EXTRCTION AND PHYTOCHEMICAL SCREENING OF *ALLIUM SATIVUM*

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ABSTRACT

Ginger is a plant with leafy stems and yellowish green flowers. The ginger spice comes from the roots of the plant. Ginger is native to warmer parts of Asia, such as China, Japan, and India, but now is grown in parts of South American and Africa.Liposomes containing high concentrations of glycerol are known as glycerosomes, which are used in new drug delivery systems. Glycerosomes have greater morphological stability than liposomes.in the present study, we evaluated the phytochemicals from the ethanolic extract. In the assessment of phytochemical screening below phytochemicals were detected in *A. sativum* bulb ethanol extract. The tests for alkaloids, carbohydrates, cardiac glycosides, anthraquinone glycosides, proteins and amino acids, steroids, triterpenoids, saponins and fixed oils were given positive colour reactions.

Key words: *Allium sativum,* alkaloids, carbohydrates, cardiac glycosides.

INTRODUCTION

Garlic has been used for centuries in various societies to combat infectious disease. Garlic (*Allium sativum*) is a species in the onion genus, *Allium*. Its close relatives include the onion, shallot, leek, chive, and Chinese onion. Garlic is native to Central Asia and Northeastern Iran, and has long been a common seasoning worldwide, with a history of several thousand years of human consumption and use. It was known to ancient Egyptians, and has been used both as a food flavouring and as a traditional medicine. In Ancient Rome, it was "much used for food among the poor". China produces some 80% of the world's supply of garlic. India lies a distant second to China. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes like Alzheimer's disease, anti-arthritic, anti-microbial, acne- reducers, cold and flu, anti-fungal, reduces blood pressure etc.

MATERIAL AND METHODS

Collection and Identification of *Allium sativum*

The healthy fresh clove of *Allium sativum*(About 0.5 to 1 kg) was collected from the local market of Hamirpur District of Himachal Pradesh in the month of December 2019. The collected bulb of *A. sativum*was identified by at Department of Botany, NSCBM Government College, Hamirpur, Himachal Pradesh, India ((30°22'40s″- 33°12′40″ N to 75°45′55″-79°04′20″ E). They identified and authenticated that the plant specimen that is collected bulb is Garlic (Local name in Lasun in Hindi) having botanical name *Alliumsativum*, of family Amaryllidaceae.

Organoleptic characters of *A. sativum*

Organoleptic properties like size, shape, colour, odour, taste, fracture and surface, are the critical parameters for the rapid identification and consumer acceptance. Sensory evaluation-visual macroscopy, colour, odour, taste, fracture and surface are the common features helped in identification of the crude drug.

Results and discussion

The organoleptic properties of bulb of *A. sativum*have been mentioned in Table 1. The bulbs of *A. sativum*possesscharacteristics odour and pungent taste. This might be due to presence of characteristic chemicals like volatile oils or phenolic compounds.

Table 1: The organoleptic characteristics of *A. sativum*bulbs

S. No.	Parameters	Characters
1	Size	2.5 to 4 cm
2	Shape	Ovoid
3	Colour	Whitish to purple
4	Odour	Characteristics
5	Taste	Pungent
6	Fracture	Spongy
7	Surface	Smooth

Processing (drying) of collected *A. sativum*

The scale leaves of the collected fresh garlic bulbs were peeled offand separated from the stalks.The bulbs were washed thoroughly with fresh water 2 to 3 times. After cleaning of *A. sativum*, the bulbs were filtered through ordinary filtration process. The garlic was cutted (Weston 363601w Hand-Operated Mill, India) into small pieces by using hand operated small cutter mill. Now the small pieces of garlicwere air (shade) dried in 12 h day (Without exposing to sun light) and night cycle each for 72 h. After it the semi dried garlics were air dried in a clean, cool, dry and dark place at room temperature of 25±2 ^{0}C and 45±5 % RH (Relative Humidity) for another 7 days. The dried garlic were weighed by using electronic digital balance (Cubis® up to 500 g capacity, Sartorius, Germany) periodically to measure weight loss and accordingly the moisture loss was determined. In due course of drying the garlics were critically observed no fungal growth should develop in the garlic. The dried garlic was stored in air tight container in dark place for further study.

