

Studies on Antimicrobial and Phytochemical Properties of *Allium sativum* Extracts

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ABSTRACT: Investigations were carried out to study the inhibitory effect of garlic (*Allium sativum*) extracts at 5%, 10%, 20%, 30%, 40%, 50% level in vitro on *E.coli* and *S.aureus*. Garlic extract exhibited bacteriostatic effect against both the test organisms. The inhibitory effect increased with the increase in the concentration of extracts. The garlic extract was more effective against *S.aureus* than *E.coli*. Phytochemical screening of the different extracts showed that garlic contains important compounds such as carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, and Tannins in the extracts under the conditions of this study. The significance of these results is discussed. It was concluded that *Allium sativum* possess a good potential to act as antimicrobial activity

KEYWORDS: *Allium sativum*, Antimicrobial activity.

I. INTRODUCTION

Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. [1, 2]. Garlic is commonly known as *Allium sativum*. The plant is a member of the Liliaceae family and one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases [3]. Also, it is a bulbous perennial food plant of the family Alliaceae which give it a botanical name known as *Allium Sativum* and the common name Garlic. When crushed, *Allium sativum* yield allicin, an antibiotic and antifungal compound (phytoncide). It has been claimed that it can be used as home remedy to help speed recovery from strep throat or other minor ailments because of its antibiotic properties [4]. It also contains the sulphur containing compounds allinin, ajoene, diallylsulfide, enzymes, B-vitamins, proteins, minerals, saponins, flavonoids and maillard reaction products, which are not sulphur containing compounds. Actually garlic contain variety of effective compound that exhibit anticoagulant (antithrombotic) [5] antioxidant, antibiotics, hypocholesterolaemic and hypoglycemic as well as hypotensive activities [1]. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibers to protect human against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour and colour. Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example, alkyl sulfide (found in onions and garlic), carotenoids (from carrots), and flavonoids (present in fruits and vegetables) [2]. Reactive oxygen-free radicals (ROS) have been implicated in many diseases and in aging process. These free radicals, which cause tissue damage via oxidative stress, are usually generated by aerobic respiration, inflammation, and lipid peroxidation. Antioxidant systems minimize or prevent deleterious effects of the ROS. Due to its medicinal values, it is important to determine some of the phytochemicals presents.

II. LITERATURE SURVEY

Garlic antimicrobial activities have been recognized for centuries being many of its therapeutic properties first mentioned in 1500 BC in an Egyptian recipe named PapyrusEbers. Currently, it is used in folk medicine for the treatment of many diseases and for the preservation of food products due to its antiseptic and disinfectant properties.

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Allium sativum L. Liliaceae or simply garlic contains various biologically active constituents, like alliin, alliinase, allicin, S-allylcystein, diallylsulphide and allmethyltrisulphide. Alliin is an amino acid, which is converted into allicin by alline-lyase catalyses when the bulbs are crushed. Allicin is the precursor of sulphur-containing compounds, which are responsible for the flavour, odour and pharmacological properties. Once exposed to air, allicin is further converted into diallyldisulphide, which has antibacterial effects, and the reduction by cysteine will disrupt the disulphide bond in microbial proteins [6]. Bacterial growth inhibition and bactericidal properties are mainly attributed to allicin and thiosulphonates found in garlic but other sulphur-containing compounds, such as ajoene, also decrease bacterial growth. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibers to protect human against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour and colour. Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example, alkyl sulfide (found in onions and garlic). metabolites such as alkaloids, flavonoids, glycosides, cardiac glycosides tannin, phenolic compounds, saponins, terpenoids and steroids. This is in agreement with the work done by Idowu et al. [7]. Secondary metabolites especially alkaloids, flavonoids, saponins, and tannins are known to have curative activity against several pathogens [8]. These compounds are known primarily for their role in reducing the permeability of blood capillaries and strengthening their resistance

III. MATERIALS AND METHOD

PLANT MATERIAL

Garlic (*Allium sativum*) used in the present study was purchased from the local market.

PROCESSING OF PLANT MATERIAL

Bulbs were peeled, washed and 50 g of cloves were weighed. These cloves were smashed in surface sterilized mortar and pestle. Fresh juice was squeezed from the paste and filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate obtained was used as crude extract (100%) kept in sterile bottle under refrigerated condition until use.

PREPARATION OF THE DIFFERENT DILUTIONS OF CRUDE EXTRACT

The crude filtrate was then used for testing antibacterial activity at different concentrations of 5%, 10%, 20%, 30%, 40% and 50 % w/v with sterile distilled water as solvent.

