such as *Candida* species which is responsible for oral and vaginal thrush. A recent study conducted by Dr Khounganian (Oral Medicine and Diagnostic Sciences, College of Dentistry, King Saud University, SAU), Nakshabandi (Oral Medicine, Harvard University, USA), and colleagues showed that garlic extract was significantly associated with inhibiting the growth of *Candida albicans*. Similar antifungal properties of garlic extract were supported by other researchers as well (Carreón-Delgado, D. F., et al., 2022)

Garlic extract interferes with fungal growth by reducing the oxygen uptake efficiency of the fungal cells, arresting growth, and inhibiting enzymes. Reduced enzymatic activity fails to produce fungal lipids, proteins, and DNA for viable growth and reproduction. Allicin, DADS, and DATS in the aged garlic extract provide effective antifungal activity. The inhibitory action is due to the thiol group-containing bioactive compounds binding with enzymes or penetrating the cells and then damaging the cell membranes which ultimately results in cell death.

Dr Xin Yan from Yanbian University Hospital, China, reported that allicin in the garlic extract possesses systemic antifungal properties (Yang et al 2023). Allicin induces oxidative stress in fungal cells and accelerates fungal cell death.

### 3.2. Antibacterial Activity

Drug-resistant bacteria are often treated by combining multiple antibiotics at once. Garlic extract is naturally a combination of many anti-bacterial bioactive compounds such as (allicin, diallyl sulfide, etc.) acting as a natural combination of antibiotics. Gut bacterial outgrowths often occur when the normal microbiome is compromised, which in turn

allows opportunistic bacteria to spread infectiously.

Bacteria normally living in the gut (probiotics) are drug-resistant by natural selection or due to oral intake of prescription antibiotics for an extended duration. Small quantities of antibiotics coming from food sources are also responsible for producing drug-resistant strains in the gut. According to the American Society for Microbiology, these drug-resistant bacteria transfer the information to nearby bacteria through gene transfer which becomes easier during bacterial overgrowth as bacteria are closely packed. The bioactive compounds in garlic extract effectively solve all these problems.

Garlic improves gut health through multiple effects including direct killing of bacteria as well as modulating gut immunity. It also influences associated organs such as the liver to produce sufficient bile which promotes the growth of the normal microbiome while discouraging the growth of infectious bacteria. A report published in the journal

“Experimental and Therapeutic Medicine” by Dr Toshio Maeda (Maeda et al 2019) demonstrated that aged garlic extract was effective in treating fatty liver and improving the gut normal microbiome. A recent placebo-controlled preclinical trial (Heimesaat et al 2021) demonstrated that garlic organosulfur compounds were not only effective against drug resistance bacteria but also effective in reducing inflammation on the intestinal wall and promoting healing of the internal lining of the intestinal wall. These results are also supported by several preclinical studies as shown in Table 3. It can be observed that garlic bioactive compounds not only inhibit the growth of pathogenic bacteria but also promote the growth of beneficial probiotics such as actinobacteria and firmicutes (Zhai et al 2018, Zhang et al 2019) as it is indicated from the results of several preclinical studies (Table 3).

The anti-inflammatory properties of garlic extract are impressive as well. The gut is under frequent chemical and pathogenic insults

*Table 3: List of selected pre-clinical trials using garlic extract as a whole or its purified bioactive compounds with specific effects on gut microbiota.*

| Study               | Garlic Compound | Dose         | Study Type        | Findings                                                                                                          |
|---------------------|-----------------|--------------|-------------------|-------------------------------------------------------------------------------------------------------------------|
| Zhai, et al., 2018  | Alliin          | 0.1 mg/mL    | Preclinical Trial | Increases <i>Actinobacteria</i> and <i>Firmicutes</i><br>Decreases <i>Bacteroidetes</i> and <i>Proteobacteria</i> |
| Zhang et al., 2019  | Alliin          | 80 mg/kg     | Preclinical Trial | Increases <i>Firmicutes</i> and <i>Allobaculum</i><br>Decreases <i>Bacteroidetes</i> and <i>Candidatus</i>        |
| Chen et al., 2019   | Garlic extract  | Raw material | Preclinical Trial | Increases bacterial diversity in the gut                                                                          |
| Satora et al., 2020 | Garlic extract  | 10 mL        | Preclinical Trial | Decreases pathogenic bacteria                                                                                     |
| Rabelo et al., 2021 | PTSO            | 60 mg/kg     | Preclinical Trial | Increases <i>Lactococcus</i> in the ileum and cecum                                                               |

