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Phytochemical and Antimicrobial Evaluation of Dried *Allium cepa* Bulb

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ABSTRACT

Allium cepa (onion) is an important plant utilized in everyday foods and is valued by the community for its traditional therapeutic applications. Although it is cultivated all over the world, the plant is thought to be native to Asia. The purpose of the study was to use qualitative phytochemistry to screen *Allium cepa* extracts against specific bacterial strains. The homogenized, fresh bulbs were extracted in two concentrations of 5mg/ml and 10mg/ml. Ciprofloxacin was used as the standard drug. Further, the phytochemical analysis of plant extracts was also carried out. All the extracts exhibited efficacy against the selected bacterial strains by showing the zones of inhibitions ranging between 15 to 30 mm. Positive results for steroids, glycosides, alkaloids, phenolic compounds, flavonoids, resins, oils, carbohydrates, and protein were obtained via phytochemical examination. The extract had 91.05% moisture, 80.6% carbohydrates, 3.2% total sugar, 3.90% protein, and an acidity of 2.1%. The results support usage of this plant to treat a variety of infections.

1. INTRODUCTION

Onions (*Allium cepa* L.) have been a valuable food source and a subject of medical concern since ancient times. With 450 species, the genus *Allium* is the largest and most significant representative of the Liliaceae family. The bulbous plant known as onion (*A. cepa*) is extensively grown in practically every nation. Onions may spread quickly, both in transit and storage. Onions help relieve sore throats, coughs, diabetes, heart disease, osteoporosis, and common colds. Proteins, carbs, salt, potassium, and phosphorus are all abundant (Irfan *et al.*, 2023). Throughout Europe throughout the Middle Ages, onions were widely consumed, and because of their potent smell, they were later believed to be against plague and evil spirits. Traditional healers have

long employed onions to ward against diseases. The plant has therapeutic value because it is used as a traditional medicine to treat illnesses. Numerous secondary metabolites, including flavonoids, tannins, terpenoids, and alkaloids, are abundant in plants and have been shown to have antibacterial qualities in vitro. Many phytochemicals (Afzal *et al.*, 2023), primarily hydrocarbons, and their derivatives, are found in onion bulbs. *A. cepa* extracts have been shown by several writers to have anti-tumor, anti-diabetic, antioxidant, antibacterial, antiallergic, and molluscicide properties. Onion has been demonstrated to have antibacterial, antiparasitic, and antifungal action in vitro. Plants with therapeutic properties are those that contain auxiliary typical medications, such as opium poppies and fox gloves, in addition to commonplace

vegetables, like onions (Serrentino and Serrentino, 1991). Using plants as the primary source of medication is a widespread practice. Every country has relied on the enormous diversity of natural chemistry found in medicinal plants for their therapeutic benefits through written or oral tradition (Mirani *et al.*, 2014). Onion bulbs are thought to have been utilized as a food source for ages. An onion comprises of both edible bulb parts and herbaceous plant parts. It may be indigenous to southwest Asia (Mirani *et al.*, 2014). There are three primary varieties: those with white, red, or purple skin (Lim, 2012). Both ecological and genetic factors influence onion's relative spiciness. Onion sulfur compounds have also been shown to have anti-inflammatory properties by preventing the synthesis of thromboxanes and by blocking the PAF, or platelet-activating factor, at work. Benefits of thiosulfates against thrombosis, include antioxidant activity (Qin and Xu, 2008). The objective of this study is to ascertain the antibacterial activity of ethanolic, dichloromethane, and water extracts, as well as qualitative phytochemical testing and proximate analysis of the *A. cepa* Linn, a botanical name for the onion used as a medicinal plant locally.

Onion varieties have 166 KJ of energy and are mostly made of water (about 89%). They also contain 9% carbohydrates and 1% protein. Onions have several health-benefiting compounds, such as flavonoids, phenolic acids, sterols (including cholesterol), and sulfur compounds like S-methyl-cysteine and S-propyl cysteine. These sulfur compounds, like dipropyl disulfide, give onions their special smell and taste. Onions also contain sugars, sterols (like sitosterol), saponins, and a small amount of oil that has sulfur compounds. These compounds have anti-inflammatory and antimicrobial properties. They can be extracted using liquids like methanol, petroleum ether, and water (Dinkecha and Muniye, 2017).

