

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF *ALLIUM SATIVUM*

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ABSTRACT

Natural products have been an integral part of ancient traditional medicine system. the objective of the study was investigate the phytochemical constituent and proximate composition of garlic (*A.sativum*) extracts. The phytochemical screening of the garlic for various phytochemical constituents was conducted using laboratory method. The proximate and mineral composition was determined by using standard method. the qualitative phytochemical screening of the *Allium sativum* aqueous and ethanol extract indicate the presence of the alkaloids, terpenoids, flavonoids, steroids, saponin, tannins and phenol. The qualitative proximate composition of the bulb in g/100g showed the extract contain carbohydrate, protein, fats. The chemical analysis revealed that the sulphated ash value of the compound was found to be 0.20%, the total ash value of the compound was found to be 3.20% and the loss on drying showing the 6% which resemblance of the standard value of that compound itself. The alcoholic extract of the compound shows the zone of inhibition while comparing with the streptomycin as the standard it showing 10mg/ml, it was shown approximately 13mm it shows *Allium sativum* having antimicrobial activity.

KEYWORDS: *Allium sativum*, alkaloids, terpenoids, flavonoids, steroids, saponin, tannins and phenol.

1. INTRODUCTION

Garlic (*Allium sativum* L.) and its derived products have been widely used for medicinal purposes by many culture. Research has demonstrated that garlic has a wide range of biological activities, including antihypertensive, lipid-lowering, antibacterial, antifungal, and antiviral activities, among others.^[1]

Garlic has a long tradition as a food and as a medicinal plant. Therapeutic effects, routes of application and modes of preparation of garlic are very varied. In order to be able to assess which mode of preparation is suitable for which application, it is necessary to explain certain substance characteristics of garlic.

The garlic bulb contains cytokine sulphoxides belonging to the secondary plant components. During the processing these compounds undergo a rapid enzymatic respectively non-enzymatic conversion. Depending on the mode of preparation, different substances with different effects can be found in the resulting products. Most of the data available on the chemo preventive and curative effects of garlic is based on the use of the freshly prepared garlic.^[1,3]

Garlic is a widely distributed plant. Nowadays, it is cultivated all over the world. In our region, it is the most

important preventive remedy, a universal folk spice and food, a well-trusted remedy. In the past, garlic has been utilized as a remedy during the various epidemics such as typhus, dysentery, cholera, influenza, and whenever an epidemic has emerged, garlic has been the first preventive and curative remedy. In the ancient and middle centuries and a long time during the modern period, garlic has been appreciated as a remedy by physicians from different nations. Recently there has been scientific research into garlic, and good results have been obtained in healing many diseases, from which for thousands years nations from various continents had been protecting themselves and healing by using garlic. Therefore, there is an increased necessity of research on the history of garlic for the sake of reinforcing the ability of pharmacists and physicians to respond to the challenges arising in the provision of professional services in order to facilitate human life.^[2,3]

Conventional extraction techniques have several drawbacks including the degradation of sensitive compounds due to the use of high temperatures, the consumption of large amounts of solvent, the toxicity of some solvents, the long processing times and the low selectivity, among others. Thus, more environmentally friendly techniques that do not present health risks and provide high-quality extracts are being utilized.

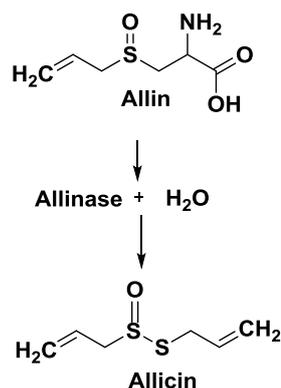


Fig. 1: Formation of *allicin* in fresh garlic.^[3]

1.1 Properties of garlic

1. Lower the cholesterol and lower the blood pressure.
2. Inhibit tumour growth and also effective in gastric cancer.
3. Antioxidant property is useful in cardiovascular disease and antibiotic property use to treat wounds.
4. Medicinal intake, specially those used to control sugar contents in blood and some anti-inflammatory.
5. Garlic in external use can produce dermatitis. It must be use cautiously. It is never recommended for the skin of young children.
6. Diuretic remedy for rheumatic arthritis, fluid retention and obesity.

1.2 Drug profile



Figure 2: *Allium sativum*.

