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Research Article

Phytochemical Profile, Antioxidant and Antibacterial Potential of Methanolic Seed Extract from *Pisum sativum*.

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Abstract

Natural remedies have been integral to alternative medicine for thousands of years, with numerous drugs now derived from plants and animals. Pisum sativum, known as "Motorsuti" in Bangladesh, is one such medicinal plant recognized for its healing properties. This study aimed to investigate the phytochemical composition of methanol extract from P. sativum seeds grown in Bangladesh and to evaluate its antibacterial and antioxidant activities using validated experimental methods. The antioxidant activity was assessed in vitro using the 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, while the antibacterial efficacy was determined via the well diffusion method against various pathogenic bacterial strains. Phytochemical screening of the P. sativum seed extract revealed the presence of proteins, terpenoids, glycosides, alkaloids, carbohydrates, flavonoids, saponins, quinine, gum, and alkaloids. The DPPH radical scavenging activity of the methanol extract was measured as the percentage inhibition of DPPH at different extract doses. The extract demonstrated significant free radical scavenging activity, with an IC_{50} value of 178.65 µg/ml, compared to 60.19 µg/ml for the standard antioxidant ascorbic acid. The methanol extract also exhibited notable antibacterial activity against some gram-negative bacteria, including Pseudomonas spp., Citrobacter spp., and Enterobacter spp., with zones of inhibition measuring 11.50 mm, 15 mm, and 24 mm, respectively. These results suggest that the methanol extract of P. sativum seeds contains valuable secondary metabolites with potent antibacterial and antioxidant properties, highlighting its potential nutritional and pharmacological benefits.

Keywords: Antioxidant Assay, DPPH, Pisum sativum, Methanol, phytochemicals, free radicals, antibacterial agents.

1. INTRODUCTION

The population of the globe greatly depends on plants and plant derived-products for not just food and shelter, but also medicine from ancient times (Alam, 2016). Secondary metabolites in many plants have been shown to possess antioxidant, anti-diabetic, anti-malarial, and anti-nociceptive properties (Adebayo JO, 2011). Notably, many of these plants and their derivatives also function as nutraceuticals. The term "nutraceutical" combines "nutrition" and "pharmaceutics" and refers to herbal extracts (Carole, 2018). The global demand for herbal medicines is steadily rising, and their study might give useful clues for the creation of alternative pharmaceuticals and treatment procedures, which in turn could contribute to the eradication of many illnesses and the satisfaction of this need.

As many as 250,000 unique species of higher plants have been identified worldwide. World Health Organization estimates that over 21,000 different plant species might be employed as therapeutic agents (Joy, 2001). However, it is claimed that around 70,000 species have been used for medical reasons in various societies (Von Gadov, 1997). From only these 94 plant species, a total of 122 biologically active chemicals have been isolated and characterized (Shekhar, 2015). WHO estimates that over 80% of the world's population uses traditional healing methods and herbal remedies as their major source of healthcare and well-being (Zhang & WHO, 2002). Large segments of the population in several industrialized nations rely on alternative medical approaches, particularly the usage of medicinal plants, for their health (Karunamoorthi, 2013). In established nations like the United States, plant-based medicines are expected to be used about 25% of the time, while in rapidly growing countries like India and China, plant medicines account for up to 80% of treatments (Kong, 2003). (Kong, 2003). Moreover, it has been reported that up to 80% of the population in Africa, 40% of Colombia (Shaikh, 2005), 70% of Ethiopia (Bekele, 2007), and 80% of South Asia (Roberson, 2010) utilize plants as medication.

Oxidative stress, which generates a flood of free radicals and contributes to the development of diseases including cancer, atherosclerosis, and cardiovascular disease, is thought to be a major factor in the emergence of many modern ailments (Braca A, 2002). Any agent that substantially retards or suppresses oxidative stress when present at low concentrations is said to be an antioxidant (Rahman, 2015).