Results and discussion

From the above drying experimental study, it was assessed and calculated that the approximately water content present in the freshly collected bulbs of *A. sativum*was 42.3 %.

Size reduction of *A. sativum*bulbs:

The objective for powdering the plant material is to rupture its organ, tissue and cell structures so that its medicinal ingredients are exposed to the extraction solvent. Furthermore, size reduction maximizes the surface area, which in turn enhances the mass transfer of active principles from plant materials to the solvent.

The dried plant material (Bulbs) was disintegrated by feeding it into a hammer mill (Weston 363601w Hand-Operated Mill, India) which had in built sieves. The particle size was controlled by varying the speed of the rotor clearance between the hammers and the lining of the grinder and also by varying the opening of the discharge of the mill. Usually, the bulbs were reduced to a very coarse powder particle size between 30 and 40 meshes. The very coarse powder form of garlicbulb was stored in air tight closed amber coloured glass container in dark place away from light at low controlled temperature and relative humidity.

Extraction of *A. sativum* by soxhlation

The very coarse powder form of crude garlic was extracted by Soxhlation method by using ethanol (Analytical grade) as solvent. The solvents, ethanol AR was procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. The very coarse powder form of dried garlic was packed in Soxhlet extractor (Model no 3840013, Borosil Glass work Limited, Mumbai, India). A total amount of 150 g very coarse powder was extracted with 300 ml of ethanol solvent.

At first the extractor was properly cleaned and dried. In the extractor, the lower end of siphon tube was blocked with a thick bed of surgical cotton. Then the very coarse powder of *A. sativum*bulbs was packed in the extractor up to upper end of siphon tube. Another thick bed of surgical cotton was placed above crude drugs just below the lower end of siphon tube. Then, the extractor was placed over round bottom flask of 500 ml capacity. The solvent was poured into the extractor unless until one solvent siphoning will be taken place. Care was taken that after first siphoning of solvent; the addition of solvent to extractor must be continued up to that extent that the solvent will remain up to that level marked in siphoning tube. Now the condenser was connected to cool water inlet and outlet system, was placed over the extractor. The whole set up was fitted to stand and round bottom flask was kept over the heating mantle (Arson Heating Mantle 500 ml capacity, Arson Scientific Works, Mumbai, India).

The extraction was done at a temperature of 30 to 34 °C, depending on boiling point of solvent. Extraction was continued unless until the solvent in siphoning tube becomes colourless. For each solvent, around four to five cycles were run to obtain thick slurry. The thick slurry was then concentrated under reduced pressure using rotary evaporator (Model: R-124, Max; Buchi, Mumbai, India) to obtain crude extract.

Result and discussion

The yield of the extract obtained irrespective to total amount of crude drug in very coarse powder form taken in the extractor was calculated by using formula given below.

$$\textbf{Yield of extract (\%)} = (W_1 / W_2) \times 100$$

Where,

W_1 is the weight of extract in almost towards dried form in g,

W_2 is the initial weight of crude drug (g) in very coarse powder form.

The yield of *A. sativum* bulb extract using ethanol as solvent was found to be 4.7 ± 1.05 % (Data calculated in the form of mean ± standard deviation, n = 3).

Pharmacognostic (physical) evaluation of dried powder

Physical standards of crude ethanol extracts of bulbs of *A. sativum* are to be determined for drugs, wherever possible. These are rarely constant for crude drugs, but may help in evaluation, especially with reference to moisture content, foreign organic matter, volatile oil content, ash (Total ash, acid insoluble ash, water soluble ash and sulphated ash) and ethanol value.

Chemicals and reagents

The chemicals that are HCl and H_2SO_4 were purchased from Himedia Laboratory Pvt. Ltd., Mumbai. N-butanol, Chloroform and Petroleum ether were procured from S.D. Fine Chemical, India. All chemicals used for physical evaluation of crude drug that is very coarse powder form of bulbs of *G. officinalis*, are of analytical grade and procured from authorised dealer.

