Phytochemical analysis, antioxidant and antimicrobial activity assessment of *Allium sativum* (garlic) extract

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Abstract
Garlic (Allium sativum) is recognized for its potential to treat and prevent various diseases including cardiovascular problems, common cold, bacterial and fungal infections. Garlic is bestowed with an array of organosulphur compounds rich in phytochemicals which act as antioxidant agents. Present study was undertaken for qualitative as well as quantitative analysis of phytochemical constituents of Allium sativum.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of methanol and chloroform extracts of garlic was found to be better than other extracts used in the study. These extracts showed good antibacterial activity against selected pathogenic bacterial cultures including Pseudomonas, Bacillus, Shigella and Salmonella. Sliver nanoparticles were also synthesized with the aid of garlic extract and were characterized by X-Ray diffraction. Synthesized silver nanoparticles showed good antibacterial activity against all Salmonella followed by Bacillus, Shigella and Pseudomonas.

Keywords: *Allium sativum*, Phytochemicals, Antioxidant, Antimicrobial, Silver nanoparticles.

Introduction

Allium is a genus of perennial bulbulous plants that produces chemical compounds which are a characteristic of onion or garlic flavour and aroma.[1] *Allium sativum* commonly known as garlic, is a member of Alliaceae family.[2] When crushed, *Allium sativum* yields allicin and phytonicide which serve as antimicrobial compounds.[3] It also contains other compounds including allinin, ajoene, diallylsulfide, B-vitamins, proteins and many phytochemicals.[4] Phytochemicals are the bioactive compounds found in plants that work with nutrients and dietary fibres to protect human against diseases.[5]

Many phytochemicals have antioxidant activity and reduce the risk of many diseases. The biological synthesis of silver nanoparticles emerges as an eco-friendly and exciting approach in the field of nanotechnology.[6] Silver nanoparticles (AgNPs) have been known to have inhibitory and bactericidal effects.[7,8] Use of biological organisms, plant extracts or plant biomass for the synthesis of silver nanoparticles serves as an alternative to the chemical and physical methods which are expensive and hazardous to the environment.[15] The present study demonstrated *in-vitro* evaluation of antimicrobial properties of different solvent extracts of garlic and the synthesized silver nanoparticles.

Material and Methods

Plant material and preparation of extract: *Allium sativum* (garlic) cloves were collected from local market of Shimla, Himachal Pradesh. Bulbs of *Allium sativum* were first washed with tap water followed by distilled water and ground to fine paste using mortar and pestle. 50 g each was soaked in 100 ml of different solvents i.e. distilled water, methanol, chloroform and dimethyl sulfoxide for 24 hours.[9] The extracts were then transferred to four different plates and kept at 40-60℃ to evaporate the solvents. Powdered form was obtained after 24-72 hours.[10]

Phytochemical analysis: The qualitative phytochemical analysis was carried out to detect the presence of different phytoconstituents including carbohydrates, tannins, flavonoids, amino acids, saponins and cardiac glycosides according to the standard method described by Arora and Kaur[11] and Gayathri and Kiruba[8]. General reactions revealed the presence or absence of these compounds.

Quantitative analysis of different solvent extracts of *Allium sativum*: To determine the amount of a substance per unit volume or unit weight, quantitative assay was performed. Crude samples prepared in different solvents were used for the determination of amino acid concentration.

Thin Layer Chromatography: The thin layer chromatography (TLC) was used to determine the retention factor (R_f) value of separated compounds. Aliquots of the extract and standard were applied on the TLC plate. The plate was placed in a glass beaker containing n-butanol:acetic acid:distilled water in ratio of 2:2:6 as a mobile phase.[12]

Anti-oxidant assay: DPPH free radical scavenging activity assay was performed by slightly modified method of Veluman.[13]

Assessment of Antibacterial Activity: Antibacterial activity of different solvent extracts was tested against *Pseudomonas*, *Shigella*, *Salmonella* and *Bacillus* by using well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) protocol.

Minimum Inhibitory Concentration (MIC) against selected pathogenic cultures: MIC was performed by using the method of Teh et al.[14]

Biosynthesis of silver nanoparticles: Silver nanoparticles were synthesized by following the methods of Moodley et al.[15]

Characterization of silver nanoparticles: Synthesized silver nanoparticles were characterized by XRD on PAN analytical X'Pert PRO X-ray diffractometer.

