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TO SCREEN OUT THE PHYTOCHEMICALS, ANTIBACTERIAL ANDANTIOXIDANT ACTIVITIES DETERMINATION FOR THE PLANT "ALLIUM FISTULOSUM"

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ABSTRACT

Introduction: Allium fistulosum, commonly known as Welsh onion, is widely used intraditional medicine for its purported health benefits. Despite its extensive use, scientific studies exploring its phytochemical properties, antioxidant activity, and antimicrobial potential are limited. **Objectives:** This study aimed to investigate the phytochemical composition, antioxidant activity, and antimicrobial properties of Allium fistulosum ethanol extracts. **Method:** The ethanol extract of Allium fistulosum was subjected to phytochemical screening toidentify the presence of bioactive compounds. The antioxidant activity was evaluated using the DPPH scavenging assay, with ascorbic acid serving as the standard. The antimicrobial properties were assessed against Bacillus subtilis, Escherichia coli, and Staphylococcus epidermidis using the disc diffusion method. **Result:** Phytochemical screening revealed the presence of flavonoids, tannins, terpenoid, and phenol in the extract. The antioxidant activity showed a moderate effect with an IC50 value of 0.760 mg/ml, compared to ascorbic acid's IC50 value of 0.322 mg/ml. However, the antimicrobial tests indicated that the extract did not exhibit significant activity against the tested bacterial strains, as no zones of inhibition were observed. **Conclusion:** Allium fistulosum ethanol extract contains various bioactive compounds with moderate antioxidant properties, suggesting potential health benefits. However, the extract did not show significant antimicrobial activity against the selected bacterial strains, highlighting theneed for further research. Future studies should explore higher concentrations, different extraction methods, and a broader range of microorganisms to fully elucidate the therapeutic potential of Allium fistulosum.

KEYWORDS: Allium fistulosum, phytochemical, antioxidant, antimicrobial, DPPH assay.

INTRODUCTION

Plants have proven to be a valuable reservoir of various bioactive substances, including phytochemicals that possess promising medicinal capabilities. Allium fistulosum, also referred to as Welsh onion or green onion, has attracted

considerable interest due to its culinary applications and unique taste, amidst the vast array of botanical specimens. Allium fistulosum, in addition to being often used in kitchens, has a rich history of being used for medical purposes in different cultures. This has led to scientific curiosity in identifying the specific bioactive components that are responsible for their pharmacological benefits. This plant is a member of the Allium genus, which is known for its sulfur-containing compounds. It is closely related to garlic (Allium sativum) and onions (Allium cepa), all of which have been widely researched fortheir health benefits. Figure 1 shows the Allium fistulosum.

Figure 1: Allium Fistulosum.

The genus Allium has long been recognized for its medicinal and culinary significance, with ancient civilizations employing these plants for their purported health benefits. The pungent aroma and distinct taste associated with Allium fistulosum are attributed to the presence of various phytochemicals, including allicin, flavonoids, and other sulfur-containing compounds (Vlase et al., 2012). While Allium fistulosum shares some phytochemical constituents with its botanical relatives, its unique chemical profile may harbor novel compounds that contribute to its specific biological activities. The Welsh onion (Allium fistulosum) is believed to have originated in Asia, particularly in China (Brewster & Rabinowitch, 1990). Rank, Scientific andcommon names are listed in Table 1.

Table 1: Taxonomy of Allium fistulosum L (Wikipedia contributors, 2024).

Rank	Scientific Name and Common Name
Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Liliopsida* - Monocotyledons
Subclass	*Liliidae*
Order	Asparagales
Family	*Amaryllidaceae*
Genus	Liliacea
Species	*Allium fistulosum* L.- welsh onion

Allium fistulosum L., known as Japanese bunching onions, welsh onions or spring onions is oneof Allium species known which has similarities to scallions and smells and tastes similar to A cepa L. Unlike other species, this plant does not form bulbs and has hollow leaves (Immaculateet al., 2020). In Japan and China, this plant is used as a vegetable or as a traditional medicine to improve the function of internal organs and metabolism and treat several diseases such as headaches, diarrhea, stomach pains and colds (Bede and Zaixiang, 2020; Hirayama et al., 2019). Welsh onion (Allium fistulosum L.) is a major vegetable product widely cultivated from Siberiato tropical Asia as well as in Japan, Korea and

