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NEW INSIGHTS FOR EXPLORING THE BIOACTIVE NATURE AND BIOMEDICAL ACTIVITIES OF *ALLIUM CEPA*

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Abstract

Medicinal plants are a rich source of bioactive compounds and have been used for the treatment of infectious diseases. *Allium cepa* exhibited several pharmacological activities including antimicrobial, antioxidant, hemolytic, cytotoxic, and thrombolytic effects. However, a comprehensive understanding of its therapeutic properties is still lacking. Therefore, this study was conducted to investigate the medicinal significance and biological activities of *Allium cepa*. The extraction was carried out through fractions of methanol, ethanol, and distilled water. The antimicrobial activity was assessed through disc diffusion method, while antioxidant activity of extract was determined through DPPH free radical scavenging assay. Hemolytic activity was evaluated to assess the toxicity of onion extract, and thrombolytic activity was performed to examine its clot lysis potential. Our results revealed that methanolic extract of *A. cepa* showed high zone of inhibition (23.3 mm) than n-hexane (9.2 mm) against *E. coli*. Methanolic extract revealed high potential of DPPH free radical scavenging (40.6%) than n-hexane (23.3%). While on the other hand, methanolic extract showed high (10.1%) thrombolytic activity than distilled water (8.3%). N-hexane fraction showed higher hemolytic activity(45.4%) than distilled water (8.2%). The results demonstrated that the extract of *Allium cepa* exhibited antimicrobial, antioxidant, and thrombolytic potentials due to the presence of several antioxidants and flavonoids. This study could be used for discovering the novel bioactive compounds and also helpful for exploring the therapeutic applications of medicinal plants that could be used for biological target of emerging diseases.

Key words: DNA damage, Antioxidant, Antimicrobial, Flavonoids, Infections, Biological activities.

Introduction

Allium cepa is used as a valuable medicinal plant in the world. It exhibits many bioactive and volatile compounds that possessed a high potential for targeting of diabetic and infectious diseases (Cvjetko *et al*., 2017). These compounds with high biodiversity can be separated through chemical and biological methods with changes in their physical and chemical characteristics. Biological methods are more suitable for the sepration of novel compounds. Common features in onion species include flavour and special compound types (Datta *et al*., 2018).

Different bioactive compounds particularly flavonoids in onion show the main bioactivities against bacteria, free radicals, and parasites. These flavonoids are responsible for the blockages of free radicals (Chakraborty *et al*., 2022). Anthocyanin is the common active flavonoid in onions that is much more significant during pigment formation (Ma *et al*., 2018). The red onions showed more anthocyanin and prominent in outer layer. Kaempferol is also common in some medicinals including the onions that is involved for reducing oxidative stress. It also particularly showed binding with cancer cells and used as a therapeutic drug for cancer treatment (Mahmood *et al*., 2022). The other bioactive compound in onions is quercetin which shows major part of the onion's composition (Akash *et al*., 2014).

Onion also possesses several bioactive compounds, so large number of infectious diseases have been treated with special onion treatment. Its extract also possesses anti-inflammatory and anticancer properties used for therapeutic applications. Onion is a source of commonly used food worldwide (Fredotović *et al*., 2020). It gains more importance than other plants used to treat metabolic diseases (Adeyemi *et al*., 2022). It contains flavonoids, proteins, and carbohydrates (Kumar *et al*., 2020).

Several onion species contain bioactive products and used for industrial preparation of food products. Allium species contain various organ sulfoxides, particularly alkyl cysteine sulfoxides are responsible for their cytotoxic activity. Organosulfur compounds in onions are used for treating the digestive tract and stomach problems as these compounds showed excellent affinity with gut microbiota (Ahmed *et al*., 2023). Recent studies revealed that di-propyl-disulfide in onion reduced oxidative stress and used for anaemia (Kishor *et al*., 2023). One of the recent study revealed that sodium n-propyl thiosulfate which makes the membrane of erythrocytes was more

flexible for the RBCs formation and reduced the risk of hemolytic disorders. However, some of medicinals plants are also used for toxicological and immunological studies (Boakye *et al.,* 2023).

