Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

Emergent Roles of Garlic-Based Nanoparticles for Bio-medical Applications -A Review.

Suresh Babu Naidu Krishna[1*]

Research and Postgraduate Support, Durban University of Technology, Durban-4000, South Africa.
Corresponding author; https://orcid.org

Abstract

Garlic (*Allium sativum* L.) is well renowned for its antimicrobial potential and broadly consumed spice globally for its medicinal properties. Moreover, several studies advocate that garlic contains many bioactives that demonstrate strong therapeutic properties in treating cardiovascular diseases, digestive system, diabetes, cancer, obesity, hypertension, inflammation, antioxidant, and viral infection. Presently, therapeutics of natural origin are drawing major interest among consumers due to their antimicrobial efficacy and safety. Garlic extracts, chitosan by- products, several spices and antimicrobial peptides are illustrations for such products. The present review paper inspects briefly the major active ingredients and key biological functions of garlic-based nanoparticles discussing the relevant mechanisms of actions.

Keywords: Antimicrobial, bioactives, antioxidant, antibiofilm, health benefits, Natural products.

Introduction

Garlic (*Allium sativum* L.) is a renowned medicinal herb, acknowledged from primeval times and recognized for its culinary, flavoring, seasoning, nutraceutical, medicinal and insecticidal properties [1-3]. All preparations against various health problems are well-practiced and recorded amongst different folk cultures and literature. The health benefits of garlic derive from the synergistic activity of present complex chemical components. It is a rich source of various mineral components such as potassium, phosphorus, sulphur, zinc, selenium, germanium, major amino acids, and moderate amounts of vitamin A and C [4, 5].

The development and extensive predominance of multi-drug resistant bacteria are foremost public health burden across global [6]. The difficulties to control severe contagions occurrences due to high antibiotic resistance have led to increased health care expenditures as well as patient ill health and death. Currently, there is great demand for naturally derived product-based therapeutics such as garlic, spices, chitosan-based derivatives, and antimicrobial peptides which are considered as major alternatives for their antimicrobial usefulness and for low drug resistance amongst popular human pathogens. Lately, medicinally important garlic has attracted worldwide attention due to its antimicrobial potential and for its capability in not to accelerate drug resistance in clinically significant human pathogens. [7]. Garlic is a case of such naturally derived therapeutic compound.

Garlic is produced next to onion plant grown worldwide where China has major

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

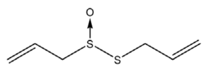
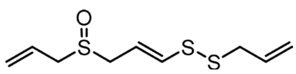
manufacturer share of 75% globally [8]. Furthermore, garlic is generally consumed for its antimicrobial properties against various kinds of bacteria, fungi, parasites, and execute biological activities which include anti-MDR, antibiofilm and fungicidal properties [3-5]. Moreover, recently there is rapid development in drug delivery using nanoparticles [9, 10] and the perspective of biomedical applications of garlic based nanoparticles has improved. Moreover, silver based nanoparticles (AgNPs) are extensively used in contrast to comprehensive range of bacterial pathogens [11]. Therefore, biosynthesis of silver nanoparticles particularly engaging plants has attracted a lot of attention as major alternatives in treatment and control of infection by human pathogens [12].

Nanotechnology entails the use and management of nanometer-size materials (1-100 nm) and their applications. Different forms of garlic extracts (Table 1) and their isolated bioactives are being used as a constituent in biosynthesis of nanoparticles owing to their exceptional properties appropriate to different applications. However, due to its strong odour, lipophilic nature, high volatility, low physicochemical stability, the extensive use of garlic oil has limited applications among people and communities[13-15]. Therefore, there is strong need for use of modern techniques which can enhance functional properties and preserve beneficial properties. To increase the industrial applications of garlic oils, developing into nanoscale microemulsions by reducing their normal dosage levels will be more advantageous [16]. Garlic based nanoparticles find extensive applications as therapeutics and to lesser extent in agriculture and food. There is great demand for nano based products which are biocompatible, sustainable, and less noxious to environment. In this setting, there is a rising attention in using plant phytoconstituents such as garlic and its extracts as a source of representatives to counter many phytopathogens that pose threat to agriculture production. The present review intends to provide informed support on garlic-based nanoparticles structures, and formulations with unique emphasis on allicin for usage in treatment of human diseases to highlight their biological effects (i.e., antibacterial, antibiofilm and antifungal) emphasizing biosafety and limitations of garlic-based nanoparticles use.

Active ingredients of Garlic

Allium sativum (bulbs) are designated to be enriched with numerous phytochemicals including sulfur-containing compounds. However, allicin and ajoene are two major chief active components of garlic, marking similarities and differences are shown in Table 1 [17]. The main active sulfur-containing compound of garlic is allicin [S-(2-propenyl)-2-propene-1-sulfinothioate] which is formed during tissue damage and is accountable for typical smell and taste of garlic [1]. Allicin is lipid soluble sulphur compound, deemed as the most abundant thiosulfinates found in freshly crushed garlic

Table 1 The comparison between garlic allicin and ajoene

Compound	Chemical name	Molecular Formula	Structure	Biological activity	Odour	Content
Allicin	Diallylthiosulphinate	$C_6H_{10}OS_2$	 allicin	Unstable	Sour or spicy	High
Ajoene	4,5,9-trithiadodeca-1,6,11-triene-9-oxide	$C_9H_{14}OS_3$		Stable	No distinctive smell	low