coming with food. This leads to inflammation of the inner lining of the gut which can lead to the formation of polyps or lesions if neglected for long. A common example is the ulcers caused by the activity of *Helicobacter pylori*. The anti-inflammatory bioactive compounds in garlic extract often reduce inflammation and restore normal gut function.

### 3.3. Antiprotozoal and Antiviral Properties

Protozoan outgrowth is often responsible for abnormal bowel moments, diarrhea, and constipation. Ajoene and diallyl sulfide are potent anti-protozoans. Garlic extract in CanXida RMV regulates bowl moments by checking on protozoans.

Common protozoan parasitic species are Entamoeba species (*E. histolytica* and *E. dispar*). *E. dispar* causes infections that are generally asymptomatic but chronic while *E. histolytica* causes acute diarrhea and dysenteries. These parasites produce toxins during the infectious period as well as when attacked by the immune system. These toxins are pore-forming proteins that destroy the epidermal layer of the intestine, causing cramps and severe bleeding. As a result, the normal intestinal activity of food digestion and nutrient absorption is reduced, and the risk of systemic infections is increased.

Besides the **Microbiome**, which is referred to probiotic bacterial strains in the gut, the digestive system also contains "**Eukaryome**" which is a collection of beneficial protozoans which not only help in nutrients acquisition but also help protect intestinal homeostasis as well as promote immune maturation.

Garlic extract is effective against pathogenic protozoans. A book titled "Medicinal Plants of South Asia" (publisher: Elsevier) described garlic extract with a potent antiprotozoal activity due to the presence of allicin, DTS,

and ajoene. It is also worth mentioning that besides intestinal protozoans, garlic demonstrates exceptional systemic anti-protozoal activity and can eliminate muscular larvae of *Trypanosoma*.

Protozoan infection not only causes intestinal damage but also induces malnutrition by reducing the absorption of essential nutrients which in turn weakens the immune system and encourages pathogen growth.

The pore-forming toxins also reduce the absorption of antibiotics as well.

Although viral infections of the intestine are not common, viral infections of other parts of the digestive system such as the liver (e.g., hepatitis) can be facilitated through the intestinal or oral route.

Similarly, garlic extract also provides a protective effect against viral infections which transmit through mucosal surfaces such as those of the intestine, oral cavity, and nose. This protective effect is either through the inhibition of viral enzymes or through the upregulation of interferon- $\gamma$  (IFN- $\gamma$ ) which inhibits the virus from infecting healthy cells. The results from the clinical trials have shown that garlic extract demonstrates effective antiviral activity. (Clinical trials NCT04449731, NCT04718519)

### 3.4. Antioxidant & Anti-inflammatory Properties

Oxidative stress is induced by free radicals or chemical compounds called oxidizing agents which can cause damage to cellular proteins, lipids, and nucleic acids. This leads to altered cellular composition, forcing cells to undergo apoptosis (cell death). These types of reactions



are more damaging to the liver which is a metabolic center of the body and can also induce damage to the cardiovascular system as well.

The major bioactive compounds in garlic extract that provide antioxidant support to cells and tissues are as follows:

- Tetrahydro-beta-carbolines
- $\beta$ -D-fructofuranosyl
- L-Phenylalanine
- L-Tryptophan
- Ascorbic Acid
- Fructans

Garlic extracts also possess antioxidant properties. Besides thiols (organosulfur compounds), it is also rich in flavonoids and carotenoids which have immense anti-oxidative potential. The antioxidative activity of garlic is done by:

- Inhibiting the formation of free radicals.
- Support cells in scavenging free radicals and oxidizing species.
- Enhancing the activity of antioxidant enzymes in the body such as superoxide dismutase, catalase, and glutathione peroxidase
- Protecting vulnerable biomolecules (such as lipoproteins) from oxidation.
- Promoting activation of nuclear factor kappa B (NF- $\kappa$ B) which is a transcription factor and activates genes that can counter oxidative stress.