There are certain various biochemical compounds in onion that work against most of the pathogenic bacterial species. Previous studies revealed that quercetin showed activity against *S. aureus* (Bukhari *et al.,* 2023). These antimicrobials showed binding with the ribosomal binding domain of bacteria and breakdown the protein (Barathikannan *et al.,* 2023. These antimicrobials have also been involved in the degradation of the bacterial peptides involved in causing the different diseases(Bordin *et al.,* 2023). Some other bioactives are common that are involved for targeting the pathogens. Quercetin showed binding with the ribosomal binding domain of *B. cereus* and thus showed high activity against them (Adam *et al.,* 2023). *H. pylori* attacks on the digestive tract and increases the risk of ulcers. So, targeting the gene involved for ulcer formation is another potential challenge. Another recent investigation revealed the (Ansari *et al.,* 2023). Quercetin is an active volatile compound that is separated through spectroscopic analysis. Its molecular structure revealed the presence of the sulphur element. Due to the presence of sulphur and amino groups in their binding domains, it is wildly helpful for controlling bacterial infections (Baran *et al.,* 2023).

It was hypothesized that the extract of *Allium cepa* may possess antimicrobial, antioxidant, hemolytic, cytotoxic, and thrombolytic effects. This type of study was not reported in the literature before. Therefore, this study was conducted to investigate the medicinal significance and biological activities of *Allium cepa*. The extraction process involved three chemicals: methanol, ethanol, and distilled water.

Material and Methods

This study was carried out to evaluate the antimicrobial, toxicological, antioxidant, and thrombolytic activities of *Allium cepa.* The study was conducted in the Medicinal Biochemistry Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Sample collection and preparation: Onion samples were collected from the local market of Faisalabad. Falcon tubes were used for the collection of samples. The powder form of the *Allium cepa* was obtained after several grinding. Onion extracts were prepared using organic solvents such as n-hexane, methanol, and distilled water. All samples were protected to avoid any contamination.

Antimicrobial activity: The disc diffusion method measured the antimicrobial activity. It employed a specific antibiotic and a variety of discs to differentiate the bacteria. In this method, bacteria were usually grown on the petri plates in the presence of an agar medium. For inoculation, nutrient broth powder in the amount of 13 g/L was put into the distilled water, then mixed properly for homogenization. Then, the growth medium was autoclaved at 120°C for 20 minutes. The growth medium was inoculated with suitable bacterial strains, including *E. coli* and *S. aureus* to a shaker for proper shaking 37°C for 24 hours. This inoculate was stored at 4°C, and the zone of inhibition was noted to measure the antimicrobial activity of the onion extract (Balouiri *et al.*, 2016).

Cytotoxicity studies by hemolytic activity: The cytotoxic potential of the extract was evaluated by placing the 3 mL blood into the sampling tubes with an anticoagulant such as heparin. It was usually added to prevent coagulation and inhibit protein clotting. The tubes were mixed carefully to avoid the destruction of blood cells and finally transferred into falcon tubes that could store 15 mL samples. Then, samples were again incubated for 40 minutes at 37°C, and tubes were placed on ice and again centrifuged for five 5 minutes at 1400 X g. When incubation was completed, falcon tubes containing supernatant were initially taken and washed primarily with phosphate buffer. These dilutions were principally maintained, and tubes were finally placed in low temperatures, such as on ice. The extracts were incubated at 37ºC for 30 minutes with diluted blood suspension and centrifuged. The triton X-100 and phosphate buffer were used as positive and negative controls, respectively, for the complete lysis of RBCs. The results were presented in percentages relevant to the lysis of RBCs. The absorbance was noted at 576nm. The hemolytic activity was evaluated to determine the cytotoxic behavior of samples (Tang *et al*., 2017). The experiments were repeated thrice, and the hemolytic inhibition was calculated with the following chemical formula as a percentage.

$$\text{Lysis of RBCs (\%)} = \frac{\text{A sample} - \text{A negative control}}{\text{A positive control}} \times 100$$

DPPH free radical scavenging assay: Radical scavenging activity was performed to determine the scavenging activity of DPPH radical. The onion extract in the amount of 10 µL was mixed properly with the methanolic biochemical solution of the DPPH that was 1mL (Nunes *et al*., 2014). The incubation occurred in the darkness after 3 minutes, and the spectrophotometer measured absorbance at 517nm. BHT was used as a standard for running this experiment. DPPH was used to determine the inhibition activity. It was calculated for the following formula and was expressed in percentage.

