

Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region

Vandana Singh¹, Ramesh Kumar^{2*}

¹M.Sc. Student, Department of Biochemistry, Bundelkhand University, Jhansi, India

²Associate Prof. & Head, Department of Biochemistry, Bundelkhand University, Jhansi, India

*Address for Correspondence: Dr. Ramesh Kumar, Assoc. Prof. & Head, Department of Biochemistry, Bundelkhand University, Jhansi (UP)-284128, India

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ABSTRACT- Secondary metabolites found in the medicinal plants play an important role in curing different diseases and used as important raw materials for the manufacturing of traditional and modern medicine. One of these medicinal plants, Garlic (*Allium sativum*) member of the Amaryllidaceae family reduces various risk factors associated with several diseases. Garlic has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotensin converting enzyme. It also reduces cholesterol, inhibits platelet aggregation, reduces blood pressure, and increases antioxidant status. Therefore, our aim was to compare the different secondary metabolites present in the aqueous and methanolic extracts of the garlic leaves, root, developed bulbs and undeveloped bulbs. Phytochemical screening revealed the results that alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannin and phenolic compounds, saponins, amino acid & triterpenoids aqueous extract and methanolic extract of garlic leaves and fully developed bulb but garlic fully developed showed negative result in reducing sugar. Methanolic extract of undeveloped bulbs of garlic shown positive a result for all expects carbohydrate, flavonoids, cardiac glycosides it showed negative results for them. Garlic roots shown also the same result as garlic undeveloped bulbs but there was the difference in flavonoids it shown negative for it.

Key-words- *Allium sativum*, Antioxidant, components, Medicinal plants, Phytochemical TLC

INTRODUCTION



The therapeutic potential of plant products can be traced back to over five thousand years ago as there is evidence of its use in the treatment of diseases and for revitalizing body systems in Indian, Egyptian, Chinese, Greek and Roman civilizations^[1]. India is one of the mega diversity hot spots with rich heritage of traditional knowledge of folk medicines. Therefore in India, plants of therapeutic potential are widely used by all sections of people both as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda, and Unani and also as processed product of the pharmaceutical industry^[2]. India has about 4.5 million plant species and among them estimated only 250,000–500,000 plant species, have been investigated phytochemically for biological or pharmacological activity.

A. sativum commonly known as garlic belongs to family Amaryllidaceae.

Name of garlic is poondu in Tamil, veluthulli in Malayala, vellulli in Telugu, rasoon in Bengali, lasan in Gujrati, lasun in Marathi, lissan in Punjabi & lissan in urdu. Its close relatives include the onion, shallot, leek, chive^[3] and Chinese onion^[4]. With a history of several thousand years of human consumption and use, garlic is native to Central Asia and has long been a common seasoning worldwide. It was known to Ancient Egyptians has been used both as a food and as a traditional medicine^[5-6]. Garlic one of the oldest plants used throughout history for both culinary and medicine ranks the highest of all the herbal remedies consumed for its health benefits. The bulbs of the plant have been used in many parts of the world as a stimulant, antiseptic, anthelmintic, antihypertensive, carminative, diaphoretic, expectorant, diuretic, antiscorbutic, aphrodisiac and antiasthmatic and for the relief of rheumatic pains^[7]. Physicians prescribed the herb during the middle ages to cure deafness and the American Indians used garlic as a remedy for earaches, flatulence, and scurvy. Recent research revealed that garlic is not only beneficial as a medicinal plant, but it can be used as a repellent to some plant pests and diseases^[8].

A. sativum is a versatile herb that contains numerous vitamins, minerals, and trace elements. The presences of two trace elements, germanium and selenium have been

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postulated to play a role in the herb's antitumor effect^[9]. The volatile oils present in garlic possess flavonoid containing compounds such as diallyl disulphide, di allyl trisulphide and methyl allyl trisulphate^[9]. Allicin, derived from amino acid allin gives the pungent characteristic odour of crushed garlic and is believed to be responsible for some of the pharmacologic activity of the plant^[10-11]. Scientific and clinical studies have shown that garlic can enhance immunity, protect against infection and inflammation and help lower the risk of cancer, heart disease and dementia^[12]. Evidence supports the fact that regular consumption of garlic can reduce factors associated with cardiovascular diseases^[13]. It has been established that garlic is rich in organosulphur compounds such as allicin that has been extensively reported to have beneficial effects on risk factors associated with cardiovascular disease^[14] that include normalization of plasma lipids, lowering of systolic blood pressure and reduction of atherosclerosis development^[15]. Garlic contains many phytochemicals but their role has not been extensively characterized, however, it has been established that the potential bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds. Keeping in view of the above beneficial effects of garlic, we sought to analyze the phytochemicals present in methanolic as well as in aqueous extract. Anti-oxidant activity was also evaluated. Further, TLC was conducted to monitor the number of bioactive components (spots) present in the extracts.