Antibacterial activity of synthesized silver nanoparticles: The antibacterial activity of the synthesized silver nanoparticles was tested by using well diffusion method.[16]

Results and Discussion

Phytochemical screening of different solvent extracts of *Allium sativum*: Present study revealed the presence of terpenoids, amino acids and cardiac glycosides in different extracts [methanol extract (ME), chloroform extract (CE), DMSO extract (DE) and distilled water extract (DWE)] of garlic. In contrast, tannins, phenols, alkaloids and saponins were completely absent in all the extracts (Table 1). A recent study on phytochemical analysis of *Allium sativum* (garlic) revealed the presence of saponins, terpenoids, flavonoids, amino acids and cardiac glycosides.[17] Another study on phytochemical profile of aqueous extract of *Allium sativum L.*, bulbs showed the presence of saponins, steroids, tannins, carbohydrates and cardiac glycosides.[18]

Quantitative analysis of amino acids in different solvent extracts: Present study showed a high amino acid content of 2.9 mg/ml in the chloroform extract of *Allium sativum* followed by methanol, distilled water and DMSO extracts (Fig. 1). In a previous study on quantitative and qualitative analysis of amino acids in *Morinda citrifolia* (Rubiaceae), total amino acid content in aqueous extracts from the fruit of *Morinda citrifolia* was found to be 0.61 in relation to glutamic acid.[19]

TLC analysis of different solvent extracts: Thin layer chromatography is commonly used for the detection of compounds through a separation process. R_f values of 0.44 and 0.45 were calculated for chloroform extract (CE) and methanol extract (ME) respectively. Tryptophan (trp) used as standard showed R_f value of 0.54 (Fig. 2). A previous study done on separation of amino acids based on thin layer chromatography by a novel quinazoline based antimicrobial agent, amino acid tryptophan showed R_f value of 0.55.[20]

Antioxidant assay of *Allium sativum* extracts: *In vitro* antioxidant activity exhibited by various solvent extracts of *Allium sativum* with varying percentage of scavenging activities is shown in fig. 3. Methanol extract showed maximum scavenging activity of 49.77% followed by chloroform extract (40.80%), distilled water extract (12.87%) and DMSO extract (7.05%). A previous study on antioxidant and antimicrobial activities of fresh garlic by-products extracted with different solvents showed similar scavenging activities.[21]

Assessment of antibacterial activity: *Allium sativum* solvent extracts were tested for antibacterial activity against pathogenic bacteria (*Pseudomonas*, *Shigella*, *Bacillus* and *Salmonella*).