Determination of moisture content (Loss on drying)

The percentage of active chemical constituents in crude drug is mentioned on the basis of air-dried material. Hence, the moisture content of a drug should be determined and should also be controlled. The presence of moisture in crude drugs can lead to its deterioration due to either activation of certain enzymes or growth of microbes. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Methodology

About 1.5 g of crude drug (Very coarse powder form of bulb of *A. sativum*) was weighed into a flat and thin porcelain dish. The drug was dried in an oven at 105 °C up to a constant

weight. The drug was cooled and reweighed. The loss in weight is usually recorded as moisture content of crude drug and it was calculated by using following equation.

$$\textbf{Moisture loss (\%)} = [(W_1 - W_2)/W_1] \times 100$$

Where,

W_1 is the initial weight of crude drug in g, and

W_2 is the final weight of crude drug in g after drying.

Determination of foreign organic matter:

The parts of the organs other than those named in descriptions of the drug are defined as foreign organic matter. If maximum limit of foreign organic matter exceeds, deterioration in quality of the drug takes place.

Methodology

Accurately 100 g of sample was weighed and it was spread on a white tile uniformly without overlapping. Then the sample was inspected visually using a lens (Magnification is 5X). The foreign organic matter was separated manually. After complete separation, the collected foreign organic matter was weighed and percentage in w/w present in sample was calculated by using following formula.

$$\textbf{Foreign Organic matter (\%)} = (W_1/ W_2) \times 100$$

Where,

W_1 is the weight of foreign organic matter in g, and

W_2 is the initial weight of crude drug (100 g).

Determination of volatile oil contents

The Pharmaceutical significance of aromatic drugs is due to their odorous principle i.e., volatile oils. Such crude drugs are standardized on the basis of their volatile oil content. The official method for the estimation of volatile oil in a crude drug is hydro-distillation based on distilling the drug with water and distillate is collected in the graduated tube, from which the aqueous portion of the distillate automatically returns to the distillation flask.

Methodology

The volatile oil content of crude drug was determined by using Clevenger apparatus (Borosilicate Clevenger Apparatus, SCA-35200710, Radial Scientific Equipment Pvt. Ltd., India).About 25 g of very coarse powder drug was taken with 250 ml of water in distillation flask. Few pieces of porcelain were added to avoid bumping during distillation. The

apparatus was set in position. The receiver and return tubes were filled with water. Then the flask was heated through heating mantle. The heating was continued till no more oil was collected. On completion of distillation, the heat was removed and the receiving tube was allowed to cool up to room temperature. The volatile oil and water layers were allowed to separate and the volume of volatile oil was recorded.

Determination of Ash values

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it, as a form of adulteration. Ash contains inorganic radicals like phosphates, carbonates and silicate salts of sodium, potassium, magnesium and calcium. The importance of knowing ash value is to assess the quality and purity of a crude drug.

Determination of total ash value

Accurately 5 g of air-dried powdered drug was accurately weighed in tarred silica crucible. It was incinerated gradually by increasing the heat (Temperature not more than 450 ^{0}C) until free from carbon, cooled and weighed accordingly. It was then kept in a desiccator (Esel Desiccator, 711/195, Esel International, Mumbai, India). The percentage of total ash with reference to the air-dried powdered drug was calculated using the below mentioned formula.

$$\textbf{Total ash value (\%)} = (w/W) \times 100$$

Where,

w is the weight of obtained ash in g, and

W is the weight of crude drug in very coarse powder form taken in g.

Determination of acid insoluble ash value

The ash from the dish used for total ash was washed with 25 ml of dilute hydrochloric acid using 100 ml beaker and boiled for 5 min. The residue was filtered through an ash-less filter paper.

Then the residue was washed twice with hot water. The crucible was ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried sample of crude drug, as per the equation mentioned below.

$$\textbf{Acid insoluble ash value (\%)} = (w_1/w_2) \times 100$$

Where,

w_1 is the weight of obtained acid insoluble ash in g, and

w_2 is the weight of crude drug in very coarse powder form taken in g.