1.2.1 Synonyms: Lushun, Lasun, Belluli, Garlic

1.2.2 Biological source: Garlic consists of the fresh compound bulb of *Allium sativum linn*

1.2.3 Family: Amaryllidaceae

1.2.4 History: *Allium sativum* is supposed to originate from Central Asia, from where its cultivation has spread to Southwest Asia and the Mediterranean region. The use of the garlic bulb as a food as well as a medicinal plant has a very long tradition. In all ancient civilization of the Eastern Mediterranean garlic was known and highly respected, as with the Egyptians where the plant was attributed to certain deities. Today garlic is cultivated in regions with moderate or subtropical climate all over the world mainly for the use of its bulb as a spice or as a vegetable *Allium sativum*.

Prefers a well-fertilized open ground with sandy or loamy soil. As the plant is very sensitive to excessive moisture, the location should be warm and sunny.

1.2.5 Geographical source: Garlic is cultivated in Indian, Russia, USA, Italy and Southern Europe.

1.2.6 Chemical constituents: Fresh or crushed garlic yields the sulfur-containing compounds allicin, ajoene, diallyl polysulfides, vinylthiins, S-allylcysteine, and enzymes, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds.

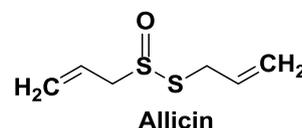


Figure 3: Structure of Allicin.

1.2.7 Uses

- a) Hardening of the arteries (atherosclerosis)
- b) **Colon cancer, rectal cancer.** Research suggests that eating garlic can reduce the risk of developing colon or rectal cancer. Also, in people diagnosed with a certain type of colon and rectal tumors, taking high doses of aged garlic extract daily for 12 months seems to reduce the risk of developing new tumors.
- c) **High blood pressure.** Some research shows that garlic by mouth can reduce blood pressure by as much as 7% or 8% in people with high blood pressure. Most studies have used a specific garlic powder product (Kwai, from Lichtwer Pharma).
- d) Garlic is very best treatment of flu cough and cold.
- e) Garlic mostly used in cancer, heart disease, liver function and stress.
- f) It is as an antibiotic, antifungal, anti-inflammatory, antioxidant, immune system bug.

1.2.8 Side effect

- a) **Pregnancy and breast-feeding:** when used in medicinal amounts during pregnancy and when breast-feeding. There is not enough reliable information about the safety of applying garlic to the skin if you are pregnant or breast feeding. Stay on the safe side and avoid use.
- b) **Bleeding disorder:** Garlic, especially fresh garlic, might increase the risk of bleeding.
- c) **Stomach or digestion problems:** Garlic can irritate the gastrointestinal (GI) tract. Use with caution if you have stomach or digestion problems.
- d) **Low blood pressure:** Garlic can lower blood pressure. In theory, taking garlic might make blood pressure become too low in people with low blood pressure.
- e) **Surgery:** Garlic might prolong bleeding and interfere with blood pressure. Stop taking garlic at least two weeks before a scheduled surgery.

2. MATERIALS AND METHODS

2.1 Collection of material

Garlic bulb was collected obtained from market of Nagpur and the collected bulb was taxonomically and authenticated by Department Of Biology And Biotechnology, RTMNU Nagpur by Mr. Dorle, professor of Department Of Biology And Biotechnology RTMNU,

Nagpur Authentication number 10139 was deposited at Herbarium, Department of Plant Biology in pharmaceutical department. Mainly use of ethanol for extraction while all other chemical used are of A grade quality.

2.2 Extraction Method Of *Alivum Sativum*: (Alcoholic extract of garlic)^[2]

The epicarps of the bulbs were peeled off and separated from the stalks for easy assessment. The peeled bulbs were thoroughly washed and dried under the sun for 3 days after which the bulbs were grinded into a fine powder.

95% ethanol was used for the extraction of bioactive from the garlic clove according to the method. 150g of grinded garlic powder was dissolved in 300ml of ethanol. The mixture was kept at room temperature for 24 hours in a sterile flask covered with aluminium foil to avoid evaporation, it was then filtered through a glass wool filter, and the extract evaporated in a rotary evaporator until about 25ml of extract was left in the container. This was further allowed to air dry in a vacuum desiccator until all the ethanol had evaporated. The solidified extracts were diluted by ethanol to make different concentrations and stored (at 4°C). Three different concentration of the extract; 50mg/ml, 100mg/ml and 200mg/ml, were used in this research.

2.3 Selection of bacterial strain

Medically important bacterial strain used in this study were staphylococcus *Aureus lacto bacilli*, and enterobacterarogenes.

2.4 Standard of refererance antibiotic

Streptomycine.