China (Hirayama et al., 2019). According to Waghulde et al. (2021). Its leaves have nutritional value, and they can be fresh consumed over the year, still green over the winter. Welsh onion is a clumping, slowly spreading, evergreen perennial type that is mainly grown as a vegetable because of its onion-scented leaves. This species is very similar in taste and smell to the related common onion, Allium cepa, but does not develop bulbs. It somewhat has hollow leaves (fistulosum meaning "hollow"). Welsh onion can reproduce by forming an evergreen clump. Phytochemical studies reported that Welsh onions contain organosulfur compounds and polyphenolic compounds (Vlase et al., 2013). Sulfur-containing compounds from the Allium plant exhibit various biological activities. Several studies have shown Welsh onions have anti-fungal, antioxidative, anti-hypertensive, anti-platelet, and anti-obesity effects (Sung et al., 2011; Yamamoto et al., 2005; Sang et al., 2002; Chen et al., 2000). Welsh onions are mainly used in traditional Chinese medicine because of its natural chemical compounds which are beneficial for human health. Bulbs, pseudo stem juice, leaves, flowers, seeds and roots have medicinal values, as antibacterial, antitumor, antioxidant, antihypertensive, anti-obesity, antiplatelet aggregation, and regulation of immune function (Liang et al., 2021; Tigu et al., 2021; Hirayama et al., 2019; Sung et al., 2018). The main active compounds include essential oils which mostly contain sulfides, oleic acid, linoleicacid, pectin, allicin, and vitamin C (Tigu et al., 2021; Sung et al., 2018; Zhang et al., 2017). It was reported that the stem, leaf and root extracts of Welsh onions all had an antioxidant effect, and the stem extract showed the strongest antioxidant effect (Wang, 2017). Phytochemical screening, aimed at identifying and characterizing the diverse array of secondary metabolites present in plants, provides a systematic approach to understanding the chemical composition of Allium fistulosum. This screening process involves the detection of various classes of bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and terpenoids, among others. Investigating the phytochemical composition of Allium fistulosum not only adds to the wealth of knowledge concerning this plant's chemical diversity but also lays the groundwork for exploring its potential pharmacological applications.

In addition to phytochemical screening, the evaluation of antibacterial and antioxidant activities is paramount in uncovering the health-promoting potential of Allium fistulosum. The antimicrobial properties of Allium species, attributed to compounds like allicin, have been acknowledged in traditional medicine. Understanding the antibacterial activity of Allium fistulosum can provide insights into its potential role as a natural antimicrobial agent, offering alternatives or complements to conventional antibiotics, especially in the face of emerging antibiotic resistance (Shaira J. Limpahong, 2019; Bede & Lou, 2020). Furthermore, the antioxidant properties of Allium fistulosum can be pivotal in elucidating its potential contribution to oxidative stress mitigation. Antioxidants play a crucial role in neutralizing reactive oxygen species, thereby safeguarding cells from oxidative damage. Given the association between oxidative stress and various chronic diseases, exploring the antioxidant potential of Allium fistulosum becomes particularly relevant in the context of preventive healthcare and nutraceutical development (Bede & Lou, 2020; Xie et al., 2023). This comprehensive research endeavor, encompassing phytochemical screening, antibacterial, and antioxidant activity determination for Allium fistulosum, aims to bridge existing knowledge gaps and unlock the full spectrum of bioactive compounds present in this plant. The outcomes of this study have the potential to not only deepen our understanding of the chemical composition of Allium fistulosum but also to unravel its therapeutic applications in the realms of antimicrobial and antioxidant interventions. As we embark on this scientific exploration, we seek to unravel the intricate tapestry of phytochemicals within Allium fistulosum, shedding light on its multifaceted contributions to human health and well-being (Bede & Lou, 2020).

METHODOLOGY

- Extraction process and phytochemical screening
- Antimicrobial activity determination
- Antioxidant activity screening

COLLECTION AND PREPARATION OF ALLIUM FISTULOSUM

Allium fistulosum plant was purchased from fresh local market. Figure 2 shows the fresh plantof Allium fistulosum.

Figure 2: Fresh Allium Fistulosum.

The spring onion was washed with distilled water to remove the unwanted impurities and dust. Then the plant was chopped into small pieces and washed again with distilled water. After washing it, the weight of the allium fistulosum weighed and value recorded.

MACERATION

The extraction of spring onion was carried out through maceration process.

1. Eighteen 250 ml of conical flasks were prepared and washed by acetone.
2. 2.75 L concentrated ethanol was prepared and poured 150 ml carefully in each conical flask.
3. The minced spring onion leaves inserted into prepared conical flasks to immerse themselves into concentrated ethanol.
4. The mixture was kept in the orbital shaker to ensure the mixture mix thoroughly during theprocess. The duration needed for the maceration process was around seven days.

Figure 3: Maceration process of the mixture in the orbital shaker for seven days.

5. After seven days, the mixture from each conical flask was poured out to a 500 ml beaker, making it into a total of five 500 ml beakers and the extract residues were removed from the flask.
6. The extract residues were put on the surface of clean muslin cloth and the solution remained the extract residues were squished out by compressing the muslin cloth. The solution was collected in another 250ml beaker.
7. The collected plant extract solution including from extract residue was 2750 ml.
8. The collected plant extract solution was transferred to two big glass bottles and to a small glass bottle, wrapped, labelled and stored in a dark place with normal room temperature.

