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Morphological and Phytochemical Studies on *Pisum Sativum L.* (Fabaceae)

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ABSTRACT

Pisum sativum L. has been essential in human nutrition and agriculture due to its rich nutritional content, which includes proteins, fibers essential vitamins and minerals. Beyond its importance as a food crop, *P. sativum* has garnered significant interest in scientific research due to its potential in medicinal and therapeutic applications. Information on its bioactive compounds is available but the vegetative and floral characteristics and the basic mitotic and meiotic chromosomal investigations are lacking or rather scanty. This study examined the taxonomic and the fertility potentials of *P. sativum* with regards to morphological and phytochemical characteristics. Morphological characters were studied by visual observation and use of hand lens. Mitotic and meiotic chromosomal investigations were examined using root tip and flower buds squash techniques respectively. Phytochemical analysis was done using standard gravimetric and spectrophotometric methods. Analysis of variance was used to determine the level of significance, and results were presented in mean ± standard deviation. Morphological result revealed the habit of the *P. sativum* to be herbaceous annual climbing plant, leaf-alternate, entire, simple, compound, acute, cuneate, glabrous, smooth and Inflorescence- racemose, flower- papilionaceous, zygomorphic, five-lobed, superior, self-pollinated, pollen-tricolpate, fruit-legume (dry and dehiscent), seed-non-endospermic. Cytological result revealed the plant to be "diploid" with the mitotic chromosome counts of 2n=2x=14 and the meiotic chromosome counts of n=7. The pollen fertility result indicated that P. *sativum* is a highly fertile species. Qualitative and quantitative results indicated varied quantities of the secondary metabolites in the part (seed) of the plant investigated. The cytological studies indicated that *P. sativum* has a very high fertility and viability level and could be easily perpetuated and amenable to genetic manipulation thus justified its uses in various hybridization programmes. The overall data are valuable for taxonomic characterization and identification of species in the genus.

Keywords: Cytological, fertility, hybridization, Morphological, *Pisum sativum*, Phytochemical, taxonomic.

INTRODUCTION

Pisum sativum L., the pea, of family Fabaceae is among the most ancient and extensively cultivated herbaceous annual leguminous crops in the world. It is cultivated nearly for its delectable and wholesome seeds [1]. Dried peas are normally added to soups and they can be bought fresh, frozen or canned. There are some species such as sugar peas and snow peas that are used in East Asian cuisine and yield edible cooked or raw pods similar to green protein beans [2]. The seed yields a source of dietary fiber and the crop is relatively simple to grow. As it is native to the mediterranean region, the crop has been of prime significance in human diets and agriculture because of its high value in terms of proteins, fibers, vitamins, and minerals [2]. Besides being a significant food crop, *P. sativum* has also attracted extensive scientific interest in research because of its relevance in medicinal and therapeutic purposes [3]. Peas are legumes and are able to fix atmospheric nitrogen in the soil. They are a beneficial crop to include in rotations, building soil fertility and lowering the use of synthetic fertilizers [4]. Peas are being exported all over the world, and demand has been boosted by increasing interest in plant-based diets [5]. Pea, and more so pea starch is being investigated in the production of biodegradable plastics and other green materials. Peas are grown as a cash crop in most regions of the globe, providing economic gains to rural populations, particularly in developing countries [6]. It is a model in plant biology, being widely used to research genetics, to formulate biology and economic relations. Root rot, powdery mildew, and viral diseases are among the most