Table 1. Dilution table for different concentrations of crude extract

CONCENTRATION (%)	5	10	20	30	40	50
VOLUME OF CRUDE EXTRACT (ml)	4.75	4.5	4.0	3.5	3.0	2.5
VOLUME OF DILUENT (ml)	0.25	0.5	1.0	1.5	2.0	2.5

BACTERIAL STRAINS SELECTED FOR SUSCEPTIBILITY ASSAY

Two bacteria namely *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria) were screened for present investigation. These pure bacterial cultures were maintained in nutrient agar slants.

PREPARATION OF INOCULUM

About 18-hour broth culture of the test bacteria isolate was suspended into sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standards by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml.

TESTING FOR ANTIBACTERIAL ACTIVITY

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described [9]. Cup-plate method was used to test the antibacterial activities of the aqueous *Allium sativum* against the test bacteria isolates, 0.2 ml of standardized bacterial stock suspensions of 10^8 - 10^9 colony-forming units per ml was

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thoroughly mixed with 20 ml of sterile MH agar and poured into sterile Petri dishes. The agar was left to set and in each of these plates, 4 cups, 10 mm in diameter, were cut using a sterile cork borer. Alternate cups were filled with 0.1ml of each extract using micropipette and allowed to diffuse at room temperature for one hours. The plates were then incubated in the upright position at 37°C for 24 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the growth inhibition zones were measured, averaged and the mean values were tabulated (Table 2).

PHYTOCHEMICAL ANALYSIS OF ACTIVE CRUDE EXTRACT

Aqueous extract of garlic was evaluated for its photochemistry by standard methodology. [10]

a. Test for carbohydrates: Benedict's test

Equal volumes of Benedict's reagent and crude extract were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

b. Tests for Proteins: Xanthoprotein test:

To 1ml of extract, 1ml of conc.H₂SO₄ was added. This resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH₄OH, yellow ppt. turned orange.

c. Test for glycosides: Borntrager's Test:

To the 3ml of aqueous extract, dil.H₂SO₄ was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. This solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

d. Test for Steroids: Salkowski Test:

To 2ml of aqueous extract, 2ml of chloroform and 2ml of conc.H₂SO₄ was added. The solution was shaken well. As a result, chloroform layer turned red and acid layer showed greenish yellow fluorescence.

e. Tests for alkaloids: The aqueous extract was evaporated in a test tube. To the residue dilute HCl was added, shaken well and filtered. To the filtrate add Hager's reagent. Yellow precipitate shows the presence of alkaloids.

f. Tests for flavonoids- To small quantity of aqueous extract 10% of lead acetate solution was added. Formation of yellow precipitate showed the presence of alkaloids.

h. Test for anthraquinones- 0.5 gm of the extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

i. Test for Tannins- For 2ml of extract add few drops of 1% lead acetate. A yellowish precipitate showed the presence of tannins.

j. Test for Terpenoids- 2ml of aqueous extract was added to 2ml of acetic anhydride and concentration of H₂SO₄. Formation of blue, green rings indicated the presence of terpenoids.

IV.EXPERIMENTAL RESULTS

Table 2, Fig 1 showed the diameter of zone of inhibition of the extracts at different concentrations against the test organism. The sensitivity of the previously mentioned bacteria gradually increased with the increment of concentration of extract. The zone of the inhibition was 32 mm and 24 mm was recorded for the concentration of 50 mg/ml, for *S.aureus* and *E.coli*. The concentrations 5-10 mg/ml were rather low active in preventing the growth of *S. aureus*, the concentrations 20-30 mg/ml were moderate active, while the concentrations 40-50mg/ml were highly active compared to ampoxcin as a control .. There was a proportional relation between the concentrations of extract and the diameters of inhibition zones of the growth of *Staphylococcus aureu* and *E.coli*. It was proved from the present investigation that both test organisms were sensitive and inhibited by aqueous garlic extract.

