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PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHYSICOCHEMICAL STUDIES OF *ALLIUM SATIVUM* LINN. BULB (LILIACEAE)

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ABSTRACT: *Allium sativum linn* (Garlic) belong to family Liliaceae has been widely known since ancient times for medicinal use and for flavouring food. It contains large amount of sulphur containing compounds like Alliin, Allicin, Ajoene, Diallyl sulphide, Diallyl disulphide Diallyl trisulfide, Vinyldithiines which has beneficial activity including Antithrombotic, Anticancer, Antiviral, Antifungal and Antimicrobial, Anti-inflammatory etc. Ajoene is known as an inhibitor of platelet aggregation induced by all known agonists which also a good inhibitor of tumour and antifungal property. The present study deals with pharmacognostical parameters for bulb of *Allium sativum* which mainly consist of the detailed macromorphology of cloves and bulb, microscopy and micrometry of transverse section of cloves of *Allium sativum linn* which has been carried out by using MOTIC Digital Microscope. The other parameters like physicochemical constant and phytochemical screening were studied. The study will provide referential information for the correct identification and standardization of crude drug.

INTRODUCTION: Medicinal plants from ancient time were considered as the God's gift to human beings as they are having profound use in the treatment of various dreadful diseases and disorders. Genus *Allium* is formally classified in the family *Liliaceae*, represented by 280 separate genera and 4000 species. Approximately 700 species of *Allium*, the edible members, including Onion (*Allium cepa L.)*, Garlic (*Allium sativum L*.), Chives (*Allium schoenoprasum L.)*, Leek (*Allium porrum L*.) and Welsh onion (*Allium fistulosum L*.) are highly prized.[1]

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Garlic bulb contains more than 200 chemical compounds. Once it is crushed or chopped create dozens of new compound through many chemical reactions in which most important are sulphur-containing compounds: Allicin, Ajoene, Vinyldithiines and Enzymes; allinase, peroxidase and myrosinase, also contains citral, a-phellandrene, geraniol, β-phellandrene and linalool [2].

The main pharmacological activity of *Allium sativum* are fibrinolytic, Antimicrobial, Anticancer, Antioxidant, Antiadrogenic, Hepatoprotective, Anticoagulant, Hypocholestermic, Antiarthritic, Antihypertensive, Hypoglycaemic, Larvicidal [1, 3, 4, 5, 6, 8].

The process of standardization can be achieved by stepwise pharmacognostical and phytochemical studies.

These studies help in identification and standardization of the plant material.[7] Correct identification and quality assurance is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.[3] The current article describes pharmacognostical, physicochemical and phytochemical characteristics. The Main objective of this study is to supplement constructive information with regards to its identification, characterization and standardization of bulb of *Allium sativum Linn*.

MATERIAL AND METHOD:

Collection of sample: The bulb of *Allium sativum* was collected from the local market of Pune. Their identity and authentication was done by Department of Pharmacognosy, Marathwada Mitra Mandal's College of Pharmacy, Pune, by correlating their macromorphological characters with those given in literatures. The fresh bulbs were used for the study of macromorphology and microscopy, where the dried bulb powder was used for determination of powder microscopy, physicochemical characterization and phytochemical analysis.

Macromorphological Description [9, 10, 11, 12]**:** The bulb of *Allium sativum* was subjected to macroscopic studies which comprised of organoleptic characteristics viz colour, odour, appearance, taste, shape, texture of the drug. These parameters are considered as quit useful in quality control of the drug and were evaluated as per standard WHO guidelines

Microscopical characteristics [9, 10, 12]**:** Fresh bulb of *Allium sativum* were selected for microscopical studies and Free hand transverse sections (T.S.) and dry bulb powder were taken and stained with different but specific staining reagents. Microphotographs of the sections were taken with the help of MOTIC Digital Microscope, provided with MOTIC IMAGE PLUSE 2.0 software.

Micrometry [12]**:** The study of quantitative microscopy of transverse section and dry bulb powder of *Allium sativum* were carried out to determine the size and dimensions of tissues, cells, and cell content.

Physicochemical Evaluation [7, 13]**:** Physico-chemical parameters such as foreign organic matter, moisture content, ash value, extractive values and pH were determined according to WHO guidelines on quality control methods for medicinal plant materials.

Florescence Analysis [7, 12, 14]**:** Many herbs show fluorescence when the cut surface is exposed to UV light and this can be useful in their identification. The fluorescence character of plant powder was studied both in daylight and UV light (254 nm and 366 nm) by treating with different chemical reagents like Chloroform, hydrochloric acid, nitric acid, sodium hydroxide, etc.

