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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 used in alternative medicine for thousands of years, and now an astonishing variety of drugs have been isolated from plants and animals. Pisum sativum, also known as "Motorsuti" in Bangladesh, is one of the various medicinal plants that have the ability to heal a variety of ailments. The current study was carried out to screen the phytochemical properties of methanol extract of P. sativum plant seeds cultivated in Bangladesh as well as to study the antibacterial and antioxidant efficacy using several validated experimental methods. In vitro antioxidant activity was evaluated using the 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, while antibacterial activity was evaluated using the well diffusion method against a panel of pathogenic bacterial strains. Screening tests of the P. sativum seed extract demonstrated the presence of proteins, terpenoids, glycosides, alkaloids, carbohydrates, flavonoids, saponins, quinine, gum, and alkaloids. The DPPH radical scavenging capabilities of a pea seed methanol extract were evaluated as the % inhibition of DPPH at different doses of the extract. The methanol extract of Pisum sativum seeds was reported for considerable free radical scavenging activity. The extraction's IC50 value was 178.65 g/ml, compared to the standard anti-oxidant ascorbic acid's IC50 of 60.19 g/ml. The methanol extract of P. sativum seeds has also shown significant antibacterial activity against a subset of gram-negative bacteria in the current investigation namely Pseudomonas spp., Citrobacter spp., and Enterobacter spp. (zone of inhibition around 11.50 mm, 15 mm and 24 mm respectively). These findings, therefore, imply that the test substance is a herb with certain nutritionally and pharmacologically significant secondary metabolites that have good antibacterial and antioxidant properties.

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

1. Introduction

The population of the globe greatly depends on plants and products generated from plants for not just food and shelter, but also medicine from ancient times (Alam, 2016). Antioxidant, anti-diabetic, anti-malarial, and anti-nociceptive effects have been shown in secondary metabolites found in many plants (Adebayo, 2011). It's worth noting that several of these plants and their byproducts do double duty as nutraceuticals. The word "nutraceutical" is an amalgamation of the words “nutrition" and "pharmaceutics," and it is used to describe extracts of herbs (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 between 35,000 and 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 a number of industrialized nations rely on alternative medical approaches, particularly the usage of medicinal plants, for their health (Karunamoorthi, 2013). Established nations like the United States are expected to rely on plant medicines as little as 25% of the time, while plant medications account for up to 80% in rapidly expanding nations like India and China (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.

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 (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 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. Several investigations have been done to evaluate the potentialities of pea plant seeds grown in Bangladesh. This study sought to identify the phytochemical makeup of *P. sativum* 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. Sun dried seeds were grinded into a coarse powder by a grinding machine 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, 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 pipette down the tube's side. 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. Two minutes were spent boiling the mixture. Testing for reducing sugars yields a brick-red copper (i) oxide precipitate as a positive result.

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 development of a crimson or reddish violet ring at the interface between the two layers after adding molish reagent and sulphuric acid to a 5 ml solution of the extract reveals the presence of gums.

Test for steroid

About 1 ml 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

1ml of the extract solution and 0.1 of diluted hydrochloric acid were put into a test tube. Mayer's reagent (a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) in 100.0 ml water) was poured at an amount of 1 ml. Alkaloids are present when a creamy white or yellow precipitate forms.

Test of proteins

In order to verify the presence of peptide linkage molecules in the sample extract, we mixed 2 ml of extract with 1 ml of 40% sodium hydroxide and a few

drops of 1% copper sulphate, which resulted in a violet color.

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 ingredients were vigorously agitated and then let to sit for a while. The formation of a yellow or brownish-red precipitate indicates the possible existence of triterpenoids.

Test for Cardiac Glycosides

1 ml of each extract was combined with 0.5 ml of glacial acetic acid and 3 drops of a 1% aqueous ferric chloride solution to test for the presence of cardiac glycosides. The presence of deoxy sugar, a hallmark of cardiac glycosides, is shown by the production of a violet hue.

Test for Glycosides

In 1 ml of water, a little quantity of an alcoholic extract of the plant material was dissolved. After that, some sodium hydroxide solution was added. The presence of glycosides is often indicated by the appearance of a yellow tint.