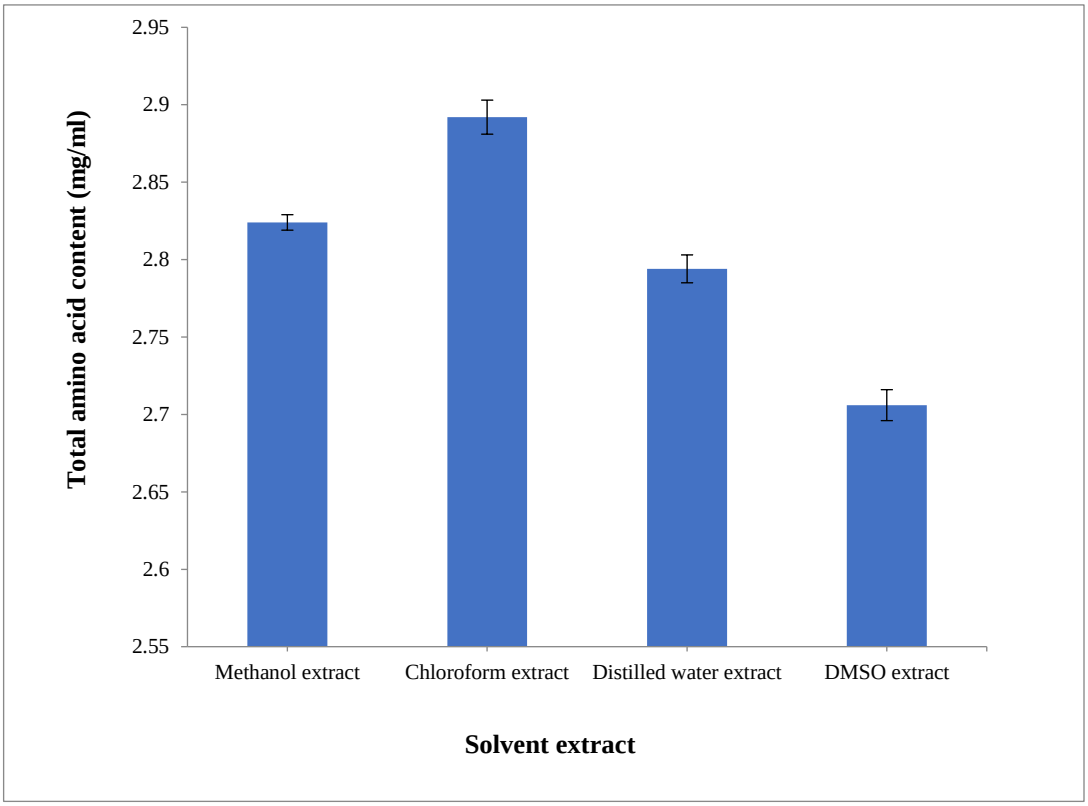

Fig. 1: Total amino acid content in different solvent extracts of *Allium sativum*.

Table 1
Phytochemical screening of different solvent extracts of *Allium sativum*.

Phytochemicals	Chloroform (CE)	Methanol (ME)	DMSO (DE)	Distilled Water (DWE)
Carbohydrates	+	-	+	-
Tannins	-	-	-	-
Flavonoids	+	+	+	-
Phenols	-	-	-	-
Alkaloids	-	-	-	-
Terpenoids	+	+	+	+
Amino acids	+	+	+	+
Saponins	-	-	-	-
Cardiac glycosides	+	+	+	+

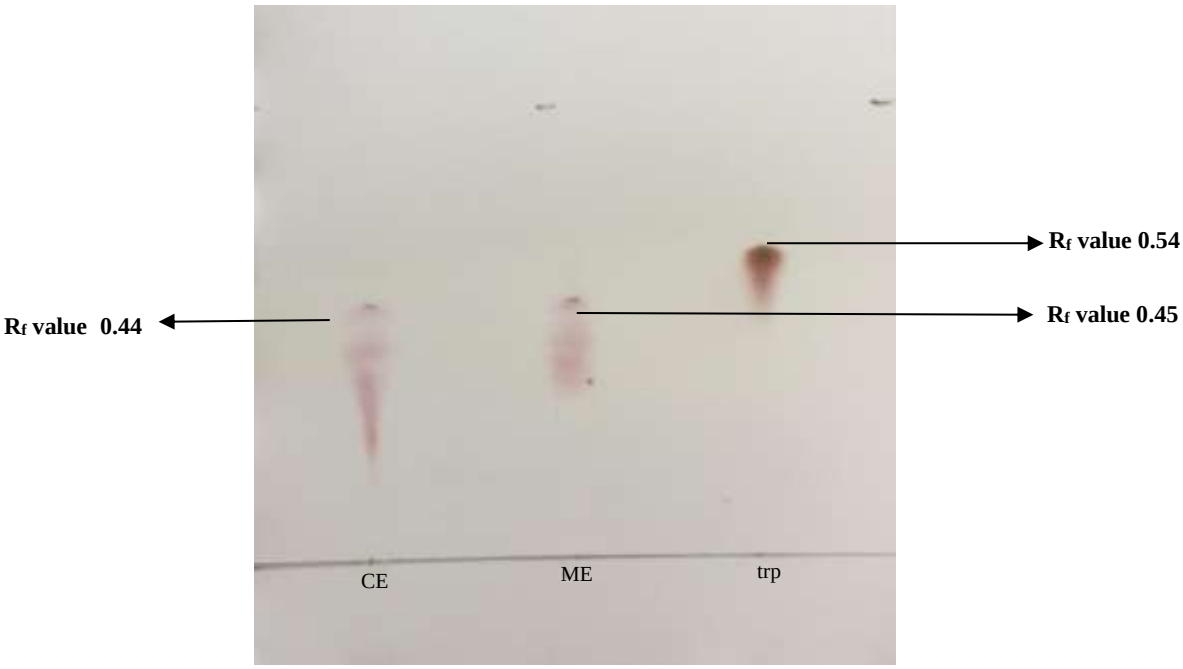

Fig. 2: TLC analysis of amino acids.