Current Trends in Biotechnology and Pharmacy
Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

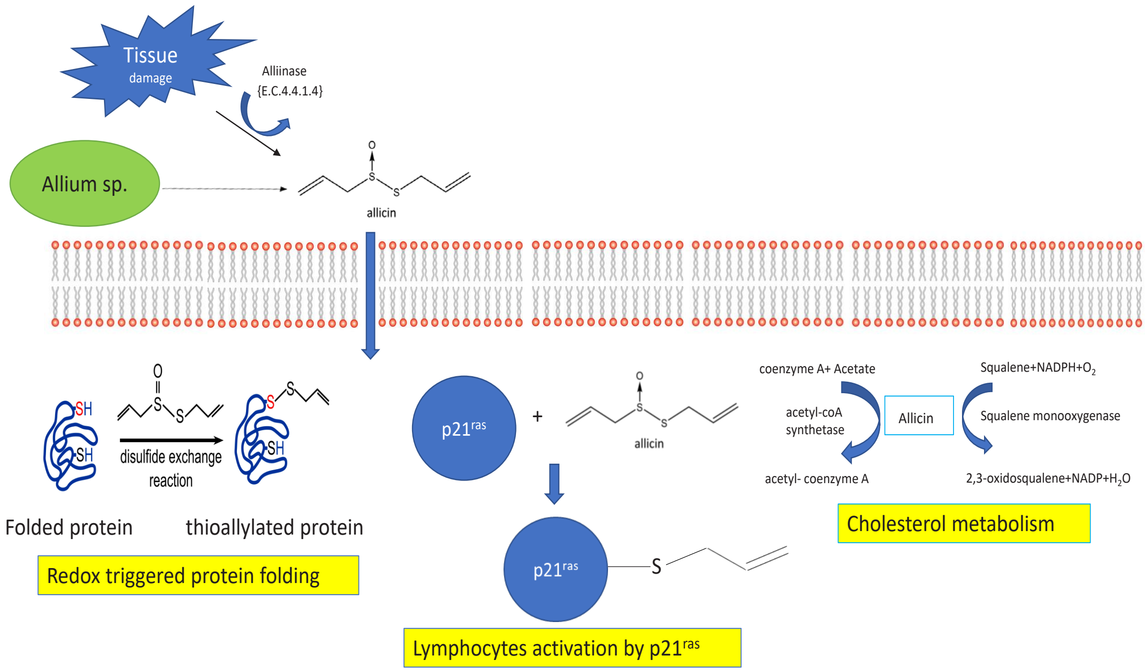


Fig.1. Overview of possible influence of allicin on protein synthesis and immunomodulation