The onion is widely used in cooking and is a healthy, fat-free, high source of calcium, salt, phosphorus, and fiber in both mature bulbs and green leaves. Clinical and epidemiological research have demonstrated that eating this vegetable can help prevent and treat several illnesses, including some types of cancer, diabetes, osteoporosis, cardiovascular diseases, cholera, skin disorders, cough, anemia, and diseases caused by peroxynitrite (Pareek *et al.*, 2017). In terms of vitamins and minerals, onions are high in folic acid, vitamin B6, magnesium, calcium, potassium, and phosphorus, and low in sodium. An onion possesses a minimal lipid level, except for glutamic acid and arginine in terms of amino acid concentration. Onions are abundant in dietary fiber and contain 90% water by weight in sugar. In terms of vitamins and minerals, onions are high in folic acid, vitamin B6, magnesium, calcium, potassium, and phosphorus, and low in sodium (Chakraborty *et al.*, 2022). In a perennial crop, onions can be eaten raw, mature, pickled, or powdered when still tender. They can be red, white, or yellow. Usually white or purple, the bushes have small flowers. The bloom stalks of common onions are leafless and reach a height of 75180 cm (2.56 ft) (Kumar *et al.*, 2024). It has a fibrous adventitious root system. The fleshy, cylindrical underground bulb emerges from a cluster of progressively larger leaves (Figure 1 A). It has an underground bulb for a stem. It has a parallel venation and a sheath leaf base. All flowers have the same length pedicels since they are derived from the peduncle apex. Flowers are tiny, complete, golden, actinomorphic, trimerous, bracteate, hypogynous, and ebracteolate (Chakraborty *et al.*, 2022). Six stamens and six tepals are present, grouped opposite the tepals in two whorls of three each. *A. cepa* are common culinary elements in our cuisine. Additionally, due to its hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, anti-thrombogenic, and anti-hyperhomocysteinemia





Figure 1. (A) Onion Bulbs

(B) Onion Bulbs Test

effects, onion extract has recently been shown to be beneficial in the treatment of cardiovascular disease (Figure 1 B). It also possesses many other biological activities, such as antimicrobial, antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory, and prebiotic properties (Sagar *et al.*, 2022). Onion's pharmacological effects include lowering blood levels of triglycerides, cholesterol, and thromboxanes, the substances that inhibit the processes of oxidation and inflammation as well as the release of histamine linked to asthma, suppresses the immune system, lowers osteoporosis, and acts as a hypoglycemic, neuroprotective, anticonvulsant, anti-hypertensive, anti-depressant, and diuretic agent. It also hinders the development of cardiovascular disease and inhibits platelet aggregation and platelet-mediated thrombosis, a process that leads to heart attacks and strokes (Teshika *et al.*, 2019). It also serves as a hypoglycemic, neuroprotective, anticonvulsant, antihypertensive, antidepressant, and diuretic agent (Sobenin *et al.*, 2019).

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The white onion bulb, *A. cepa*, was purchased from the vegetable market of Bahawalpur. The

experiments were conducted in the laboratory of The Islamia University of Bahawalpur. The onion bulbs were rinsed under running tap water to get rid of dust and other debris, then they were properly cleaned and dried.

2.2 Extraction of Bioactive Constituents

Recently prepared sterile distilled water was used to wash the onion bulb. The bulb's outer layer was peeled off, and the onion was then thoroughly cleaned with freshly made, sterile, distilled water. The onion bulb was squashed after chopping it into little pieces. The smashed preparation was soaked for 24 hours in 40 ml of methanol. Whatman No. 1 filter paper was used to filter the extracts before being placed in a plastic sample vial (Likos and Lu, 2002).

2.3 Tested Microorganisms

E. coli, *K. pneumonia*, *P. aeruginosa*, *Staphylococcus epidermidis* and *S. aureus* were tested against the extracts. In the case of fungi, *Pythium*, *Fusarium oxysporum*, and *Colletotrichum* spp. were used. These were provided by the Department of Microbiology, Islamia University of Bahawalpur. Before being used in the study, the pure cultures were sub-cultured on nutrient agar slants. Antimicrobial susceptibility was evaluated using solid (agar) media in petri plates.