Determination of water insoluble ash value:

The ash from the dish used for total ash was washed with 25 ml of water and boiled for 5 min. The insoluble matter was collected on an ash-less filter paper. Then the residue was washed twice with hot water. The crucible was ignited to a constant weight at low temperature, cooled and weighed. The percentage of water insoluble ash was calculated with reference to the air-dried sample of crude drug using following formula.

$$\textbf{Water insoluble ash value (\%)} = (W/w) \times 100$$

Where,

W is the weight of obtained water insoluble ash in g, and

w is the weight of crude drug in very coarse powder form taken in g.

Determination of sulphated ash value

About 5 g of powdered drug was taken in a silica crucible and ignited thoroughly. The residue was cooled and moistened with 1 ml of dilute sulphuric acid. It was heated gently until white fumes were no longer evolved and again ignited until all black particles have disappeared. The crucible was allowed to cool and the weight of the residue was taken. The percentage of sulphated ash with reference to the air-dried drug was calculated as per the formula given below.

$$\textbf{Sulphated ash value (\%)} = (P1/P2) \times 100$$

Where,

P1 is the weight of obtained sulphated insoluble ash in g, and3

P2 is the weight of crude drug in very coarse powder form taken in g.

Determination of extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives.The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired.

Extractive value determination may help in evaluation, specifically with reference to solubility in different solvents.Evaluation of a drug basically needs its identification and can be done by morphological or microscopical characters. Many a times, the drug identified by its diagnostic characters is of substandard quality due to either faulty collection or incorrect storage. So, to prove its acceptability as a drug, extractive value determination can be applied, wherever possible. The determination of extractive value helps for evaluation of crude drugs,

give idea about the nature of chemical constituents present in crude drugs and also useful for the estimation of specific constituents, soluble in that particular solvents used for extraction.

Methodology

About 5 g of powdered drug (Very coarse form) of *A. sativum*bulb was transferred into a dry 250 ml conical flask. It was macerated with 100 ml of each solvent (Water, Ethanol, n-butanol, Petroleum ether and Chloroform respectively) in stoppered flask for 24 h. In first 6 h, the flask was frequently shaken and then allowed to stand for 18 h. Then the mixture was filtered into 50 ml of cylinder.

When sufficient filtrate was collected, 25 ml of filtrate was transferred to a weighed, thin porcelain dish. The filtrate was evaporated to dryness by placing the dish on a boiling water bath.

The drying was completed in a Hot air oven (Remi RDHO-80, RemiElektrotechnik Ltd., India) at 100 °C. The extract was cooled in a desiccator and weighed. The percentage (w/w) of extractive with reference to the air-dried drug was calculated.

Results and discussion

The results of physical evaluation of crude drug i.e.,very coarse powder form of *A. sativum*bulbs are shown in Table 2 and 3. The moisture content of very coarse powder form of *A. sativum*bulbs was 4.5 %. This result explains that this crude drug is less prone to microbial decomposition and enzymatic deactivation, suggesting more stable for longer periods.

The crude drug of *A. sativum* showed foreign organic matter of only 0.28 %. This study assures that this crude drug is purer and more qualitative. The volatile oil content of the crude drug was found to be very less i.e.,1.8 % only. The ash study revealed that total, acid insoluble, water soluble and sulphated ash values were 3.8, 0.51, 8.55 and 0.78 % respectively.

Table 2: Physical evaluation of crude drug (Very coarse powder form of *A. sativum*bulbs)

S. No.	Parameters	Values in percentage (w/w) (X ± S.D.)
1	Moisture content	4.5 ±0.08
2	Foreign organic matter	0.28 ± 0.02
3	Volatile oil content	1.8 ± 0.17
4	Total ash	3.8 ± 0.22

5	Acid insoluble ash	0.51 ± 0.11
6	Water soluble ash	8.55 ± 0.16
7	Sulphated ash	0.78 ± 0.12

All values are expressed as mean ± standard deviation (n = 3).

The colour of *A. sativum*bulbs was slightly brownish. All the extracts showed a characteristic odour. The appearance of *A. sativum*bulbs extracts were very slightly sticky. The extractive values were 18.6, 5.7, 3.3, 1.1 and 0.25 % for water, ethanol, n-butanol, Petroleum ether and chloroform respectively. The ethanol extracts showed maximum extractive yield, whereas chloroform exhibited minimum extractive yield.