Phytochemical Investigations [11, 12]**:** The qualitative chemical tests carried out for the identification of the natural phytoconstituents present in the powdered crude drug. The tests were carried out using conventional protocols.

RESULT AND DISSCUSSION:

Macromorphological Description [5, 16]**:** Garlic is bulbous perennial herb. It has tall, erect flowering stem that reaches 2-3 feet in height. The plant has pink or purple flowers that bloom in mid to late summer. The part used medicinally is the bulb. Bulb 4-6 cm in diameter, consisting of 8-20 cloves (bulblets), surrounded by 3-5 whitish papery membranous scales attached to a short, disc-like woody stem having numerous, wiry rootlets on the underside; each clove is irregularly ovoid, tapering at upper end with dorsal convex surface, 2-3 cm long, 0.5 - 0.8 cm wide, each surrounded by two thin papery whitish and brittle scales having 2-3 (**figure 1**) yellowish green folded leaves contained within two white fleshy, modified leaf bases or scales; odour is Characteristic, taste is pungent gives warmth to the tongue . The results of macromorphology were depicted in **Table 1**.

Microscopical characteristics: The transverse section of bulb of *Allium sativum* has cuticle, epidermis, cortex, endodermis and scattered vascular bundles. Epidermis consists of narrow thin walled continuous single layered with rectangular cells, surrounded by cuticle (**Fig. 3c**). The cortex region have parenchymatous and homogenous cell having large prismatic crystals of calcium oxalate and vascular bundles (**Figure 2a**).

The vascular bundles are bicollateral (**Figure 3d**) small, occur as a ring and scattered in the median part of cortex and circularly arranged below endodermis (**Figure 2b**).

The powder of *Allium sativum* bulb is pale buff to white, with characteristic aromatic Characteristic odour and taste consist of vessels, fibers, fragments of Vascular bundle cells and cortex cells. (**Figure 4**)

Micrometry: The result of micrometric characters of tissues, cells and cell content were depicted in **Table 2**. Measurements of different cells are frequently necessary for the quantitative identification of closely allied substances. In most cases, these allied substances are mixed with the original drugs as adulterants and substituent. Thus, the adulterants and /or substituent present in crude drug can be distinguished by this way with the aid of optical microscopy (**Figure 5, 6, 7**).

FIGURE 1: MORPHOLOGY OF *ALLIUM SATIVUM* LINN.

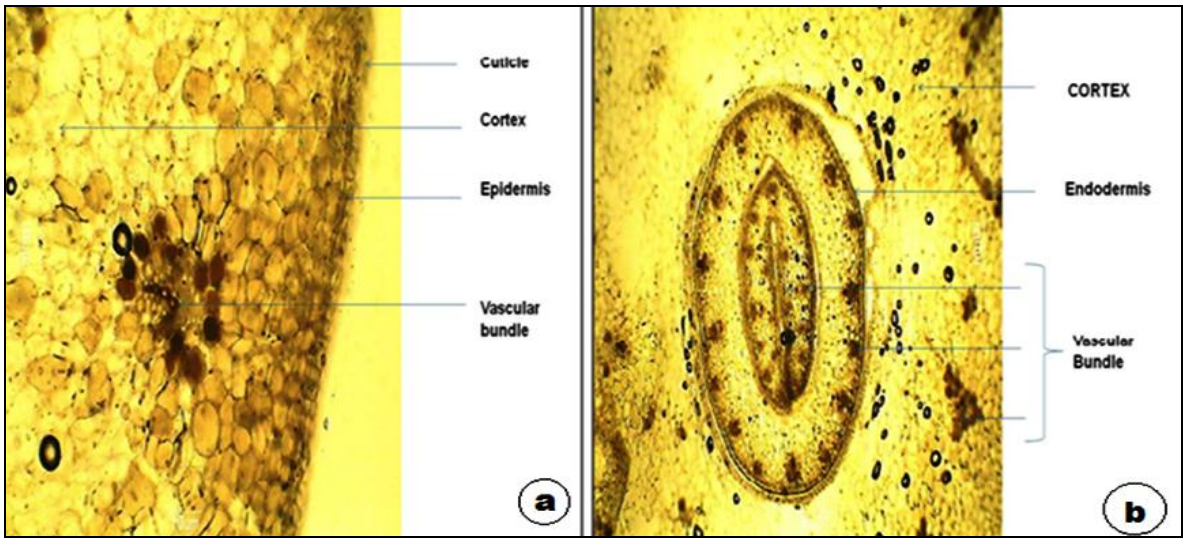

FIGURE 2 [A, B]: T.S. OF *ALLIUM SATIVUM* LINN.