A variety of aerobic gram-negative bacteria are the causative agents of a number of life-threatening diseases, often treated with a broad array of antibiotics (Tumah, 2005). Due to the widespread misuse of antibiotics, many bacteria have developed resistance to many drugs. This has accelerated research into new drugs and nutritional supplements that may combat this problem. Since ancient times, many plant components, herbs, and spices have been utilized to prevent diseases. These are readily accessible and may be used for self-medication in a residential situation. (Saeed, 2005)

Herbs include a diversity of compounds with substantial therapeutic benefits (Craig, 1999; Dureja, 2003) within the plant kingdom. The green pea (*Pisum sativum*), is a plant of the Fabaceae family that has been used medicinally for centuries. This plant has shown some efficiency against diabetes (Marinangeli, 2011), obesity (Abete, 2009), and the generation of free radicals (Abete, 2009). In addition, it has been proven to improve cardiovascular, gastrointestinal, and homeostatic functioning (Dahl, 2003). For thousands of years, *P. sativum* seeds have been exploited as nourishment, appetizers, astringents, coolants, and laxatives. It has been effective in treating a variety of conditions, including hyperglycemia, phlegm, acne, and intestinal irritation (Alasalvar, 2002). It has been shown that the seeds possess antioxidant, antibacterial, and hypoglycemic characteristics (Rehman, 2011).

As Bangladesh is a low-income nation, many individuals cannot afford to purchase synthetic medications. As a consequence, the economic importance of medicinal plants in Bangladesh is much greater than in many other countries. Limited investigations have been done to evaluate the phytochemical potentialities of pea plant seeds grown in Bangladesh. This study sought to identify the phytochemical makeup of seed extracts as well as their antibacterial and antioxidant properties. As a result, this research may help generate new and medicinally helpful components, which might lead to a wide range of treatment options.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Material

The pods of *Pisum sativum* were collected from the local market of Nowabganj, Savar, Dhaka. Seeds had been separated from the pods and directly exposed to sunlight until completely dehydrated. Finally, fully dried seeds were ground into a coarse powder by a grinding machine (Jaipan 1000W Hotel King Mixer Grinder, India), then filtered using a thin sieve and kept in an airtight container for later use.

2.2. Preparation of Plant Extract

The powder was extracted with methanol by dissolving powdered pea seeds in methanol at an 80% (W/V) concentration in a sealed glass container. The bottle was stored at room temperature for 15 days while being regularly shaken. When the solvent was concentrated, a Soxhlet extractor was used to filter off the liquid alcohol. A water bath heated at the temperature 55-60 °C was then used to thoroughly evaporate the solvents. After complete evaporation, a highly concentrated, greenish, gelatinous methanol extract crude was obtained. Finally, the residue was kept in sterile vials at 4^0C until use.

2.3. Phytochemical Profile

Methods for Screening

Standard protocols outlined by Harborne were followed to perform a qualitative phytochemical screening of the plant extract (Harborne, 1998)

Test for Carbohydrate (Molish Test)

In a dry test tube, 2ml of the extract (10% w/v) was added along with a few drops of Molisch's reagent. Then, 1 ml of concentrated H_2SO_4 was carefully added to the mixture. Carbohydrates may be detected by a violet or purple ring at the intersection of two layers.

Test for Reducing Sugar

Fehling's solutions A and B were combined in an equal amount with 2 ml of plant extract in a test tube. The mixture was then boiled for two minutes. A positive test for reducing sugars is indicated by the formation of a brick-red copper (I) oxide precipitate.

Test for Tannins

In a 5:1 ratio, the extract was combined with 10% potassium dichromate. The presence of tannin is indicated by a yellow precipitate.

Test for Saponin

In a graduated cylinder, the mixture of 2 ml of extract and 2 ml of distilled water was stirred for 15 minutes. Foam formation is indicative of the presence of saponin.

Test of Quinone

One milliliter of concentrated H_2SO_4 was added to one milliliter of extract in a test tube. The development of a red hue shows the presence of quinine.