Table 3. Physical and extractive values of bulb extracts of *A. sativum*

Sl. No.	Specifications	Values in percentage (w/w)
01	Colour	Creamy whitish
02	Odour	Characteristics
03	Appearance	Slightly sticky
04	Water soluble extractives	18.6 ± 0.55
05	Ethanol soluble extractives	5.7 ± 0.38
06	n-butanol soluble extractives	3.3 ± 0.29
07	Petroleum ether	1.1 ± 0.29
08	Chloroform	0.25 ± 0.31

All values are expressed as mean ± standard deviation (n = 3).

Preliminary phytochemical screening

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. The plants may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of

compounds like glycosides, alkaloids, volatile oils, tannins, etc., that exert a pharmacological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites.

Chemicals and reagents

Dragendorff's reagent, Mayer's reagent and Biuret reagent were procured from LobaChemiePvt. Ltd., Mumbai. Wagner reagent, Benedict's reagent, Fehling's solution A & B and Ninhydrine were obtained from MerkPvt. Ltd., Mumbai. Hager's reagent was obtained from Ozone International Ltd., Mumbai. Tollen's reagent was obtained from Nice Chemical Pvt. Ltd., Cochi. Molisch's reagent was procured from Stan Bio, Calcutta. Millon's reagent was obtained from Otto KemiPvt. Ltd., Mumbai. All other chemicals and reagents used were of Analytical grade and procured from authorized dealer.

Plant extracts

The ethanol extracts of *A. sativum*bulbs thus obtained were subjected to qualitative chemical tests for the identification of various plant constituents.

Detection of alkaloids

About 20 mg of ethanolic crude extract of *A. sativum*bulbs was taken and stirred separately with a few drops of dilute hydrochloric acid. The solution was filtered through Whatman filter paper No.1. The filtrates were used for the following tests.

***Wagner's test*:**

The filtrate (1 ml) was treated with few drops of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

***Mayer's test*:**

The filtrate (1 ml) was treated with few drops of Mayer's reagent (Potassium Mercuric Iodide solution). Formation of a yellow-coloured precipitate indicates the presence of alkaloids.

***Dragendorff's test*:**

Thefiltrate (1 ml) was treated with few drops of Dragendorff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

***Hager's test*:**

The filtrate was treated with few drops of Hager's reagent (Saturated solution of picric acid). The presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates:

The ethanolic crude extract of *A. sativum*bulbs dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

***Molisch's test*:**

The filtrate was treated with 2 drops of 10 % solution of Molish's reagent (Alcoholic α-naphthol solution) in a test tube followed by the addition of 1ml of concentrated sulphuric acid. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Fehling's test:

The filtrate was hydrolyzed with 0.5 ml of dilute hydrochloric acid. The solution was neutralized with alkali and heated with equal volume of Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedict's test:

The filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

***Barfoed test*:**

The Barfoed reagent was added to a small amount of each extract and boiled on water bath for few minutes. Reddish precipitate indicates the presence of carbohydrate.

Detection of cardiac glycosides:

The ethanolic crude extract of *A. sativum*bulbs was stirred separately with a few drops of dilute hydrochloric acid. The solution was filtered through Whatman filter paper No.1 and then subjected to test for glycosides.

***Keller killiani test*:**

A few mg of the extract was taken separately boiled with 5 ml of 70 % alcohol for 2 to 3 min and filtered. To the filtrate, 2 ml of water and 1 ml of strong lead acetate solution were added. The chloroform layer was separated and evaporated slowly in a porcelain dish. To the resultant residue, 1 ml of glacial acetic acid containing one drop of Ferric chloride was added and this was carefully transferred to the test tube containing 1 ml of sulphuric acid. A reddish-brown ring at the junction of two layers indicates a positive result for the deoxy sugars.

***Legal test*:**

The extracts was treated with 1 ml sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

***Kedde's test*:**

A small quantity of residue of the extract was taken in alcohol, decolorized with lead acetate and filtered. The filtrate was evaporated to dryness and residue was dissolved in a few drops of methanol. Two to three drops of Kedde's reagent (1 % 3.5 ml dinitro-benzoic acid in methanol) followed by two drops of 2N methanolicNaOH were added. Blue or violet colour indicates the presence of cardiac glycosides.