$$I\ (\%) = 100 \times (\text{A blank} - \text{A sample}/\ \text{A blank})$$

***Ln vitro* clot lysis activity:** Clot lysis potential of the extract was evaluated and 500uL of blood was initially taken and then put into the microcentrifuge tubes, cleaned with distilled water, and properly labeled for experiments. The blood under investigation was optimized by placing them in an incubator for about forty minutes at 7ºC, maintaining the blood temperature. Blood samples were processed in micro-centrifuge tubes that were properly labelled and 100 µL of sample was added to the blood clot in the tube. After adding the drug, blood clots were placed in the stand and again incubated at 37°C for 90 minutes to complete the process of clot lysis. After 90 minutes, all the micro centrifuge tubes were invented and left for one night in the incubator (Prasad *et al.*, 2006).

Statistical analysis

Statistical analysis was performed through the data obtained through experiments in the laboratory and analyzed by ANOVA (Analysis of Variance). MS Excel was used for designing the data graph. Values were reported in the tables.

Results

Allium cepa is used for diabetic patients due to bioactive compounds such as flavonoids, antioxidants, and phenolics. The presence of these compounds in onions significantly reduced the risks of many diseases, such as cancer and diabetes. This study performed different tests to evaluate the plant extract's biological activities, such as onion. Antimicrobial activity of the onion extract was performed against the two strains of the bacteria viz *Escherichia coli* and *Staphaphlocoocuus aureus*. The inhibition zones were presented along the y-axis in millimeters. In contrast, the concentration of the parameters such as distilled water, n-hexane, methanol and ciprofloxacin were finally presented along the x-axis.

Figure 1 shows the interpretation of the antimicrobial activity of the onion extract again *E. coli.* Figure 2 shows that the zone of inhibition was presented along the y-axis in the form of millimeters. In contrast, the concentration of the parameters such as distilled water, n-hexane, methanol, and ciprofloxacin was finally presented along the x-axis. Ciprofloxacin showed a zone of inhibition 30.7 mm as a standard, while methanol had a zone of inhibition 23.3 mm. Similarly, distilled water had a zone of inhibition 14.1 mm the, while n-hexane had 9.2 mm. Therefore, the antimicrobial activity of the onion extract against the *E.coli* in which ciprofloxacin shows the maximum inhibition zone, methanol shows a moderate zone, and n-hexane shows the smallest zone.

The antimicrobial activity was evaluated to determine nature of the compounds in extracts of the onion. This activity was evaluated against *E. coli* with the extraction through the n-hexane, methanol, and distilled water. Table 1 shows that F-value with 166.09 and Fcrit that showing 4.06 with the N=3. So, the F-value is critically greater than the Fcrit. These values interpret the results that compounds showing significance between the groups from other groups while investigating the antimicrobial activity.

Antimicrobial activity against *S. aureus* was also investigated. Figure 3 shows the antimicrobial activity of the onion extract against the *S. aureus*. Ciprofloxacin had the maximum zone of inhibition as a standard, while n-hexane had the smallest zone. Figure 4 shows the activity against *S. aureus* in which ciprofloxacin had a zone of inhibition 29.3 mm while methanol had a zone of inhibition 18.1 mm. Similarly, distilled water had a zone of inhibition 10.5 mm, while the n-hexane showed 4.2 mm. The zone of inhibition was presented along the y-axis in millimeters. In contrast, the concentration of the parameters such as distilled water, n-hexane, methanol, and ciprofloxacin was finally presented along the x-axis.

DPPH free radical scavenging assay: Antioxidant activity of the onion extract was performed by using the DPPH free radical scavenging assay. The scavenging activity was presented along the y-axis in the form of a percentage. In contrast, the concentration of the parameters viz distilled water, n-hexane, and methanol was finally presented on the x-axis. Figure 5 shows that methanol had a high potential or scavenging activity of 40.6% while n-hexane had a moderate scavenging activity of 23.3%. Similarly, distilled water had a low value or low potential for scavenging activity such as 12.1 %. Overall, methanol had the maximum scavenging activity, while distilled water had the low potential of scavenging.

The antioxidant activity was evaluated to determine the nature of the compounds in the onion extracts. This activity was evaluated using the DPPH assay to extract n-hexane, methanol, and distilled water. The data was obtained through experiments in the laboratory and analysed by ANOVA. Table 3 shows the F-value 236.7727 and Fcrit which shows the 5.143253 with the N=3. So, the F-value is critically greater than the Fcrit. These values interpret the results that compounds showing significance between the groups from other groups to determine the antioxidant activity.