Test for Gums

The presence of gums is indicated by the development of a crimson or reddish-violet ring at the interface between the two layers after adding Molisch's reagent and sulfuric acid to a 5 ml solution of the extract.

Test for steroid

About 1 ml of extract solution was treated with 2 ml of Libermann-Burchard reagent. A structure with a greenish hue suggests the presence of steroids. The presence of steroids is indicated by a greenish coloration.

Test for alkaloid

1 ml of the extract solution and 0.1 ml of diluted hydrochloric acid were placed into a test tube. Then, 1 ml of Mayer's reagent (a mixture of 1.36 g of mercuric chloride and 5.00 g of potassium iodide in 100.0 ml of water) was added. The formation of a creamy white or yellow precipitate indicates the presence of alkaloids.

Test of proteins

To verify the presence of peptide linkages in the sample extract, we mixed 2 ml of the extract with 1 ml of 40% sodium hydroxide and a few drops of 1% copper sulfate. The appearance of a violet color indicates the presence of peptide linkages.

Test of terpenoids

One milliliter of the plant extract was mixed with five milliliters of chloroform and a few drops of strong hydrogen peroxide. The mixture was vigorously shaken and then allowed to sit for a while. The formation of a yellow or brownish-red precipitate indicates the possible presence of triterpenoids.

Test for Cardiac Glycosides

To test for the presence of cardiac glycosides, 1 ml of extract was combined with 0.5 ml of glacial acetic acid and 3 drops of a 1% aqueous ferric chloride solution. The formation of a violet hue indicates the presence of deoxy sugar, a hallmark of cardiac glycosides.

Test for Glycosides

A small quantity of the plant extract was dissolved in 1 ml of water. Then, some sodium hydroxide solution was added. The presence of glycosides is typically indicated by the appearance of a yellow tint.

2.4. Antioxidant Activity Assay

DPPH free Radical Scavenging Activity

The antioxidant capacity of the test sample was evaluated using the DPPH assay (Braca, 2001). A DPPH solution (0.004% w/v) was prepared in methanol. The plant extract was first prepared as a stock solution in methanol (0.5 mg/ml) and then serially diluted to obtain concentrations of 7.81 µg/ml, 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml. One milliliter of the DPPH solution was mixed with three milliliters of each diluted plant extract solution, stirred, and allowed to react for thirty minutes. For comparison, standard ascorbic acid was dissolved in methanol to make a solution with the same concentrations. A control solution was prepared using equal parts methanol and DPPH. The free radical scavenging activity was calculated using the following equation, with the absorbance measured at 517 nm to determine the percentage of inhibition of DPPH discoloration:

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Then, the IC_{50} was calculated by graphing the percentage of inhibition versus the log concentration ($y = mx + e$) (Viturro, 1999).

2.5. Evaluation of Antibacterial Activity

Antibacterial activity was investigated using a well-diffusion screening method (Kivanc & Kunduhoglu, 1997). The antibacterial properties of seed extract were tested against six different gram-negative bacterial strains: (*E. coli, Pseudomonas, Klebsiella, Serratia, Citrobacter, Enterobacter*). At first, the test microorganisms were cultured on Mueller-Hinton agar (MHA) plates. The inoculum was evenly spread across the entire plate using a sterilized glass spreader. Using a cork borer, wells with a consistent diameter of 8 mm were made in the agar, and then 50, 100, and 150 µl of the methanol seed extract were added to each well. The plates were then incubated at 37°C for 24 hours to allow for bacterial growth. The antibacterial activity of the seed extract was indicated by the presence of a clear, defined zone of inhibition surrounding the wells, where bacterial growth was prevented. The effectiveness of the antibacterial activity was determined by measuring the diameter of the inhibition zones in millimeters.

3. RESULT

3.1. Phytochemical

The results of phytochemical screening are shown in Table 1. Our experiment identified the existence of carbohydrates, flavonoids, saponins, quinine, gum, alkaloids, proteins, terpenoids, and glycosides in the methanolic extract of *P. sativum*.