prevalent diseases that infect pea plant [7]. Plant morphology refers to the physical characteristics of a plant that are observable. Morphological traits are those characteristics pertaining to external appearances or forms [8]. They now supply characters employed for useful plant identification and some of those employed for hypothesizing phylogenetic relationships [9], these characters have been utilized for a longer period than anatomical evidence at the start of plant systematic. Morphological characterization of a species is a very useful in the separation of population into different morphotypes and proper utilization of genetic resources in plant breeding programme.The pea plant presents wide morphological variation of traits depending on variety, environmental conditions and cultivation practice. Morphological analysis often focuses on traits like seed length, shape and pod length, which are important for selection in breeding programs [10]. Plants are primarily identified according to their external morphological characteristics, for instance, flowers and fruits. However, these characteristics might not always be available in plants since their development is typically seasonal. Thus, there is a necessity to combine other modes of identification and classification, such as cytology and phytochemistry. By offering extra characters, cytology has proven to be especially helpful in resolving some taxonomic issues. Cytology offers insights into cell structure, primarily chromosomes, which are important for taxonomy [11], [7]. Cytological studies shed light on its genetic composition and breeding potential, especially with regard to the chromosome structure and division processes. According to [12]. chromosomal number and homology play a major role in determining pairing behavior during meiosis, which in turn influences hybrid fertility levels and, consequently, breeding behavior and population variation patterns. The number of chromosomes in each cell, or the chromosome number, is typically constant across all members of a single species. Furthermore, with the exception of that number, the more closely related species are, the more probable.Understanding plant genetics and chromosomal behavior has greatly benefited from cytology studies of *P. sativum*. Peas have 14 diploid chromosomes (2n=14), and cytogenetics research procedures are well-established. The species has long been employed in studies of plant genetics, particularly in the work of Gregor Mendel, who established the fundamentals of inheritance using peas. The vast array of organic materials that plants accumulate is the subject of phytochemistry, which also examines the chemical structures, biosynthesis, metabolism, natural distribution, and biological roles of these materials. One methodical line of evidence that leads to a natural classification is phytochemistry [11]. It investigates the bioactive substances found in the plant, including saponins and alkaloids, which are thought to have therapeutic qualities. Numerous bioactive substances, such as alkaloids, phenolic compounds, flavonoids, tannins, saponins, and glycosides, have been found in pea seeds by studies [13]. These substances have anti-inflammatory, anti-cancer, antimicrobial, and antioxidant qualities [7]. *P. sativum* L. is a species of immense importance, with applications ranging from nutritional, agricultural, industrial, medicinal, economic, cultural, and culinary fields. However, there is currently no comprehensive study or rather scanty that covers its morphological, cytological, and phytochemical characteristics. Hence, the need for the present study. The aim of the study was to evaluate the morphological, cytological and phytochemical characteristics of *P. sativum*.

MATERIALS AND METHODS

Area of the Study

The research was carried out in the year 2024 at Docchy Analytical Laboratories Service Center, Awka and Botany Laboratory, Nnamdi Azikiwe University Awka.

Collection and Identification of Plant Material

The plant materials (seeds, immature unopen flower bud, mature flower bud, *pisum* twig) of P. sativum were collected at horticuture center of Amobia area of Awka in Anambra State and kept in sterile paper bags. The species was identified at the Botany Department Nnamdi Azikiwe University, Awka by a taxonomist Dr. C.A. Ezeabara.

Sterilization of Materials

In cytological research, tools used were sterilized using 70–95% ethanol, autoclaving at 121°C (15 psi for 15–20 min), to maintain chromosomal integrity. For phytochemical studies, glassware and extraction equipment are disinfected with 70–80% ethanol, while autoclaving and 0.1–0.5% sodium hypochlorite was used for culture media and plant samples. Heat-sensitive extracts were sterilized via 0.22 μm membrane filtration. In morphological analysis, scalpels, petri dishes, and measuring instruments are cleaned with 70–90% ethanol, while 0.1–1% sodium hypochlorite was used to remove contaminants from plant samples.

Procedure for Morphological study

Observations on vegetative and floral characteristics were done using samples collected from mature plant. The 3rd and 4th fully open leaves from the species stem tip were used. A meter rule was used for measuring internodes and leaf stalk lengths, fruits, seeds, and other parts. Observation tools such as a magnifying lens or microscope are used to examine small structures like tendrils and flowers.

Cytological Studies

Materials for cytological studies (mitotic and meiotic events) were *P. sativum* root tips, immature flower buds, photomicroscope, reagents and stains used were carnoy's fluid, 1: 3(v/v) glacial acetic acid and 95% ethanol, 70% ethanol, 18% hydrochloric acid, F. L. P. orcein, distilled water and 0.002 M 8- hydroxyl-quinoline.