EVAPORATION

1. After the extract obtained from extraction process, the extract was evaporated by using rotatory evaporator to remove the solvent. The temperature of rotatory evaporator was set to 45℃, below the boiling point of ethanol to avoid overheating.
2. When there were no further changes observed, the extract was removed and transferred into China dish. The China dish was heated by placing on water bath at 68℃ for further evaporating. After a few hours, the extract became concentrated and removed from the water bath.
3. The China dish covered with the aluminum foil and few holes formed on the surface. The extract was kept in refrigerator to protect the extract from contamination.

PRE-PREPARATION

Dilution of extract sample was carried out before phytochemical process started. 1 ml of extract solution was prepared and inserted in a 50.0 ml volumetric flask. The ethanol was added to makeup to 50 ml of solution.

Preparation of Allium fistulosum ethanol extract

A stock solution of 3 mg/ml was prepared by dissolving 300 mg of extract in 100 ml of ethanol.Only once concentration of each extract was prepared and used for the test.

Preparation of difference concentration of gallic acid standard

Gallic acid was used as standard for this assay. Six difference concentrations of standard solution were prepared in 3, 2.4, 1.8, 1.2, 0.6, 0.3 mg/ml. The concentration was prepared by serial dilution methods. A stock solution of 3 mg/ml

was prepared by dissolving 300 mg of gallic acid in 100 ml ethanol. Then, from the freshly prepared stock solution of gallic acid, 10, 8, 6, 4, 2, 1 ml were pipette out into the volumetric flask and make up to 10 ml by ethanol to produce 3, 2.4, 1.8, 1.2, 0.6 and 0.3 mg/ml respectively.

Table 2: Preparation of various gallic acid standard concentrations for TPC by serialdilution.

Concentration(mg/ml)	Volume of Stock Solution (ml)	Volume of ethanol(ml)
3	10.0	0
2.4	8	2
1.8	6	4
1.2	4	6
0.6	2	8
0.3	1	9

Total Phenolic Content Test

The total phenolic content was performed according to experimental protocol given in standard reference to slight modifications. A 2.5% sodium carbonate solution was prepared by dissolving 2.5 g of sodium carbonate in 100 ml of distilled water. 1 ml of extract was added into a test tube by using a micropipette. The testtube containing extract was added with 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5% sodiumcarbonate solution. Standard gallic acid of different concentrations was prepared by using the same procedure by replacing extract with different concentration of standard gallic acid. Then followed by addition of 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5% sodium carbonate solution. Similarly, control solution was prepared by replacing the extract with 1 ml of ethanol and addition of 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5% sodium carbonate solution. Ablank solution was prepared only with 3 ml of ethanol. All the test tube containing different concentrations of standard gallic acid, extract, control as well as blank were allowed for stand for two hours. Next, all the test tubes containing different concentrations of standard gallic acid,extract, control and blank were subjected to UV visible analysis. Approximately 2 ml content of each test tube was transferred into the quartz cuvette and measured for the absorbance at 760nm in UV-Visible spectrometer. The absorbance values of each different concentration of standard gallic acid, extract, control and blank were recorded. The total phenolic content was expressed in mg of gallic acid equivalents per g dry extract (mg GAE/g). All points were plottedto line graph where x-axis was concentration of gallic acid and y-axis was absorbance. The following formula was applied to calculate total concentration of phenolic content:

$$\text{Total phenolic content, C} = \frac{c \times v}{m}$$

Whereas,

C = Total phenolic content mg GAE/g dry extract

c = Concentration of gallic acid obtained from calibration curve in mg/ml

V = Volume of extract in ml,

m = Mass of extract in gram.

ANTIOXIDANT ACTIVITY

Preparation of different concentration of Allium fistulosum ethanolic extract

The extract was prepared according to experimental protocol. Six different concentrations of extract solution were prepared in 0.3, 0.6, 1.2, 1.8, 2.4 and 3 mg/ml. A stock solution of 3 mg/mlwas prepared by dissolving 300 mg of extract

in 100 ml of ethanol. The concentrations were prepared by serial dilution methods. Then, from the freshly prepared extract solution, 1 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were pipette out into the volumetric flask and make up to 10 ml by ethanol individually to produce 0.3, 0.6, 1.2, 1.8, 2.4 and 3 mg/ml respectively. Table 3 gives the preparation of various concentrations of extract for DPPH serial dilution.

Table 3: Preparation of various concentrations of extract for DPPH by serial dilution.

Concentration(mg/ml)	Volume of stock solution(ml)	Volume of ethanol (ml)
3	10	0
2.4	8	2
1.8	6	4
1.2	4	6
0.6	2	8
0.3	1	9

Preparation of different concentration of ascorbic acid standard

Ascorbic acid was used as the standard for this assay. Six different concentrations of standard solution were prepared in 0.3, 0.6, 1.2, 1.8, 2.4 and 3 mg/ml. The concentration was prepared by serial dilution method. A stock solution of 3 mg/ml was prepared by dissolving 100 mg of ascorbic acid in 100 mg ethanol. Then, from the freshly prepared stock solution of ascorbic acid,1 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were pipette out into the volumetric flask and make up to 10 ml by ethanol individually to produce 0.3, 0.6, 1.2, 1.8, 2.4 and 3 mg/ml respectively. Table 4 shows the preparation of various concentrations of ascorbic acid standard for DPPH byserial dilution.