Nutrient agar was used for this assay (Olurinola, 1996).

2.4 Extraction

After being ground into a fine powder, fresh onion bulbs were immersed in 100 ml of 95% ethanol for 48 hours. The extracted juice was transferred into a glass beaker after fractioning the dried extract using dichloromethane. Three plant fractions were obtained: water, dichloromethane, and ethanol in ratio of 3:3:8. Several extracts' antimicrobial properties were assessed using the agar well diffusion method (Versalovic, 2011).

2.5 Agar Well Diffusion Method

Using the Balouiri method, the dried leaf extract of *A. cepa* was tested for antibacterial activity. Two 6 mm holes were drilled into nutrient agar petri plates using an aseptic cork-borer spaced equally apart. Each petri plate had one hole filled with ciprofloxacin as a positive control and one hole filled with an *A. cepa* crude extract. Each petri plate contained an extract concentration of 5 mg and 10 mg. Petri plates were incubated for 24 hours at 37°C. The clear zones (also known as the inhibition zones) around each well was measured following the incubation, which indicated the antibacterial activity of the dried leaf extract (Balouiri *et al.*, 2016).

2.6 Phytochemical Analysis of *A. cepa*

The extract was proceeded through phytochemical examination to detect alkaloids, flavonoids, steroids, volatile oil, carbohydrates, glycosides, saponins, and proteins.

2.6.1. Detection of Alkaloids

Wagner's test was used to detect alkaloids. 2ml of 1% HCl was combined with crude extract and heated gradually. The combination was then supplemented with Mayers and Wagner's

chemicals. The turbidity of the resultant precipitate was taken as proof of the presence of alkaloids.

2.6.2. Detection of Flavonoids

To identify flavonoids, 3 ml of extract was mixed with 10 ml of distilled water. To the mixture, 1 ml of 10% NaOH solution was added. The appearance of yellow colour indicated the presence of flavonoids.

2.6.3. Detection of Saponins

A test for foaming was carried out. For the frothing test, 2 ml of distilled water was added to 3 ml of extract. The mixture was given a good shake. The presence of saponins is shown by frothing on top of the solution. For another test, 5ml of distilled water and crude extract were combined, and the mixture was vigorously agitated. It was believed that the production of stable foam indicated the presence of saponins.

2.6.4. Detection of Steroids

The Salkowski test was used for the detection of steroids. A small amount of strong sulfuric acid and an equal volume of chloroform was added to one ml of test extract. A brown ring's appearance suggests the presence of steroids.

2.6.5. Detection of Tannins

The lead acetate test was used to detect the presence of tannins. 5ml of the extract was combined with a few drops of freshly made 1% lead acetate. Presence of tannins was indicated by yellow precipitates.

2.6.6. Detection of Glycosides

Using the Erdmann's test, glycosides were identified. The Erdmann's reagent was prepared by adding 10 drops of HNO₃ to 100 ml of distilled water. Two or three drops of Erdmann's reagent were added to two ml of



alcoholic extract. When phenolic glycosides were present, the hue turned brilliant red. Concentrated H_2SO_4 was added sidewise after the crude extract and 2 ml of chloroform were combined. A crimson hue emerged in the lower part of the test tube. Two ml each of acetic acid and chloroform were combined with crude extract and chilled on ice. A precise concentration of H_2SO_4 was introduced. Green, blue, and violet hue shifts suggested the presence of glycosides.

The crude extract was combined with two ml of a 2% FeCl_3 solution. Phenols and tannins were characterized by a blue-green or black coloring.

3. RESULTS

3.1 Antibacterial Activity

Ethanol extract's antibacterial activity was assessed by measuring the zone of inhibition at two different concentrations (10 mg/ml and 5 mg/ml). The ethanol extract resulted in a zone of inhibition of 30 mm at 10 mg/ml and a zone of 15 mm at 5 mg/ml. These data demonstrate the extract's capacity to suppress microbial growth, however its efficacy varied with concentration.