Procedure for Meiotic Studies

Young flower buds of Pisum sativum were collected at 9am and fixed in Carnoy's solution (glacial acetic acid and 95% ethanol, 1:3 v/v) for 24 h. Pollen mother cells (PMCs) were extracted from the anthers in a drop of acetic orcein stain. A cover slip was placed over the material and gently tapped using the blunt end of a biro pen to ensure even dispersion of the cells. Tapping continued until the sample was adequately spread and nearly transparent. To further enhance cell separation, the slide was positioned between folded filter paper on a flat, hard surface, and thumb pressure was carefully applied to the cover slip. Excess stain was removed using filter paper. Slides were examined under high-power light microscopy, and photomicrographs were taken for documentation [14].

Procedure for Mitotic Studies

Root tips from newly germinated *Pisum sativum* seedlings were harvested and pretreated with 8-hydroxyquinoline for 5hrs to arrest cell division at metaphase. The pretreated roots were then fixed in Carnoy's solution (glacial acetic acid and 95% ethanol, 1:3 v/v) for 24 h. Following fixation, the samples were hydrolyzed in 18% HCl for 5 min to soften the middle lamella and facilitate cell separation during squashing. The hydrolyzed roots were rinsed in 70% ethanol to prevent stain crystallization. Approximately 1 mm of the apical region was excised using a mounted needle and transferred to a clean glass slide. A drop of FLP orcein stain was applied, and a cover slip was gently placed over the material. The sample was squashed by tapping the cover slip with the blunt end of a biro pen to ensure proper cell dispersion. For improved spreading, the slide was placed between folded filter paper on a hard, flat surface, and gentle thumb pressure was applied to the cover slip. Excess stain was removed using filter paper. The slides were examined under high-power light microscopy for chromosomal observation, and photomicrographs were taken for documentation [14].

Pollen Fertility Study

Pollen fertility was determined by teasing out anthers onto a clean slide. The slide was stained with cotton blue and small thumb pressure applied. The deeply stained blue ones were regarded as viable while lightly stained ones were regarded as non- viable. Percentage fertility was calculated as follows:

$$\text{Fertility (\%)} \frac{\text{No. of viable pollen grains}}{\text{Total no. of pollen grains}} \times \frac{100}{1}$$

Phytochemical Studies

Six phytochemicals investigated were: flavonoids, tannins, saponins, alkaloids `phenols and sterols

Materials and Chemicals for Phytochemical Analysis

The equipment and materials utilized in this study included a plant sample (dried *Pisum* seeds), (grinder), masking tape, mortar and pestle, moisture cans, crucibles, Whatman No. 42 filter paper, burettes, volumetric flasks, beakers, conical flasks, sample tubes, desiccators, spectrophotometer, muslin cloth, oven, measuring cylinder, spatula, electric balance, Bunsen burner (stove), funnels, aluminum foil, test tubes, syringes, pipettes, and cotton wool, among others. The chemicals and reagents employed in the analysis consisted of ethanol (alcohols), concentrated acetic acid, sulfuric acid, diluted ammonia, distilled water, ferric chloride, potassium ferrocyanide, ethyl acetate, hydrochloric acid, petroleum ether, sodium hydroxide, potassium hydroxide (potassium permanganate), hydrogen peroxide, sodium chloride, copper sulfate, sodium picrate, methyl red, cresol green, Folin-Ciocalteu reagent, Folin-Denis reagent, Erichrome black, and Solechrome dark blue.

Ethanol Extraction of the Plant Sample

A total of 100 g of grounded seeds was placed in a Soxhlet apparatus and extracted thoroughly using 500 mL of absolute ethanol for duration of eight hours. The resulting ethanol extract was then concentrated by evaporating the solvent in a water bath (GFL, Germany) at 40°C for two hours, followed by further evaporation at room temperature overnight to remove residual ethanol. This process yielded semi-solid extracts, which were subsequently used for further phytochemical analysis.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the extracts was conducted to determine the presence of phytochemicals according to [15] and [16].

Quantitative Phytochemical Determination

Flavonoid Determination

Flavonoid content was measured according to the method of [15]. Five grams of the sample were boiled in 50 mL of 2M HCl solution under reflux for 30 minutes, then cooled and filtered using Whatman No. 42 filter paper. The

filtrate was treated with an equal volume of ethyl acetate, and the resulting precipitate was collected, dried, and quantified gravimetrically.