***In vitro* clot lysis activity:** Thrombolytic activity of the onion extract was performed to evaluate the *In vitro* clot analysis. Figure 6 shows the thrombolytic activity of the onion extract with clot lysis in which streptokinase had the maximum thrombolytic activity as a standard, and methanol had high activity. In contrast, n-hexane showed the least thrombolytic activity. Then results were finally obtained that presented in the form of a graph. The thrombolytic activity was presented along the y-axis in the form percentage while the concentration of the parameters such as distilled water, n-hexane, methanol, and triton-x as control was finally presented in the x-axis. Figure 6 shows the thrombolytic activity in which streptokinase had the highest at 90.2 %, while methanol had a moderate value of 10.1%. Similarly, distilled water had a low activity of 8.3 % compared to the methanol. Overall, n-hexane had the least possible activity at 5.1 %.

The thrombolytic activity was evaluated to determine the nature of the compounds in the onion extracts. This activity was evaluated with n-hexane, methanol, distilled water, and blood taken from healthy patients. The data was obtained through experiments in the laboratory and analysed by ANOVA. Table 4 shows the F-value 1642.324074 and Fcrit that shows the 4.066181 with the N=3. So, the F-value is critically greater than the Fcrit. These values interpret the results that compounds showing significance between the groups from other groups while investigating the thrombolytic activity.

Cytotoxicity studies by hemolytic activity: Hemolytic activity of the onion extract was performed for the cytotoxic studies. Then results were finally obtained that presented in the form of graph. The hemolytic activity or blood lysis was presented along the y-axis in the form percentage. In contrast, the concentration of the parameters viz distilled water, n-hexane, methanol, and triton-x as control was finally presented in the x-axis. Figure 7 shows that triton-X had the highest value as the standard, such as 87.8%. N-hexane shows a high value such as 45.4%, which means it critically has high capacity for blood lysis.

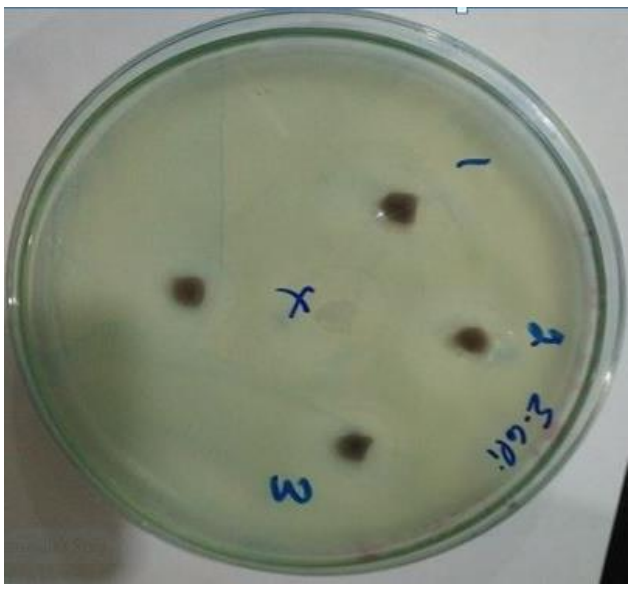

Fig. 1. Zone of inhibition of onion extract against the *E. coli*.

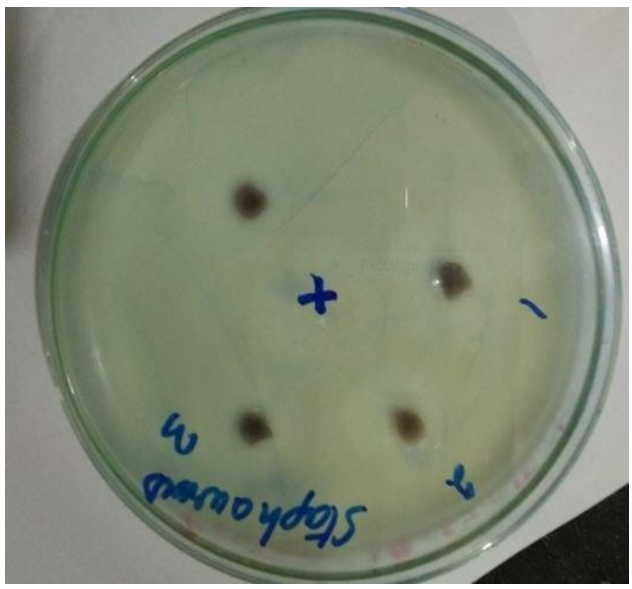

Fig. 3. Zone of inhibition of onion extarct against the *S. aureus*.