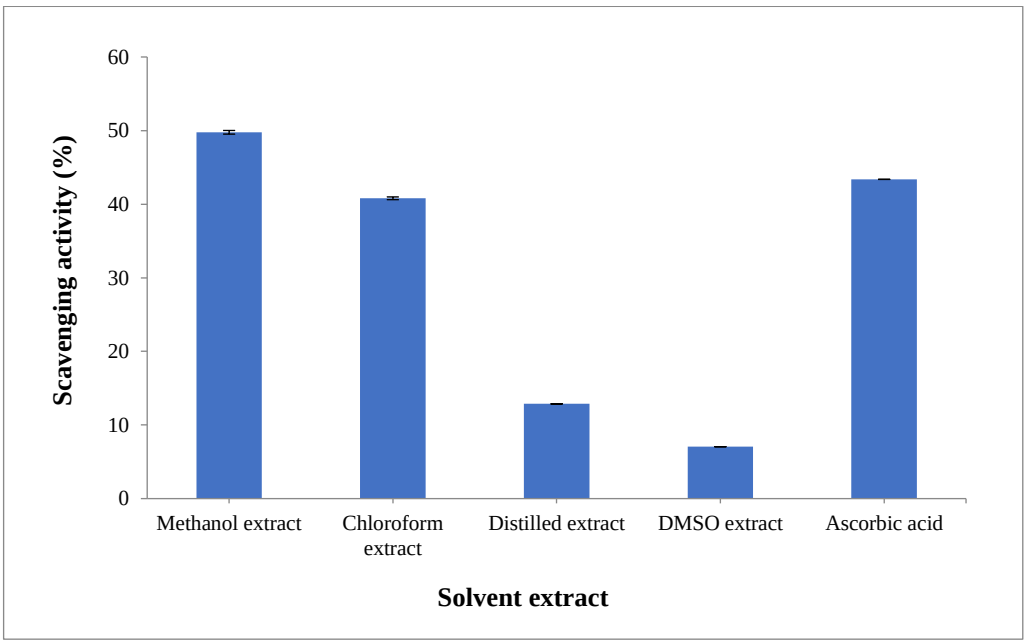

Fig. 3: Percent scavenging activity of different solvent extracts of *Allium sativum*.

Methanol and chloroform extracts showed good antibacterial activity against all bacterial cultures at all concentrations whereas extracts of DSMO and distilled water showed less antibacterial activity when used in low concentrations against bacterial cultures. The antibacterial activity pattern of solvent extracts by agar well diffusion method against selected bacterial cultures has been shown in table 2.

Allium sativum extract showed a broad spectrum activity against all the Gram-positive and Gram-negative bacteria used in the present study. The reason for the different sensitivity of the Gram-negative bacteria compared to that of Gram-positive could be due to differences in their cell wall composition[22]. A study conducted on antioxidant and antimicrobial activities of fresh garlic by-products extracted with different solvents showed good activity against *Bacillus cereus*.[21]

Minimum inhibitory concentration: *Allium sativum* extract showed good minimum inhibitory concentration against *Shigella* (Table 3). In a previous study on antimicrobial activity and minimum inhibitory concentration of essential oils of spices, among the bacterial pathogens tested, *Campylobacter* was found to be most sensitive with a minimum inhibitory concentration of 1:1300 followed by *E. coli*, *Salmonella* and *Staphylococcus* with a minimum inhibitory concentration of 1:1000. Among the two strains of *Staphylococcus*, *Staphylococcus aureus* was most sensitive (1:1000) than the Methicillin resistant *Staphylococcus aureus* (1:700).[23]

