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Phytochemical Screening and Invitro Antibacterial Activity of Allium Cepa Extracts Against Methicillin Resistant *Staphylococcus* Species

KEYWORDS: *Allium cepa*, disc diffusion, methicillin resistant organism, phytochemicals

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ABSTRACT *Onions (Allium cepa) 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 antibacterial activity of onion against three MRS species. Various solvent extracts of onion inhibited the growth of MRS species at the concentrations of 200,300,400 and 500µg. Chloroform extract and petroleum ether extract of Allium cepa (O1) was found to be highly effective against MRS1sp. Methanol and petroleum ether extract of O2 showed good activity against MRS3 species. Chloroform extract of O3 was found best towards MRS 3 species. The extract showed concentration dependent antibacterial activity against methicillin resistant Staphylococcus species (MRSA). The traditional use of onion for infectious diseases and for controlling MRS species infection appears to be justified.*

Introduction

The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Hsueh et al 2005; Mora et al 2005). Onions (*Allium cepa*) possess strong, characteristic aromas and flavours, which have made them important ingredients of food. Onions and onion flavours are important seasonings widely used in food processing. Recent research has demonstrated that onions possess several biological properties, such as antibacterial (Griffiths et al 2002), antimutagenic (Singh et al 2009) and antioxidant activities (Dini et al 2008). It is well known fact that vegetables belonging to *Allium* species are strongly resistant to diseases caused by nematodes (Tada et al 1988). These vegetables are characterized by a specific flavour and are used for cooking.

Onion maybe the first cultivated crops in the world due to their long storage time and portability. They could be dried and preserved for several months. At the present time, the *Allium* family has over 500 members, each differing in appearance, color and taste, but close in biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess antibacterial and antifungal activities and contain the powerful sulphur and other numerous phenolic compounds which arouse great interest (Rivlin, 2001; Griffiths et al 2002). Onions composed mainly of water (85-90 g/100g and 60-70 g/100 fresh weights, respectively) and the most significant components, medicinally, are the organosulfur containing compounds. In our study, we determined the invitro susceptibility of methicillin resistant *Staphylococcus* species for organic extracts of onion collected from three different places and performed the qualitative analysis of phytochemicals.

Materials and Methods

Collection of plant materials

Allium cepa from different cultivation site such as Surandai (O1), Alankulam (O2) and Vilathikuklam (O3) were collected and brought to the laboratory for further analysis.

Processing of plant materials

The collected *A.cepa* bulb from different cultivation sites were 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.cepa* 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 (1982).

Collection of pathogens

The pus samples were collected from Government Hospital, Tirunelveli and methicillin resistant *Staphylococcus* species were isolated and identified.

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 (100 – 500µg) 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.

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

Result

In preliminary phytochemical analysis of onion, non polar solvent petroleum ether yields only flavonoids, terpenoids, phenolics and aminoacids. Saponin, alkaloids, tannins and flavonoids were determined in chloroform extract of onion. Analysis of methanol extract showed the presence of phytochemicals flavonoids, saponin, anthroquinones, reducing sugar and phenolics. Alkaloid, terpenoid, saponin, reducing sugar and tannins were identified in water extract of onion.

Isolation and Identification of clinical pathogens

Three methicillin resistant *Staphylococcus* species from pus samples and named as MRS1 sp., MRS2 sp. and MRS3 sp. based on the ability to tolerate salt concentration. MRS1 sp. had the ability to tolerate higher concentration of salt (5.5%), MRS2 sp. tolerated 4.25% of salinity and MRS3 sp. tolerated only 3.0% of salt.