2.5 Chemicals

Ethanol, Sodium Hydroxide, Hydrochloric Acid, Sulphuric Acid, Phloroglucinol, Mayers Reagent, Benedict Reagent, Hagers, Reagent, Wagner Reagent, Dragendorff Reagent. Biuret Reagent, Ninhydrin Reagent, Acetic Acid, 5% Ferric Chloride Reagent, Potassium Dichromate, Fehling Solution A And Fehling Solution B, Streptomycin.

3. Pharmacognostic Evaluation

3.1 Macromorphological Description

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 quite useful in quality control of the drug and were evaluated as per standard WHO guidelines.^[5]

3.2 Microscopical characteristics

Fresh bulb of *Allium stadium* 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.

3.3 Physicochemical Evaluation

Physico-chemical parameters such as foreign organic matter, moisture content, ash value and pH were determined according to WHO guidelines on quality control methods for medicinal plant materials.

3.4 Loss of drying (moisture contain)

About 1 g of garlic powder was heated at 100–105 C in a hot air oven. After getting a constant weight, percentage loss on drying was calculated with reference to the initial weight of powder.

Moisture contain = $\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}$

3.5 Total ash

Powdered drug (2–5 g) was taken in a silica dish and incinerated at temperature not exceeding 450°C until free from carbon, cooled and weighed. Total percentage of ash with reference to the air dried drug was then calculated.

Total ash value = $\frac{100(Z-X)}{Y}$ %

Where, weight of empty crucible = X

weight of empty crucible + ash = Z

weight of ash = (Z – X)

weight of drug taken = Y

3.6 Sulphated ash

Powder drug (1-2g) accurately weight was ignite gently at first time. cool moisture the residue 1ml of sulphuric acid heat gently until white fumes are no longer evolved and ignite at 800 C +25 C until black partical and allow to cool and weight. Repeat the operation until two successive weighing do not differ by more than 0.5.

4. Phytochemical Test Of Garlic Extract^[5,6,8]

4.1 Test For Carbohydrate

- **Fehling's test:** Take 2ml sample was add fehling's solution A & B boil for min then observe brick red precipitate.
- **Benedict test;** Take volume of test with reagent and test solution in test tube heat in boiling water.

4.2 Test For Amino Acid

- **Ninhydrin test:** Take 3ml of test sample was heatadd 3drops of 5% ninhydrin solution in boiling water bath for 10 min purpule bluish color appear.

4.3 Test For Alkaloids

- **Dragendorffs test-** Take 2-3 ml filtrate was add few drop Dragendorffs reagent. Orange brown precipitate is formed
- **Mayer's test-** Take 2-3 ml filtrate was added few drops mayer's reagent gives precipitate
- **Hager's test** – Take 2-3 ml filtrate was add in few drops Hager's reagent gives yellow precipitate
- **Wagner's test** – Take 2-3 ml filtrate was add few drops Wagner's reagent gives reddish brown precipitate

4.4. Test For Terpenoids

About 0.5g of extract in separate in test tube was take with 2ml of chloroform; 5ml of concentrated sulphuric acid was carefully added to form layer and observed to form reddish brown color.

4.5. Test For Saponins

- Foam test –shake the drug extract in 2ml of water. Persistent foam observed

4.6. Test For Tannin & Phenolic

- Potassium dichromate- Drug extract was add few drops of potassium dichromate. red precipitate was observed.
- Lead acetate test- Drug extract was add few drops Lead acetate solution. White precipitate observed.
- Acetic acid solution: - Drug extract was add few drops Acetic acid solution, red colour solution.

4.7. Test For Flavonoids

- To small quantity of extract was add 1 ml of 10% sodium hydroxide was added. a yellow colour indicated the presence of flavonoids
- Shinoda test: To dry powder or extracts add 5ml of 99% ethanol few drops of HCl and 0.5 magnesium turning orange red pink color appear.

4.8 Test For Steroids

- Libermann's reaction: mix 3 ml of extract was add 3ml acetic anhydride. heat and cool few drops of concentrated H₂SO₄. Blue colour appears.

- Salkowski reaction: To 2ml of extract was add 2ml of chloroform and 2ml concentrated H₂SO₄. shake well. chloroform layer appears red add acid layer show greenish yellow fluorescence.

4.9 Test For Glycosides

- Keller-killianis test: TO 2ml extract was add glacial acetic acid, one drop FeCl₃ and concentrated H₂SO₄. Reddish brown colour appears at junction of the two liquid and upper layer appear bluish green.
- Legal's test: Drug extract was add 1ml pyridine and 1ml sodium nitroprusside. pink to red colour appears.