Damages induced by oxidative stresses activate proinflammatory responses from the immune system. Reduction in oxidative stress downregulates inflammatory responses. Bioactive compounds in garlic also provide anti-inflammatory assistance in removing the

existing inflammation or cellular damage at the molecular level. It is achieved through the inhibition of enzymes such as COX-2 (Jeong et al 2016).

#### 4. Mechanism of Action

Garlic antimicrobial activity is either by compromising the structural integrity of the pathogen or recruiting immune cells to the infection site.

##### 4.1. Bactericidal Effect

Chen and colleagues from Chengdu Normal University, China, (Chen et al 2018) observed that the broad-spectrum antimicrobial activity of garlic extract is due to damage to bacterial cell walls. The bioactive compounds from garlic extract were able to penetrate bacterial cells, disintegrated the cell membranes, and cause leakage of bacterial proteins that resulted in bacterial cell death. This effect became more pronounced as more time was allowed for garlic extract to act in a dose-dependent manner.

##### 4.2. Immunomodulatory Effect

Furthermore, garlic extract has immune modulatory properties where bioactive compounds in garlic extract can recruit different immune cells at the site of infection, increase antibody production by regulating B-cells, or activate cytotoxic T-cells.

A report published in the Journal of Immunology Research demonstrated that alliin in garlic extract can reduce inflammatory cytokines such as interleukin 6 (IL-6) and monocyte chemoattractant protein 1 (MCP-1). This allows the involvement of the adaptive immune system to eliminate the infection (Arreola et al 2015). In parallel, caffeic acid, uracil, DATS, and DAS from garlic extract also supported the anti-inflammatory activity of the alliin by activating NF- $\kappa$ B, a master regulator which controls several genes important for immune function.

In addition to alliin, allicin also has a comprehensive immune modulatory profile. Allicin helps in controlling the parasitic load in an already infected host by activating T Helper Cells 1 (Th1) which then activate CD4+ T cells (a subpopulation of T-lymphocytes) as well as macrophages which can act together to remove the pathogen through phagocytosis (directly eating the pathogen). In a similar fashion, allicin along with other bioactive compounds in the extract also activates Th2 cells which are involved in the activation of B-lymphocytes as well as T-lymphocytes. B-cells produce pathogen-specific antibodies to eliminate infectious organisms.

### 4.3. Immunostimulatory Effect

Garlic extract contains fructo-oligosaccharides which have immune stimulatory activity. It also contains lectins and agglutinin proteins which are not only immunostimulatory but also effective against lesions and ulcers in the gut. These substances may initiate signaling at the site of infection to immune cells such as macrophages to initiate phagocytosis as well as stimulate different probiotic bacteria as an accessory to immune response.

### 5. Pharmacokinetics

The antimicrobial characteristics of a therapeutic substance are not only limited to its interaction with the pathogen or immune cells but also to its behavior within the host body, such as how much time it stays in significant concentration in the vicinity of the pathogen or how it affects host cells. If it is metabolized too rapidly, it might not get sufficient time to interact with the pathogen and if it stays too long, it might produce some undesirable side effects. The behavior of a therapeutic substance within the host is termed its pharmacokinetics.

The pharmacokinetics of garlic extract is highly complicated as it is a mixture of many substances. However, the pharmacokinetics of S-allyl-L-cystine (SAC), a derivative of allicin, is well documented and is often used as a

general reference for the pharmacokinetics of garlic extract.

SAC is readily absorbed in the digestive system with 90% bioavailability and is distributed to blood plasma, liver, and kidneys. Oral bioavailability increases with the increase in dosage and can reach up to 98%. In the liver, an enzyme called acetyltransferase converts allicin into S-allyl-L-cysteine sulfoxide (SACS) which is then removed through the urine.