Alkaloids Determination

The alkaloid content in Pisum sativum seeds was measured using the alkaline precipitation gravimetric method as described by [15]. A known weight of the seed sample was mixed with a 10% acetic acid solution in ethanol at a ratio of 1:10 (10%). The mixture was left to stand for 4 hours at 28°C before being filtered using Whatman No. 42 filter paper. The filtrate was then evaporated to one-fourth of its original volume, followed by the gradual addition of concentrated aqueous NH_4OH to induce alkaloid precipitation. The resulting precipitate was collected on pre-weighed filter paper, rinsed with 1% ammonia solution, and dried at 80°C in an oven. The total alkaloid concentration was determined and expressed as a percentage of the sample weight.

Phenolic acid Determination

The phenolic content was determined using the Folin-Denis method as described by [15]. The extract was reacted with Folin-Denis reagent and Na_2CO_3, incubated at 28°C for 90 minutes, and analyzed at 260 nm using a spectrophotometer.

Saponin Determination

According to the method outlined by [17], 10 mL of each extract was transferred into 250 mL separator funnels and washed with 20 mL diethyl ether. Two layers were separated: the aqueous layer and the ether layer. The aqueous layers were recovered, and the ether layers were discarded. The purification process was repeated. Sixty milliliters of n-butanol was added into the extracts, and they were washed twice with 10 mL of 5% aqueous NaCl. The remaining solutions were heated over a water bath. After evaporation, the samples were dried in pre-weighed beakers in an oven to constant weights. The final weights were obtained, and the saponin content was calculated as a percentage.

Tannin Determination

Tannin content was determined by the Folin-Denis colorimetric method described by [15]. Five grams of the sample were dispersed in 50 mL of distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28°C before being filtered through Whatman No. 42 filter paper. Two milliliters of the extract were dispersed into a 50 mL volumetric flask. Similarly, 2 mL of standard tannin solution (tannic acid) and 2 mL of distilled water were placed in separate volumetric flasks to serve as the standard and reagent blank. The Folin-Denis reagent was added to each flask, followed by 2.5 mL of saturated Na_2CO_3 solution. The contents were made up to 50 mL with distilled water and allowed to incubate at 28°C for 90 minutes. Their respective absorbance values were measured in a spectrophotometer at 260 nm, using the reagent blank to calibrate the instrument to zero.

Steroids Determination

This was determined by the method described by [18]. A measured weight of each sample was dispersed in 100 mL of freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered, and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). Two milliliters of the eluate were placed in a test tube and mixed with 2 mL of chloroform. Three milliliters of ice-cold acetic anhydride were added to the mixture in the flask, and 2 drops of concentrated H_2SO_4 were cautiously added while cooling. A standard sterol solution was prepared and treated as described above. The absorbance of the standard and prepared sample was measured in a spectrophotometer at 420 nm.

Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD) from triplicate measurements. A one-way Analysis of Variance (ANOVA) was conducted to assess statistical significance of the sample means, with results or difference in means values considered significant at $p < 0.05$.

RESULTS

Morphological Studies of *Pisum sativum*

Table 1a: Morphology of *Pisum sativum* Leaf (Plate 1 and 2)	
Phyllotaxy	Alternate
Margin	Entire
Venation	Pinnate
Apex	Acute
Base	Cuneate
Colour	Green
Lamina	Pinnate with 3 pairs
Leaf Form	Compound
Shape	Ovate to lanceolate
Surface	Glabrous
Texture	Smooth
Stipules	Large, semi sagittate
Tendrils	Spirally coiled
Leaf Width	1.0-3.0 cm
Leaf length	2.0-6.0 cm
Internode length	5.5 cm
Leaf Stalk	4.5-5.3 cm

Table 1b:	**Morphology of *Pisum sativum* Root and Stem (Plate 1)**
Root	Tap root with a primary and numerous lateral roots
Root nodules	Present
Stem habit	Herbaceous (soft, non-woody)
Mode of life	annual
Colour	green
Shape	cylindrical and somewhat smooth
Size	0.5 to 2 m, depending on the variety
Branching	branched
Node	distinct
Apex	terminal
Texture	smooth