5. Antimicrobial Assay

Garlic extracts activity test well diffusion method screening

Screening of the anti-microbial activity was performed by well diffusion technique. agar plates were seeded with 0.1 ml of the standardized inoculums of bacteria. The inoculums were spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard crack border of 9 mm diameter was used to cut uniform wells on the surface of the plates, and 0.1 ml of each concentration was introduced in the well with streptomycin a control. The inoculated plates were incubated at 37°C for 24 hrs and zone of inhibition diameter was measured to using nearest millimeter (mm).

6. RESULTS AND DISCUSSION

6.1 PHARMACOGNOSTICS EVALUATION

6.1.1 Morphological characters

Table 1: Macromorphological Description of Bulblets (Cloves of *Allium Sativum L.*)

Characters	Observation
Colour	Whitish and faint yellow
Odour	Characteristics
Taste	Pungent
Shape & Texture	Ovoid, Smooth
Quantitative Macromorphology:	
Length	2.0-3.0 cm
Width	0.6-0.9 mm
Thickness	0.5-0.8 mm

6.1.2 Microscopical characters

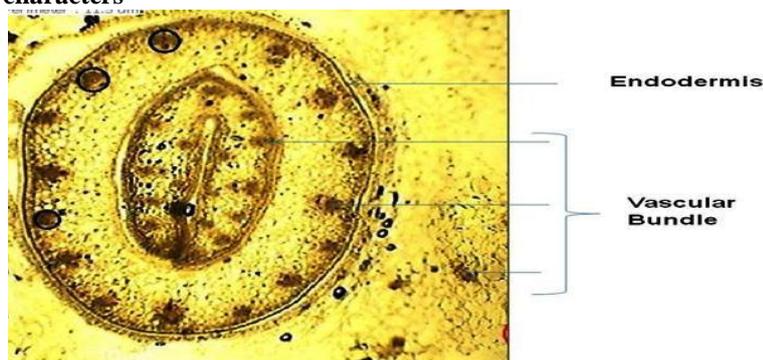


Figure 4: T.S. of *Allium Sativum*.

The transverse section of bulb of *Allium sativum* consist of cuticle, epidermis, cortex, endodermis and vascular bundles. Epidermis consists of narrow thin walled

continuous single layered with rectangular cells, surrounded by cuticle.

6.2 Physicochemical test of *Allium sativum*

Table 2: Physicochemical Test of *Allium Sativum*.

Physicochemical properties	Obtained value	Standard value As per USP	Standard value As per Ayurvedic pharmacopoeia
Loss on drying	6%	Not more than 7%	Not more than 1%
Total ash value	3.20%	Not more than 5%	Not more than 5%
Sulfated ash	0.20%	Not more than 0.90 %	

6.3: Phytochemical test of garlic

Table 3: Phytochemical Test of Garlic Extract.

Phytochemical tests	Result
Carbohydrates	+
Terpenoids	+
Saponins	+
Alkaloids	+
Tannins and Phenolics	+
Carbohydrates	+
Amino acid	+
Flavonoids	+
Steroids	+

The phytochemical evaluation of ethanolic extract shows following result in extract. The presence of alkaloid, phenoliccarbohydrate, amino acid, flavonoids and steroids with some amount of saponin, tannin compound.

that of standard was found to be 23mm. While that of streptomycin drug of reference standard forms the zone of inhibition of about 19mm in dose of 10mg/ml. While that of standard is more than 19 mm.



Figure 5: Antimicrobial assay of garlic extract and standard drug.

6.4 Antimicrobial assay

The test sample extract of *Allium sativum* form the zone of inhibition of about 13mm in the dose of 10mg/ ml but

Table 4: Antimicrobial Assay of Garlic Extract and Standard Drug (Zone of Inhibition).

Drug	Dose	Zone of inhibition (mm) <i>Staphylococcus aureus</i> .	Standard
Streptomycin	10mg/ml	19mm	More than 19mm
Garlic extract	10mg/ml	13mm	18mm

CONCLUSION

The result showed the ethanolic extract of *Allium sativum* exhibited significance antimicrobial activity on *staphylococcus aereus* and could be a good candidate for antimicrobial drugs also study revealed that the pharmacognostic, phytochemical study was comparatively same as that of satandard value so being non toxic economical and easily available and due to herbal mature of *Allium sativum* have more preference.

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