The half-life of SAC is around 10 hours, and it is cleared from the body within 30 hours. It can be found in most of the body tissues such as the liver, spleen, kidneys, lungs, heart, and brain after 8 hours of ingestion. Its excretion time from the liver is 2.2 hours while in the brain it is less than 1.2 hours (Rais et al 2023). Only trace amounts of SAC (2.9%) have been detected in urine in pre-clinical studies on mice models while the rest is converted into different metabolites through enzymatic activity. Its low renal excretion is also because most of it is reabsorbed in the urinary tract. This provides an optimal retention rate for effective drug action.

Excellent oral availability, sufficient retention time in blood plasma, wider systemic distribution throughout the body in different tissues, and efficient metabolism of the SAC give it an exceptional pharmacokinetic profile as a pharmaceutical compound.

### 6. Effective Targets

Garlic extract as a whole or its purified bioactive compounds have well-established antimicrobial properties.

Among **fungal pathogens**, garlic extract is effective against:

- ***Candida species*** (*C. albicans*, *C. tropicalis*, and *C. glabrata*) – This group of yeast is responsible for oral and vaginal infections, and skin infections.

- ***Dermophytes fungi*** – These fungi grow on keratin protein which is present in skin, nails, and hair. Toenails, dandruff, and ringworm infections are caused by these fungi.
- ***Trichophyton fungi*** – This group of fungal pathogens infects skin causing athlete's foot, and tinea (ringworm). It is also responsible for skin rashes and itching.

Among **bacterial pathogens**, garlic extract is effective against:

- ***Escherichia coli*** – It is an opportunistic bacterium, that causes diarrhea or stomach cramps when the gut microbiome is disturbed. Its outgrowth can produce intestinal gas.
- ***Bacillus subtilis*** – It is non-pathogenic bacteria found in the human gut. Garlic

*Table 4: Antimicrobial targets of Allicin in the garlic extract. Source: Oosthuizen et al (2018).*

| Allicin        |                                    |
|----------------|------------------------------------|
| Antimicrobial  | <i>E. coli</i>                     |
|                | <i>Cytomegalovirus</i>             |
|                | <i>Influenza B virus</i>           |
|                | <i>Herpes simplex virus type 2</i> |
|                | <i>Parainfluenza virus type 3</i>  |
|                | <i>Vaccinia virus</i>              |
|                | <i>Vesicular stomatitis virus</i>  |
|                | <i>Human Rhinovirus type 2</i>     |
|                | <i>Common cold virus</i>           |
| Antifungal     | <i>C. albicans</i>                 |
|                | <i>Saccharomyces cerevisiae</i>    |
|                | <i>Aspergillus fumigatus</i>       |
| Anti-protozoal | <i>G. lamblia</i>                  |
|                | <i>Schistosoma mansoni</i>         |
|                | <i>Trypanosoma brucei</i>          |

extracts have a positive effect in maintaining the healthy colonies of this bacteria.

- ***Salmonella*** – It infects the intestinal tract and causes food poisoning which is characterized by vomiting, bloody diarrhea, and stomach cramps.
- ***Klebsiella*** – It normally lives in the intestine but can travel to other parts of the body causing pneumonia (lungs), meningitis (brain), and urinary tract infections during leaky gut syndrome.
- ***Helicobacter pylori*** – Infamous bacteria that live in acidic conditions of the stomach and cause gastric ulcers chronic gastric inflammation if remain untreated. Often causes heartburn and bloating after eating.

Among **protozoal pathogens**, garlic extract is effective against:

- ***Entamoeba histolytica*** – It is responsible for diarrhea and dysentery. It often produces resistant spores to spread to other hosts. It produces toxins that cause tissue damage to the intestinal wall and severe inflammation leading to characteristic symptoms of dysentery with cramps and bloody diarrhea.
- ***Giardia intestinalis*** – It causes intestinal infections and diarrhea. It is responsible for 30% of all infections caused by the protozoans in the human intestine.
- ***Toxoplasma gondii*** – It is responsible for life-threatening infections among immune-compromised people. It causes chronic infections in pregnant mothers leading to birth defects in children. It is transmitted through oral ingestion of food contaminated

with the cysts of *T. gondii*. Gut mucosa acts as a first-line defense against it. Garlic extract has a supportive effect on gut mucosa against the *T. gondii* infection.