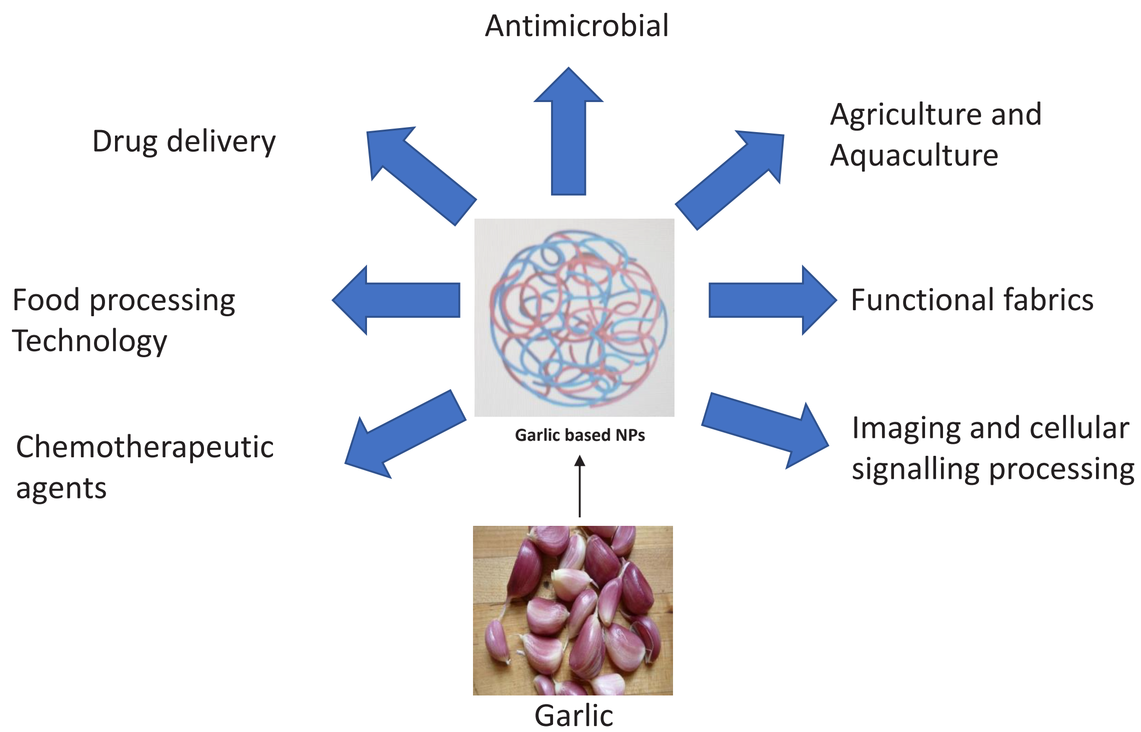


Fig. 2. Biomedical applications of garlic based nanoparticles

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

and is known to induce allergic reactions [18]. Furthermore, allicin is known to exhibit diverse biological properties comprising antimicrobial, antifungal and antibiofilm [19]. Besides, allicin is highly volatile, unstable, and rapidly oxidised with limited applications. The antimicrobial properties of allicin is exerted through multiple mechanisms which include 1) membrane disruption and thereby causing damage to cell structure [20, 21] 2) modify gene expression of microorganisms [22] 3) interact with thiol containing enzymes thereby inducing oxidative stress [23, 24](Fig.2) 4) immunomodulation [9]. Ajoene, on the other hand, an organosulfur compound found in garlic, is a molecule that is significantly stable and biologically active and is documented for its deep antimicrobial activity which specifically inhibits quorum sensing in pathogenic bacteria such as *Pseudomonas aeruginosa* [25, 26].

Garlic based biogenic nanoparticles and its bioactives applications

Antibacterial effect of garlic

Garlic based nanoparticles have multitude of biomedical applications as shown in Fig. 2. Allicin is the chief component of garlic that attributes towards diverse clinical pathogens which include antibiotic resistant, Gram-positive and Gram-negative bacteria such as *Shigella*, *Escherichia coli* [27], *Staphylococcus aureus*, *Pseudomonas aeruginosa* [28], *Streptococcus mutans*, *S. faecalis*, *S. pyogenes*, *Salmonella enterica*, *Klebsiella aerogenes* [29], *Vibrio*, *Mycobacteria*, *Proteus vulgaris*, and *Enterococcus faecalis* [30] (Table 2).

Tomisk et al. [31] reported preparation of wild garlic (*Allium ursinum* L.) extract encapsulated by means of spray congealing technology to safeguard its bioactive compounds with enhanced oral bioavailability. The encapsulation of microparticles led to enrichment of the extract dissolution performance and improve its solubility by more than 18-fold over pure extract. Furthermore, stability of microparticles was boosted over three months period displaying a minor reduction in bioactives (allicin and S-methyl methanethiosulfonate) whilst upholding determined antimicrobial properties. Consequently, the findings suggested that encapsulation of wild garlic (*A. ursinum*) extract by above technology is a trusted method toward enhancing properties of the extract without conceding its antibacterial properties alongside *P. hauseri*, *E. coli* and *S. enteritidis* for biopharmaceutical applications. Another study established a proteome model to identify allicin's cellular mechanism of action in *Escherichia coli* [32]. In this study, after treatment of cells with 0.79 mM allicin, 73 S-thioallylated proteins were isolated and studied further for its inhibition mechanism. It was demonstrated that, allicin modifies low-molecular weight cellular thiols such as glutathione (GSH), altering the GSH-based cellular redox-potential towards more oxidized state for set of cytoplasmic proteins. The candidate protein isocitrate lyase AceA, is mainly inhibited by allicin treatment.

The large surface area of nanoparticles (NPs) makes them more efficient against bacteria [33]. The efficacy of NPs counter to bacteria is principally due to electrostatic attraction between negatively charged membranes and positively charged NPs. Wu et al. [34] investigated the chemically synthesis of extremely active molecule, (2E, 2E)-4,4-trisulfanediylbis(but-2-enoic acid) (TSDB) using comparative molecular field analysis (COMFA) with diallyl trisulfide structure of garlic against *Staphylococcus aureus*. The authors concluded that TSDB presented strong inhibitory effect against *S. aureus* at low inhibitory and low bactericidal concentrations of 16 and 128 µg/mL, respectively by damaging the integrity of *S. aureus* cell membrane structure and weakening bacterial cell wall. Additionally, TSDB correspondingly enhanced the conductivity and protein expression in microbial broth but had little influence on leakage of extracellular alkaline phosphatase from cell wall. In another study, Zheng et al.[35] prepared monodispersed garlic oil microspheres in

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

Table 2 Garlic based extracts and their bioactives potential role as therapeutics.