Table 4: Preparation of various concentrations of ascorbic acid standard forDPPH by serial dilution.

Concentration(mg/ml)	Volume of stock solution(ml)	Volume of ethanol(ml)
3	10	0
2.4	8	2
1.8	6	4
1.2	4	6
0.6	2	8
0.3	1	9

DPPH ANTIOXIDANT TEST

The DPPH (1,1-Diphenyl2-picryl hydrazyl) Radical Scavenging Assay was performed. According to experimental protocol given in standard references with slight modifications 0.3mM of DPPH (1,1-Diphenyl2-picryl hydrazyl) was prepared by dissolving 11.83 mg in 100 ml of ethanol. 2.5 ml of different concentrations of extract was added into different test tubes respectively. All the test tubes were then covered with aluminum foil as DPPH is light photosensitivity reagent as shown in Figure 12. The aluminum foil covered test tube containingdifferent concentrations of extract were added with 1 ml of 0.3 mM of alcoholic solution of DPPH reagent in each test tube. Standard ascorbic acid of different concentrations was prepared by using the same procedure by replacing extract with different concentration of standard ascorbic acid. Then followed by addition of 1 ml of 0.3 mM of alcoholic solution of DPPH. Similarly, a control solution was prepared by replacing the extract with ethanol and addition of 1 ml of 0.3 mM of alcoholic solution of DPPH. A blank solution was prepared only with 3 ml of 95% ethanol. All the test tubes containing different concentrations of standard ascorbic acid, extract, control, as well as blank were allowed for stand for 30 minutes in dark cupboard. Next,all the test tubes containing different concentrations of standard ascorbic acid, extract, control and blank were subjected to UV visible analysis in a dark condition. Approximately 2 ml contentof each test tube was transferred into the quartz cuvette and measured for the absorbance at 518 nm in UV-Visible spectrometer. The

absorbance values of each different concentration of standard ascorbic acid, extract, control and blank were recorded. The percentage of DPPH scavenging activity (SA) is calculated using the following equation:

$$\% SA = (A0-A1/A0) \times 100$$

Whereas = % SA = percentage scavenging activity,

A0= Absorbance of control

A1= Absorbance of sample

Then, curves are constructed by plotting percentage of inhibition against concentration in mg/ ml. The equation of this curve allowed to calculate IC50 corresponding to the sample concentration that reduced the initial DPPH absorbance of 50%.

ANTIMICROBIAL ACTIVITY

PREPARATION OF AGAR MEDIUM

1. 19g of Mullen-Hinton agar powder was prepared and mixed with 1L of sterile distilled water in a 2L beaker. The mixture poured into two 500 ml sterile bottles. Before pouring, the mixture was stirred regularly to ensure they mixed well.
2. The solution poured into two sterile bottles slowly and accordingly. The solution avoided to pour directly into one sterile bottle followed by another bottle. After pouring into the sterile bottles, the Mullen-Hinton agar allowed to settle down. The concentration of Mullen-Hinton agar sedimented should be approximately same in both sterile bottles to ensure their effectiveness of solidification of agar.
3. After the pouring process, both sterile bottles were sent to autoclave for 2 hours.
4. After autoclave, both sterile bottles were kept at 80 for storage to prevent the solidification of agar. Sterile cell plates prepared and placed inside the laminar flow cabinet to prevent contamination. The solution poured into the cell plate slowly and half of the cell plate was covered by the solution. Gloves wore prior pouring due to high temperature of solution.
5. The lid of the cell plates opened to let the solution become dry and solidification of agar occurred. After the agar solidified, the lid of the cell plates closed, and the water vapor removed by sterile tissues to prevent the growth of microorganisms during storage.

PREPARATION OF BACTERIAL STRAINS

Bacillus subtilis, Escherichia coli and Staphylococcus epidermidis were microorganisms used for the study.

PREFORMATION OF ANTIMICROBIAL ASSAYS

1. Marker pen used to divide the cell plate into four region which were positive control, negative control, and two different concentrations of plant extract. Nine cell plates were prepared and labelled for each tested bacterial strain. Each region was maintained at approximately the same distance. Ciprofloxacin used as positive control, sterile distilled water used as negative control and two different concentrations of plant extract labelled in another two areas.
2. 5mg/ml and 10mg/ml of plant concentration were prepared in a sterile bottle and placed in the laminar flow cabinet. The tested bacterial strains swiped by the sterile cotton swab onto the surface of the agar plates. One tested bacterial strain applied to three agar plates.