In the same test, ciprofloxacin, a well-known antibiotic, served as the benchmark. Ciprofloxacin displayed a zone of inhibition of

70 mm at 10 mg/ml and 35 mm at 5 mg/ml. (Table 1). These are much greater zones of inhibition. These findings show that although ethanol extract possesses antibacterial qualities, at the same concentrations, its activity is far less potent than ciprofloxacin's. The results showed promise for the extract but also emphasized that the regular antibiotic is more effective in these tests.

3.2 Phytochemical Analysis

Two types of extracts—ethanol and aqueous—were used for the phytochemical analysis of the dried bulb of *A. cepa*. This investigation aimed to use many conventional procedures to assess the presence of different bioactive chemicals. Tests for alkaloids, flavonoids, saponins, steroids, tannins, glycosides, reducing sugars, and volatile oils were performed on both extracts (Figure 2).

However, in both the ethanol and aqueous extracts, the results for tannins, glycosides, and reducing sugars were negative (Table 2). The absence of glycosides, which are frequently associated with cardiovascular benefits, and tannins, which are generally recognized for their astringent qualities, raises the possibility that these substances are not the main constituents of the *A. cepa* bulb. Reducing sugars, which are generally involved in the metabolism of energy, were also not found.

Table 1. Zone of Inhibition of Ethanol Extract

Extract/Solution	Concentrations	Zone of inhibitions
Ethanol	10mg/ml	30 mm
	05mg/ml	15 mm
Standard antibiotic (Ciprofloxacin solution)	10mg/ml	70 mm
	05mg/ml	35mm



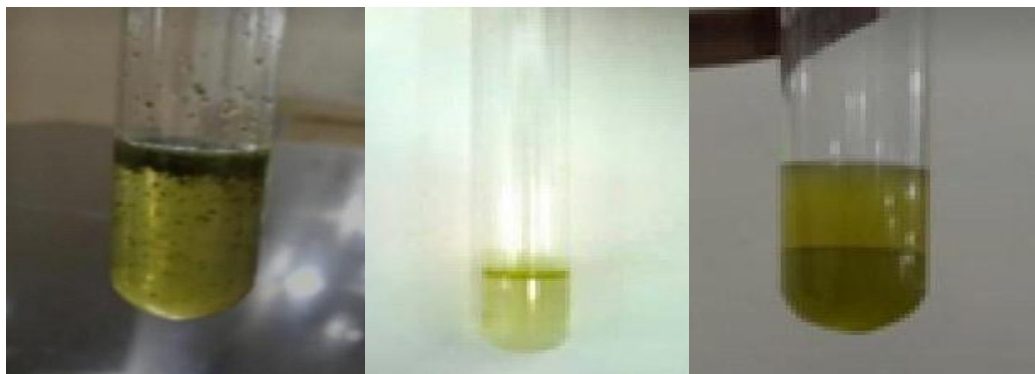


Figure 2 (A) Detection of flavonoids (B) saponins (C) steroids

Table 2. Results of phytochemical analysis

Sr. no.	Tests	Ethanollic extract	Aqueous extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponins	+	+
4	Steroids	+	+
5	Tannins	-	-
6	Glycosides	-	-
7	Reducing sugar	-	-
8	Volatile oil	-	+

Interestingly, the two extracts produced different results in the volatile oil test. The ethanolic extract did not exhibit volatile oil content, although the aqueous extract did. The presence of volatile oils in the aqueous extract is noteworthy because of their aromatic qualities and their links to antibacterial and anti-inflammatory activities.

Additionally, the leaf extract was examined phytochemically in a variety of polar aqueous and ethanol-based solvents. Table 3 displays the presence of flavonoids, tannins, alkaloids, saponins, and volatile oils in both the extracts. Components of phytochemistry of onion bulb extracts have been shown to include flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, and terpenoids in previous studies (Jeeva *et al.*, 2014).

4. DISCUSSION

Flavonoids are recognized for their antioxidant and anti-cancer qualities, alkaloids are known for their pharmacological capabilities, which include antibacterial and anti-inflammatory effects. Steroids are necessary substances with a wide range of medical uses, while saponins are well known for their ability to lower cholesterol and strengthen immunological responses. The fact that both extracts passed these tests with positive results suggests that *A. cepa* contains a variety of bioactive substances that may have health advantages.