Antimicrobial activity of onion against methicillin resistant *Staphylococcus* species

Onion collected from various cultivation sites exhibited good antibacterial activity against methicillin resistant *Staphylococcus* species. The antibacterial activity of chloroform extract of O1 against MRS1 sp. ranges between 9.33±0.58mm and 16.17±0.29mm zone of inhibition in 100 and 500 µl concentration of extract followed by petroleum ether extract ranges from 9.5±0.5m to 16±0.5mm zone. Both methanol and water extract showed no activity against MRS1 sp. at low concentration. Methanol extract showed 9.67±0.29mm zone at 200 µl concentrations and water extract showed 9mm zone of inhibition at 300 µl concentrations. The least activity was given by water extract (11.83±0.29mm) at 500 µl concentration was given in table 1 and figure 1.

Table 1. Antimicrobial activity of O1 against MRS1 species

Extract	Zone of inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleumether	9.5±0.5	11±0	12.5±0.5	14.17±0.29	16±0.5
Chloroform	9.33±0.58	9.67±0.28	12.17±0.29	15.5±0.5	16.17±0.29
Methanol	0	9.67±0.29	12.17±0.29	12.5±0.5	13.83±0.29
Water	0	5.67±4.90	9±0	10.17±0.29	11.83±0.29

The best activity of O1 against MRS2 sp. was found in methanol extract and the bioactivity range from 11.83±0.29mm to 15.83±0.29mm followed by water extract (8.67±0.29mm to 14mm zone) in 300 µl to 500 µl concentrations. The antibacterial activity of O1 against MRS2 was given in table 2.

Table 2. Antimicrobial activity of O1 against MRS2 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	0	0	0	12±0	13.83±0.29
Chloroform	0	5.83±5.06	9.67±0.29	11.83±0.29	12.33±0.29
Methanol	0	6±5.19	11.83±0.29	12.17±0.29	15.83±0.29
Water	0	0	8.67±0.29	10.5±0.5	14±0

The maximum activity by petroleum ether and chloroform extract was 13.83±0.29mm and 12.33±0.29mm of zone respectively at 500µl concentration. Methanol extract was found as effective and petroleum ether extract was found as ineffective to MRS2 sp.

MRS 3 sp. was highly sensitive to petroleum ether extract of O1. The bioactivity of petroleum ether extract ranges between 9.83±0.29mm and 14.17±0.29mm zone of inhibition in 200 µl and 500 µl concentration. Chloroform and water extract showed no activity even in 200 µl concentration and showed minimum activity in 300 µl concentration (10±0.5mm and 8.67±0.29mm zone of inhibition respectively). Among all the three MRS sp., MRS2 sp. was sensitive to all the extracts of O1 at higher concentration. This study showed concentration dependent activity (table 3).

Table 3. Antimicrobial activity of O1 against MRS3 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	0	9.83±0.29	10.33±0.29	12.17±0.29	14.17±0.29
Chloroform	0	5.67±4.90	10±0.5	10.5±0.5	12.33±0.58
Methanol	0	8.67±0.29	10.17±0.29	11.5±0.5	12.33±0.29
Water	0	0	8.67±0.29	10.83±0.29	12.33±0.58

The petroleum ether extract of onion from Alankulam (O2) showed a maximum activity against MRS1 sp., ranges from 9.83±0.29mm to 16.17±0.29mm followed by chloroform extract (8.83±0.29mm to13.83±0.29mm) in 100 µl to 500 µl concentration. Water and methanol extract showed no activity in low concentrations. A maximum zone of 12.5±0.5mm was recorded for water extract and 9.83±0.29mm for methanol extract in 500 µl concentrations (table 4 and figure 1).

Table 4. Antimicrobial activity of O2 against MRS1 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	9.83±0.29	12±0	14.16±0.29	15.83±0.29	16.17±0.29
Chloroform	8.83±0.29	11.17±0.29	11.5±0.5	12.17±0.29	13.83±0.29
Methanol	0	0	0	8.67±0.29	9.83±0.29
Water	0	0	8.67±0.29	12.33±0.58	12.5±0

The petroleum ether extract of O2 exhibited a zone of 12.33±0.58mm and 16.17±0.76mm towards MRS2 sp. in 400 µl and 500 µl concentration. Petroleum ether extract showed no activity in 100 to 300 µl concentrations and it showed good antibacterial activity in 500 µl concentrations compared to other three solvent extracts. The results were given in table 5. The maximum activity recorded in chloroform extract and water extract was 14.5±0.5mm and 14mm respectively in 500 µl concentrations. The least activity was exhibited by methanol extract (11.67±0.58mm) at 500 µl concentration.