Table 1c: Morphology of *Pisum sativum* flower (Plate 1, 2, 3)	
Type	papilionaceous
Symmetry	zygomorphic
Calyx	five-lobed
Corolla	papilionaceous (butterfly-shaped)
Ovary	superior
Pollination	self-pollinated
Inflorescence	racemose

Table 1d: Morphology of *Pisum sativum* pollen, fruit and seed (Plate 1, 2)	
Pollen	tricolpate
Fruit type	legume (dry and dehiscent)
Fruit nature	simple
Fruit shape	elongated
Fruit length	5 to 10 cm
Fruit width	1 to 2 cm
Seed shape	oval or globular
Seed length	6-8 mm
Seed width	4-6 mm
Seed type	non-endospermic
Colour	green and yellow

Plate 1: *Pisum sativum* in its habitat showing the habit

Plate 2: showing the fruit, leaf, seed and flower of *Pisum sativum*.

Plate 3: *Pisum sativum* twig

Cytological Analysis

a. Mitotic analysis

The diploid chromosome number of *P. sativum* observed is 2n=14 (Plate 4)

b. Meiotic analysis

The meiotic chromosomes showed that *P. sativum* has n= 7 (Plate 5).

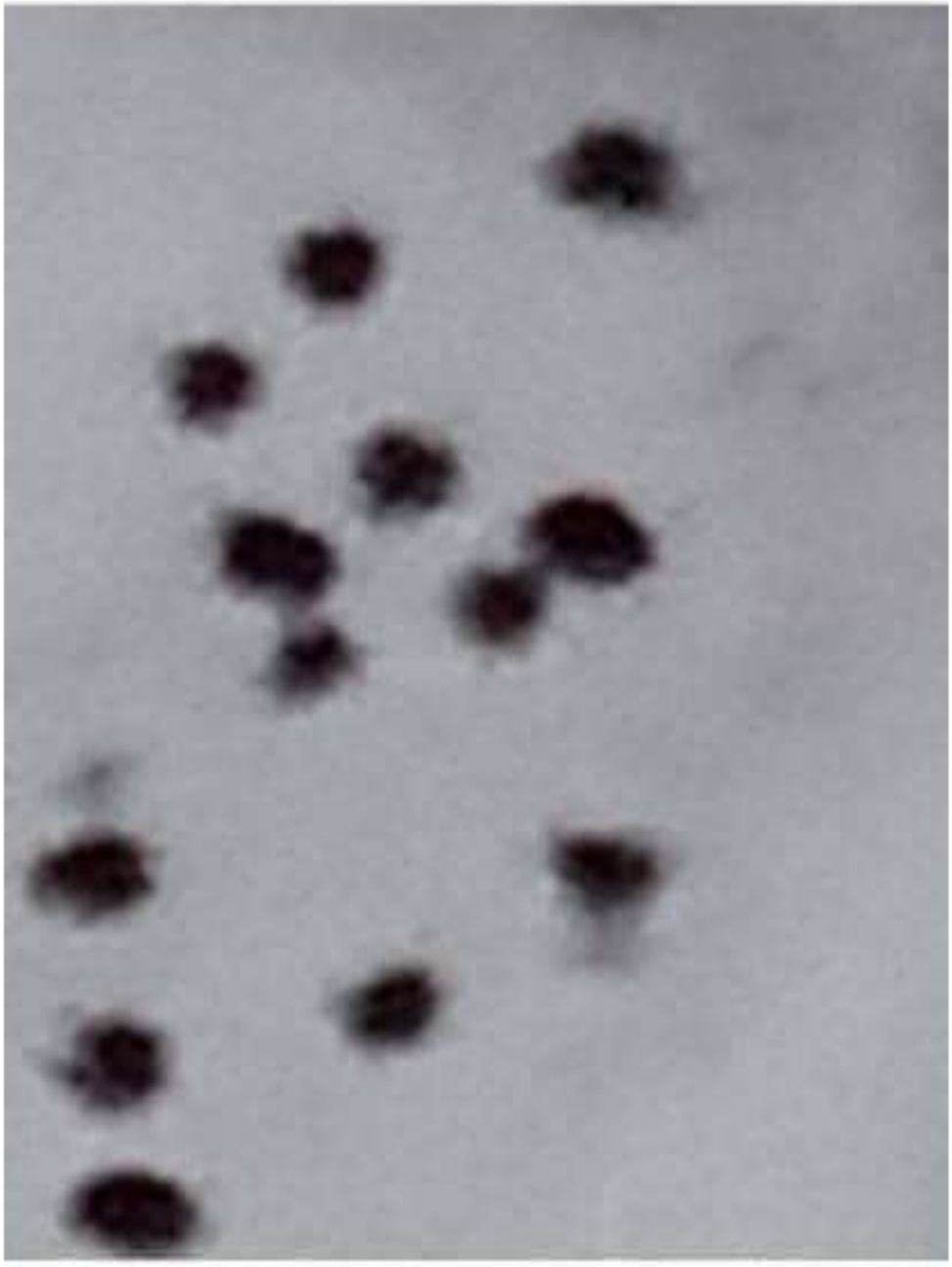

Plate 4: Mitotic Chromosomes of *P. sativum* x 1000

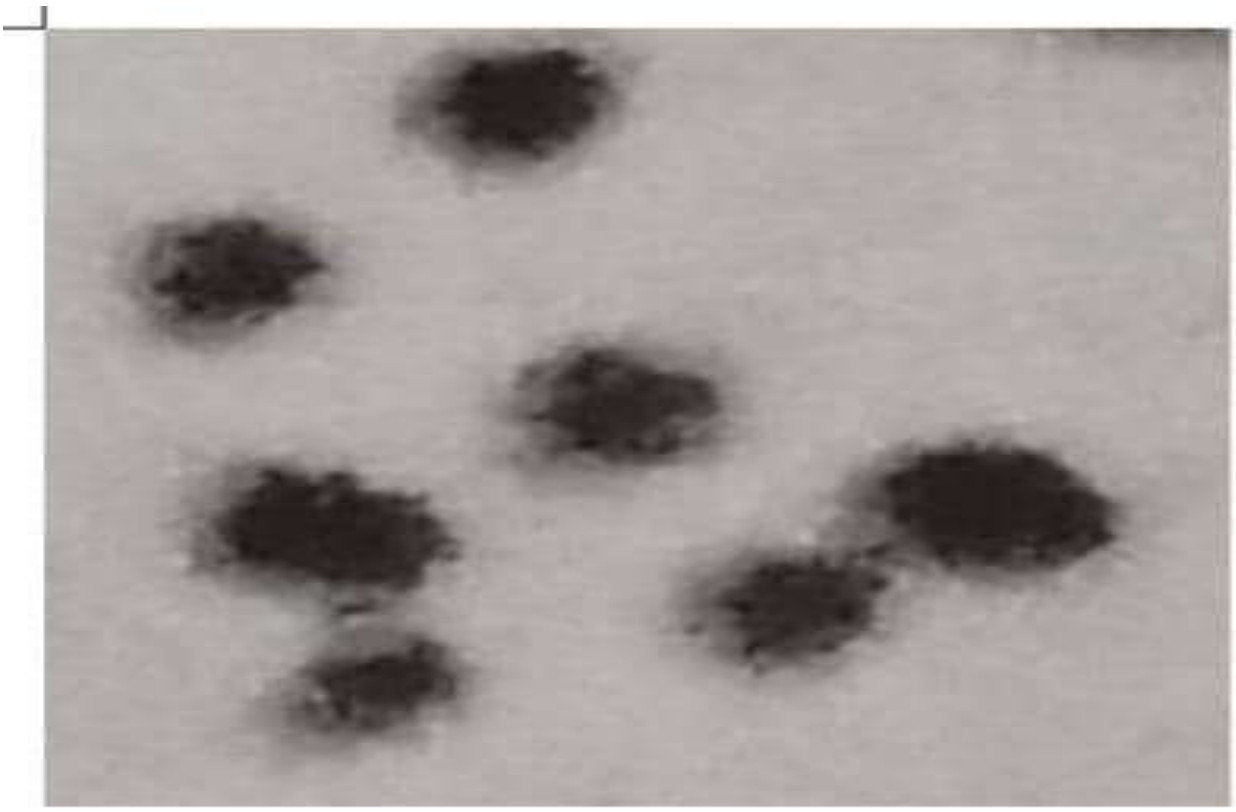

Plate 5: Meiotic Chromosomes of *P. sativum* x 1000

Pollen Fertility Study