3. The agar plates were punched to make holes in each region by using sterile cork borer. Thecork borer sterilized by passing it through the flame after completing of making holes on one test bacterial agar plate to avoid contamination. The cork borer was allowed to cool before continuing the process to avoid agar damage.
4. The agar removed from the holes made by other side of sterile cork borer. The removed agartransferred to the beaker prepared. After removing agar from all holes made, the water vaporwas removed from the lid of agar plate.
5. All agar plates incubated at 37 for 24 hours to promote the growth of tested bacterial strains.The presence of zone of inhibition observed and the results recorded.
6. The diameter of zone of inhibition was calculated.
7. 1-6 steps were repeated for 50 mg/ml and 100 mg/ml of allium fistulosum extract on theagar plates with the same bacterial strains.

RESULTS

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Table 5: Results of phytochemicals present in filtrate extract by maceration.

S. No.	Analysis Test	Maceration (Filtrate)
1	Test for Alkaloids	-
2	Test for Reducing Sugars	-
3	Test for Saponins	-
4	Test for Terpenoids	+
5	Test for Anthraquinones	-
6	Test for Glycosides	-
7	Test for Tannins	+
8	Test for Flavonoids i) NaOH and HCL ii) Lead acetate test	 - +
9	Test for Phenols	+
10	Test for Quinone	-
11	Test for Carbohydrate	+
12	Test for Amino Acids	+
13	Test for Fatty Acids	-

DETECTION OF TOTAL PHENOLIC CONTENT(TPC)

Gallic acid (standard solution)

Table 6: Table of standard solution concentration and absorbance values.

Concentration(mg/ml)	Absorbance (WL765nm)
0.3	0.167
0.6	0.253
1.2	0.359
1.8	0.38
2.4	0.483
3	0.567

Allium fistulosum ethanol extract

The absorbance value of extract with 3mg/ml obtained was 0.369 which is shown in Figure 4.

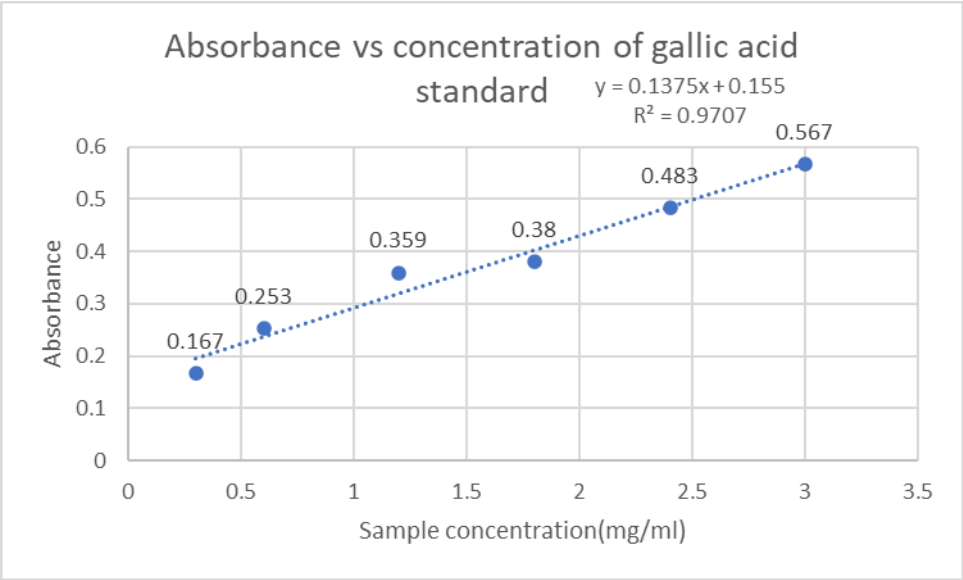

Figure 4: Graph of absorbance against standard concentration.

ANTIOXIDANT ACTIVITY OF ETHANOLIC ALLIUM FISTULOSUMDPPH SCAVENGING ASSAY

Tables 7 and 8 shows the concentration of ascorbic acid, specific absorbance and percentage scavenging and the concentration of Allium fistulosum ethanolic extract, specific absorbance and percentage scavenging, respectively. Figures 5 and 6 show the graph of percentage scavenging against ascorbic acid, standard solution concentration and graph of percentagescavenging against sample concentration, respectively.

Table 7: Table of the concentration of ascorbic acid, their absorbance and percentagescavenging.

Concentration(mg/ml)	Absorbance	SA (%)
0.3	0.608	59.06
0.6	0.601	59.5
1.2	0.546	64.48
1.8	0.484	68.51
2.4	0.274	82.17
3	0.222	85.56

Table 8: Table of the concentration of Allium Fistulosum ethanolic extract,specific absorbance and percentage scavenging.