Sl.No.	Bioactive	Bacteria	Fungi	Virus	Experimen-tal model/ Source used	Mode of action	Therapeutic role	Reference
1	Fresh garlic extracts	MRSA, Pseu-domonas aerug-inosa	Candida albicans		Clinical isolates and type cultures	Synergistic antimicrobial effect of aged garlic extracts with fungistats and antibiotics on bacterial and fungal growth	Antifungal	[37]
2	Garlic extract (Solgel)	methicillin-resistant Staphy lococcus aureus (MRSA) biofilms				slow and sustained release of garlic componentsii) sta-bilization of the active com-ponent and iii) significant enhancement of antimicro-bial and antibiofilm activity	Treatment of chronic infections due to biofilm forming drug-resist-ant bacteria	[38]
3	Allicin (Polybutyl cyanoacrylate) nanoparticles		Candida albicans			Prolonged release effect and nano-scale dimen-sions of PBCA nanoparti-cles	Antifungal	[39]
4	Allicin (Novel gel Formulation)	Lance-field group B strepto-cocci (GBS)	clinical isolates of Can-dida		Clinical isolates of Can-dida		Antimi-cro-bial	[40]
5	Diallyl disulfide [Solid lipid nanopar-ticle]				breast cancer cell lines (MCF-7 and MCF-10)	Enhanced anticancer effect through induction of apoptosis	Anticancer	[41]
6	Allicin [Nano-composite hydrogel]	Candida albicans (ATCC10231), As-pergillus fumiga-tus (PTCC5009), Tri-cho-phyton rubrum (PTCC5143), Epi-der-mo-phyton floccosum (PTCC52063) and Micro-sporum canis (PTCC5069) rum can-is (PTCC5069)			Superior wound dressing materi-als		Wound healing	[42]
7	Allicin				AS Mice	Allicin signifi-cantly de-crease TNF-α, IL-6, and IL-8 secre-tion and HLA-B2704 protein ex-pres-sion	Anti-inflam-matory	[43]

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

water by microemulsion method to increase volatile characteristics and poor aqueous solubility of garlic oil. The study concluded that the water-dilutable microemulsion formed by garlic oil encapsulation in a nanoparticle vector is active in inhibiting *Staphylococcus aureus* than *Escherichia coli*. Sheppard et al.[36] screened chemical library containing nineteen synthesized pyridyl disulfides that emulate the chemical reactivity of allicin for antimicrobial properties against Gram-positive species including vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. The results evidenced that pyridyl disulfides are stable alternatives to allicin with a comparable narrow spectrum profile and are believed to function as pro-oxidants in a manner suggestive of allicin.

Abbreviations: Ankylosing Spondylitis Mice; MRSA-Methicillin resistant *Staphylococcus aureus;* PBCA- Polybutylcyanoacrylate

Antibiofilm effect of garlic

Biofilm refers to normal bacterial growth across all environmental surfaces which include tissues, medical devices, natural aquatic systems, or potable water system. Microorganisms such as *E coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungi belonging to *Candida* sp. are clinically important and known for forming biofilms. The biofilm development indicates increased resistance to antibiotics and antimicrobial agents [38]. Lately, Sekar et al. [12] reported facile and low-cost garlic clove extract-based silver nanoparticles biosynthesis and its wide spectrum of therapeutic action comprising antibiofilm, antiparasitic and anti-breast cancer activity. The results demonstrated that biosynthesized garlic clove extract silver nanoparticles (G-AgNPs) presented high anti-bacterial and antibiofilm activity on clinically significant pathogens including methicillin-resistant *S. aureus* and *P. aeruginosa* at 100 µg ml^{-1}concentartions. Furthermore, nanoparticles revealed promising anticancer effect by inhibiting MCF-7, human breast cancer cell viability at 100 µg ml^{-1} after 24 h incubation. It is concluded that garlic clove extract-based silver nanoparticles to have promising biomedical applications and can be eco-friendly having no side effects to environment.

A series of allicin analogues were developed for evaluation of antimicrobial potential and thermal stability against bacteria and fungus *S. cerevisiae* by Roman et al. [44]. Interestingly, dimethyl- (DMTS), diethyl- (DETS), diallyl- (DATS, allicin), dipropyl- (DPTS) and dibenzyl- (DBTS) thiosulfinates form a series of increasing molecular mass and hydrophobicity which were anticipated to distress physical properties such as volatility, diffusion and membrane permeability all contributing towards disturbance in bio properties of molecules. In this study, the authors noted that more volatile compounds indicated antimicrobial properties via the gas phase. Additionally, a chemogenetic screening with yeast mutants shown that the mode of action of these analogues was like that of allicin and that glutathione pool and glutathione metabolism were of central importance for resistance against them. The results demonstrated by allicin analogues could be ideal for biomedical and agricultural application either singly or in combination for developing new novel antimicrobial agents. Reiter et al.[45] examined the removal behavior and antibacterial action of allicin aerosols and vapor in a lung model. The Axial velocity air flow distribution model associated with areas showing bacterial inhibition demonstrated that it has remarkable value and can lower the need of animal sacrifice in clinical studies of novel antibiotics in future. Moreover, a synergistic effect of allicin vapor and ethanol in the direction of bacterial growth inhibition was presented. Von White et al [46] examined “green” synthesis of monodisperse silver nanoparticles using *Allium sativum* (garlic) extract as reducing and stabilizing agents. This study proved that garlic extract has stabilizing effect on silver nanoparticles and are resistant to aggregation in biological media with high

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

oxidative resistance in presence of hydrogen peroxide in comparison to citrate stabilized silver nanoparticles. Also, cytotoxic studies established no decrease in cellular proliferation suggesting improved biocompatibility of silver nanoparticles biosynthesized using garlic extract. Due to many beneficial medicinal properties of garlic, the biosynthesized silver nanoparticles find biomedical applications as therapeutics and aid in progress of novel technologies in human health and safety. Nguyen et al. [47], established nanostructured mesoporous silicon (pSi) derivative of silicon-accumulator plant Tabasheer (Bambuseae) to assist as a potential carrier matrix as carrier and stabilizing agent for naturally active garlic oil encompassing phytochemicals (namely, allicin) which can inhibit growth of Staphylococcus aureus. The study resolute that under prolonged UV irradiation (λ = 365 nm, 24 h) exposure, free extract completely loses all quantifiable antibacterial activity versus *S. aureus*.