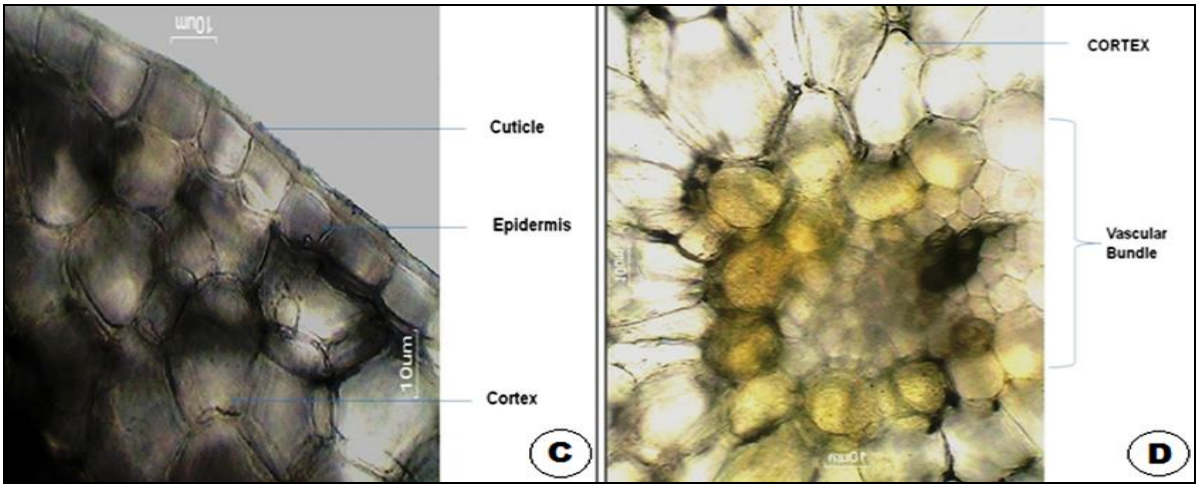

FIGURE 3 [C, D]: T.S. OF *ALLIUM SATIVUM* LINN. (C – Cuticle & Epidermis; D – Vascular bundle)

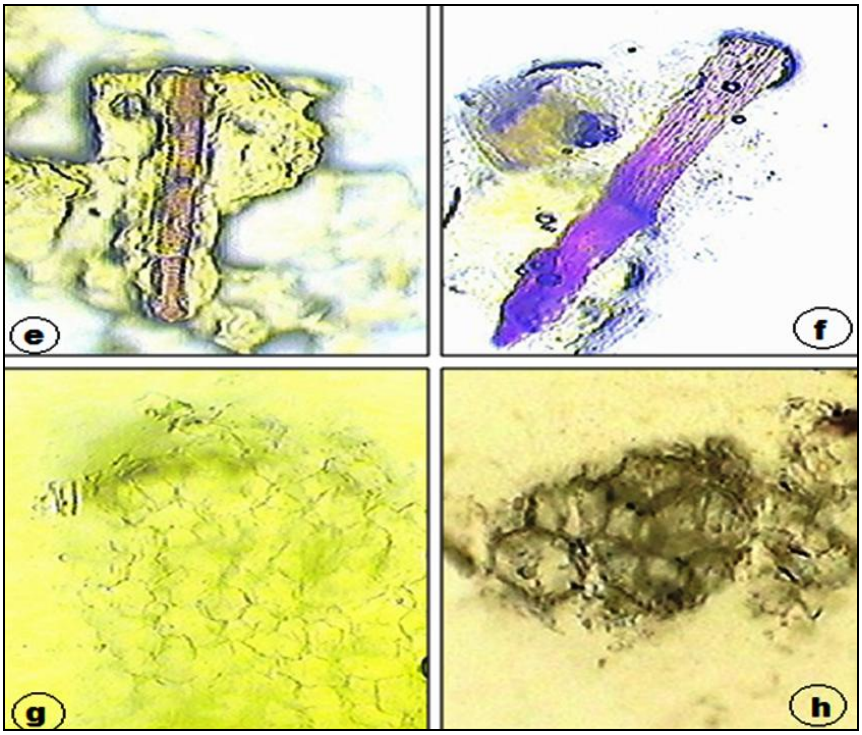

FIGURE 4 [E, F, G, H]: POWDER MICROSCOPY OF *ALLIUM SATIVUM* LINN. (e – Xylem Vessel, f – Fragment of fibres, g – Fragments of Cortex cells, h – Fragments of Vascular Bundle)