Concentration(mg/ml)	Absorbancevalue	SA (%)
0.3	1.018	33.77
0.6	0.682	55.63
1.2	0.605	60.64
1.8	0.575	62.58
2.4	0.387	74.82
3	0.362	76.44

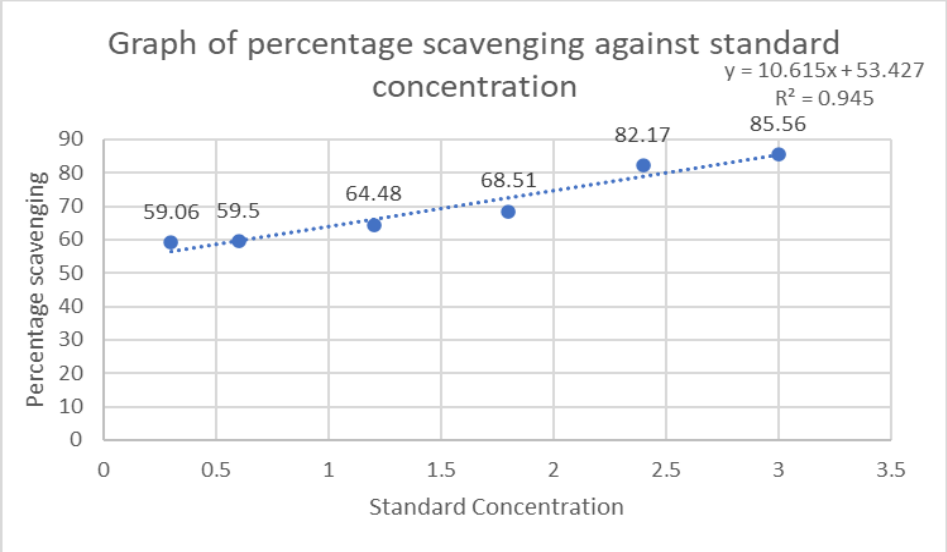

Figure 5: Graph of percentage scavenging against ascorbic acid, standard solutionconcentration.

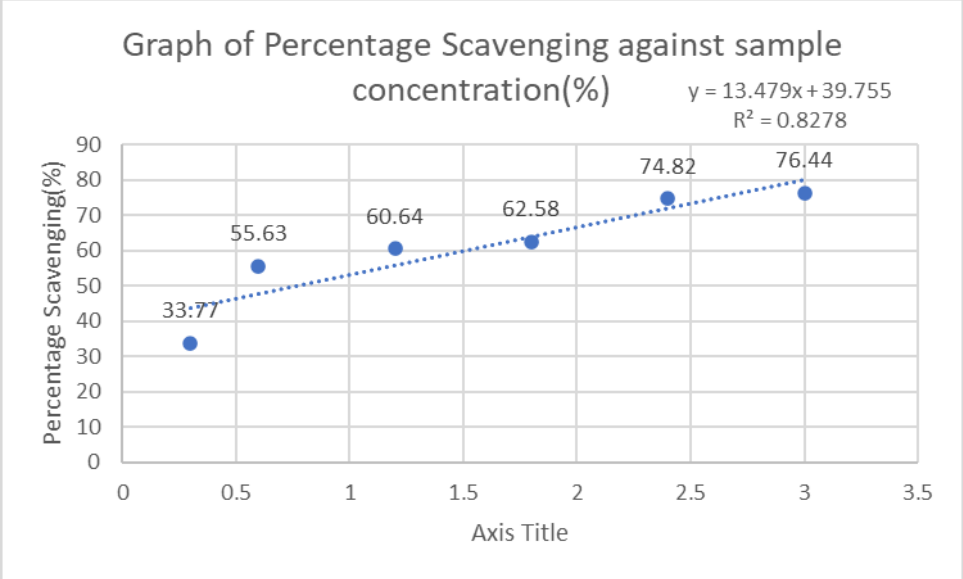

Figure 6: Graph of percentage scavenging against sample concentration IC50 of antioxidant activity of ascorbic acid:

y= 10.615x + 53.427 50=10.615x + 53.427

X=0.322mg/ml

IC50 of antioxidant activity of allium fistulosum extract:

y=13.479x + 39.755

50= 13.479x + 39.755

X=0.760mg/ml

ANTIMICROBIAL SUSPECTIBILITY TEST

Bacillus Subtilis

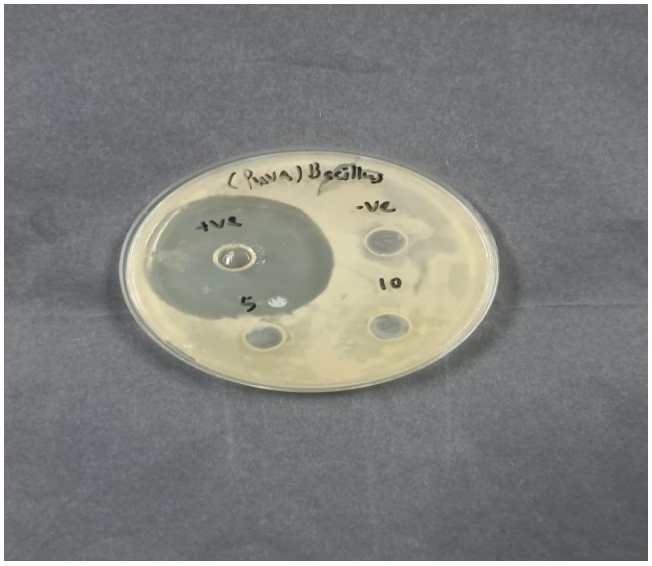

Figure 7: Result of antimicrobial activity of filtrate extract, concentration of 5 mg/ml and 10 mg/ml toward Bacillus Subtilis.