The mean pollen fertility study of *Pisum* was found to be 90.07% ± 0.31%

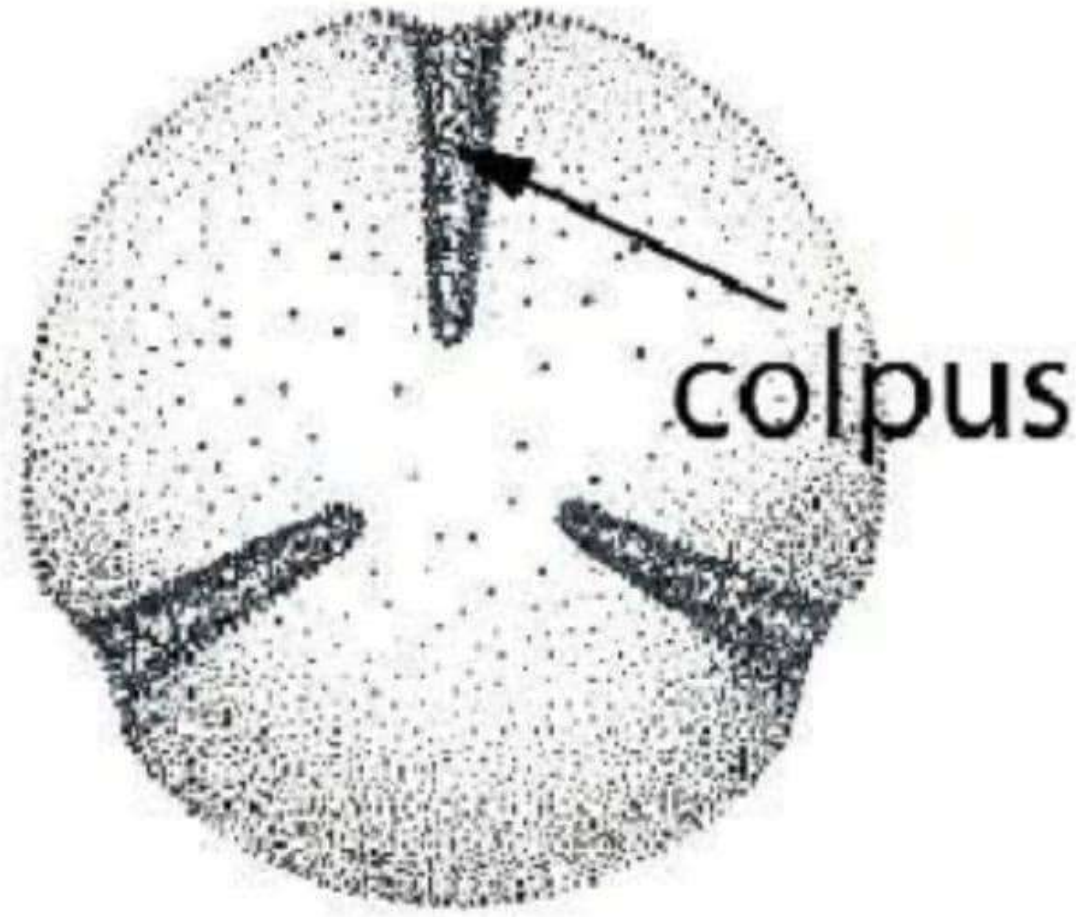

Plate 6: Tricolpate pollen of *P. sativum* x 1000

Phytochemical Analysis	
Qualitative Phytochemical Analysis of *Pisum sativum*	
Table 2: Qualitative Phytochemical Analysis of *Pisum sativum* Seed	
Phytochemicals	**Seeds**
Phenolic acids	+
Flavonoids	+
Steroids	+
Saponins	+
Tannins	+
Alkaloids	+
Keys:	
Positive (+ve) = presence;	
Negative (-ve) = absence.	
Qualitative phytochemical result showed that, the phytochemicals were present in all the part examined (Table 2).	