***Guignard's test*:**

About0.2 g of extract was taken in a test tube, followed by the addition of few drops of chloroform to enhance the enzymatic activity. The strip of filter paper was moistened with the Sodium picrate solution. The strip was removed, dried and inserted between the split cork stoppers that were fitted into the neck of the test tube containing reaction mixture. The test tube and contents were then warmed at 30 to 35 °C for 0.5 h. The appearance of a red colour on the paper indicates the presence of HCN evolution and hence indicates the presence of cyanogenetic glycosides.

Detection of anthraquinone glycosides:

***Borntrager's test*:**

About 0.1 g of extract was boiled with 5 ml of 10 % sulphuric acid for 2 min. It was filtered while hot, cooled and the filtrate was shaken with equal volume of benzene. The benzene layer was allowed to separate completely from the lower layer, which was pipetted out and transferred out to a clean test tube. Then half of its volume of aqueous ammonia (10 %) was added, shaken gently and the layers were allowed to separate. The lower ammonia layer will show red pink colour due to presence of free anthraquinone.

Modified Borntrager's test:

The extracts were treated with 1 ml of ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with a few drops of ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthracene glycosides.

Detection of gums and mucilage's:

***Ruthenium red test*:**

Theextractwas added to a solution containing 0.08 g of ruthenium red in 10 ml of 10% solution of lead acetate; it stains the mucilage to red colour.

Molisch's test:

Theaqueous or alcoholic solution (2 ml) of the extract was shaken with 10 % alcoholic solution of α-naphthol and concentrated sulphuric acid was added along the side of the test tube. A violet ring at the junction of two liquids confirms the presence of carbohydrates, gums and mucilages.

Test with 95 % alcohol:

When 95 % alcohol was added to the extract, gums get precipitated out. The precipitate was insoluble in alcohol.

Detection of proteins and amino acids:

Biuret test:

About 2 ml of the extract, 2 ml of 10 % NaOH solution and 2 to 3 drops of 1 % $CuSO_4$ solution were mixed together. The appearance of violet or purple colour confirms the presence of proteins.

Ninhydrintest:

To the extract, 0.25 % w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acids.

Xanthoproteic test:

About 2 ml of the extract was boiled with 1 ml of conc. HNO_3, cooled and subsequently 40 % NaOH solution was added drop by drop to it. Appearance of coloured solution indicates the presence of proteins.

Millon's test:

About2 ml of the extract and 2 ml of Millon's reagent were boiled, subsequently cooled, and then few drops of $NaNO_2$ were added to it. Appearance of red precipitate and red coloured solution indicates the presence of proteins.

Detection of tannins and phenolic compounds:

Lead acetate test:

Thelead acetate was added to the extract. Tannins get precipitate.

Ferric chloride test:

The extract was treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence phenols.

***Gelatin test*:**

Theextract was added with 1 % gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of steroids and sterols:

Salkowski's test:

The extract was treated with 1 ml of chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicates the presence of sterols.

Liebermann-Burchard test:

The extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. The cooled solution was mixed with few drops of concentrated sulphuric acid. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of triterpenoids:

Tin and thionyl chloride test:

For the detection of triterpenoids, the extract was dissolved in chloroform. A piece of metallic tin and 1 drop of thionyl chloride were added to it. The presence of pink colour confirms the result.

Salkowski test:

Few drops of concentrated sulphuric acid are added to the ethanol extract and shaken. On standing lower layer turns to golden yellow colour indicating positive results.

Liebermann Burchard test:

To the chloroform solution of the extract few drops of acetic anhydride are added and mixed well. One ml of concentrated sulphuric acid is added from the side of test tube. A reddish-brown ring indicates triterpenes.

Detection of saponins:

***Foam test*:**

A few mg of residue of the extract was taken separately in a test tube with small amount of sodium bicarbonate along with water and shaken vigorously. Formation of stable froth indicates the presence of saponins.

