ABSTRACT

Objective: Plants offer a novel source for the isolation of a wide variety of medicinal agents. *Allium cepa* commonly known as onion is very well known medicinal plants and we investigated the antibacterial activity of different extracts and their phytochemical analysis by gas chromatography mass spectrometry (GCMS).

Methods: The extracts of *A. cepa* prepared in six different solvents was analyzed for antibacterial activity against nine American type cell culture (ATCC) reference bacterial strains i.e. *Shigella flexneri*, *Enteroxoccus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Serratia marcescens*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* by using the agar well diffusion method. GCMS analysis also has been carried out for their phytochemical analysis.

Results: The results obtained from agar well diffusion assay showed the zone of inhibition ranging from 10±0.76 to 26±0.76 mm for different extracts. The methanol extract was found most potent against *K. pneumonia* and *S. marcescens* with the zone of inhibition of 26±0.76 mm for both strains. Minimum inhibitory concentration (MIC) values were in the range of 1.87 to 7.5 mg/ml and the MIC values for *K. pneumonia* and *S. marcescens* were 1.87 mg/ml. A total of 43 compounds were identified by GCMS analysis. Out of them dodecanoic acid was found common in all extracts.

Conclusion: It is concluded that *Allium cepa* have good antibacterial activity so it can be used for the treatment of various infectious diseases.

Keywords: Antimicrobial activity, GCMS, *Allium cepa*, Bioactive compounds

INTRODUCTION

Medicinal plants are being used for the treatment of different kind of diseases since ancient times. Plants synthesize a number of chemical compounds which are not directly involved in plant growth, but responsible for the different biological activities and provide protection from predators such as insects, fungi and herbivorous [1]. These chemicals are known as secondary metabolites. The secondary metabolites isolated from plants act in the human body in the similar way of the chemical compounds of allopathic drugs in their mechanism of action [2]. Moreover, day by day the pathogens are becoming resistant against the synthetic drug, due to which herbal medicines can be an effective source for treatment of diseases with lesser side effects [3-4].

*A. cepa* has been used as spices, vegetables, ornamentals and as medicines for curing and treatment of various diseases. The *Allium* genus comprises of more than 700 species, widely distributed all over the world [5] and known for their flavor, easy growth and long storage time. It is one of the civilization’s oldest medicines and described as the dynamite of natural foods. *Allium* species are characterized by their rich content of sulphydryl compounds that are responsible for their organoleptic characteristics [6-7] and contributes to the antioxidant and antimicrobial activities [8]. Many studies have been done for its uses in the treatment of different diseases. The bulb is the main and most commonly used part of the onion. The bulb of *A. cepa* has been reported to possess various activities like antithelmintic, antibacterial, anti-inflammatory, antiseptic, antiapomasic, carminative, diuretic, expectorant, febrifuge, hypoglycemic, hypotensive, lithintropic, stomachic and tonic [9-11]. Quercetin is one of the major flavanol beside other phytochemicals present in the *A. cepa* which has antioxidant, antibacterial, urease inhibition and anti melanogenesis activities [12-13]. Diallyl disulphide, polyphenols and anthocyanins are the other major components present in *A. cepa* [14].

Keeping in view of the importance of this plant, the present study has been carried out to check the antibacterial activity of different extracts of *A. cepa* against different bacteria. GCMS analysis also has been carried out for their phytochemical analysis.

MATERIALS AND METHODS

Chemicals and reagents

Nutrient agar, peptone water and streptomycin discs were purchased from HiMedia, India. Resazurin was purchased from Sigma-Aldrich Chemicals Private Limited, India. The solvents used for the preparation of plant extracts were purchased from Sisco research laboratory (SRL), India. All solvents and chemicals, purchased were of analytical grade.

