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PHYTOCHEMICAL SCREENING AND INVITRO ANTIBACTERIAL ACTIVITY OF *ALLIUM SATIVUM* EXTRACTS AGAINST BACTERIAL PATHOGENS

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ABSTRACT

Garlic (*Allium sativum*) is a important plant used in diet and in siddha medicines. *Allium* species are used in traditional medicine for centuries. The present study aimed at assessing the preliminary phytochemical analysis and antibacterial activity of garlic by disc diffusion method against ten MTCC bacterial species. Various solvent extracts of garlic inhibited the growth of bacterial species at the concentrations of 100, 200, 300, 400 and 500µg. All the four extracts showed maximum activity against *Staphylococcus aureus.* Among the solvent extracts, methanol extract of garlic showed better result in antimicrobial activity. This may be due to the extraction of all the major phytochemicals such as flavonoids, phenolics, saponin, alkaloids and tannins in methanol. The extracts showed concentration dependent antibacterial activity against bacterial cultures. The traditional use of *Allium* species for infectious diseases and for controlling bacterial infection appears to be justified.

Keywords:*Allium sativum*, Disc diffusion, Bacterial pathogens, Phytochemicals.

INTRODUCTION

Allium is a genus of perennial bulbulous plants that produce chemical compounds (mostly cysteine sulfoxide) that give them a characteristic onion or garlic flavour and aroma. Many are used as food plants, though not all members of the genus are equally flavourful. In most cases, both bulb and leaves are edible. Their taste may be strong or weak, depending on the species and on ground sulphur (usually as sulphate) content (Block E, 2010). In the rare occurrence of sulphur-free growth conditions, all *Allium* species will lack their usual pungency altogether.

About two dozen other *Allium* species are locally cultivated or collected as highly esteemed vegetables, seasonings, and/or medicinal plants (Hanelt P, 2001; Fritsch R & Friesen N, 2002; Fritsch RM, 2007). *Allium sativum* is one of the best studied medicinal plants.

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As mentioned, antibacterial and antiseptic properties were already described by Egyptians, Greeks and Romans. The various effects of garlic on bacteria, fungi, protozoa and viruses have been shown in vitro as well as invivo. The antibiotic activity is mainly due to allicin (Koch HP Lawson LD, 1996; Ankri S & Mirelman D, 1999).

Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella* sp., *Shigella* sp. and *Staphylococcus aureus* (Teferi G & Hahn HJ, 2002). Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. For example, antifungal, antiviral, antibacterial, antihelmantic, antiseptic and anti-inflammatory properties of garlic are well documented. Moreover, garlic extracts exhibited activity against both gram negative (*E.coli, Salmonella* sp. and *Citrobacter* sp., *Enterobacter* sp., *Pseudomonas* sp. and *Klebsiella* sp.) and gram positive (*S.aureus, S.pneumonia* Group A *Streptococcus* and *Bacillus anthrax*) all of which are

causes of morbidity worldwide. In our study, we determined the phyto chemicals and antimicrobial activity of four different solvent extracts of garlic.

MATERIALS AND METHODS

Collection of plant materials

Allium sativum used in this study was collected from Poomparai village of Kodaikanal district and brought to the laboratory for further analysis.

Processing of plant materials

The collected *A.sativum* bulb was cleaned thoroughly and dried under shade. The dried bulb was blended into fine powder and stored in air tight container at room temperature.

Preparation of extracts

The organic solvents such as petroleum ether, chloroform, methanol and distilled water was used for the extracting the bioactive compounds from *A.sativum* bulb. The extraction was done using soxhlet apparatus. The extract dried using vacuum evaporator and stored in air tight containers.

Qualitative analysis of phytochemicals

Qualitative analysis of phytochemicals from the organic solvents was done by following Brindha *et al*., (Block E, 2010).

Steroids

The presence of steroids in the extracts was determined by adding 2 ml of extract with minimum quantity of chloroform, 3-4 drops of acetic anhydride and one drop of concentrated sulphuric acid. The change of colour from purple to blue or green indicates the presence of steroids.