Antifungal effect of garlic

Garlic extracts (GE) are well known to show antifungal properties by changing membrane structure by varied membrane permeability and conductivity in fungal mycelia leading to leakage of protein and there by disruption in cell membrane integrity [48] and by modify gene expression of microorganisms [49]. Antimycotic activity of garlic-based silver nanoparticles was demonstrated by Robles-Martinez et al. [50]. Fungal infection (dermatophytic onychomycosis) of skin was tested by synthesized GE AgNPs (AsExt-AgNPs), which exhibited complete inhibition of *Tricophyton rubrum* a dermatophyte, making them more operative antimycotic candidates at all-natural concentrations of AgNPs. The projected mechanism for their antifungal properties has been the cytoplasmic membrane disruption by garlic extract. Polybutylcyanoacrylate (PBCA)-Allicin NPs (wrapped with allicin by polymerization) have presented successful inhibition of fungal pathogens including *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubum*, *Microsporum gypseum*, *M. Canis* and *Epidermophytone floccosum* comparative to pure allicin, which may be credited to the sustained release of allicin after wrapping into PBCA NPs and given their nanoscale dimensions [51].

Biosafety of Garlic extract

There is considerable literature available on GE toxicity and allicin which is major barrier against its clinical usefulness though it exhibits potential antimicrobial and antibiofilm activities [16, 52, 53]. Zhu and Zeng [54] tested the safety of garlic extract (administered at dose of 250 mg/kg of GE daily) were given to rats intraperitoneally for 38 days. With increasing dose of garlic extract, serum creatinine and liver enzymes were also elevated. Concurrently, the structure and function of related organs were also damaged. Therefore, it was concluded that GE administration was safe at low levels (250–350 mg/kg/day) while high doses(>400 mg/kg/day) will present severe danger to selected organs. In an alternative study by Lawal et al. [55] recommended that the safe dose of GE was 350 mg/kg. Regular doses of a garlic supplemented with allicin being the major constituent ranging from 0.05 g to 1.5 g, were evaluated during 1–24 weeks in type 2 diabetes patients[53]. No significant benefits were noticed in a variety of biochemical parameters, including total cholesterol (LDL and HDL) progress, glucose levels and blood liquid profiles (triglyceride, total cholesterol, LDL and HDL) regulation. Regarding side effects, a low rate of gastric disturbances was observed, such as indigestion and heartburn. Several side effects of using garlic containing supplements are registered in the literature, which include and not limited to perspiring, vertigo, reflux, menstrual disorders, anaphylaxis in some isolated cases, liver toxicity and abdominal pain. Nevertheless, these consequences are primarily observed in people who are allergic to garlic constituents, such as allicin [10, 56]. Therefore, taking the above results in confidence, the allicin amount should not be

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

consumed at higher doses. In general, garlic is regarded as a safe product, but its copious consumption should be prevented in noticeable cases of reaction due to its consumption.

Limitations of garlic extract use

Considerable progress has been made on use of garlic extract as potential phytotherapeutic agent which proves to be highly efficient with minimum side effects nonetheless low in vivo efficacy due to its low solubility, lipophilic properties, weak absorption and less bioavailability [15]. Nevertheless, there are still many barriers to be answered, including the following issues: (i) how to improve biological activity of GE to be efficiently maintained? (ii) which is safe route of administration (local or systemic) for GE to be safer and more compelling? (iii) Pharmacokinetics of GE under different conditions of administration? (iv) what would be ideal administration frequency for GE to be more successful?

Conclusion

Garlic is a widely used spice with a distinct fragrance that has been linked to a variety of health benefits for humans around the world. Chemical sulfides, saponins, phenolic compounds, and polysaccharides are among the bioactive materials. Garlic and its bioactive compounds (allicin) have potential as functional foods or nutraceuticals for disease prevention and treatment. In the future, garlic applications should be more directed on pharmaceutical formulations; additional biological functions of garlic should be evaluated, and the comparative compounds of garlic need to be separated and ascertained. Nonetheless, future research on garlic should be directed towards pharmaceutical formulation, instability, reactivity, volatility, biocompatibility, and its delivery. In conclusion, this review, excitedly, will support to bring around scientific literature on the potential effects of garlic extract based nanoparticles on antibacterial, antibiofilm, antifungal and biosafety concerns to the attention of interested scientific community who may then further contribute to our understanding of this fascinating molecule in biomedical applications.

Acknowledgement:

We extend our sincere acknowledgement to Prof Linda Zikhona Linganiso (Research Director, DUT); Prof JK Adam (DUT); Prof. Sibusiso Moyo, DVC Research Innovation Engagement, Durban University of Technology, South Africa for awarding Research fellowship to Dr. K. Suresh Babu Naidu.

Conflict of interest: Author declare no conflict of interest.

Funding: The author received no funding/ grants for this work

References

1. Ankri S, M. T., Rabinkov A, Wilchek M, Mirelman D. (1997). Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of Entamoeba histolytica. *Antimicrob Agents Chemother, 41*, 2286-2288.