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Table 1. Garlic extract concentrations showed zone of inhibition (mm) against E.coli and S.aureus

CONCENTRATIONS OF CRUDE EXTRACT	ZONE OF INHIBITION (mm)	
	<i>S.aureus</i>	<i>E.coli</i>
5%	16	13
10%	17	18
20%	20	19
30%	24	20
40%	27	20
50%	32	24
Crude extract	35	30
Positive control [Drug (ampoxin)]	32	29
Negative control [Distilled water]	00	00

Figure 1. The inhibitory effect of different concentration of garlic extract on the growth of *S.aureus* and *E.coli*

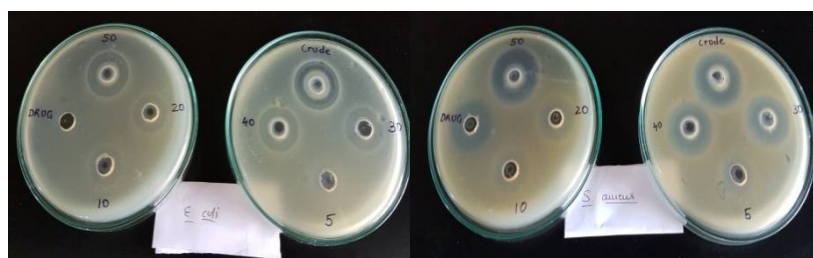
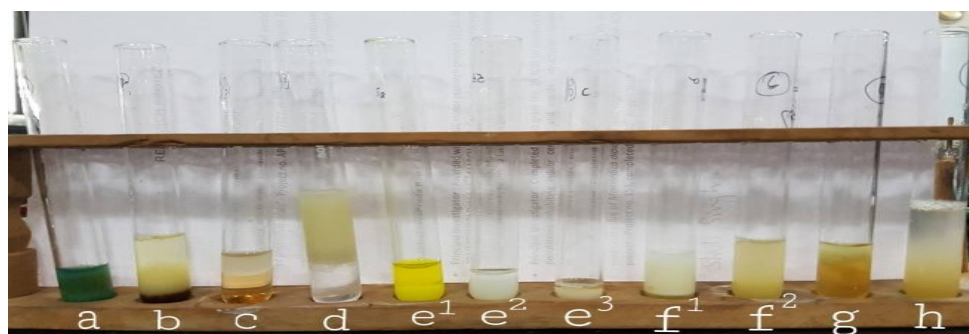


Table 3, Fig 2 shows the results of preliminary phytochemical screening of garlic (*Allium sativum*). Tannins, flavonoids, terpenoids, alkaloids and saponin, carbohydrates, protein, and cardiac glycosides were found in the extracts while anthraquinones, steroids, and anthocyanin was absent.

Figure 2. Phytochemical screening of crude extract of *allium sativum*.



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Table 3. Phytochemical profile of aqueous extract of *Allium sativum*

PHYTOCHEMICAL CONSTITUENT	AQUEOUS EXTRACT
Carbohydrates	+
Proteins	+
Glycosides	+
Steroids	-
Alkaloids	+
Flavonoids	+
Anthraquinone	-
Tannins	+
Terpenoids	-
Anthocyanin	-

V. DISCUSSION

The study focused on the garlic antibacterial activity on *S. aureus* and *E. coli* has shown that different concentrations of garlic can completely inhibit the growth of *S. aureus* and *E. coli*. This could be due to the action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action [3].

One previous study showed the antimicrobial properties of garlic against oral streptococci. In this previous study, 25% garlic extract was able to inhibit oral streptococci (*S. mutans*, *S. milleri* and *S. sanguis*) ranging from 1/64 to 1/128 dilutions (11). These results were similar to the results of the present study. There are a greater number of studies showing antimicrobial activity of garlic against other microorganisms than against oral bacteria including yeast, virus and human intestinal protozoan parasites. It was observed that garlic showed potent antimicrobial activity in vitro against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. Some scientists [12] observed a significant bactericidal effect of garlic extract against *Staphylococcus epidermidis*, *Salmonella typhi* and various yeasts. Even bacteria resistant to antimicrobial agents were sensitive to extracts of garlic. In the present study, we found similar values, showing that garlic in small concentrations could inhibit a number of different microorganisms. Allicin is another important active constituent of freshly crushed garlic homogenates. Pure allicin exhibits antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant and enterotoxigenic strains of *E. coli*, antifungal activity (particularly against *C. albicans*), antiparasitic activity (including against *Entamoeba histolytica* and *Giardia lamblia*) and antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase and RNA polymerase.

The presence of alkaloids in *Allium sativum* extract in this study shows the potential of the extract to have an analgesic, anti-inflammatory and adaptogenic effects, which may help the host (man and animal) to develop resistance against disease and endurance against stress. Flavonoids are water soluble polyphenolic molecules and therefore belong to the polyphenol family. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs. Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Flavonoids detected in *Allium sativum* could be used in the treatment of various disease conditions like edema, toothache, fever, common cold, diarrhea and dental caries. These could be possible as the root extracts contain some antibacterial activities. The flavonoids are acting on bacteria by inhibiting its protein synthesis. Saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties and their hemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also

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exhibit structure dependent biological activities. Saponins cause a reduction of blood cholesterol by preventing its reabsorption. Plants produce saponins to fight infections by parasites and in humans saponins help the immune system and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases. Tannins may elicit antibacterial activities via cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions. They are also reported to have physiological effects like anti-irritant, anti-secretolytic and anti-parasitic effects. Plants containing tannins are used to treat non specific diarrhea and inflammation of the mouth.

