Kitchen phytochemicals from *Allium cepa – their role in multidrug resistance*

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Abstract: Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Current antibiotics have failed to bring desirable results to many bacterial infections due to multi-resistant strains. So, concerted efforts are being made to curve this with the help of dietary phytohemicals. A number of dietary phytochemical are being put to trials for antimicrobial activity; however it is worthwhile to search such a part of diet that is very frequently used by major population. Keeping this view in mind an effort has been made to evaluate the antimicrobial activity of most common vegetable component Allium *cepa*. Methanolic and aqueous extracts were prepared from *Allium cepa* and antibacterial activity was tested on four pathogenic bacteria (Gram-positive and Gram negative) *Staphylococcus aureus, Bacillus Subtilis, Escherichia coli* and *Pseudomonas aeruginosa* using Disc diffusion method and Minimum inhibitory concentration by the Microtitre well plate method. Conventional antibiotic discs of Tetracycline and Gentamycin were used as positive control. Among the two extracts, methanolic extract exhibited a significant antimicrobial activity on the test organisms *Ps. aeruginosa* (17.5mm) and *B. subtilis* (11.3mm). The minimum inhibitory concentration value for *Ps. aeruginosa* was 500µl and for *B. subtilis* was 250µl for methanolic extract. The growth of other two bacteria i.e. *E. coli* and *S. aureus* were not inhibited by methanolic as well as aqueous extract that indicates non susceptibility to aqueous and methanol extract of *Allium cepa*. From the study, it was concluded that the commonly used *Allium cepa* possess potent antibacterial property against *Bacillus Subtilis* and *Pseudomonas aeruginosa*.

Keywords: Antibacterial activity, *Allium cepa*, Pathogenic bacteria

INTRODUCTION

Bacterial resistance to antibiotics is registering at a very high rate and has increasingly become a concern to public health. Current antibiotics are proving inefficacious to many bacterial infections due to multi- resistant strains. It is believed that the history of herbal medicine began with the earliest man of all era for treating different diseases (Caceres *et al.*, 1991; Nweze *et al.*, 2004; Vineela *et al*., 2005; Shinwari *et al*., 2011) because of the advantages of herbs i.e. they typically have fewer side effects and are inexpensive compared to formulated drugs and are readily available and can be put to medicinal use (Grunnet J, 2010). Plants are a rich source of secondary metabolites like tannins, terpenoids, alkaloids and flavonoids that have been found *in vitro* to have antimicrobial properties (Serrentino J, 1991). So, concerted efforts are being made to curve this menace with the help of dietary phytochemical that are used in routine. Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms (Shinkafi SA *et al*., 2013).

Allium cepa L. (Onion) also known as 'garden onion' or 'bulb' is a good example of such plants found effective locally against infections (Cowan M, 2001). Onions were used as early as 5,000 years ago in Egypt. Flavonoids are a second class of health enhancing compound produced by onions and active against microorganisms (Ekwenye UN *et al*., 2005). Since ancient times onions (*Allium cepa, L.*) have been an important dietary resource and have also been of interest for medical purposes (Rose P *et al*., 2005). Onion has a proven antitumor activity, enhance healing of stomach ulcers; hypocholesterolemic activity, inhibit platelets aggregation and anti-inflammatory activity associated with asthma (Augusit K, 1996; Dorsch W *et al*., 1991). It is also used for many other common ailments like coughs, flu, burns, toothaches, intestinal infections, blood purifier kidney infections and heart failure. Raw onion can also act as a complete sterilizer for the mouth and throat (Mehrabian S. *et al*., 2005, Lanzotti V, 2006). Onions were consumed throughout Europe during the middle ages and were also thought to protect against evil spirits and the plague, probably because of their strong odor (Blumenthal M. *et al*., 1998).

The development of antibiotic resistance is leading to increase in diseases and death rate (Adeshina GO *et al*., 2011). A number of dietary phytochemicals are being put to trials for antimicrobial activity; however it would be worthwhile to search and evaluate antimicrobial potential of such a part of diet that is very frequently used by major population. Keeping this view in mind an effort has been made to authenticate the antimicrobial activity of very commonly and routinely used vegetable *Allium cepa* against multi drug resistant bacteria- *Pseudomonas*

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aeruginosa, Escherichia coli, Staphylococcus aureus, and *Bacillus subtilis.*

MATERIAL AND METHODS

Material

Fresh and healthy red onions were purchased from the market, washed in water to remove the dust and then dried for two hours. The plant was authenticated by taxonomist at Department of Botany, Punjabi University, Patiala (Punjab). The outer covering of the onions were removed. All the chemicals used for preparing reagents were procured from Hi-media and were of analytical grade. All the glassware's like test tubes, beaker and Erlenmeyer's flask were of Borosil grade.

Test organism

Microbial strains used *Escherichia coli* (MTCC 443*), Pseudomonas aeruginosa* (MTCC 2297), *Staphylococcus aureus* (MTCC 7443) *and Bacillus subtilis* (MTCC 1427) were procured from Institute of Microbial Technology, Chandigarh, India. The stock cultures were maintained on nutrient agar slants.