2. Barros, C. H. N., & Casey, E. (2020). A Review of Nanomaterials and Technologies for Enhancing the Antibiofilm Activity of Natural Products and Phytochemicals. *Acs Applied Nano Materials, 3*(9), 8537-8556. doi:10.1021/acsanm.0c01586

3. Chen, C., Liu, C.H., Cai, J., Zhang, W., Qi, W.L., Wang, Z., Liu, Z. Bin & Yang, Y. (2018). . (2018). Broad-spectrum antimicrobial activity, chemical composition and mechanism of action of garlic (Allium sativum) extracts. *Food Control, 86, 117–125. 564, 86*, 117–125. 564.

4. Corzo-Martínez, M., Corzo, N., & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends in Food Science & Technology, 18*(12), 609-625.

5. Cutler, R. R., Odent, M., Hajj-Ahmad, H., Maharjan, S., Bennett, N. J., Josling, P.

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

D., . . . Dall'Antonia, M. (2009). In vitro activity of an aqueous allicin extract and a novel allicin topical gel formulation against Lancefield group B streptococci. *Journal of antimicrobial chemotherapy, 63*(1), 151-154.

6. Cutler, R. W., P. . (2004). Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant Staphylococcus aureus. *Br. J. Biomed. Sci, 61*, 71-74.

7. El-Saber Batiha, G., Magdy Beshbishy, A., G Wasef, L., Elewa, Y. H., A Al-Sagan, A., El-Hack, A., . . . Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. *Nutrients, 12*(3), 872.

8. Fahmy, S. A., & Mamdouh, W. (2018). Garlic oil–loaded PLGA nanoparticles with controllable size and shape and enhanced antibacterial activities. *Journal of Applied Polymer Science, 135*(16), 46133.

9. Farrag, H. A., Hosny, A. E.-D. M., Hawas, A. M., Hagras, S. A., & Helmy, O. M. (2019). Potential efficacy of garlic lock therapy in combating biofilm and catheter-associated infections; experimental studies on an animal model with focus on toxicological aspects. *Saudi Pharmaceutical Journal, 27*(6), 830-840.

10. Girish, V. M., Liang, H., Aguilan, J. T., Nosanchuk, J. D., Friedman, J. M., & Nacharaju, P. (2019). Anti-biofilm activity of garlic extract loaded nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine, 20*, 102009. doi:https://doi.org/10.1016/j.nano.2019.04.012

11. Gu, X., Wu, H., & Fu, P. (2013). Allicin Attenuates Inflammation and Suppresses HLA-B27 Protein Expression in Ankylosing Spondylitis Mice. *BioMed Research International, 2013*, 171573. doi:10.1155/2013/171573

12. H. Wang, X. L., D. Shen, Y. Oiu, J. Song. (2014). Diversity evaluation of morphological traits and allicin content in garlic (Allium sativum L.) from China. *Euphytica, 198*, 243-254.

13. Hussein, H. J., Hameed, I. H., & Hadi, M. Y. (2017). A Review: Anti-microbial, Anti-inflammatory effect and Cardiovascular effects of Garlic: Allium sativum. *Research Journal of Pharmacy and Technology, 10*(11), 4069-4078.

14. J.S. Kim, J. S. K., K. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, M.H. Cho,, . (2007). Antimicrobial effects of silver nanoparticles. *Nanomed. , 3*, 95-101.

15. Jakobsen, T. H., Gennip, M. Van, Phipps, R.K., Shanmugham, M.S., Christensen, L.D., Alhede, M., Skindersoe, M.E., Rasmussen, T.B., Friedrich, K., Uthe, F., Jensen, P.Ø., Moser, C., Nielsen, K.F., Eberl, L., Larsen, T.O., Tanner, D., Høiby, N., Bjarnsholt, T. & Givskov, M. . (2012). Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrobial Agents and 640 Chemotherapy, 56*, 2314-2325.

16. K. Hirsch, M. D., J. Giat, T. Miron, A. Rabinkov, M. Wilchek, D. Mirelman, J. Levy, Y. Sharoni. (2000). Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation,. *Nutr. Cancer, 38*, 245-254.

17. Kuda, T. I., A.; Yano, T. . (2004). Effect of red pepper Capsicum annuum var. conoides and garlic Allium sativum on plasma lipid levels and cecal microflora in mice fed beef tallow. *Food Chem. Toxicol.,*

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

42, 1695–1700.

18. Lawal, B., Shittu, O. K., Oibiokpa, F. I., Mohammed, H., Umar, S. I., & Haruna, G. M. (2016). Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. *Journal of Acute Disease, 5*(4), 296-301.

19. Li, G., Ma, X., Deng, L., Zhao, X., Wei, Y., Gao, Z., . . . Sun, C. (2015). Fresh garlic extract enhances the antimicrobial activities of antibiotics on resistant strains in vitro. *Jundishapur journal of microbiology, 8*(5).

20. Li WR, S. Q., Dai HQ, et al. (2016). Antifungal activity, kinetics and molecular mechanism of action of garlic oil against Candida albicans. *Sci Rep, 6*, 22805.