Preparation of plant extracts

Onions (red type) were purchased from the local market of Rohtak (28.9909°N and 76.5796°E), Haryana, India. These were peeled off, followed by washing and subjected to shade dry. The dried material was ground in an electrical grinder to obtain powder form. The powdered material (50 gms) was extracted with six organic solvents i.e. acetone, benzene, chloroform, ethyl acetate, methanol, and petroleum ether (1:10) using cold percolation for 48-72 h. The obtained extracts were filtered using Whatman No. 1 filter paper and then concentrated using rotary evaporator at 40 °C.

Antibacterial activity

The antibacterial activity of the extracts was analyzed against nine different american type cell culture (ATCC) reference bacterial strains i.e. *Shigella flexneri* ATCC 12022, *Enteroxoccus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis* ATCC 43071, *Salmonella typhi* ATCC 13311, *Serratia marcescens* ATCC 27137, *Klebsiella pneumonia* ATCC 706063, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 by using agar well diffusion method of Perez et al. (1990) with slight modifications [15]. The bacterial strains were obtained from Department of Microbiology of Pt. B. D. Sharma University of Health Sciences, Rohtak, Haryana, India.

Minimum inhibitory concentration (MIC)

MIC was determined using micro broth dilution method using 96 multi-well microtitre plates following the method of Sarker et al.
Antibacterial activity

In the present study antimicrobial activities of extracts of *A. cepa* was investigated against reference bacterial strains at different concentrations by agar well diffusion assay. All the extracts showed good antibacterial activity with the zone of inhibition diameter ranging from 10 to 26 mm for different bacteria (table 2). The methanol extract showed highest antibacterial activity at all concentration as compared to other different extracts. Methanol extract of *A. cepa* showed concentration dependent activity. The concentration of 40 mg/ml was found to be more effective. Highest zone of inhibition was reported against *K. pneumonia* ATCC 700603 and *S. marcescens* ATCC 27137. Similarly, acetone extract showed 17 mm and 15 mm zone of inhibition against *S. flexneri* ATCC 12022 and *E. coli* ATCC 25922 respectively. The outcomes of our study indicate that *A. cepa* have significant antimicrobial potential which supports the study of Pakasha et al. [17]. It has been reported that the antimicrobial activity of *A. cepa* due to the presence of organosulfur and phenolic compounds [18].

Antibacterial activity of extracts of *A. cepa* has been analyzed against *Bacillus subtilis* and *S. aureus* by Sable et al. and the zone of inhibition measured were 8 mm and 9 mm respectively. *A. cepa* extract in combination with *Zingiber officinale* extract showed the zone of inhibition of 13 mm and 11 mm against *B. subtilis* and *S. aureus* respectively [19]. Thus, the synergistic effects may increase the potential of the *A. cepa*. Antagonistic effect of the onion extracts has been checked against bacterial isolates i.e. *E. coli*, *S. aureus*, *Streptococcus pneumonia* and *Streptococcus pyogenes* with the inhibition zone of 17, 19, 17 and 20 mm respectively [20]. Kim et al. have studied the effect of *A. cepa* extracts on the oral pathogenic bacteria *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis* and *Prevotella intermedia* and found that extracts were active against all of these bacteria [21]. Shakur et al. have checked the antibacterial activity of ethyl acetate extract of *A. cepa* against four Gram+ve bacteria (Bacillus cereus, *S. aureus*, Micrococcus luteus and *Listeria monocytogenes*) and two Gram-ve bacteria (*E. coli* and *P. aeruginosa*).

They observed that the extract inhibit the Gram+ve bacteria effectively while Gram-ve were found resistant [23]. On the contrary, in the present study the methanol extract of *A. cepa* was more effective against the Gram-ve bacteria.