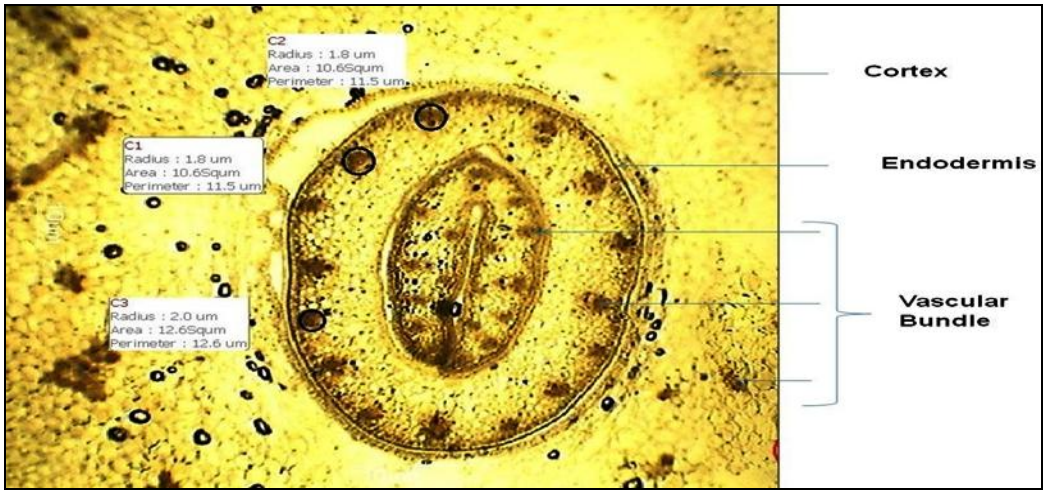

FIGURE 5: MICROMETRY OF T.S OF *ALLIUM SATIVUM* LINN.

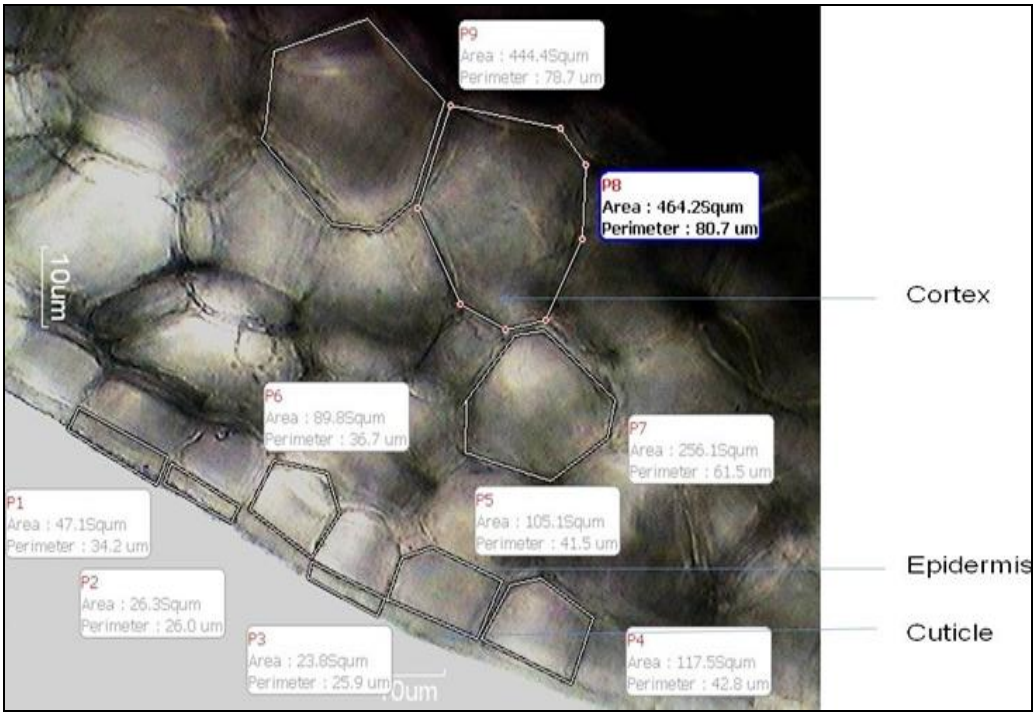

FIGURE 6: MICROMETRY OF T.S OF *ALLIUM SATIVUM LINN* (CUTICLE, EPIDERMIS, AND CORTEX)