3.2. Antioxidant Activity

The free radical scavenging experiment was performed to determine the antioxidant capability of green peas' seed extract at varying concentrations. To determine the percentage inhibition induced by the plant extract, we evaluated the actual reduction in absorption at 517 nm and utilized that value to

Table 1. Result of the qualitative phytochemicals screening

Phytochemicals	Methanolic extract
Carbohydrate	+
Reducing Sugar	-
Tannins	-
Flavonoids	+
Saponins	+
Quinine	+
Gum	+
Steroids	-
Alkaloids	+
Proteins	+
Terpenoids	+
Cardiac Glycosides	-
Glycosides	+

Note: The "Plus" sign indicates the existence of Phytochemical, whereas the "minus" sign indicates its absence.

compute the percentage inhibition, this was then compared to a standard vitamin C benchmark. The antioxidant properties of standard ascorbic acid and *P. sativum* extract are shown in Tables 2 and 3, respectively. *P. sativum* methanolic extract shows a mediocre level of antioxidant activity compared to standard. The extraction has an IC_{50} of 178.65 µg/ml, whereas ascorbic acid has an IC_{50} of 60.19 µg/ml (Fig. 1). Scavenging activity increased successively with increasing concentrations of both the extract and ascorbic acid, but reached a plateau at the highest concentrations.

3.3. Antibacterial Activity

The in vitro antimicrobial activities of *P. sativum* seed extracts are shown in Table 4. The plant seed extract showed moderate antimicrobial efficacy against *Enterobacter spp.* (11.50 mm), whereas no activity was observed against *Escherichia coli, Klebsiella, Serratia, Citrobacter*.

The in vitro antimicrobial activities of *P. sativum* seed extracts are presented in Table 4. The methanolic seed extracts exhibited significant activity against *Pseudomonas* spp. and *Citrobacter* spp, with 24 mm and 15 mm inhibition zones, respectively (Figure 2).

Table 2: Antioxidant activity of Ascorbic Acid

Concentration (µg/ml)	Absorbance (nm)	% Inhibition	% DPPH Remaining
7.81	0.41	2.33	97.67
15.62	0.21	51.16	48.84
31.25	0.12	72.09	27.91
62.5	0.094	78.14	21.86
125	0.058	86.51	13.49
250	0.056	86.98	13.02
500	0.055	87.21	12.79

Table 3: Antioxidant activity of Extract Solution

Concentration (µg/ml)	Absorbance (nm)	% Inhibition	% DPPH Remaining
7.81	0.76	0	100
15.62	0.38	11.63	88.37
31.25	0.14	67.44	32.56
62.5	0.13	69.77	30.23
125	0.14	67.44	32.56
250	0.13	69.77	30.23
500	0.14	67.44	32.56