A comprehensive list of target pathogens is given in Table 5.

## 7. Significance of Aged Garlic Extract

Garlic has a natural defense mechanism to combat bacteria and fungi which might cause damage to the garlic bulb during dormant stages when the plant is not growing. The vacuoles in the cells are filled with alliin, allicin, and inactive enzymes. Upon damage to garlic cells, either physically induced or caused by bacteria and fungi, its enzyme become active and break the alliin and allicin into more potent bioactive compounds such as DAS, DATS, ajoene, etc. These bioactive compounds are not present if the extract is fresh or if garlic is cooked. The aging process of garlic is to activate the natural defense mechanism and associated enzymes of garlic to produce those potent antimicrobial products which are then used later in the formulations.

The aging process involves soaking garlic in a 15 – 20% solution of ethanol for 10 – 20 months at room temperature. This process activates the enzymatic degradation of alliin and allicin into organosulfur and other bioactive compounds that are characteristic of garlic extract. This process utilizes allicin and hence removes the strong odor of garlic present in the fresh extract. The aging process removes chemical irritants which might otherwise worsen the condition of the inflamed gut. Some of the by-products of the enzymatic degradation of allicin are antioxidants such as allixin, selenium organic compounds, and mercapto-cysteines.

During the aging process, enzymes utilize all the allicin and alliin to produce organosulfur compounds. To compensate for this, allicin and alliin can be added separately to aged garlic

extract to obtain the benefits of both aged and fresh garlic extracts collectively.

## 8. Garlic Extract in Canxida RMV

Canxida RMV contains aged garlic extract as well as standardized quantities of allicin in a controlled released tablet. Standardized extracts contain known quantities of bioactive compounds which makes it easy for dosing and biosafety assessment. The aged extract, bioactive compounds, and allicin have been analyzed qualitatively and quantitatively through recommended high-end analytical procedures as well.

Garlic extract supports all other ingredients in a synergistic way to provide a comprehensive antimicrobial formulation for the gut as well as systemic infections caused by bacteria, fungi, and protozoans and prevents transmission of several others. The inclusion of aged garlic extract in the formulation was done after rigorous research and is backed by clinical trials, pre-clinical studies, and numerous lab reports supporting the antimicrobial activity of the garlic extract.

*CanXida RMV contains standardized aged garlic extract which is supplemented with 2% allicin.*

Garlic extract has exceptional biosafety and pharmacokinetic properties which makes it a desirable active pharmaceutical ingredient in medicines. Being used for thousands of years as a food as well as a folk medicine, human digestive system and metabolism has evolved well to efficiently utilize it.

Table 5: Antimicrobial targets of garlic extract. Source: Oosthuizen et al (2018).