Characterization of synthesized silver nanoparticles by X-Ray Diffraction (XRD): X-Ray diffraction patterns recorded for the synthesized silver nanoparticles have been shown in fig. 4. The results showed 2θ intense values with various degrees (18.038°, 30.731° and 72.906°) revealing the crystalline nature of the particles. The average crystalline size of the synthesized silver nanoparticles was 17.63 nm. A recent study on phytochemical analysis, antimicrobial and antioxidant activity assessment of orange peels showed the synthesis of silver nanoparticles having size equal to 12.32 nm.[5]

A previous study on synthesis of silver nanoparticles using plant extract and analysis of their antimicrobial property showed that average particle size of the silver was found to be 26 nm, 26 nm, 59 nm, 20 nm and 24 nm corresponding to *O. tenuiflorum, S. cumini, C. sinesis, S. tricobatum* and *C. asiatica* respectively.[24]

Table 2
Antibacterial activity of garlic extracts against different pathogenic bacteria

Pathogenic Bacteria	Zone of Inhibition (mm)/ Concentration of extract (mg/ml)												Positive control	Negative control
	0.3				0.5				0.7					
	Methanol	Chloroform	DMSO	Distilled water	Methanol	Chloroform	DMSO	Distilled water	Methanol	Chloroform	DMSO	Distilled water		
Salmonella	13± 0.2	15± 0.2	11± 0.5	14± 0.2	15± 0.2	14± 0.5	13± 0.5	16± 0.2	17± 0.2	16± 0.2	14± 0.5	15± 0.2	21± 0.5	0
Pseudomonas	17± 0.5	14± 0.2	12± 0.5	11± 0.5	20± 0.2	15± 0.2	14± 0.2	12± 0.2	16± 0.5	14± 0.5	13± 0.2	12± 0.5	16± 0.5	0
Shigella	14± 0.2	19± 0.5	12± 0.5	13± 0.2	15± 0.2	17± 0.2	11± 0.2	14± 0.2	13± 0.2	20± 0.2	12± 0.5	15± 0.5	21± 0.5	0
Bacillus	14± 0.2	15± 0.2	14± 0.2	14± 0.2	17± 0.5	17± 0.5	15± 0.2	13± 0.5	16± 0.5	16± 0.2	17± 0.5	13± 0.2	19± 0.5	0

Table 3
MIC of different solvent extracts of *Allium sativum* against pathogenic bacteria.

Pathogenic Bacterium	MIC Value (mg/100 µl)			
	Methanol	Chloroform	DMSO	Distilled water
Salmonella	0.44	0.44	1.75	1.75
Pseudomonas	1.75	0.88	1.75	0.88
Shigella	0.03	0.11	0.11	0.88
Bacillus	1.75	0.44	0.44	0.44

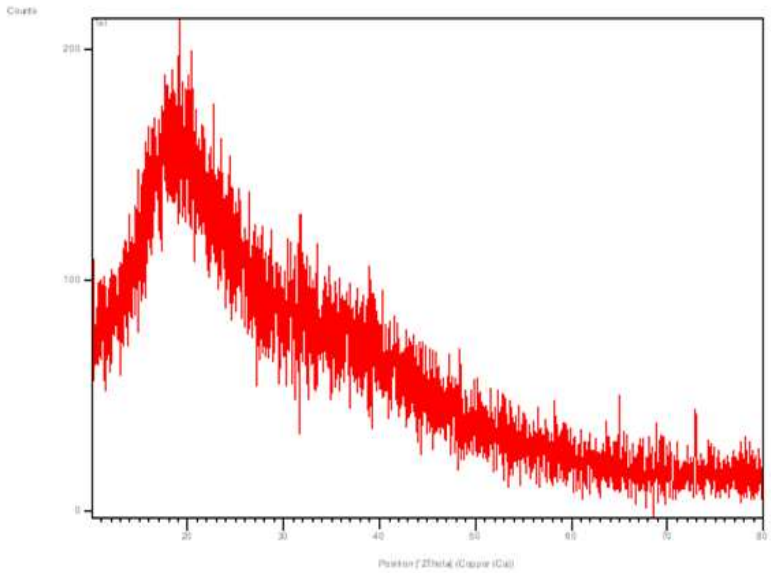

Fig. 4: X-Ray Diffraction pattern of the silver nanoparticles synthesized from *Allium sativum* extract.