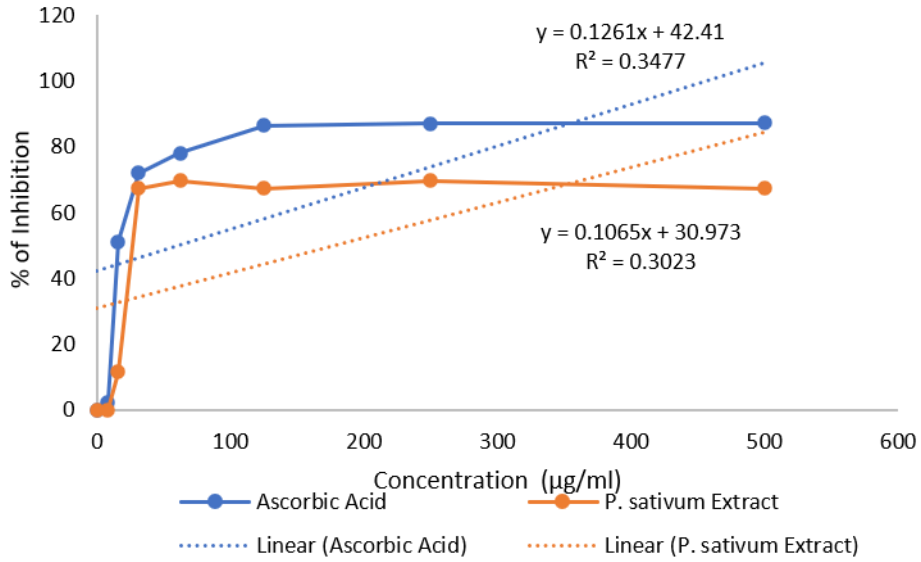

Fig. 1. Calculation of the IC_{50} value by comparing the extract's % radical scavenging activity with the standard. (Here, y=mx+e, x can be calculated by x= y-e/m).

showed high activity against *Pseudomonas spp.* and *Citrobacter* (inhibition zone of 24 and 15 mm, respectively) from its methanol extract (Figure 2). Since these bacteria are pathogenic, the methanolic extract of the plant seeds may be somewhat effective against them. The plant extract showed moderate antimicrobial efficacy against *Enterobacter* spp. with an inhibition zone of 11.50 mm. However, no activity was observed against *Escherichia coli*, *Klebsiella*, and *Serratia*.

Table 4: Antibacterial activity of *P. sativum* seed extracts

Bacterial Strain	Zone of Inhibition (mm)
Pseudomonas spp.	24
Citrobacter spp.	15
Enterobacter spp.	11.50
Klebsiella spp.	00
Serratia spp	00
Escherichia coli	00

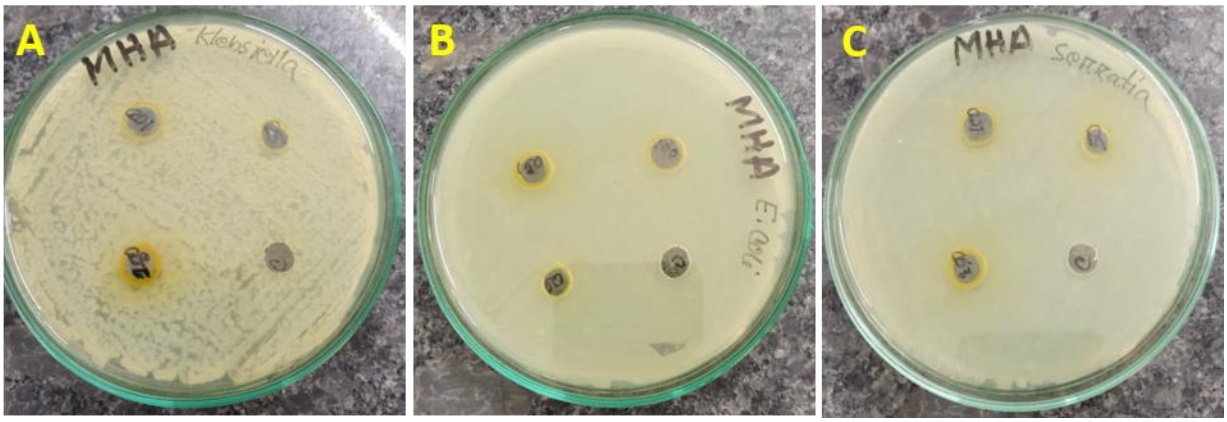

Fig. 2a: Antibacterial activity of *P. sativum* seed extracts against different gram-negative bacteria A) *Klebsiella* spp. B) *E. coli* C) *Serratia* spp.