Haemolytic test:

About 2 ml of 1.8 % NaCl was taken in two test tubes. To one test tube, 2 ml distilled water was added, where as to another test tube, 2 ml of 1% extract was added. About 5 drops of blood was added to each tube and gently mixed the contents. The appearance of haemolysis under the microscope in the tube containing the extract indicates the presence of saponins.

Detection of flavonoids:

Magnesium turning method:

About20 mg of crude extract was mixed with 10 ml of methanol and filtered; 2 ml of filtrate solution was taken and mixed with concentrated HCl. Magnesium ribbon was added until a pink- tomato red colour was gauged, indicating the presence of flavonoids.

***Ferric chloride test*:**

Few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract. A blackish green colour production indicates the phenolic nucleus.

Lead acetate test:

Few drops of lead acetate solution (10%) were added to alcoholic solution of test extract. Appearance of yellow precipitate indicates positive test.

Zinc hydrochloric acid reduction test:

The alcoholic solution of the extract was treated with pinch of zinc dust and few drops of concentrated HCl; magenta colour is produced after a few min.

***Sodium hydroxide test*:**

For the detection of flavonoids, the extract was first dissolved in water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colour confirms the presence of flavonoids.

Sulphuric acid test:

The extract was first dissolved in water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colour confirms the presence of flavonoids. A drop of H_2SO_4 when added, the yellow colour disappears.

Results and Discussion

Table 4 shows the phytochemicals detected in *A. sativum*bulb ethanol extract. The tests for alkaloids, carbohydrates, cardiac glycosides, anthraquinone glycosides, proteins and amino acids, steroids, triterpenoids, saponins and fixed oils were positive. The phytochemicals that are gums and mucilages, tannins, phenols and steroids are absent in ethanol extract of *A. sativum*bulb. Thus *A. sativum*bulbethanolic extract contains alkaloids and saponins in abundant manner, cardiac glycosides and saponins in moderate form and carbohydrates, anthraquinone glycosides, proteins, amino acids, fixed oils and triterpenoids in fair manner.

Table 4: Phytochemical constituents detected in bulb ethanol extracts of *A. sativum*

Phytochemicals	Test	Result	Remark
Alkaloids	Wagner's test	+	+
	Mayer's test	+	
	Dragendorff's test	+	
	Hager's test	-	
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	
	Benedict's test	-	
	Barfoed test	+	
Cardiac glycosides	Keller killiani test	+	+
	Legal test	+	
	Kedde's test	+	
	Guignard's test	-	
Anthraquinone glycosides	Borntrager's test	-	-
	Modified Borntrager's test	-	
Gums and mucilages	Ruthenium red test	-	-
	Molisch's test	-	
	Test with 95% Alcohol	-	
Proteins and amino acids	Biuret test	-	-
	Ninhydrin test	-	

	Xanthoproteic test	-	
	Millon's test	-	
Tannins	Lead acetate test	-	-
	Ferric chloride test	-	
	Gelatin test	-	
Phenols	Ferric chloride test	+	+
	Lead acetate test	+	
	Gelatin test	-	
Steroids and sterols	Salkowski's test	+	+
	Libermann-Burchard test	+	
Triterpenoids	Tin and thionyl chloride test	+	+++
	Salkowski test	+	
	Liebermann Burchard test	+	
Saponins	Foam test	+	++
	Haemolytic test	+	
Flavonoids	Magnesium turning method	+	+++
	Ferric chloride test	+	
	Lead acetate test	+	
	Zinc hydrochloric acid reduction test	+	
	Sodium hydroxide test	+	
	Sulphuric acid test	+	
Fixed oil and fats	Fat test	+	+

(+) Sign indicates present and (-) sign indicates absent. +++ (Abundantly present), ++ (Moderately present and + (Fairly present)

CONCLUSION

From the above study, in the assessment of phytochemical screening below phytochemicalswere detected in *A. sativum* bulb ethanol extract. The tests for alkaloids, carbohydrates, cardiac glycosides, anthraquinone glycosides, proteins and amino acids, steroids, triterpenoids, saponins and fixed oils were positive.