Triterpenoids

Triterpenoid was determined by adding 2 ml of extract with a piece of tin and 2 drops of thionyl chloride. The development of purple or violet colour denotes the presence of triterpenoids.

Reducing sugar

Reducing sugar in the extract was analysed by adding 2 ml of fehlings reagent and 3 drops of water with 2 ml of extract. Red or orange colour indicates the presence of reducing sugar.

Sugars

Very small quantity of anthrone and very small drops of concentrated sulphuric acid was added with 2 ml of extract and heated. Development of green or purple colour denotes the presence of sugars.

Alkaloids

Two ml of extract was mixed with 2N hydrochloric acid and mixed well. The aqueous layer formed was decanted and one or few drops of Mayer's reagent were added. Development of white precipitate or turbidity confirmed the presence of alkaloids.

Phenolic compounds

Intense blue colour was developed when a drop of neutral ferric chloride was added with 2 ml of extract in alcohol indicates the presence of phenolic compounds.

Catechins

Two ml of extract was mixed with Ehlrich reagent and few drops of concentrated HCl. Formation of pink colour denotes the presence of catechins.

Flavonoids

Two ml of extract was mixed with bit of magnesium and one or few drops of concentrated HCl and heated. Formation of red or orange colour confirmed the presence of flavonoids.

Saponins

Two ml of extract was added with water and shaked well. Foamy leather formation indicates the presence of saponins.

Tannins

Two ml of sample was mixed with water and lead acetate to form white precipitation confirmed the presence of tannins.

Anthroquinones

Two ml of sample was mixed with magnesium acetate. Formation of pink colour denotes the presence of anthroquinones.

Aminoacids

Two ml of sample was mixed with 1% ninhydrin in alcohol. Development of blue or violet colour indicates the presence of aminoacids.

Collection of bacterial cultures

Ten different bacterial cultures used in this study were collected from microbial type culture collection (MTCC). The cultures used were *Klebsiella pneumoniae, E.coli, Streptococcus pyogenes, Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Bacillus typhi* and *Aeromonas hydrophila.* The cultures were revived in nutrient agar medium and stored as slant cultures.

Determination of antimicrobial activity

The Muller Hinton agar (MHA) plates were swabbed with bacterial pathogens and well of 8mm diameter was punched into the MHA medium and filled with 10-50µl (200-1000µg) of solvent extract. The plates were incubated at 37˚C for 24 hours. After incubation period, the diameters of zone of inhibition produced by the extract with different human bacterial pathogens in different plates were measured and recorded.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of garlic revealed the presence of terpenoids, phenolics, aminoacids in petroleum ether extract, saponins, reducing sugar in chloroform extract. Phenolics, flavonoids, saponin, alkaloids, tannins and anthroquinones were found in methanol extract and terpenoids, saponin, tannins, flavonoids, alkaloids and reducing sugar were identified in water extract. Phenolics, flavonoids, saponin, alkaloid, tannins were also determined in *Allium* sp. by Udu-Ibiam *et al.,* (2014), Rekha and Shruti, (2014) and Huzaifa *et al*., (2014) in their study.