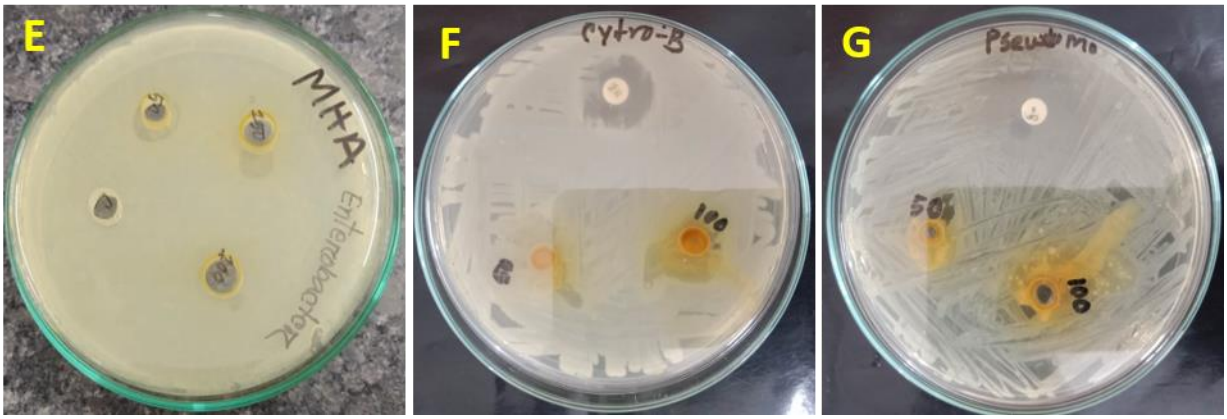

Fig. 2b: Antibacterial activity of *P. sativum* seed extracts against different gram-negative bacteria D) *Enterobacter* spp. E) *Citrobacter* spp. F) *Pseudomonas* spp.

4. DISCUSSION

Nutrition and healthcare are closely related in ancient civilizations, and many plants have been used both as food and medicine (Rahman, 2015). All human cultures have used medicinal herbs at one point or another in their development (Viturro, 1999). The world's poorest countries still rely significantly on medicinal plants to cure a variety of illness issues (Carole, 2018). Given the diverse and abundant vegetation in our biosphere this is not unexpected (Van Vuuren, 2008). Numerous human diseases have been partially or completely treated using different medicinal plants. They have still been utilized as diabetics, malaria preventives, narcotic pain relievers, antioxidants, and antilipidemics, etc. (Adebayo, 2011). A significant category of nutraceuticals also includes these therapeutic herbs. A nutraceutical is a supplement that combines medications and nutrition. Using nutrition and bioactive herbal ingredients to prevent illness is a novel idea (Lifongo, 2014). Nutraceuticals and medicinal plants are increasingly being sought after due to their efficacy, low cost, and lack of side effects in comparison to standard medical treatments (Nasir, 2015).

The green pea (*Pisum sativum*), in its vegetative form, is a delicious treat. Due to its high nutritional density (carbohydrates, protein, fiber, and other nutrients), it is a common ingredient in many of the foods we eat, such as salads and vegetarian meals. Anti-diabetic (Marinangeli, 2011), anti-oxidant (Duenas, 2004), and anti-obesity (Abete, 2009) properties have all been attributed to it. Positive modulation of cardiovascular, gastrointestinal, and homeostatic systems (Dahl, 2003) has also been described. The goals of this study were to profile the phytochemical composition (qualitative analysis) of *P. sativum* seeds cultivated in the Bangladeshi area and examine the antibacterial and antioxidant activities of the methanol extract of these seeds.

The qualitative chemical examinations displayed the presence of various phytoconstituents like carbohydrates, flavonoids, saponins, quinine, gum, alkaloids, proteins, terpenoids, and glycosides. In the study on *P. sativum* seed extract, Alam *et al.* discovered remarkably identical phytochemicals except for the tannin group (Alam, A. K., 2016). However, in another experiment of phytochemical screening, where fresh green pea methanol extract was analyzed, quinine, alkaloids, and glycosides were not observed, but steroids and cardiac glycosides were detected (Carole, 2018).