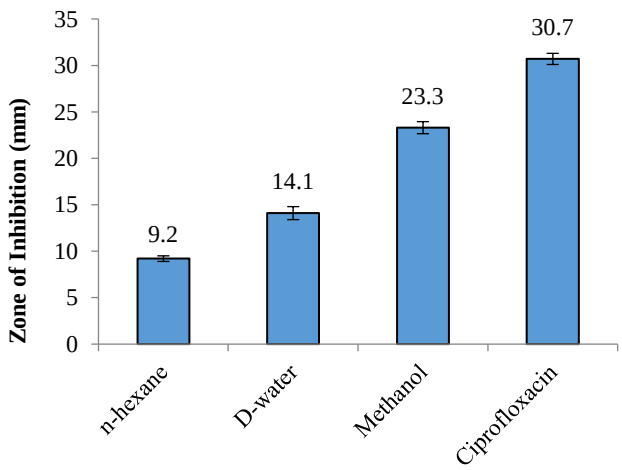

Fig. 2. Shows the antimicrobial activity of the onion extract against the *E. coli.*

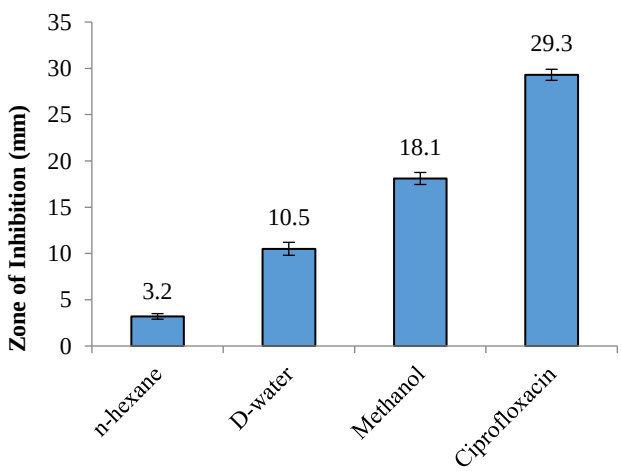

Fig. 4. Interpretation of antimicrobial activity of the onion extract against *S. aureus.*

Table 1. ANOVA table showing antimicrobial activity against *E. coli.*

Source of variation	SS	df	MS	F-value	F crit
Between groups	778.5625	3	259.5208333	166.0933**	4.066181
Within groups	12.5	8	1.5625		
Total	791.0625	11			

** Represents the significant results at 0.05 level of significance

Table 2. ANOVA table showing antimicrobial activity against *S. aureus.*

Source of variation	SS	df	MS	F-value	F crit
Between groups	1009.456	3	336.4852778	259.0009**	4.066181
Within groups	10.39333	8	1.299166667		
Total	1019.849	11			

** Represents the significant results at 0.05 level of significance

Table 3. ANOVA table showing antioxidant activity of the onion extracts.

Source of variation	SS	df	MS	F-Value	F crit
Between groups	1157.556	2	578.7778	236.7727**	5.143253
Within groups	14.66667	6	2.444444		
Total	1172.222	8			

** Represents the significant results at 0.05 level of significance

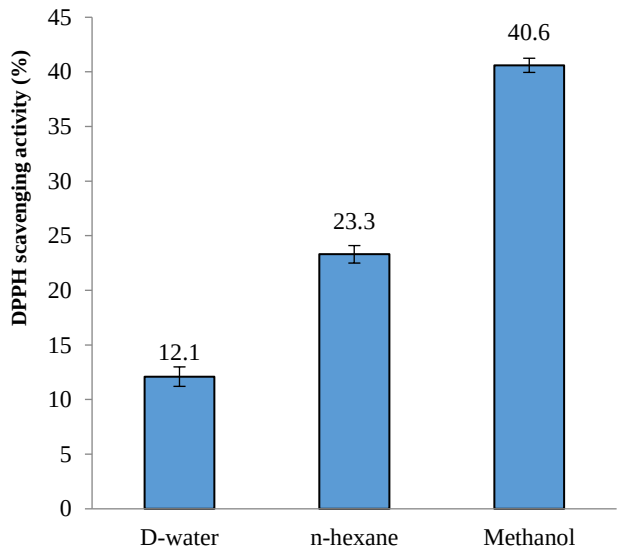

Fig. 5. Interpretation of DPPH scavenging activity of the onion extract.