Antimicrobial activity of garlic extracts against bacterial pathogens

The solvent extracts of garlic exhibited good antibacterial activity against all the ten MTCC cultures tested. Methanol, petroleum ether, water and chloroform extract showed maximum activity against *Staphylococcus aureus* and the antibacterial activity observed was 13.17±0.29mm, 17.17±0.29mm, 19.3±0.2mm, 21.8±0.2mm and 24.2±0.2mm; 11.83±0.29mm, 12.8±0.2mm, 16±0.4mm, 19.3±0.4mm and 23.8±0.2mm; 11.1±0.2mm, 13.1±0.2mm, 15.3±0.2mm, 18.1±0.2mm and 22.1±0.2mm and 9.8±0.2mm, 11.3±0.4mm, 13.5±0.4mm, 15.6±0.4mm and 17±0.4mm zone of inhibition respectively in 100, 200, 300, 400 and 500 µl concentrations. The study of Daka D (2011) revealed that antibacterial activity of the fresh *Allium* extract showed greater effectiveness against tested organisms. He studied on the antibacterial activity of garlic on *S.aureus.* The dilute solutions of garlic can completely inhibit the growth of *S.aureus* at the concentration of more than 7.5mg/ml. According to Onyeagba and his colleagues (Onyeagba, RA et al., 2014; Brindha P *et al*., 1982), the crude extract of garlic did not exhibit any invitro inhibition on the growth of test organisms including *Staphylococcus* sp.

Methanol extract showed maximum activity against *Enterobacter aerogenes* in the range of 14.6±0.2mm, 16.3±0.2mm, 17.3±0.5mm, 20.5±0.5mm and 22.3±0.5mm zone of inhibition in 100, 200, 300, 400 and 500 µl concentrations. Petroleum ether and water extract of garlic showed maximum of 17±0.4mm and 17.3±0.2mm zone of inhibition in 500 µl concentrations respectively. Chloroform extract showed only 11.3±0.2mm zone against *Enterobacter aerogenes* in 500 µl concentrations (table 1). Among the gram negative organisms, the maximum zone was observed against *S.typhi* (50mm) and minimum zone against *Proteus* sp. (20mm) in Hindi NK (2012) study. The maximum inhibition zone for garlic extract was observed against *Enterbacter* sp. (40mm) and minimum zone was observed against *S.aureus* (25mm).

Methanol extract showed about 19.3mm zone against *Pseudomonas aeruginosa* and *Proteus vulgaris* in 500 µl concentrations. Water and petroleum ether extract showed bioactivity in the range of 12.1±0.2mm to 16.3±0.2mm zone in 100 to 500 µl concentrations and 9.3±0.4mm to 13.5±0.4mm zone of inhibition in 200 to 500 µl concentrations respectively. *Klebsiella pneumonia* showed maximum sensitivity to water extract (14.5±0.5mm zone of inhibition) and methanol extract (14.3±0.2mm zone) in 500 µl concentrations.

Water and methanol extract of garlic exhibited 11.1±0.2mm, 14.3±0.2mm, 15.1±0.2mm, 15.3±0.2mm and 17.1±0.2mm; 9.8±0.2mm, 11.1±0.2mm, 11.5±0.5mm, 13.1±0.2mm and 13.3±0.5mm in 100, 200, 300, 400 and 500 µl concentrations against *Streptococcus pyogenes*. *Streptococcus pyogenes* was sensitive to petroleum ether and chloroform extracts in the range of 8.6±0.2mm to 15.8±0.2mm inhibition zone and 8.8±0.2mm to 15±0.4mm zone of inhibition in 100 to 500 µl concentrations.

The wide spectrum of antibacterial activity of methanol, petroleum ether, water and chloroform extract was 10.8±0.2mm, 14.8±0.2mm, 15.3±0.2mm, 16±0.5mm and 17.3±0.2mm; 8.8±0.2mm, 11±0.4mm, 12.1±0.2mm, 15±0.4mm and 16.1±0.2mm; 10.1±0.2mm, 11.1±0.2mm, 11.3±0.2mm, 12.5±0.5mm and 13.3±0.2mm zone of inhibition in 100, 200, 300, 400 and 500 µl concentrations; 9.8±0.2mm, 10.8±0.2mm, 11.3±0.2mm and 12.1±0.2mm zone in 200, 300, 400 and 500 µl concentrations respectively against *E.coli.* In Karuppiah and Rajaram, (2012) investigation, the garlic cloves extracts exhibited high degree of inhibitory activity against most of the seven tested organisms. Among the clinical pathogens, *P.aeruginosa*, *E.coli*, *Bacillus* sp., *S.aureus* and *Enterobacter* sp. were the least inhibited by garlic extracts. The diameter of zone of growth inhibition varied between 7mm and 19mm in garlic. *Proteus vulgaris* was found sensitive to water and chloroform extract in the range of 12.1±0.2mm, 12.3±0.2mm, 13.1±0.2mm, 14.3±0.2mm and 15.5±0.5mm; 10mm, 11mm, 12mm and 13mm in 100, 200, 300, 400 and 500 µl concentrations respectively. Petroleum ether extract showed maximum of 14.3±0.4mm zone against *Proteus vulgaris* in 500 µl concentration.