![Table 1: The yield of *A. cepa* extracts and %age of extracted value](image1)

| S. No. | Extracts     | Yield of extracts (g) | %age of extracted value (Quantity of extract obtained × 100/Weight of dried powder) |
|--------|--------------|-----------------------|----------------------------------------------------------------------------------|
| 1      | Methanol     | 5.68                  | 11.36                                                                             |
| 2      | Acetone      | 4.69                  | 9.38                                                                              |
| 3      | Ethyl acetate| 3.45                  | 6.9                                                                                |
| 4      | Chloroform   | 0.370                 | 1.5                                                                                |
| 5      | Benzene      | 0.746                 | 1.4                                                                                |
| 6      | Petroleum ether | 0.480                 | 0.96                                                                               |

![Table 2: The zone of inhibition in mm against different bacterial strains](image2)

| Bacterial strain | Methanol | Acetone | Ethyl acetate | Chloroform | Petroleum ether | Benzene | Streptomycin |
|------------------|----------|---------|---------------|------------|----------------|---------|--------------|
| *S. flexneri*    | 20±0.76  | 17±1.00 | 10±0.57       |            | 14±0.57        |         | 21±0.57      |
| *E. faecalis*    | 14±0.57  | 13±1.00 | 10±0.76       |            | 11±1.00        | 12±1.00 | 11±0.57      | 23±0.57 |
| *S. aureus*      | 16±0.76  | -       | 12±0.76       |            | 12±1.00        |         | 26±0.76      |
| *P. mirabilis*   | 20±0.57  | -       | -             |            | 11±0.57        | 11±0.76 | 23±1.00      |
| *S. typhi*       | 15±1.00  | 12±0.57 | -             |            | -              |         | 19±1.00      |
| *S. marcescens*  | 26±0.76  | 11±0.76 | 10±1.00       |            | 12±0.57        | 10±0.76 | 12±0.76      | 20±0.57 |
| *K. pneumonia*   | 26±0.76  | 10±0.57 | 17±1.00       |            | -              | 10±0.57 | 19±0.57      |
| *E. coli*        | 23±0.76  | 10±0.57 | 12±0.57       | 10±0.76    | -              |         | 18±0.57      |
| *P. aeruginosa*  | 20±0.57  | 15±1.00 | 10±0.57       | 12±0.57    | -              |         | 23±0.76      |

*The zone of inhibition showed as mean±Standard deviation (n=3)  

MIC values of different extracts against tested microbes have been shown in table 3. The results showed that MIC values of *A. cepa* extracts varied from 1.87 mg/ml to 7.50 mg/ml. Lowest MIC value was observed for methanol extract (1.87 mg/ml) against *K. pneumoniae* and *S. marcescens*. It means that *A. cepa* methanol extract possess the highest antimicrobial activity as compared to other extracts.
Table 3: MIC values (in mg) of different extracts against different bacteria

| Bacterial strain | Methanol | Acetone | Ethyl acetate | Chloroform | Petroleum ether | Benzene |
|------------------|----------|---------|---------------|------------|----------------|---------|
| S. flexneri       | 5.0      | 3.75    | 7.5           | 3.75       | -              | -       |
| E. feaecalis      | 3.75     | 5.0     | 7.5           | 5.0        | -              | 5.0     |
| S. aureus         | 7.5      | -       | 5.0           | -          | 5.0            | -       |
| P. mirabilis      | 3.75     | -       | -             | 7.5        | -              | 5.0     |
| S. typhi          | -        | 7.5     | -             | -          | -              | -       |
| S. marcescens     | 1.87     | 7.5     | 7.5           | 5.0        | 7.5            | 5.0     |
| K. pneumonia      | 1.87     | 7.5     | 3.5           | 7.5        | -              | -       |
| E. coli           | 7.5      | 7.5     | 5.0           | 7.5        | -              | -       |
| P. aeruginosa     | 5.0      | 5.0     | 3.75          | 5.0        | -              | -       |

The MIC values are showed as mean (n=3)