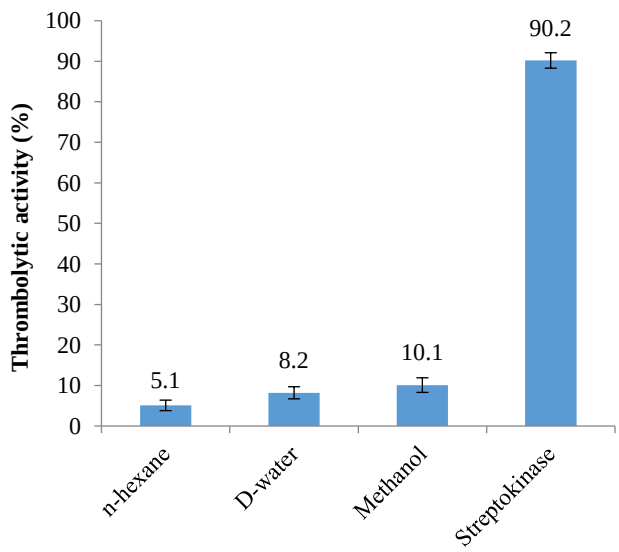

Fig. 6. Interpretation of the thrombolytic activity of onion extract.

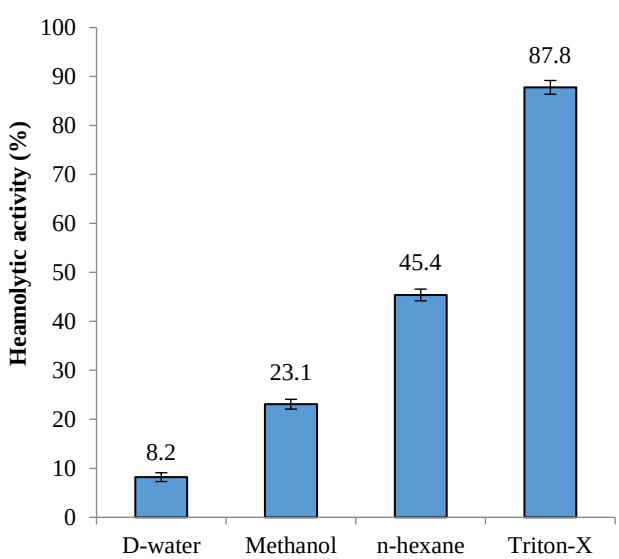

Fig. 7. Interpretation of the hemolytic activity of the onion extract.

In comparison, methanol had a moderate value of 23.1%, which means it critically has normal capacity for blood lysis. Similarly, distilled water had the lowest value, such as 8.2%, which means it critically has critically low capacity for blood lysis. Overall, n-hexane had the maximum possible value for the lysis of the blood, and distilled water had a low value indicating the important parameter in the lysis of the blood.

The hemolytic activity was evaluated to determine the nature of the compounds in the onion extracts. This activity was evaluated on the n-hexane, methanol, distilled water, and blood extracts. The data was obtained through experiments in the laboratory and analysed by ANOVA. Table 5 shows the F-value that shows the 1409.129 and Fcrit that the 4.066181 with the N=3. So, the F-value is critically greater than the Fcrit. These values interpret the results that compounds showing significance between the groups from other groups while investigating for the hemolytic activity.

Discussions

The radical scavenging potential of the onion extracts was accessed for determination of scavenging activity to DPPH radical and methanol shows the maximum activity and n-hexane shows moderate while the low potential of the scavenging to the free radicals and distill water shows the low potential of the scavenging to the free radicals. The cytotoxicity studies and n-hexane show that hemolytic activity has the highest value while methanol shows a moderate value. The thrombolytic activity accessed the clot lysis property, and methanol shows a high value as compared to the other compounds for investigating the thrombolytic activity. The current findings revealed the discovering the novel bioactive compounds and also helpful for exploring the therapeutic applications of medicinal plants that could be used for biological target of emerging diseases.