| Biological Activity   | Effect                                                                   | Preparation                                |
|-----------------------|--------------------------------------------------------------------------|--------------------------------------------|
| <b>Antibacterial</b>  | <i>Staphylococcus aureus</i>                                             | Whole extract                              |
|                       | <i>Escherichia coli, Salmonella typhi</i>                                | Whole extract                              |
|                       | <i>Klebsiella pneumoniae</i>                                             | Whole extract                              |
|                       | <i>Helicobacter pylori</i>                                               | Extract                                    |
|                       | <i>Salmonella enteritidis</i>                                            | Extract                                    |
|                       | <i>Shigella spp, Proteus mirabilis</i>                                   | Extract                                    |
|                       | <i>Actinobacillus pleuropneumoniae serotype 9</i>                        | Extract                                    |
|                       | <i>Streptococcus mutans</i>                                              | Extract                                    |
| <b>Antifungal</b>     | <i>Candida albicans, Candida tropicalis, Blastoschizomyces capitatus</i> | Extract                                    |
|                       | <i>Botrytis cinerea, Trichoderma harzianum</i>                           | Extract                                    |
|                       | <i>Ascosphaera apis</i>                                                  | Essential oil vapors                       |
|                       | <i>Paracoccidioides brasiliensis</i>                                     | Extract                                    |
|                       | <i>Aspergillus niger</i>                                                 | Extract                                    |
|                       | <i>Dermatophytes, saprophytes</i>                                        | Ethanol extract                            |
|                       | <i>Cryptococcal spp.</i>                                                 | Alcoholic extract                          |
|                       | <i>B. cinerea, Mycosphaerella arachidicola, Physalospora piricola</i>    | Extract                                    |
| <b>Anti-protozoal</b> | <i>Trypanosoma sp, Entamoeba histolytica, Giardia lamblia</i>            | Extract                                    |
|                       | <i>Trypanosoma cruzi</i>                                                 | Extract                                    |
|                       | <i>Plasmodium spp, Giardia spp</i>                                       | Whole extract                              |
|                       | <i>Trypanosoma, Plasmodium, Giardia, and Leishmania spp.</i>             | Extract                                    |
|                       | <i>Hymenolepiasis, Giardiasis</i>                                        | Aqueous extract                            |
|                       | <i>Haemonchus contortus</i>                                              | Ethanol, dichloromethane and water extract |
| <b>Immune system</b>  | Immunomodulation                                                         | Extract                                    |
|                       | Antioxidant properties                                                   | Organosulfur compounds                     |

## References

- Arreola, R., Quintero-Fabián, S., López-Roa, R. I., Flores-Gutiérrez, E. O., Reyes-Grajeda, J. P., Carrera-Quintanar, L., & Ortuño-Sahagún, D. (2015). Immunomodulation and anti-inflammatory effects of garlic compounds. *Journal of immunology research*, 2015.
- Ashrit, P., Sadanandan, B., Shetty, K., & Vaniyamparambath, V. (2022). Polymicrobial Biofilm Dynamics of Multidrug-Resistant *Candida albicans* and Ampicillin-Resistant *Escherichia coli* and Antimicrobial Inhibition by Aqueous Garlic Extract. *Antibiotics*, 11(5), 573.
- Bakri, I. M., & Douglas, C. W. I. (2005). Inhibitory effect of garlic extract on oral bacteria. *Archives of oral biology*, 50(7), 645-651.
- Carreón-Delgado, D. F., Hernández-Montesinos, I. Y., Rivera-Hernández, K. N., del Sugeyrol Villa-Ramírez, M., Ochoa-Velasco, C. E., & Ramírez-López, C. (2023). Evaluation of pretreatments and extraction conditions on the antifungal and antioxidant effects of garlic (*Allium sativum*) peel extracts. *Plants*, 12(1), 217.
- Chen, C., Liu, C. H., Cai, J., Zhang, W., Qi, W. L., Wang, Z., ... & Yang, Y. (2018). Broad-spectrum antimicrobial activity, chemical composition and mechanism of action of garlic (*Allium sativum*) extracts. *Food Control*, 86, 117-125.
- Chen, K., Xie, K., Liu, Z., Nakasone, Y., Sakao, K., Hossain, M. A., & Hou, D. X. (2019). Preventive effects and mechanisms of garlic on dyslipidemia and gut microbiome dysbiosis. *Nutrients*, 11(6), 1225.
- Elmowalid, G. A., Abd El-Hamid, M. I., Abd El-Wahab, A. M., Atta, M., Abd El-Naser, G., & Attia, A. M. (2019). Garlic and ginger extracts modulated broiler chicks innate immune responses and enhanced multidrug resistant *Escherichia coli* O78 clearance. *Comparative immunology, microbiology and infectious diseases*, 66, 101334.
- El-Saber Batiha, G., Magdy Beshbishy, A., G. Wasef, L., Elewa, Y.H., A. Al-Sagan, A., Abd El-Hack, M.E., Taha, A.E., M. Abd-Elhakim, Y. and Prasad Devkota, H., 2020. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. *Nutrients*, 12(3), p.872.
- Heimesaat, M. M., Mousavi, S., Weschka, D., & Bereswill, S. (2021). Garlic essential oil as promising option for the treatment of acute *Campylobacteriosis*—results from a preclinical placebo-controlled intervention study. *Microorganisms*, 9(6), 1140.
- Jabar, M.A. and Al-Mossawi, A., 2007. Susceptibility of some multiple resistant bacteria to garlic extract. *African Journal of Biotechnology*, 6(6).
- Jeong, Y. Y., Ryu, J. H., Shin, J. H., Kang, M. J., Kang, J. R., Han, J., & Kang, D. (2016). Comparison of anti-oxidant and anti-inflammatory effects between fresh and aged black garlic extracts. *Molecules*, 21(4), 430.
- Khounghanian, R. M., Alwakeel, A., Albadah, A., Nakshabandi, A., Alharbi, S., & Almslam, A. S. (2023). The Antifungal Efficacy of Pure Garlic, Onion, and Lemon Extracts Against *Candida albicans*. *Cureus*, 15(5).
- Londhe, V.P., Gavasane, A.T., Nipate, S.S., Bandawane, D.D. and Chaudhari, P.D., 2011. Role of garlic (*Allium sativum*) in various diseases: An overview. *Angiogenesis*, 12(13), pp.129-134.