GCMS analysis
Organic compounds of different extracts were identified by GCMS analysis and spectra result was matched with the National Institute of Standards and Technology (NIST) MS library. The lists of identified phytochemicals from different extracts (methanol, acetone, ethyl acetate, chloroform, benzene, petroleum ether) have been given in Table 4. The main phytochemicals identified by matching the spectra with NIST library were dodecanoic acid, methyl tetradecanoate, tetradecanoic acid, pentadecanoic acid, 14-methyl-, methyl stearate, eicosanoic acid, methyl ester, di(2-ethylhexyl) adipate, 9-octadecenamide, (Z), 9-octadecenamide, (Z), tetracontane, 3,5,24-trimethyl, beta-sitosterol. The spectra of the GCMS are given in fig. 1-6. The GCMS analysis revealed the presence of fatty acids and esters. Many fatty acids isolated from plants have been reported for their antimicrobial activity. Dodecanoic acid, also known as lauric acid has been isolated from coconut oil possesses good antibacterial activity against S. aureus, Bacillus cereus, Salmonella thypimuirum and E. coli [24]. Pthalic acid, also known as benzoic acid has been tested against bacterial strain and zone of inhibition were ranged from 15 to 18 mm [25]. Odiba et al. had extracted the beta sitosterol from honey bee Propolis and studied its antibacterial activity against P. aeruginosa, E. coli, K. pneumonia, S. aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Bacillus subtilis, Shigella dysenteriae, P. mirabilis, Candida albicans, Candida krusei and Candida tropicalis. They concluded that beta sitosterol showed the good antibacterial activity [26]. We have also reported the presence of beta sitosterol in A. cepa extracts. The GCMS analysis of extracts showed the existence of volatile compounds which are comparable to the study done by Lekshmi et al. [27]. The similar compounds reported in our study are octadecanoic acid, undecene, sitostanol, tetradecanal, dibutyl phthalate etc. Farag et al. identified the 39 volatile compounds from A. cepa and A. sativum using solid-phase micro-extraction coupled to GCMS and 38 non-volatile compounds by using UPLC/PDA/orbitrap-MS in methanol extracts [28]. In our study, most of the compounds identified in all six extracts were almost similar. However their quantities may differ as revealed from peak area and % of total. Maximum numbers of compounds (25) were identified in methanol extracts [28]. The similar compounds reported in our study are 39 volatile compounds from A. cepa and A. sativum using solid-phase micro-extraction coupled to GCMS and 38 non-volatile compounds by using UPLC/PDA/orbitrap-MS in methanol extracts [28]. In our study, most of the compounds identified in all six extracts were almost similar. However their quantities may differ as revealed from peak area and % of total. Maximum numbers of compounds (25) were identified in methanol extract using NIST library.

Table 4: Phytoconstituents of six extracts screened by GCMS analysis with area and % of total