2.4. Antioxidant Activity Assay

DPPH free Radical Scavenging Activity

Using DPPH, the antioxidant capacity of the test sample was evaluated (Braca, 2001). A DPPH solution (0.004 w/v) was made in methanol. The plant extract was first made into a stock solution in methanol (0.5mg/ml), and then that solution was diluted serially to get the desired 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 DPPH solution was combined with three milliliters of diluted solutions, stirred, and then allowed to react for thirty minutes. For comparison, a solution of the same concentration was also made by dissolving standard ascorbic acid in methanol. Equal parts of methanol and DPPH were used to create a control solution. The free radical scavenging activity was calculated using the following equation, where the absorbance at 517 nm was used to assess 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).Seed extract was put through an antibacterial test using six different gram-negative bacterial cultures (*E. coli, Pseudomonas, Klebsiella, Serratia, Citrobacter, Enterobacter*) were used in this study. To test for antibacterial properties, the test microorganisms were seeded into Mueller-Hinton agar (MHA) plates. A sterilized glass spreader was used to distribute the inoculums across the whole plate. Cutting consistent wells through the MHA's surface was accomplished using a normal 8 mm cork borer and 50, 100, and 150 µl of methanol seed extract were poured into the well. The plates were then left in an incubator at 37^0 C for 24 hours to allow the organisms to develop to their fullest. The test materials' antibacterial activity prevented the microbes from proliferating, and a definite, well-defined zone of inhibition could be seen encircling the medium. The antibacterial activity of the sample under test was determined by measuring the size of the zone of inhibition in mm.

3. Result

3.1. Phytochemical

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

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

3.2. Antioxidant Activity

The free radical scavenging experiment was performed to determine the green peas' antioxidant capability at varying doses of the methanolic extract. 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 compute the percentage inhibition; this was then compared to a standard vitamin C benchmark. The antioxidant properties of standard ascorbic acid and *Pisum sativum* extract are shown in Tables 2 and 3, respectively. *P. sativum* methanol extract shows a mediocre level of antioxidant activity. The extraction has an IC50 of 178.65 g/ml, whereas ascorbic acid has an IC50 of 60.19 g/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.

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

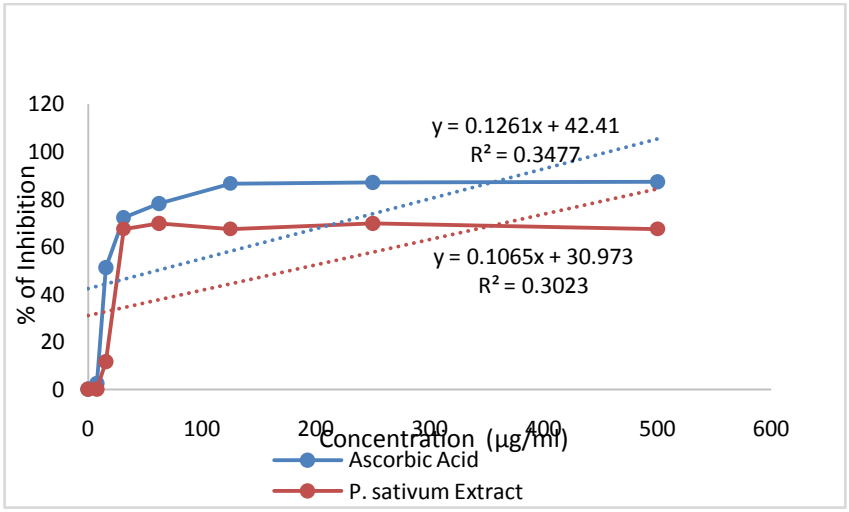

Fig. 1. Calculation of the IC50 value by comparing of the extract's % radical scavenging activity with the standard. (Here, y=mx+e, x can be calculated by x= y-e/m)

3.3. Antibacterial Activity

The in vitro antimicrobial activity of *P. sativum* seed extracts are shown in **Table 4**. The plant seed extract showed high activity against *Pseudomonas spp.* And *Citrobacter* (24 and 15 mm, respectively) from its methanol extract (Figure 2). These bacteria are pathogenic and therefore, methanolic extract of the plant seed can be useful in some extent against these bacteria. Plant extracts shown moderate antimicrobial efficacy against *Enterobacter spp.* (11.50 mm), whereas no activity was observed against *Escherichia coli, Klebsiella, Serratia, Citrobacter*.