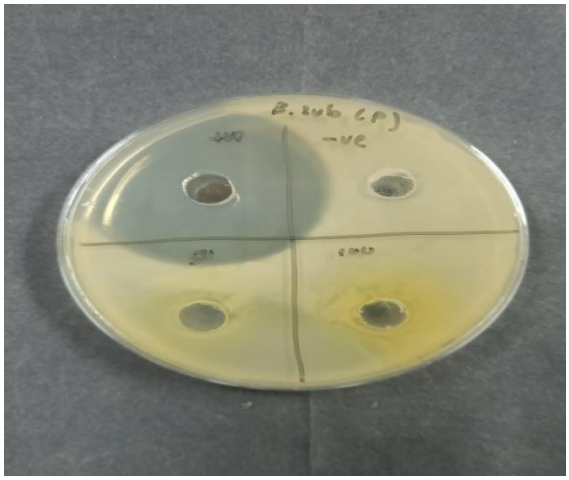

Figure 8: Result of antimicrobial activity of filtrate extract, concentration of 50 mg/ml and 100 mg/ml toward Bacillus Subtilis.

From Figures 7 and 8, negative result is shown as no zone of inhibition observed ineach concentration of plant extract.

Escherichia coli

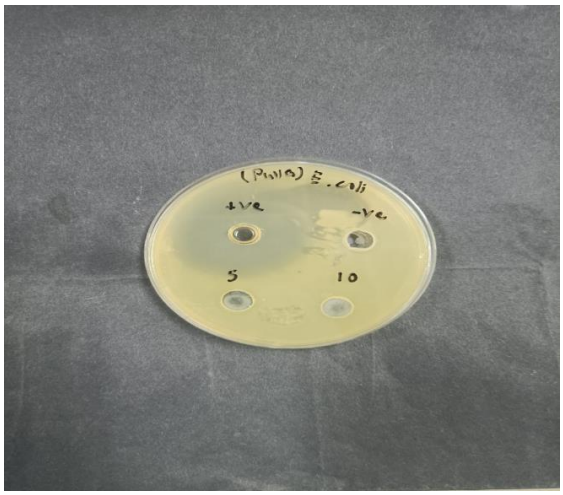

Figure 9: Result of antimicrobial activity of filtrate extract, concentration of 5 mg/ml and 10 mg/ml toward E. coli.

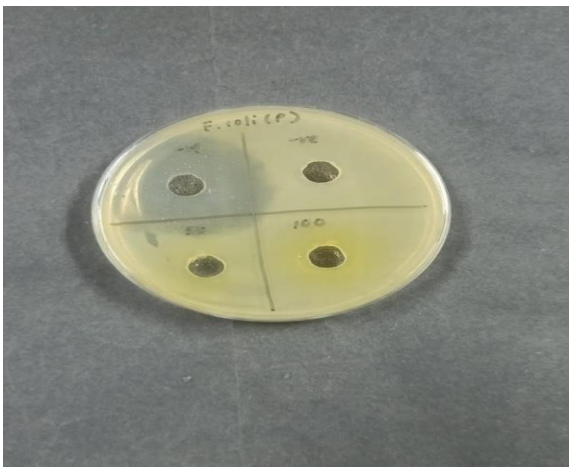

Figure 10: Result of antimicrobial activity of filtrate extract, concentration of 50 mg/mland 100 mg/ml toward E. coli.

From Figures 9 and Figure 10, negative result is shown as no zone of inhibition observed ineach concentration of plant extract.

Staphylococcus epidermidis

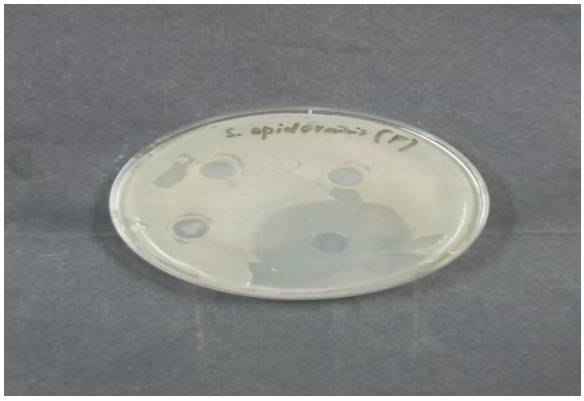

Figure 11: Result of antimicrobial activity of filtrate extract, concentration of 5 mg/ml and10 mg/ml toward S. epidermidis.