Our aim in preparing this paper was to show the traditional usage and previously confirmed pharmacological effects of garlic the most well-known medicinal plants in Iran and to illustrate their potential to be used as novel sources for development of new drugs based on the most recent associated studies. As it is shown in this study, garlic has a wide range of pharmacological effects like antimicrobial, and cardiovascular, anti-inflammatory, anticancer, and immunomodulatory activity among many other effects. Among these biologically active compounds, allicin, allyl methyl sulfide, DTS, and ajoene have been shown to be the main responsible compounds for the antifungal, antibacterial, antiprotozoal, and antiviral effects of garlic.

VI. CONCLUSION

This study has demonstrated the effectiveness of garlic against clinical isolates of *E. coli* and *S. aureus* that are associated with various infectious diseases. This has provided justification that if well processed, garlic can be used to develop bioactive substances that may have promising effect on the treatment of some diseases.

REFERENCES

1. Usman, Ali, Khan, Hazir, Rahman., Zeeshan Niaz., Muhammad, Qasim, Jafar, Khan., Tayyaba and Bushra, Rehman, "Antibacterial activity of some medicinal plants against selected human pathogenic bacteria", *European Journal of Microbiology and Immunology* 3 4, pp. 272–274, 2013.
2. M, Safi, thri., M. Bintang., and M, Poeloengan., "Antibacterial Activity of Garlic Extract Against some Pathogenic Animal Bacteria", *Media Peternakan*, pp. 155-158, 2011.
3. Atheer, Abdulhameed Khashan., "Antibacterial activity of garlic extract (*Allium sativum*) against *Staphylococcus aureus* in vitro", *G.J.B.B.*, Vol, 3 (4), 346-348., 2014.
4. Najeeb, Ullah., Abida, Parveen., Rahat, Bano., Iqra, Zulfiqar Mukharma Maryam., Sadia, Jabeeb., Amna, Liaqat., Sohail Ahmad, "In vitro and in vivo protocols of antimicrobial bioassay of medicinal herbal extracts: A review", *Asian Pac J Trop Dis*, 6(8): 660-667, 2016.
5. Huzaifa, U., Labaran, I., Bello, A.B. and Olatunde A., "Phytochemical Screening of Aqueous Extract of Garlic (*Allium sativum*) bulbs", *Rep Opinion*, 6(8):1-4, 2014.
6. Ameh, G. I., Eze, S. C. and Omeje, F. U., "Phytochemical screening and antimicrobial studies on the methanolic bulb extract of *Allium sativum*", *African Journal of Biotechnology* Vol. 12(14), pp. 1665-1668, 2013.
7. Garba, I., Umar, A.I., Abdulrahman, A.B., Tijjani, M.B., Aliyu, M.S., Zango, U.U. and Muhammad, A., "Phytochemical and antibacterial properties of garlic extracts", *Bayero Journal of Pure and Applied Sciences*, 6(2): 45 – 48, 2013.
8. Moulay, Sadiki., Saad Koraichi Ibn Souda., "Methods for in vitro evaluating antimicrobial activity: A review", *Journal of Pharmaceutical Analysis* 6 71–79, 2016.
9. Seema Rawat., "Evaluation of synergistic effect of ginger, garlic, turmeric extracts on the antimicrobial activity of drugs against bacterial pathogens", *International Journal of Biopharmaceutics*; 6(2): 60-65, 2015.
10. N. Harsha, V. Sridevi., M.V.V. Chandana Lakshmi., K.Rani, N. Divya Satya Vani, "Phytochemical Analysis of Some Selected Spices", *IJRSET*, Vol. 2, 2013.
11. Ross, Z. M., E. A. O'Gara., D. J. Hill., H. V. Sleightholme., and D. J. Maslin., "Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparison with garlic sulphides and garlic powder". *Appl. Environ. Microbiol.* 67:475-480. 2001.
12. Cutler R. and P. Wilson., "Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant *Staphylococcus aureus*". *British J. Biomed. Sci.* 61:1-4, 2004.