Petroleum ether and methanol extract of garlic showed maximum antibacterial activity against *Salmonella typhi* in the range of 9.3±0.4mm to

16.5±0.4mm and 9.1±0.2mm to 16.1±0.2mm zone of inhibition respectively in 100 to 500 µl concentrations. Water extract showed the bioactivity in the range of 9.1±0.2mm to 13.3±0.2mm zone in 200 to 500 µl concentrations and chloroform extract in the range of 9mm to 12mm zone in 300 to 500 µl concentrations against *Salmonella typhi*. *E.aerogenes* was not susceptible to aqueous extract of garlic while *S.typhi* was susceptible (22±0.4mm, 24±0.6mm and 26±0.4mm in 300, 400 and 500 µg concentration) in Shobana *et al*., (2009) study. Also they reported that alcoholic extract of *A.sativum* was highly effective against all the bacterial species that was taken for the study. Al-Delaimy and Ali (1970) reported that 4% (w/v) fresh garlic extract inhibited the growth of *S.aureus, E.coli* and *S.typhi*.

Methanol extract exhibited 9.1±0.2mm, 10.3±0.5mm, 10.5±0.5mm and 12.3±0.2mm zone in 200, 300, 400 and 500 µl concentrations against *Bacillus subtilis*. *Bacillus subtilis* was found sensitive to petroleum ether, chloroform and water extract in the range between 8.8±0.2mm and 11.1±0.2mm zone of inhibition in 300 and 500 µl concentrations; 9mm and 11mm zone in 200 and 500 µl concentrations; 9.1±0.2mm and 10.1±0.5mm zone in 300 to 500 µl concentrations respectively. All the four solvent extracts do not showed antibacterial activity against *Bacillus subtilis* in the least concentrations. *Aeromonas hydrophila* showed sensitivity pattern to chloroform, methanol and petroleum ether extract in the range of 14mm, 13.3±0.2mm and 13±0.2mm zone of inhibition in 500 µl concentrations respectively. Water extract showed only 10.5±0.5mm zone of inhibition in highest concentration against *Aeromonas hydrophila.* The aqueous extract of garlic showed maximum activity against *K.pneumoniae* (8mm), *Bacillus* sp. (7mm), *E.coli* (6mm) and *Streptococcus* sp. (6mm) and minimum antibacterial activity against *S.typhi* (4mm) in Saravanan *et al*., study. A zone of 2mm was recorded against *Bacillus* sp., *E.coli*, *S.typhi* by methanolic extract. The methanol extract exhibited a zone of 3mm towards *E.coli, K.pneumoniae* (Saravanan P et al., 2010).