Figure 12: Result of antimicrobial activity of filtrate extract, concentration of 50 mg/mland 100 mg/ml toward S. epidermidis.

From Figures 11 and 12, negative results are shown as no zone of inhibition observed in each concentration of plant extract. Table 9 gives the antimicrobial activity of Allium Fistulosum ethanolic extract of different concentrations.

Table 9: Table of antimicrobial activity of Allium Fistulosum ethanolic extract of differentconcentration

Microorganism	Concentrationof allium fistulosum extract(mg/ml)	Zone of inhibition observed on Mullen-Hinton agar, mm in diameter	Ciprofloxacin (positivecontrol), mm in diameter	Sterile Distilledwater (Negativecontrol), mm indiameter
Bacillus subtilis	5.0	-	2.5±0.2	-
	10.0	-	2.6±0.2	-
	50.0	-	2.3±0.2	-
	100.0	-	2.2±0.2	-
Escherichia Coli	5.0	-	2.4±0.2	-
	10.0	-	2.5±0.2	-
	50.0	-	2.6±0.2	-
	100.0	-	2.3±0.2	-
Staphylococcus epidermidis	5.0	-	2.4±0.2	-
	10.0	-	2.5±0.2	-
	50.0	-	2.5±0.2	-
	100.0	-	2.3±0.2	-

DISCUSSION

The study conducted on the phytochemical screening, antioxidant activity, and antimicrobial properties of Allium fistulosum ethanol extracts provides valuable insights into its potential medicinal applications. The detailed analysis of these properties helps to understand thetherapeutic potential and limitations of Allium fistulosum.

The phytochemical screening revealed that Allium fistulosum ethanol extract contains a varietyof bioactive compounds including terpenoids, tannin, phenol and flavonoid. These phytochemicals are known for their diverse biological activities and contribute to the therapeuticefficacy of medicinal plants.

The antioxidant activity of Allium fistulosum ethanol extract was assessed using the DPPH scavenging assay. The results indicated that the extract has moderate antioxidant activity with an IC50 value of 0.760 mg/ml. This is in comparison to ascorbic acid, a standard antioxidant, which exhibited a much lower IC50 value of 0.322 mg/ml, indicating higher potency. The antioxidant activity of the extract can be attributed to the presence of phenolic compounds, which are known for their ability to donate hydrogen atoms or electrons and neutralize free radicals. The moderate antioxidant activity observed suggests that Allium fistulosum can contribute to reducing oxidative stress, which is implicated in various chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. However, the extract's antioxidant potency is less than that of ascorbic acid, suggesting that while it has potential, it may not be the most effective antioxidant available. This indicates a need for further optimization of the extraction process or the use of higher concentrations to achieve more significant antioxidant effects. The antimicrobial properties of Allium fistulosum ethanol extract was evaluated against three bacterial strains: Bacillus subtilis, Escherichia coli, and Staphylococcus epidermidis. The results showed that the extract did not exhibit significant antimicrobial activity, as no zones of inhibition were observed at various concentrations.

This lack of antimicrobial activity could be due to several factors:

1. **Concentration of Extract**: The concentrations used in the study might not have been high enough to exert antimicrobial effects. Future studies could explore higher concentrations or different extraction methods to enhance the antimicrobial efficacy.
2. **Spectrum of Activity**: The study was limited to three bacterial strains. Allium fistulosum might possess antimicrobial properties effective against other microorganisms, including fungi or different bacterial species. Expanding the range of tested microorganisms could provide a more comprehensive understanding of its antimicrobial potential.
3. **Synergistic Effects**: Phytochemicals often work synergistically, and the individual compounds present in the extract may not be potent enough on their own. Combining the extract with other known antimicrobial agents could reveal potential synergistic effects.
4. **Methodological Factors**: The method used for antimicrobial testing (e.g., disc diffusion assay) might not be the most sensitive for detecting the antimicrobial activity of the extract. Other methods, such as minimum inhibitory concentration (MIC) assays, couldbe employed to provide more accurate assessments.

CONCLUSION

In conclusion, the phytochemical screening of Allium fistulosum ethanol extract indicates the presence of several bioactive compounds with potential health benefits. The moderate antioxidant activity suggests that the extract can contribute to reducing oxidative stress, althoughit is less potent than ascorbic acid. The lack of significant antimicrobial

activity against the tested bacterial strains highlights the need for further investigation, including testing against a broader range of microorganisms and using higher concentrations or different methods. Overall,Allium fistulosum shows promise as a source of bioactive compounds with antioxidant properties, but its antimicrobial potential requires further exploration. Addressing the limitationsof this study, such as expanding the microbial testing scope, optimizing extraction methods, andexploring synergistic effects, will help to fully elucidate the therapeutic potential of Allium fistulosum.

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CONFLICT OF INTERESTS

Authors state that there is no conflict of interests regarding this original article publication.

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