21. Li WR, S. Q., Dai HQ, et al. (2016). Antifungal activity, kinetics and molecular mechanism of action of garlic oil against Candida albicans. *Sci Rep, 6*, 22805. doi:DOI: 10.1038/ srep22805

22. Luo, D. Q., Guo, J. H., Wang, F. J., Jin, Z. X., Cheng, X. L., Zhu, J. C., . . . Zhang, C. (2009). Anti-fungal Efficacy of Polybutylcyanoacrylate Nanoparticles of Allicin and Comparison With Pure Allicin. *Journal of Biomaterials Science, Polymer Edition, 20*(1), 21-31. doi:10.1163/156856208X393473

23. Luo, D. Q., Guo, J.H., Wang, F.J., Jin, Z.X., Cheng, X.L., Zhu, J.C., Peng, C.Q. & Zhang, C. . (2009). Anti-fungal efficacy of polybutylcyanoacrylate nanoparticles of allicin and comparison with pure allicin. Journal of Biomaterials Science. *Polymer Edition,, 20*, 21-31.

24. Morales-González, J. A., Madrigal-Bujaidar, E., Sánchez-Gutiérrez, M., Izquierdo-Vega, J. A., Valadez-Vega, M. d. C., Álvarez-González, I., . . . Madrigal-Santillán, E. (2019). Garlic (Allium sativum L.): A brief review of its antigenotoxic effects. *Foods, 8*(8), 343.

25. Müller, A. e. a. (2016). Allicin Induces Thiol Stress in Bacteria through S-Allylmercapto Modification of Protein Cysteines. *J. Biol Chem., 291*, 11477-11490.

26. Nguyen T. L, J. R. K., Armando, L., Leigh T. C.,,and Jeffery L. C. (2017). Biogenic Nanostructured Porous Silicon as a Carrier for Stabilization and Delivery of Natural Therapeutic Species. *Molecular Pharmaceutics, 14*, 4509-4514. doi:DOI: 10.1021/acs.molpharmaceut.7b00638

27. Olad, A., Eslamzadeh, M., Katiraee, F., & Mirmohseni, A. (2020). Evaluation of in vitro anti-fungal properties of allicin loaded ion cross-linked poly (AA-co-AAm)/PVA/ Cloisite 15A Nanocomposite hydrogel films as wound dressing materials. *Journal of Polymer Research, 27*(4), 1-10.

28. Prager-Khoutorsky M, G. I., Rabinkov A, Mirelman D, Geiger B, Bershadsky AD. (2007). Allicin inhibits cell polarization, migration and division via its direct effect on microtubules. *Cell Motil Cytoskeleton, 64*, 321-327.

29. Privett BJ, B. A., Bauman SJ, Riccio DA, Schoenfisch MH. (2012). Examination of bacterial resistance to exogenous nitric oxide. *Nitric oxide, 26*, 169-173.

30. Rabinkov A, M. T., Konstantinovski L, Wilchek M, Mirelman D, Weiner L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochim Biophys Acta, 1379*, 233-244.

31. Rasul Suleria, H. A., Sadiq Butt, M., Muhammad Anjum, F., Saeed, F., Batool, R., & Nisar Ahmad, A. (2012). Aqueous

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

garlic extract and its phytochemical profile; special reference to antioxidant status. *International Journal of Food Sciences and Nutrition, 63*(4), 431-439.

32. Reiter, J., Borlinghaus,J., Dörner, P.,Wolfgang,S., Gruhlke,M.C.H.,Klaas,M and Slusarenko,A.J. (2020). Investigation of the deposition behaviour and antibacterial effectivity of allicin aerosols and vapour using a lung model. *Experimental and therapeutic medicine, 19*, 1541-1549. doi:DOI: 10.3892/etm.2019.8387

33. Robles-Martínez, M., González, J.F.C., Pérez-Vázquez, F.J., Montejano-Carrizales, J.M., Pérez, E. & Patiño-Herrera, R. . (2019). Antimycotic Activity Potentiation of Allium sativum Extract and Silver Nanoparticles against Trichophyton rubrum. *Chemistry and Biodiversity, 16*.

34. Roman, L. N., H. Claus, J. Martin C. H. G & Alan J. S. (2018). A Comparison of the Antibacterial and Antifungal Activities of Thiosulfinate Analogues of Allicin. *Sci Rep, 8*, 6763. doi:DOI:10.1038/s41598-018-25154-9

35. Ross, Z. M. O. G., E.A.; Hill, D.J.; Sleightholme, H.V.; Maslin, D.J.. (2001). Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Appl. Environ. Microbiol, 67*, 475-480.

36. Salehi, B., Zucca, P., Orhan, I. E., Azzini, E., Adetunji, C. O., Mohammed, S. A., . . . Ahmad, Z. (2019). Allicin and health: A comprehensive review. *Trends in Food Science & Technology, 86*, 502-516. doi:https://doi.org/10.1016/j.tifs.2019.03.003

37. Schwingshackl, L., Missbach, B., & Hoffmann, G. (2016). An umbrella review of garlic intake and risk of cardiovascular disease. *Phytomedicine, 23*(11), 1127-1133. doi:https://doi.org/10.1016/j.phymed.2015.10.015

38. Sekar Vijayakumara, B. M., Kandasamy Saravanakumar Esteban F. Durán-Larac Myeong-Hyeon Wang, Baskaralingam Vaseeharana. (2019). Garlic clove extract assisted silver nanoparticle – Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. *Journal of Photochemistry & Photobiology, B: Biology, 198*, 111558. doi:https://doi.org/10.1016/j.jphotobiol.2019.111558

39. Shang, A., Cao, S.-Y., Xu, X.-Y., Gan, R.-Y., Tang, G.-Y., Corke, H., . . . Li, H.-B. (2019). Bioactive compounds and biological functions of garlic (Allium sativum L.). *Foods, 8*(7), 246.