Preparation of onion extract

The onion extracts (aqueous and methanol) were prepared by using standard method (Ponnulakshmi R *et al*., 2013). Peeled onions were washed in water containing Tween-20 solution for 10 minutes and sterilized with 70% ethanol. After a wash with distilled water 2-3 times, surface sterilized onions were cut into pieces and then dried. Dried onions were powdered and 200mg onion powder was then mixed with 50ml distilled water with intermittent shaking. It was filtered twice with Whatman filter paper no.1 and concentrated on a water bath at 100°C. Finally, extract was collected in air tight bottle, covered with aluminum foil and was stored in refrigerator. Similarly, 50ml methanol extract was prepared.

Preparation of Mc farland standard

0.5Mc equivalent turbidity was prepared by using standard method (Chessbrough M, 2000).

Antibacterial activity assay

For antibacterial activity, petri plates were prepared by pouring about 25ml of Cetrimide Agar, Luria Bertanii Agar, Brain Heart Infusion Agar (BHI) and Muller Hinton Agar in each plate for *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Bacillus subtilis* respectively. One plate each for standard antibiotics tetracycline and gentamycin were prepared. Then, 100µl of standardized inoculum suspension was poured, spreaded uniformly on respective agar plates and dried for 5 minutes. The impregnated discs as well as antibiotic discs were then placed on the agar surface with the help of forceps. The plates were then kept undisturbed for 10-15 minutes followed by incubation at 37^{o}C for 24hrs. The Zone of Inhibition (ZOI) obtained is directly proportional to the sensitivity of test organisms to the extract (Bakht *et al*., 2013).

Micro dilution broth method

Micro Dilution Broth Method was used to determine the Minimum Inhibitory Concentration (MIC) values of methanol extract in 96 well micro titer plate. The methanol extract was diluted to get different concentrations i.e. 250µl, 500µl, 750µl and 1000µl and then inoculated with respective bacterial strain. Standard antibiotic tetracycline and gentamycin were taken as positive control. 96 well micro titer plate was swabbed with ethanol and then placed under UV radiation for 30 minutes for sterlization. In first well A of column 1, 100µl gentamycin was added and in well B, 100µl tetracycline was added. In well A of column 2, 3, 4 and 5, 100µl sample inoculated with *Pseudomonas aeruginosa* was added. Similarly in well B, of column 2, 3, 4 and 5, 100µl sample inoculated with *Bacillus subtilis* was added and incubated at 37^{o}C for 24 hrs (Saad *et al*., 2014). Then the absorbance was taken at 630nm using microquant microplate spectrophotometer.

RESULTS

Antimicrobial activity

From the present study, it is evident that the methanol extract of *Allium cepa* has more antimicrobial properties than aqueous extract. The methanol extract of bulbs of *Allium cepa* showed pronounced activity against *Pseudomonas aeruginosa* and high activity against *Bacillus subtilis* while inactive against *Staphylococcus aureus* and *Escherichia coli*. The activity of the methanolic extract was statistically significant and comparable to the standard drugs i.e. Tetracyclin and Gentamycin.

Minimum inhibitory concentration

It is observed that, the Minimum inhibitory concentration value of the methanol extract of *Allium cepa* is more for *Ps. aeruginosa* than for *Bacillus subtilis*. It shows that *Ps. aeruginosa* was less sensitive for methanol extract in comparison to *B. subtilis* while aqueous extract is having no effect on both of them.

DISCUSSION

The above results shows that bacteria are more susceptible to methanol extract which may be due to some active components like Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, flavonoid, phenolic acids, sterols, sugars and a trace of volatile oil composed mainly of sulfur compounds. The antibacterial activity of onion juice can be attributed to the presence of flavonoids and polyphenols which has been reported to have broad spectrum of antibacterial activity (Hendrich

Table 1: Showing zone of inhibition of onion extract against different bacterial isolates.

S No.	Microorganism	Zone Of Inhibition(mm)			
		Methanol	Aqueous	Tetracycline	Gentamycin
1.	*Pseudomonas aeruginosa (MTCC 2297)*	17.5mm	No ZOI	20.2mm	18.6mm
2.	*Bacillus subtilis (MTCC 1427)*	11.3mm	No ZOI	21.0mm	17.4 mm
3.	*Escherichia. Coli (*MTCC 443*)*	No ZOI	No ZOI	22.9mm	19.8mm
4.	*Staphylococcus aureus (MTCC 7443)*	No ZOI	No ZOI	22.4mm	20.0mm

Positive controls
1. Tetracycline: 100ul
2. Gentamycin: 100ul

Table 2: Minimum inhibitory concentrations of onion extract for *Pseudomonas aeruginosa* and *Bacillus subtilis.*

S. No.	Micro organism	MIC of methanol extract of onion (µl).
1.	*Pseudomonas aeruginosa (MTCC 2297)*	500µl
2.	*Bacillus subtilis (MTCC 1427)*	250µl

AB, 2006). Polyphenols from plants have been reported to have antibacterial activity (Ani *et al*., 2006).

CONCLUSION

From the above observations, it was concluded that the methanol extract of *Allium cepa* possess potent antibacterial property against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Thus, onion can be used as a potent antimicrobial agent to fight day to day infections without causing any harm to body. Being a part of daily diet it can be easily promoted for its use even among rural, illiterate people. The susceptibility of the multi drug resistant bacteria especially *Pseudomonas aeruginosa* and *Bacillus subtilis* to the onion extract is encouraging because of the health crisis caused by these organisms all over the world.

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