REFERENCES

1. Ademiluyi AO, Oboh G, Owoloye TR, Agbebi OJ. (2013) 'Modulatory effects of dietary inclusion of garlic (Allium sativum) on gentamycin–induced hepatotoxicity and oxidative stress in rats', Asian Pac J Trop Biomed,3:470–475.
2. Adetumbi M, Javor GT, Lau BH. (1986) 'Allium sativum (garlic) inhibits lipid synthesis by Candida albicans',Antimicrob Agents Chemother; 30:499–501.
3. Allison GL, Lowe GM, Rahman K.(2012) 'Aged garlic extract inhibits platelet activation by increasing intracellular cAMP and reducing the interaction of GPIIb/IIIa receptor with fibrinogen'. Life Sci; 91:1275–1280.

4. Amagase H, Milner JA. (1993) 'Impact of various sources of garlic and their constituents on 7, 12- dimethylbenz[a]anthracene binding to mammary cell DNA'. Carcinogenesis; 14:1627–1631.
5. Ashraf R, Aamir K, Shaikh AR, Ahmed T. (2005) 'Effects of garlic on dyslipidemia in patients with type 2 diabetes mellitus'. J Ayub Med Coll Abbottabad; 17:60–64.
6. Auer W, Eiber A, Hertkorn E, Hoehfeld E, Koehrle U, Lorenz A, Mader F, Merx W, Otto G, Schmid-Otto B, et al. (1990) 'Hypertension and hyperlipidaemia: garlic helps in mild cases'. Br J Clin PractSuppl; 69:3–6.
7. Bakhshi M, Taheri JB, Shabestari SB, Tanik A, Pahlevan R. (2012) 'Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouthwash in denture stomatitis. Gerodontology; 29:e680–684.
8. Banejee SK, Maulik SK. (2002)'Effect of garlic on cardiovascular disorders a review Nutr J; 1:4.
9. Bordia A, Verma SK, Srivastava KC.(1989) 'Effect of garlic (Allium sativum) onblood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease'. Prostaglandins Leukot Essent Fatty Acid;58:257–63.
10. Cai Y.(1991) 'Anticryptococcal and antiviral properties of garlic'. Cardiol Pract;9:ll.
11. Capasso A.(2013) 'Antioxidant action and therapeutic efficacy of Allium sativum L'. Molecules; 18:690–700.
12. Cavallito CJ, Bailey JH. (1944) 'Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action'. J Am Chem Soc; 66:1950–1951.
13. Chanderkar AG, Jain PK.(1973) Analysis of hypotensive action of Allium sativum (garlic) Ind J PhysiolPharmacol; 17:132–133.
14. Colín-González AL, Santana RA, Silva-Islas CA, Chánez-Cárdenas ME, Santamaría A, Maldonado PD.(2012) 'The antioxidant mechanisms underlying the aged garlic extract- and S-allylcysteine-induced protection'. Oxid Med Cell Longev;907162.
15. Davis SR, Penie R, Apitz-Castro R. (2003) 'The in vitro susceptibility of Scedosporiumprolificans to ajoene, allitridium and a raw extract of garlic (Allium sativum)'. J AntimicrobChemother; 5:1593–1597.
16. De Witt JC, Notermans S, Gorin N, Kampelmacher EH.(1979) 'Effect of garlic oil or onion oil on toxin production by Clostridium botulinum in meat slurry'. J Food Protect; 42:222–224.

17. Delaha EC, Garagusi VF. (1985) ‘Inhibition of mycobacteria by garlic extracts (Allium sativum) Antimicrob Agents Chemother’; 27:485–486.
18. Dini C, Fabbri A, Geraci A. (2011) The potential role of garlic (Allium sativum) against the multi-drug resistant tuberculosis pandemic’.A review. Ann Ist Super Sanita; 47:465–473.
19. Fenwick GR, Hanley AB.(1985)’Allium species poisoning’. Vet Rec. 1985; 116:28.
20. Fromtling RA, Bulmer GS. (1978) ‘In vitro effect of aqueous extract of garlic (Allium sativum) on the growth and viability of Cryptococcus neoformans. Mycologia’; 70:397–405.