40. Sharma, N., Behl, T., Singh, S., Bansal, A., Singh, S. K., & Zahoor, I. (2021). Expatiating the Therapeutic Profile of Garlic (Allium sativum): a Bench to Bedside Approach.

41. Sheppard, J. G., McAleer,J.P., Saralkar,P., Geldenhuys, W.J and Long, T.E. (2017). Allicin-inspired pyridyl disulfides as antimicrobial agents for multidrug-resistant Staphylococcus aureus. *European Journal of Medicinal Chemistry*. doi:0.1016/j.ejmech.2017.10.018

42. Silva, F., Khokhar, S.S., Williams, D.M., Saunders, R., Evans, G.J.S., Graz, M. & Wirth, T. . (2018). Short Total Synthesis of Ajoene. *Angewandte Chemie International Edition, 57*, 12290–12293.

43. Subbanna, S., TS, G., & Basalingappa, K. M. (2020). Biogenic Nanoparticles From Allium Sativum And Its Bioactives Applications. *European Journal of Molecular & Clinical Medicine, 7*(8), 212-232.

Vol. 15 (3) 349-360, July 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.3.35

44. Suleria, H. A. R., Butt, M. S., Khalid, N., Sultan, S., Raza, A., Aleem, M., & Abbas, M. (2015). Garlic (Allium sativum): diet based therapy of 21st century–a review. *Asian Pacific journal of tropical disease, 5*(4), 271-278.

45. Talluri, S. V., Kuppusamy, G., Karri, V. V. S. R., Yamjala, K., Wadhwani, A., Madhunapantula, S. V., & Pindiprolu, S. S. (2017). Application of quality-by-design approach to optimize diallyl disulfide-loaded solid lipid nanoparticles. *Artificial cells, nanomedicine, and biotechnology, 45*(3), 474-488.

46. Tomišk, A., Šarić,L., Bertoni,S., Protti,M., Albertini,B., Mercolini,L and Passerini, N. (2018). Encapsulations of wild garlic (Allium ursinum L.) extract using spray congealing technology. *Food Research International*. doi:doi:10.1016/j.foodres.2018.10.081

47. Vallerinteavide Mavelli Girish, H. L., Jennifer T. Aguilan, Joshua D. Nosanchuk, Joel M. Friedman, Parimala Nacharaju. (2019). Anti-biofilm activity of garlic extract loaded nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine, 20*, 102009. doi:https://doi.org/10.1016/j.nano.2019.04.012

48. Von White, G., 2nd, Kerscher, P., Brown, R. M., Morella, J. D., McAllister, W., Dean, D., & Kitchens, C. L. (2012). Green Synthesis of Robust, Biocompatible Silver Nanoparticles Using Garlic Extract. *Journal of nanomaterials, 2012*, 730746. doi:10.1155/2012/730746

49. Wallock-Richards, D. D., C.J.; Doherty, L.; Clarke, D.J.; Place, M.; Govan, J.R.; Campopiano, D.J. (2014). Garlic revisited: Antimicrobial activity of allicin-containing garlic extracts against Burkholderia cepacia complex. *PLoS ONE, 9*, e112726.

50. Wang J, V. W. (2016). Antimicrobial nanomaterials derived from natural products—a review. *Materials (Basel, Switzerland), 9*.

51. Wu T, H. Y., Chen Y, Zhang M. (2018). Antibacterial Effect of (2E,2E)-4,4-Trisulfanediylbis (but-2-enoic acid) against Staphylococcus aureus. *Plos One, 13*(5), e0197348. doi:https://doi.org/10.1371/journal.pone.0197348

52. Xu, L., Wang, Y.-Y., Huang, J., Chen, C.-Y., Wang, Z.-X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics, 10*(20), 8996.

53. Yadav, S., Trivedi, N. A., & Bhatt, J. D. (2015). Antimicrobial activity of fresh garlic juice: An in vitro study. *Ayu, 36*(2), 203.

54. Zheng, H. M., Li,B.H., Wang,D.W and Liu, D. (2013). Preparation Methods for Monodispersed Garlic Oil Microspheres in Water Using the Microemulsion Technique and Their Potential as Antimicrobials. *J Food Sci, 78*(8), 1301-1306. doi:doi: 10.1111/1750-3841.12208

55. Zhu, X.-Y., & Zeng, Y.-R. (2020). Garlic extract in prosthesis-related infections: a literature review. *Journal of International Medical Research, 48*(4), 0300060520913778.

56. Zhu, X. Y., & Zeng, Y. R. (2020). Garlic extract in prosthesis-related infections: a literature review. *Journal of International Medical Research, 48*(4). doi:10.1177/0300060520913778