Allium cepa is used for treatment against bacterial infections. Most of the bacterial usually attack to the skin and causing the mucluar abnormalities. Ectract of the onion is used for the infection caused by the *M. luteus* (Balamanikandan *et al*., 2015). *S. epidermidis* that increased the skin rashes and other diseases due to their braod sepctrum(Ansari *et al*., 2016). *S. auerus* is another pathogenic strain that usually attacks to the smooth surface of urinary system infections (Shafiq *et al*., 2017). Extracts of the novel medicinals are a potential source of the antifungal activities and works most of the microbial infections. Quercetin is an active volatile compound that is separated through the spectroscopic analsysis. Its molecular structure revealed the presence of the sulphur element (Baran *et al.*, 2023).

Lipid peroxidation is another cellular mechanism that increased the risk of the free radicals. The abnormal production of free radicals leads to the chain reaction through the formation of hydrogen transfer. These free radicals acts in such as way that mitochondrial memebrane damage causes the high leakage of the electrons and causes the cancer (Iqbal *et al*., 2022). However, some of medicinal plants are also used for toxicological and immunological studies (Boakye *et al.*, 2023). Use of the Allium cepa for controlling the free

radicals is an important strategy for discovering the novel bioactive phytochemicals (De Dicastillo *et al*., 2015).

Preventing the oxidation process is another challenge in the current era due to emerging infections. Different medicinals are also potentially used for lowering the oxidation and some of the experimental-based studies proved the presence of the quercetin. Its mechanism was revealed its binding with platelets through the cellular kinase. This type of signalling in the medicinals proved that quercetin as the biological therapeutic drug used for breaking the large and huge clots in highly infectious patients (Abdelrahman *et al*., 2017).

Cellular signalling revealed that quercetin has the ability for binding to the blood cells with tremendous applications in the fields of blood chemistry (Qin *et al*., 2018). Through spectroscopic analysis, it was found that quercetin showed to the large family of active and biological flavonoids that are involved for large preparation of the synthetic drugs used for inhibiting the heparin (Nile *et al*., 2017).

Different studies supported that colon cancer was considered as an infectious dieases through abnormal production of genes. Extract of the onions that are potential target the cells of the colon cancer and thus significant in targeting the high populations of the active growing cancer cells (Aruna *et al*., 2014). Similarly, lung cancer that is caused through the mutations in the subsequent cells and abnormal cellular signaling leads to the formation of highly emerging cancer cells. Some experimental studies potentially revealed that extracts of the onions that are separated through spectroscopic analysis are use for the treatment of active growing cancer cells (Qin *et al*., 2018).

Table 4. ANOVA table shows the thrombolytic activity of onion extracts.

Source of variation	SS	Df	MS	F-value	F crit
Between groups	14780.92	3	4926.972222	1642.324074**	4.066181
Within groups	24	8	3		
Total	14804.92	11			

** Represents the significant results at 0.05 level of significance

Table 5. ANOVA table shows the Hemolytic activity of onion extract.

Source of Variation	SS	df	MS	F-value	F crit
Between groups	10480.4	3	3493.465278	1409.129**	4.066181
Within groups	19.83333	8	2.479166667		
Total	10500.23	11			

** Represents the significant results at 0.05 level of significance

Conclusion

Different activities were performed to assess the nature of the compounds in extracts of the onion. Onion extract was prepared by extraction by using the solvents viz n-hexane, methanol, and distilled water. The concentration of the microbes was determined by the disc diffusion method, and methanol showed the largest zone of inhibition and also showed the maximum activity. In contrast, n-hexane showed the smallest zone when tested against the bacteria *viz*., *E. coli* and *S. aureus*. The radical scavenging potential of the onion extracts was accessed to determine the scavenging activity of DPPH radical. Methanol showed the maximum activity, n-hexane showed a moderately low potential of the scavenging to the free radicals, and distilled water showed a low potential for scavenging the free radicals. The cytotoxicity studies carried out the hemolytic activity, and n-hexane showed the highest value while methanol showed a moderate value. Similarly, distilled water showed less activity than the methanol extract. The results demonstrated that the extract of *Allium cepa* exhibited antimicrobial, antioxidant, and thrombolytic potentials due to several antioxidants and flavonoids. This study will help for discovering the novel bioactive compounds and also helpful for exploring the therapeutic applications of medicinal plants that could be used for biological targets of emerging diseases.

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