The antioxidant capacity of plants is a remarkable ability to eliminate free radical intermediates in the body, which are responsible for life-threatening disorders such as cancer, diabetes, stroke, and many others. Free radicals may speed up the chain reactions that lead to cell death once they are within the body. Antioxidants serve an important role in preventing these chain reactions by neutralizing free radical molecules (Islam, 2013). A DPPH free radical assay was used to determine whether or not the methanol

extract of *P. sativum* seeds has antioxidant properties. "The DPPH assay is one of the most used screening assays for determining the antioxidant activity of plant extracts" (Nanjo, 1996). In solution, DPPH is a stable, nitrogen-centered free radical that creates a violet color. It was converted to diphenylpicryl hydrazine in a density-dependent manner by adding the fractions. The quantity of accessible hydroxyl groups correlates with the decline in the number of DPPH molecules. Antioxidant properties are indicated by the presence of flavonoids, saponins, phenols, and aromatic molecules (Alabri, 2014). In this assay, flavonoids were identified by phytochemical screening, and these crucial molecular components are what give plants their anti-oxidant properties. Flavonoids play a significant role in detoxifying the body by neutralizing reactive oxygen species such as hydrogen peroxide, hydroxyl, peroxyl, and superoxide anion (Islam, 2013). Flavonoids have the potential to convert DPPH to the stable, non-reactive DPPH-H form by contributing an electron or hydrogen ion (Razali, 2008, Paixão, 2007). The DPPH assay's finding of radical-scavenging activity was supported by the extract's flavonoid concentration. The plant extract has shown potential DPPH-scavenging activity, this was considerable to the standard antioxidant activity. Given that the standard antioxidant agent ascorbic acid was found to have an IC_{50} of 60.19 µg/ ml, but the IC_{50} of the plant extract was 178.65 µg/ ml, the antioxidant test proved that the plant extract had significant antioxidant activity. In contrast to this research, Alam et al. found that green pea extract had extremely low antioxidant activity (the standard's IC_{50} was 16.28 µg/ml, but the plant extract's IC_{50} was 489.25 µg/ml) (Alam, 2016).

For thousands of years, various plant components (flowers, buds, leaves, stems, skins, and pulp) have been utilized to improve the taste and aroma of food. In addition, plants are abundant in secondary metabolites such as terpenoids, flavonoids, tannins, and alkaloids, which show antibacterial effects when tested in vitro (Cowan, 1999). In this regard, we evaluated the antibacterial activity of *P. sativum* seeds in our study, and this plant was discovered efficient against several gram-negative pathogenic bacteria. A similar study carried out in Karachi, Pakistan, showed that *P. sativum'*s skin and seed juice was efficient against *Klebsiella, Pseudomonas, Salmonella, Shigella, Proteus, and Enterobacter* (Saeed, 2005). The degenerative impact of *P. sativum* seeds on some bacteria is indicative of their wide range of action; hence, they might be used as a source of antibiotic chemicals for the development of pharmaceuticals, that can be used to treat certain bacterial infections. However, the literature lacks considerable studies on its antibacterial activities against other microorganisms.

As global work on the creation of herbal medicines is now underway, this research will assist in the discovery of novel products/drugs. Furthermore, it may be used for domestic self-medication.

5. CONCLUSION

The phytochemical profile of the methanolic extract of dried pea seeds showed the presence of flavonoids, glycosides, saponins, quinine, alkaloids, and terpenoids. Based on the research's results, it can be claimed that the test material demonstrated significant antioxidant and antibacterial activities, bolstering the rationale for its use in sources of antioxidant and antibacterial agents in conventional medicine. Therefore, *Pisum sativum* might be an aid in the search for nutraceuticals that may help in illness treatment and prevention. Given all of the good qualities and promising aspects of pea seeds, it is clear that there is room for more research into it.

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7. CONFLICTS OF INTEREST

Each author herein certifies that they have no competing interests relating to the publication of this work.

8. CONTRIBUTION

Rubait Hasan, Foyzur Rahman, Faruk Hossain, and Munna Kumar conceived and designed the study. Rubait Hasan and Rifat Hossain conducted the study. The data was gathered, processed, and written up by Rubait Hasan, Jamiatul Husna Shathi and, Zakerin Abedin. Rubait Hasan prepared the original draft. Mohammad Shahangir Biswas, Shahidur Rahman, and Rezaul Karim reviewed and edited the manuscript. Each author has given their stamp of approval to the final text.

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