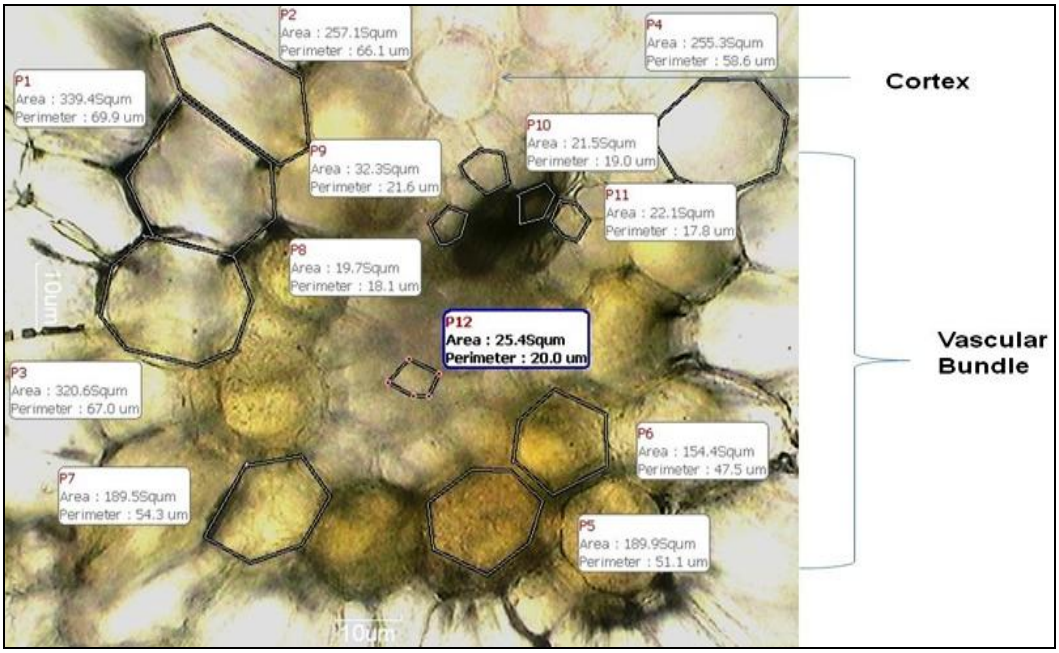

TABLE 1: MACROMORPHOLOGICAL DESCRIPTION OF BULBLETS (CLOVES) OF *ALLIUM SATIVUM L.*

Sr. No.	Characters	Observation
	Organoleptic Characters	
1.	Colour	Off White with pink shade
2.	Odour	Characteristics
3.	Taste	Pungent
	Quantitative Macromorphology	
4.	Length	2.0 – 3.0 cm
5.	Width	0.6 - 0.9 mm
6.	Thickness	0.5 – 0.8 mm
	Extra Features	
7.	Shape	Ovoid
8.	Texture	Smooth
9.	Fracture	Spongy

TABLE 2: MICROMETRY OF SOME CELLS

Sr. No.	Type of Cells	Dimension Area in Square Micrometre (Squm)
1	Cuticle	032.40 ± 12.79
2	Epidermis	104.13 ± 13.87
3	Cortex cells	415.93 ± 67.19
4	Vascular Bundle	11.26 ± 1.16
5	Phloem	21.10 ± 1.25
6	Xylem	29.40 ± 3.58

*Values are expressed as mean ± standard deviation

Physicochemical evaluation: Evaluation of crude drug ensures the identity of drug and determines the quality and purity of drugs. The main reason behind the need for the evaluation of crude drug is biochemical variation in the drug, effect of treatment, storage of drug and adulteration and substitutions [10, 17]. The results of the physicochemical parameters of bulb of powder lie within the limit which is depicted in **Table 3**. The results of foreign organic matter denote presence of foreign particulate, part or product other than that named in the specification and description of the herbal material concerned [9, 13].