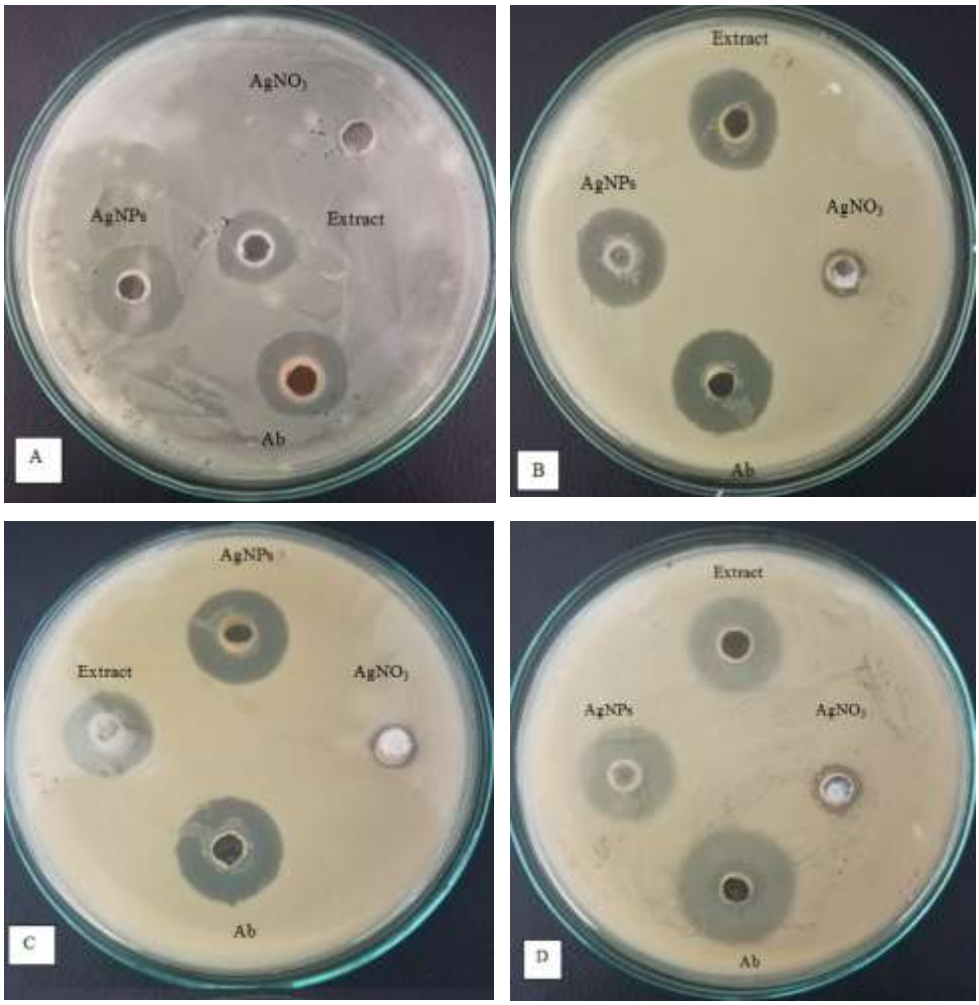

Fig. 5: Zone of inhibition shown by synthesized silver nanoparticles against pathogenic bacterial cultures (A) *Pseudomonas* (B) *Salmonella* (C) *Shigella* (D) *Bacillus.*

Antibacterial activity of silver nanoparticles: The zone of inhibition formed by AgNPs against each pathogenic bacterium was much more than that formed by the methanol extract of *Allium sativum* as shown in fig. 5. A previous

study on antibacterial activity of nanoparticles from *Allium* species also showed that silver nanoparticles exhibited good antibacterial activity against all the bacterial cultures used in the study.[1]

Conclusion

Present study revealed the presence of certain bioactive components in garlic which may have promising utilization in the field of therapeutics. The solvent extracts of garlic showed a good antimicrobial potential against all the selected bacterial isolates, so it can be used for the treatment of bacterial infections. However, further studies need be done to isolate the active constituents and to determine their toxicity, side effects and pharmaco-kinetic properties.

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