Table 5. Antimicrobial activity of O2 against MRS2 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	0	0	8±0	12.33±0.58	16.17±0.76
Chloroform	0	9±0.5	10.33±0.58	13.17±0.57	14.5±0.5
Methanol	0	8.83±0.29	10.33±0.58	10.83±0.76	11.67±0.58
Water	0	10.17±0.76	12.5±0.5	12.67±0.58	14±0

The methanol extract of O2 has the highest range of an-

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

tibacterial effect on the test organism MRS3 sp. (11mm to 21.33±0.57mm zone of inhibition in 100 to 500 µl concentrations) followed by petroleum ether extract (9.83±0.29mm to18.33±0.58mm) and chloroform extract (9.83±0.29mm to 16.33±0.58mm). Water extract of O2 showed a minimum of 9.83±0.29mm in 200 µl concentrations and maximum of 14mm in 500 µl concentrations. The activity increased with rising concentration of plant extract (table 6).

Table 6. Antimicrobial activity of O2 against MRS3 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	9.83±0.29	10.5±0.5	13.83±0.29	14.5±0.5	18.33±0.58
Chloroform	9.83±0.29	11.33±0.58	13.33±0.58	15±0.5	16.33±0.58
Methanol	11±0	14.83±0.29	16.67±0.58	20±0.5	21.33±0.57
Water	0	9.83±0.29	11.67±0.58	12.5±0.5	14±0

The best activity was shown by methanol and petroleum ether extract of O3 forming a zone range of 10.33±0.58mm to 16.33±0.58mm in 200 to 500 µl concentrations and 8.83±0.29mm to 16mm in 100 to 500 µl concentrations respectively against MRS1 sp. Zone of 13.83±0.29mm and 12.33±0.58mm was observed in 500 µl concentrations for chloroform and water extract respectively (table 7 and figure 1).

Table 7. Antimicrobial activity of O3 against MRS1 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	8.83±0.29	14.17±0.29	14.5±0.5	14.83±0.29	16±0
Chloroform	0	0	8.67±0.29	12.33±0.58	13.83±0.29
Methanol	0	10.33±0.58	10.5±0.5	12±0	16.33±0.58
Water	0	0	8.83±0.29	10.5±0.5	12.33±0.58

Methanol and petroleum ether extract of O3 showed a wider activity range of 10.5±0.5mm to 16.33±0.58mm and 8.83±0.29mm to 16mm respectively against MRS2 sp. followed by chloroform extract (9±0.5mm to 14.66±0.58mm in 300 to 500 µl concentrations). The zone range of 8.67±0.29mm to10.83±0.76mm was recorded for water extract against MRS2 sp. in 300 to 500 µl concentrations (table 8).