Maeda, T., Miki, S., Morihara, N., & Kagawa, Y. (2019). Aged garlic extract ameliorates fatty liver and insulin resistance and improves the gut microbiota profile in a mouse model of insulin resistance. *Experimental and therapeutic medicine*, *18*(1), 857-866.

Magryś, A., Olender, A., & Tchórzewska, D. (2021). Antibacterial properties of *Allium sativum* L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. *Archives of microbiology*, *203*, 2257-2268.

Oosthuizen, C. B., Reid, A. M., & Lall, N. (2018). Garlic (*Allium sativum*) and its associated molecules, as medicine. In *Medicinal plants for holistic health and well-being* (pp. 277-295). Academic Press.

Rabelo-Ruiz, M., Ariza-Romero, J. J., Zurita-González, M. J., Martín-Platero, A. M., Baños, A., Maqueda, M., ... & Peralta-Sánchez, J. M. (2021). Allium-based phytobiotic enhances egg production in laying hens through microbial composition changes in ileum and cecum. *Animals*, *11*(2), 448.

Rais, N., Ved, A., Ahmad, R., Kumar, M., Barbhai, M. D., Chandran, D., ... & Lorenzo, J. M. (2023). S-Allyl-L-Cysteine—A garlic Bioactive: Physicochemical Nature, Mechanism, Pharmacokinetics, and health promoting activities. *Journal of Functional Foods*, *107*, 105657.

Satora, M., Magdziarz, M., Rząsa, A., Rypuła, K., & Płoneczka-Janeczko, K. (2020). Insight into the intestinal microbiome of farrowing sows following the administration of garlic (*Allium sativum*) extract and probiotic bacteria cultures under farming conditions. *BMC veterinary research*, *16*(1), 1-18.

Yang, X., Bai, S., Wu, J., Fan, Y., Zou, Y., Xia, Z., ... & Yang, R. (2023). Antifungal Activity and Potential Action Mechanism of Allicin against *Trichosporon asahii*. *Microbiology Spectrum*, *11*(3), e00907-23.

Zhai, B., Zhang, C., Sheng, Y., Zhao, C., He, X., Xu, W., ... & Luo, Y. (2018). Hypoglycemic and hypolipidemic effect of S-allyl-cysteine sulfoxide (alliin) in DIO mice. *Scientific reports*, *8*(1), 3527.

Zhang, C., Xie, J., Li, X., Luo, J., Huang, X., Liu, L., & Peng, X. (2019). Alliin alters gut microbiota and gene expression of colonic epithelial tissues. *Journal of Food Biochemistry*, *43*(4), e12795.