| Name of compounds | Area | % of total | Formula |
|-------------------|------|------------|---------|
| Dodecanoic acid, methyl ester | 6.501e+7 | 6.490e+7 | 5.116e+7 |
| Dodecanoic acid | 9.935e+7 | 7.748e+7 | 7.748e+7 |
| Methyl tetradecanoate | 1.230e+8 | 7.029e+7 | 7.029e+7 |
| Tetradecanoic acid | 1.293e+8 | 4.948e+8 | 4.948e+8 |
| Phthalic acid, hex-3-yl isobutyl ester | 7.029e+6 | 7.029e+6 | 7.029e+6 |
| Pentadecanoic acid, 14-methyl, methyl ester | 5.410e+6 | 5.410e+6 | 5.410e+6 |
| 9,12-Octadecadienoic acid, methyl ester, (E,E) | 8.764e+6 | 8.764e+6 | 8.764e+6 |
| 1-Decanol, 2-hexyl | 2.405e+7 | 2.405e+7 | 2.405e+7 |
| Eicosanoic acid, methyl ester | 3.034e+6 | 3.034e+6 | 3.034e+6 |
| Hexa-2,6-ethylhexyl) adipate | 7.311e+6 | 7.311e+6 | 7.311e+6 |
| Hexa(2-ethylhexyl) phthalate | 7.311e+6 | 7.311e+6 | 7.311e+6 |
| 9-Octadecaneamide, (Z) | 1.402e+7 | 1.402e+7 | 1.402e+7 |
| Phenol, 4-propyl | 2.582e+7 | 2.582e+7 | 2.582e+7 |
| 13-Octadecenoic acid, methyl ester | 7.932e+6 | 7.932e+6 | 7.932e+6 |
| Hexadecanoic acid, 15-methyl, methyl ester | 8.674e+6 | 8.674e+6 | 8.674e+6 |
| Heptatriacontane | 9.617e+6 | 9.617e+6 | 9.617e+6 |
| Triteracontane | 1.080e+7 | 1.080e+7 | 1.080e+7 |
| Hexacosanoic acid, methyl ester | 8.505e+6 | 8.505e+6 | 8.505e+6 |
| Octadecyl | 3.350e+7 | 3.350e+7 | 3.350e+7 |
| Phenol, 2-propyl | 3.750e+7 | 3.750e+7 | 3.750e+7 |
Tridecanoic acid, 12-methyl, methyl ester
Benzenedicarboxylic acid, bis(2-methylpropyl) ester
Hexadecanoic acid, methyl ester
Hexadecanoic acid, ethyl ester
(8R,9S)-14-Methyl-8-hexadecyn-1-ol
docosanoic acid, methyl ester
9,19-Cyclooctadecan-3-ol acetate, (3, beta.)
beta-Sitosterol
9,19-Cyclooctadecan-24-en-3-ol, acetate
2-Methoxy-4-vinylphenol
3(2H)-Furanone, 2-hexyl-5-methyl
11,14-Octadecadienonic acid, methyl ester
(3S,6Z,9Z)
Pentadecanonic acid, methyl ester
Pentadecanoic acid, 12-methyl, methyl ester
Methyl 11-hexadecenoate
Methyl 12,13-tetradecadienoate
Oleic Acid
docosanoic acid, ethyl ester
9,12-Hexadecadienic acid, methyl ester
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

ACM-Allium cepa methanol extract, ACA-Allium cepa acetone extract, ACEA-Allium cepa ethyl acetate extract, ACC-Allium cepa chloroform extract, ACPE-Allium cepa petroleum ether extract, ACB-Allium cepa benzene extract

Fig. 1: GCMS spectra of methanol extract
In this study, the common compounds identified were dodecanoic acid (methyl ester), dodecanoic acid and bis (2-ethylhexyl) phthalate in all the extracts. The maximum amount of dodecanoic acid was 44.86% in methanol extract. The % of total dodecanoic acid (methyl ester) and bis (2-ethylhexyl) phthalate was comparable in all extracts which lies in the range of 6.284% to 12.91%. The highest antibacterial activity was found in methanol extract of *A. cepa* which may be due to the presence of higher amount of dodecanoic acid in comparison to other extracts. The highest antibacterial activity of methanol extract may also be due to the presence of volatile and non-volatile secondary metabolites. Further, research is required to study the effectiveness of purified active components from methanol extract of *A. cepa*.

**CONCLUSION**

It is concluded that *A. cepa* methanol extract has shown good antibacterial activity against different bacterial strains. GCMS analysis of different extracts revealed the presence of 43 phytochemicals. The findings of this work support the vision that this plant could provide biologically active natural drugs which may be useful for the treatment of bacterial infectious diseases.

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ABBREVIATIONS

ATCC: American Type Cell Culture, GCMS: Gas Chromatography Mass Spectrometry, MIC: Minimum Inhibitory Concentration.

AUTHORS CONTRIBUTIONS

Dushyant Sharma has performed experimentation work, data collection, and drafted the manuscript. Reena Rani has made significant involvement in the interpretation of data and revising the manuscript. Monika Chaturvedi participated in the design of the study and performed the statistical analysis. Jaya Parkash Yadav helped in designed the study and manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest between authors.

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