Table 8. Antimicrobial activity of O3 against MRS2 species

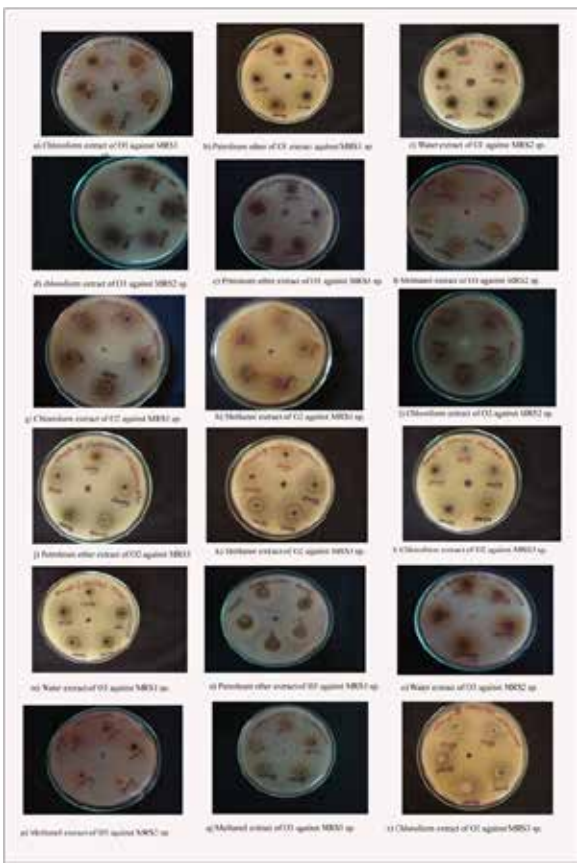
Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	0	8.83±0.29	13.67±0.58	14±0	16.33±0.58
Chloroform	0	0	10.33±0.58	13.83±0.76	14.66±0.58
Methanol	10.5±0.5	10.66±00.58	12.17±0.29	12.67±0.58	16±0
Water	0	0	8.67±0.29	10.33±0.58	10.83±0.76

A maximum zone of 20.33±0.58mm against MRS3 sp. was observed for chloroform extract in 500 µl concentrations and zone of 14.83±0.29mm, 16.83±0.29mm, 17.83±0.29mm and 19±0.5mm was observed in 100, 200, 300 and 400 µl concentrations respectively. The activity was followed by methanol extract in the range of 10.17±0.29mm to 18mm in 300 to 500 µl concentrations. The diameter of zone of inhibition obtained for water extract and petroleum ether extract against MRS3 sp. ranges from 10.5±0.5mm to 14.5±0.5mm and 8.83±0. 29mm to12.17±0.29mm respectively (table 9, figure 1).

Table 9. Antimicrobial activity of O3 against MRS3 species

Extract	Zone of Inhibition (mm)/Concentration of extract (µg)				
	100	200	300	400	500
Petroleum ether	0	8.83±0.29	10.33±0.58	10.67±0.58	12.17±0.29
Chloroform	14.83±0.29	16.83±0.29	17.83±0.29	10±0.5	20.33±0.58
Methanol	0	0	10.17±0.29	14±0	18±0
Water	0	0	10.5±0.5	13.83±0.29	14.5±0.5

Figure 1. Antimicrobial activity of three onion cultivars against methicillin resistant Staphylococcus species.



Discussion

The preliminary phytochemical investigation of onion by Shenoy et al (2009) revealed the presence of tannins in chloroform extract and none of the phytochemicals were identified in petroleum ether extract. But in this study, flavanoids, terpenoids and phenolics were identified in petroleum ether extract and saponin, alkaloids, tannins and flavanoids were found in chloroform 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.

The study of Daka (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 Onyeag-

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

ba and his colleagues (2004) the crude extract of garlic did not exhibit any invitro inhibition on the growth of test organisms including *Staphylococcus* sp. Various bacterial strains resistant to antibiotics such as methicillin resistant *Staphylococcus aureus* as well as other multidrug resistant organisms were all found to be sensitive to allicin, a major bioactive compound in garlic (Ankri and Mirelman 1999).

The results by Ye et al (2013) showed that the essential oil of onion exhibited a potent inhibitory effect against all bacteria (*E.coli, B. subtilis* and *S.aureus*) with diameter of inhibition zones ranging from 4.1mm to 19.3mm. Onions and garlic exhibited different levels of inhibition against bacterial pathogens. In the dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentration inhibited weakly on the development of bacteria. The high concentration of extracts exhibited marked inhibition activity against bacteria.

Conclusion

The use of *Allium* sp. can promote antimicrobial capability, provides a tonic to improve the immune system. The use of *Allium* sp. will reduce the side effects and cost associated with the applications of synthetic antibiotics and will also be an eco-friendly measure.

Conflict of interest

None

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