Table 1. Antimicrobial activity of Garlic against MTCC cultures

Clinical Pathogens	Zone of Inhibition (mm)/Concentration of extract (µg)																			
	100				200				300				400				500			
	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water
K.pneumoniae	0	0	9±0.5	0	0	0	10±0.5	9.1±0.2	9 ± 0.4	8.6 ± 0.2	12.8±0.2	11.1±0.2	11 ± 0.4	9.1 ± 0.2	13.3±0.5	13.3±0.2	12.1 ± 0.2	10.1 ± 0.2	14.3±0.2	14.5±0.5
E.coli	8.8 ± 0.2	0	10.8±0.2	10.1±0.2	11± 0.4	9.8 ± 0.2	14.8±0.2	11.1±0.2	12.1 ± 0.2	10.8 ± 0.2	15.3±0.2	11.3±0.2	15 ± 0.4	11.3 ± 0.2	16± 0.5	12.5±0.5	16.1 ± 0.2	12.1 ± 0.2	17.3±0.2	13.3±0.2
S.pyogenes	8.6 ± 0.2	8.8 ± 0.2	9.8 ±0.2	11.1±0.2	11.1 ± 0.2	10 ± 0.4	11.1±0.2	14.3±0.2	12.8 ± 0.2	12 ± 0.4	11.5±0.5	15.1±0.2	12.8± 0.2	13.1 ± 0.2	13.1±0.2	15.3±0.2	15.8 ± 0.2	15 ± 0.4	13.3±0.5	17.1±0.2
P.auroginosa	0	0	15.1±0.2	12.1±0.2	9.3 ± 0.4	0	17.1±0.2	13.3±0.2	11.1 ± 0.2	0	17.5±0.5	14.1±0.2	12.5 ± 0.4	8.8 ± 0.2	19.1±0.2	14.1±0.2	13.5 ± 0.4	9.8 ± 0.2	19.3±0.2	16.3±0.2
E.aerogenes	9.6 ± 0.2	8.8 ± 0.2	14.6±0.2	10.8±0.2	11.3 ± 0.4	8.8 ± 0.2	16.3±0.2	12.1±0.2	14.3 ± 0.4	10.1 ± 0.2	17.3±0.5	15.3±0.5	15.5 ± 0.4	10.3 ± 0.2	20.5±0.5	16.5±0.5	17 ± 0.4	11.3 ± 0.2	22.3±0.5	17.3±0.2
S.aureus	11.8 ± 0.2	9.8 ± 0.2	13.1±0.2	11.1±0.2	12.8 ± 0.2	11.3 ± 0.4	17.1±0.2	13.1±0.2	16 ± 0.4	13.5 ± 0.4	19.3±0.2	15.3±0.2	19.3 ± 0.4	15.6 ± 0.4	21.8±0.2	18.1±0.2	23.8± 0.2	17 ± 0.4	24.2±0.2	22.1±0.2
P.vulgaris	0	10	11± 0.5	12.1±0.2	8.8 ± 0.2	11	14.1±0.2	12.3±0.2	11.8 ± 0.2	11	15± 0.5	13.1±0.2	13 ± 0.4	12	16.1±0.2	14.3±0.2	14.3 ± 0.4	13	19.3±0.5	15.5±0.5
S.typhi	9.3 ± 0.4	0	9.1 ±0.2	0	11.1 ± 0.2	0	11.1±0.2	9.1 ±0.2	13.5 ± 0.4	9	13.8±0.2	11.3±0.2	14.8 ± 0.2	11	14.8±0.2	13.3±0.2	16.5± 0.4	12	16.1±0.2	13.3±0.2
B.subtilis	0	0	0	0	0	9	9.1 ±0.2	0	8.8 ± 0.2	10	10.3±0.5	9.1 ±0.2	10.1 ± 0.2	10	10.5±0.5	9.3 ±0.2	11.1 ± 0.2	11	12.3±0.2	10.1±0.2
A.hydrophila	8.6 ± 0.2	0	9.8 ±0.2	8.8 ±0.2	9.1 ± 0.2	9	11.1±0.2	9.3 ±0.2	9.6 ± 0.4	11	12.1±0.2	9.3 ±0.2	12 ± 0.4	13	12.3±0.5	10.3±0.2	13 ± 0.2	14	13.3±0.2	10.5±0.5

CONCLUSION

The phytochemical analysis of garlic revealed that it contains most of the phytochemicals which can be used in therapeutic purposes. Also the solvent extracts of garlic inhibited all the ten bacterial cultures used. From this study, we conclude that garlic has a good antimicrobial potential towards the bacterial pathogesn, so it can be used in treatment of such bacterial infections and also it can be used as neutraceuticals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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