TABLE 3: PHYSICOCHEMICAL EVALUATIONS

Sr. No.	Parameters (%w/w)	Observation (%w/w)
1	Foreign organic matter	00.30 ± 0.03
2	Moisture content (LOD)	05.08 ± 0.07
	Ash Value	
3	Total ash	06.20 ± 0.34
4	Acid insoluble ash	00.35 ± 0.03
5	Water soluble ash	00.50 ± 0.07
6	Sulphated ash	00.90 ± 0.09
	Extractive values	
7	Water soluble extractive value	20.3 ± 0.12
8	Alcohol soluble extractive value	06.33± 0.23
9	pH	03.00 ± 0.00

*Values are expressed as mean ± standard deviation

It was found to be 0.30±1.13; it indicates that very less amount of foreign matter may be present in crude drug. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles [12]. Not only the ultimate dryness of the drug is important, equally important is the rate at which the moisture is removed thus the determination of moisture content also provide the method of preparation of drug [18, 19] and it is observed that the moisture content of the drug was found to be 05.08±0.07%w/w which signify that drug is properly dried; which lies within the limit.

The results of Ash values signify the purity of drug that is the presence or absence of foreign matter such as metallic salt or silica present in the raw material. The total ash usually consists of carbonates, phosphates; silicates and silica which include both physiological ash and non-physiological ash [9]. The value of total ash was found to be 06.20 ± 0.34% w/w. Acid insoluble ash indicates contamination with silicious materials e.g., earth and sand, comparisons of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug [9] which was found to be 0.35± 0.03 %.w/w. The water soluble ash was found to be 0.50±0.07 % w/w, this parameter is used to detect the presence of material exhausted by water whereas the value for Sulphated ash was found to be 0.90±0.09 % w/w. As the ash values of the crude drugs lies within the fair limit which signify its quality and purity and gives idea about the total inorganic content [15, 11, 12].

The water soluble extractive value found to be 20.30±0.12 %w/w while the alcohol soluble extractive value was found to be 06.33±0.23 %w/w which signifies the nature of the phytoconstituents present in plant. The pH conventionally represents the acidity and alkalinity. [9] The pH was determined which was near to 3, indicates acidic compounds may be present.

Fluorescence Analysis: The results of fluorescence analysis were shown in **Table 4**. Fluorescence is the phenomenon exhibited by numerous phytoconstituents present in the plant material. In fluorescence the fluorescence light is always of greater wavelength than the exciting light.

Light rich in short wavelength is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substance which do not visible fluoresce in day light [14].

TABLE 4: FLUORESCENCE ANALYSIS OF POWDER OF ALLIUM SATIVUM L.

Sr. No.	Reagents	Visible light	Short UV (254 nm)	Long UV (366 nm)
1.	Dist. Water	Pale yellow	Pale green	Greenish yellow
2.	Pet Ether (60-80)	Colourless	Yellow	Greenish yellow
3.	Chloroform	Pale yellow	Light green	Dark green
4.	Methanol	Cream	Grey	Pale green
5.	Conc. HCl	Cream	Light yellow	Dark green
6.	Conc.HNO3	Yellow	Light green	Dark green
7.	Conc.H2SO4	Dark red	Dark red	Black
8.	Picric acid	Yellow	Greenish yellow	Dark green
9	Dil. Ammonia solution	Cream	Light green	Whitish green
10.	10% NaOH	Pale brown	Greenish brown	Green
11.	5% Ferric chloride	Brownish yellow	Dark green	Dark brown
12.	Ethyl acetate	Colourless	Colourless	White

Dist. – Distilled , Conc. – Concentrated, HCl- Hydrochloric acid, H2SO4 – Sulphuric acid, NaOH- Sodium hydroxide

Phytochemical Investigations: The preliminary Phytochemical Investigations of powder drug of bulb of *Allium sativum L.* were preformed which shows the presence of Carbohydrates, Proteins, Amino acids, Enzymes as primary metabolites and Terpenoids, Saponins, Steroids as major secondary metabolites which revealed their potent therapeutic activity. The results of the screening were expressed in **Table 5**.

TABLE 5: PRELIMINARY PHYTOCHEMICAL SCREENING

Sr. No.	Parameters	Observations
1	Carbohydrates	+
2	Proteins	+
3	Amino acids	+
4	Volatile oil	+
5	Saponins	+
6	Terpenoids	+
7	Steroids	+
8	Enzymes	+

***** + indicates presence

CONCLUSION: Macromorphology and microscopy along with the micrometry are one of the simplest and cheapest methods to start with establishing the correct identity of the source material.

The present study was carried out with a vision to setup standards that could be beneficial for detecting the authenticity of this vital medicinal plant. Numerical standards reported in this work could be useful for the compilation of a suitable monograph of *Allium sativum* Linn.

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