Alkaloids are naturally occurring chemical compounds having basic nitrogen atoms. They have pharmacological effects and are commonly used as therapeutic compounds.



Flavonoids increase the advantages by acting as antioxidants. They are also known to fight liver poisons, tumors, viruses, and other microorganisms. In addition to being researched for their antibacterial, anticancer, and other medicinal qualities, they are utilized in conventional herbal medicines. Tannins may possess antibacterial, antiviral, and antiparasitic qualities. According to a phytochemical examination of the components' percentage composition, *A. cepa* bulb leaves are extremely nutrient-dense. Its dry leaves have been shown to contain 23.6% of carbohydrates, an energy source, crude fiber (35.0%), which facilitates bowel movements, moisture (10.0%), which is roughly 80% of the blood, and is a necessary medium for the delivery of oxygen, and ash (10.0%), which is thought to be a measurement of the mineral content in diet that the body needs for appropriate physiological functioning (Lukaski, 1995). The amount of protein in dried leaves is determined by their crude protein content, which is 30.29%. Among its many uses, protein aids in the development and repair of bodily tissues (Chukwuebuka, 2015). The dry leaves' crude fat is 6.50%. When there is not enough glucose available for ATP synthesis, fat acts as a backup energy source. According to another research, chloroform extracts of *A. cepa* bulb contained alkaloids, tannins, and saponins (Abalaka *et al.*, 2012). The plant's ability to combat dangerous microorganisms is indicated by the presence of alkaloids and flavonoids.

The goal of the current study was to screen for phytochemicals to determine the antibacterial activity of dried *A. cepa*. The zone of inhibition of ethanolic extracts on *S. aureus* at several experimental dosages were examined. The zone of inhibition in the ethanolic extract was 10 ml (70 mm), and the normal experimental dosage was 5 ml (35 mm) and 10 ml (30 mm) of ethanolic 5 ml (15 mm). As a medicinal plant, *A. cepa* was extracted in various ways,

and the results were used to determine the antimicrobial analysis (Chipulu *et al.*, 2014). *S. aureus* was susceptible to the antibacterial properties of the stem bark juice. The growth of pathogenic microorganisms (*P. aeruginosa* and *S. aureus*), which were previously researched by (Caceres *et al.*, 1991), was found to be inhibited by the fresh leaf juice. Maximum antibacterial activity was often demonstrated by ethyl acetate and acetone extracts against *Salmonella gallinarum*, *P. aeruginosa*, *S. aureus*, and *Escherichia coli*. Among the extracts, the aqueous extract exhibited the least amount of antibacterial activity against each of the aforementioned bacteria. The outcomes also agreed with Ahmad *et al.* (2012), who found that at 200 mg/ml concentration with a 10.5 mm zone of inhibition, the methanolic leaf extract of *C. australis* had the maximum activity against *S. aureus*.

The findings of Ojiako (2014) investigation revealed that ethanol extract suppresses the activity of *S. aureus*. The outcomes concurred with those of Ahmad *et al.* (2012), who reported methanolic leaf extract with a 10.5 mm zone of inhibition, whereas *C. australis* exhibited the best activity against *S. aureus* at 200 mg/ml concentration. Similarly in a study carried out on the 70% ethanolic extract of medicinal plant *Diospyros malabarica* exhibited zone of inhibitions ranging from 13mm to 19mm against various gram-positive microorganisms including *S. aureus*. According to Hasan *et al.* (2017), methanolic crude oil has antimicrobial properties.

5. CONCLUSION

A highly helpful natural remedy for treating a variety of diseases caused by pathogenic organisms is *A. cepa* confirmed by the antibacterial activity of leaf extracts and the existence of secondary metabolites. The



extracts with organic solvents possess more potent antimicrobial properties. The activity diminished as the extract's concentration dropped. However, to draw a firm conclusion, it is required to pinpoint the specific elements causing the antibacterial activity in addition to the extract's mode of action.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AVAILABILITY OF DATA

Not applicable.

ETHICS APPROVAL AND INFORMED CONSENT

Not applicable.

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