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

Bacterial Strain	Zone of Inhibition (mm)
Pseudomonas spp.	24
Citrobacter spp.	15
Enterobacter spp.	11
Klebsiella spp.	00
Serratia spp	00
Escherichia coli	00

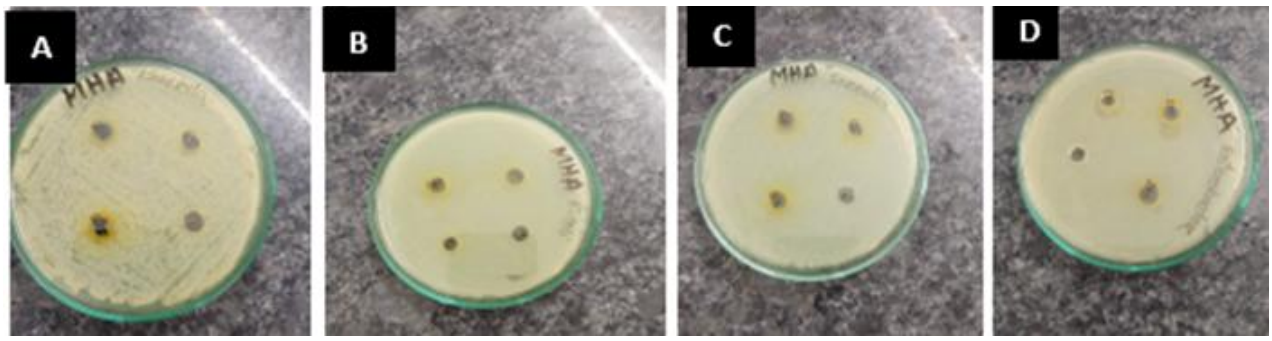

Fig 2a: Antibacterial activity of *P. sativum* seed extracts against different gram-negative bacteria A) *Klebsiella spp.* B) *E. coli* C) *Serratia spp.* D) *Enterobacter spp.*

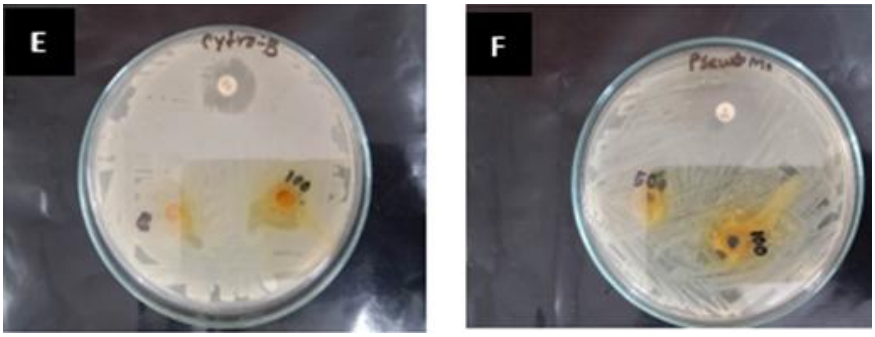

Fig 2b: Antibacterial activity of *P. sativum* seed extracts against different gram-negative bacteria E) *Citrobacter spp.* F) *Pseudomonas spp.*

4. Discussion

Nutrition and healthcare are closely related in ancient civilizations, and many plants have been used both as food and medicine. 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). Numerous diseases that have afflicted human populations have been treated using medicinal plants. They have 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 his study on *P. sativum* seed extract, Alam *et al.* discovered remarkably identical phytochemicals with the exception of 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 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). 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 considerably contrasted to the standard antioxidant activity. Given that the standard antioxidant agent ascorbic acid was found to have an IC 50 of 60.19 g/ ml, but the IC 50 of the plant extract was 178.65 g/ 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 g/ml, but the plant extract's IC 50 was 489.25 g/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 creation 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

According to their phytochemical profile, the methanol 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's extract 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.

6. Acknowledgement

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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, Mohammad Zakerin Abedin and Munna Kumar Podder conceived and designed the study. Rubait Hasan and Rifat Hossain conducted the study. The data was gathered, processed, and written up by Rubait Hasan. Each author has seen and given their stamp of approval to the final text.

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