Quantitative Phytochemical Analysis of *Pisum sativum* Seed

Table 3: Quantitative Phytochemical Composition of *Pisum sativum* Seed

Phytochemical groups	Mean conc (ppm) ± SD	P- value
Flavonoid	12.12 ± 29.33	*
Tannin	6.60 ±12.43	*
Phenolic acids	4.26 ±10.81	*
Saponins	3.18 ±3.21	*
Steroid	12.6 ±19.53	*
alkaloids	106.18±0.28	*

Results are in mean ±SD

Quantitative phytochemical result showed varying compositions of the six phytochemicals in the seeds of *Pisum sativum*. Among the examined phytochemicals, the composition of the alkaloid was highest with the value 106.18±0.28 while saponin was lowest with the value 3.18 ±3.21 in the seed extract of *P. sativum* (Table 3).

DISCUSSION

The research carried out on the morphological characteristics of *Pisum sativum* showed that it is an herbaceous, annual, soft, non-woody climbing plant. It has a fibrous root system that originates from the radicle. The roots grow to anchor the plant and absorb water and nutrients. The leaf morphology revealed phyllotaxy -alternate, margin- entire, venation-pinnate, apex- acute, base-cuneate, colour-green, lamina- pinnate with 3 pairs, form-compound, shape- ovate to lanceolate, surface-glabrous, texture-smooth, stipules-large, semi sagittate, tendrils-spirally coiled. The weak, hollow, and cylindrical stems necessitate external support, which is facilitated by terminal tendrils. The leaf sizes were 2.0-6.0 cm in length and 1.0-3.0 cm in width. The internode length was 5.5 cm. The variability in internode length suggests environmental influence on stem elongation, aligning with previous findings on plant adaptability [19]. The pinnately compound leaves, with bright green ovate leaflets and alternate phyllotaxy, enhance photosynthetic efficiency. Cytological evaluation revealed a diploid chromosome number of 2n = 14 and a haploid number of n = 7, in agreement with earlier cytogenetic reports [19]. Observed chromosomal associations included both univalents and bivalents. Although specific cytological investigations on Pisum sativum remain limited, the observed chromosome counts from both mitotic and meiotic divisions conform to values documented in existing literature and align with the basic chromosome number (x = 11) characteristic of the Fabaceae family. Pollen viability analysis, assessed through staining techniques, demonstrated approximately 90% fertility, indicating a high level of reproductive competence. This high fertility suggests efficient gamete formation and minimal chromosomal anomalies during meiosis, which are critical for successful fertilization and seed development. These outcomes are consistent with earlier findings that support the genetic stability of P. sativum, underscoring its suitability for effective reproduction and its utility in breeding programs [20]. Phytochemical analysis revealed the presence of tannins, flavonoids, steroids, phenolic acids, alkaloids, and saponins in *Pisum sativum* seeds, albeit in varying concentrations. It is well established that the chemical composition of plant extracts varies with plant variety [14]. These bioactive compounds exhibit diverse medicinal and health-promoting properties. Flavonoids, known for their strong free radical scavenging abilities, suggest potential antioxidant properties, playing a crucial role in plant defense and disease prevention, including cardiovascular diseases and cancer [14], [21]. Phenolic acids, another major secondary metabolite detected, contribute to plant defense against pathogens and environmental stress [22] and exhibit antimicrobial, anti-inflammatory, and anticancer properties [23]. Tannins, found in the highest concentration, are polyphenolic compounds with antimicrobial, anti-inflammatory, and anticarcinogenic properties [24]. Their presence aligns with their known role in plant defense against herbivores and pathogens [25]. Saponins, naturally occurring glycosides, have antimicrobial, antifungal, and immune-boosting properties [26], [14]. Commonly found in legumes, they are associated with cholesterol-lowering effects, making them valuable for cardiovascular health [27]. Steroids, detected in the lowest concentration, play vital physiological roles as hormone precursors and exhibit anti-inflammatory and therapeutic properties [28]. They are widely used in medicine for treating skin diseases, asthma, and arthritis [14].

CONCLUSION

In general, the data obtained from chromosomal and pollen studies indicated that the plant has high fertility and viability level thus could easily perpetuate, and open to genetic manipulation and variation. Result also revealed that the seed of *P. sativum* is rich in valuable secondary metabolites and could be the potential sources of these

phytochemicals, thus justified its use medicinally and nutritionally. Again, the data can be used to enhance proper taxonomic characterization and identification of the plant species.

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