MATERIALS AND METHODS

Collection of Plant Materials

The plant of *A. sativum* was collected in the month of January from the local market of Jhansi (U.P). Firstly the collected plant material was washed with tap water for 3-4 times and then with de-ionized water for two times. After washing, the plants were kept in the dark for drying at room temperature and under the constant observation to avoid any contamination. Dried leaves were crushed with the help of electric grinder. Powdered sample was stored for further use.

Study Area- Bundelkhand region covers total 13 districts, out of which 7 of Uttar Pradesh and 6 districts of Madhya Pradesh. It is fallen under 23° 8-26° 31, 78° 11-81° 31 latitude and longitude respectively. The region is characterized as hot semi arid eco region along with growing period of 90-150 days. The annual rainfall ranges from 838.6-1251 mm over the region which is often erratic^[16]. The main occupation of this region is agriculture and most people are involved in labour work and are very poor because of unemployment and education. However the region is rich in ecological and biodiversity, and also rich in medicinal plants. Some of the medicinal plants are mainly found in this region. Therefore to improve the economic status of the farmers, villagers; an extensive research work is required for their use.

Extraction procedure- The leaves, root and bulb (developed and undeveloped) of *A. sativum* was subjected to extraction. Extraction was done by two methods i.e. Aqueous and Methanolic extraction.

Aqueous extraction of Garlic- Different concentration of dry powder i.e. 5gm and 10 gm was taken in conical flasks having equal amount (100 ml) of de-ionized water. Both the flasks were heated at 90°C in water bath for 1 hour. After 1 hour flasks were taken out from the water bath and kept at room temperature for cooling purpose then the extract was filtered with the help of filter paper and stored at 4°C for further process.

Methanolic extraction of Garlic- The powdered material was extracted with absolute 80% methanol using a Soxhlet apparatus. Different parts of the plant material i.e. leaves, roots & bulb were used for extraction. After filling the soxhlet apparatus with plant material and solvent it was run at 60-80°C until it gets colorless and continuously flows of water to cool down the condenser. Finally the extract was collected in airtight bottles and stored at 4°C for further process.

Phytochemical analysis- The detailed phytochemical analysis was carried out for all the extracts i.e. leaves, developed bulbs, undeveloped bulbs, and roots as per the standard methods^[17,18] with some of the modifications.

Tests for alkaloids- To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

Mayer's reagent test- To 1 ml of filtrate, few drops of Mayer's reagent was added along sides of the tube. Formation of creamy precipitate indicates the presence of alkaloids.

Wagner's test- To 1 ml of filtrate, few drops of Wagner's reagent was added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Hager's test- To 1 ml of filtrate, few drops of Hager's reagent was added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

Molisch test- One ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 500 μ l of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Barfoed's test- One ml of extract and Barfoed's reagent were mixed in a test tube and heated on the water bath for 2 minutes. Red color due to a formation of cupric oxide indicates the presence of monosaccharide.

Tests for Reducing Sugars

Fehling's test- To 500 µl of extract, 500µl of Fehling's A and 500 µl of Fehling's B solutions were added in a test tube and heated on a water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's test- 500 µl of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5–10 minutes. The solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Tests for Flavonoids

Alkaline reagent test- One ml of the extract was treated with a few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on the addition of few drops of dilute acid, indicates the presence of flavonoids.

Lead Acetate Test- One ml of the extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Ammonia solution test- 500 µl of dilute iodine solution was added to a portion of the aqueous filtrate of each plant part extract followed by the addition of 500 µl of concentrated sulphuric acid.

Tests for Glycosides

Borntrager's test- To 1 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, 1 ml of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinones glycosides.

Legal's test- 500 µl of test solution was dissolved in pyridine. 500 µl of sodium nitroprusside solution was added and made alkaline using 500 µl of 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.

10% NaOH test- 1 ml of dilute sulphuric acid was added to 200 µl of plant extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 200 µl of fehling solution A & B was added. A brick red precipitate of reducing sugars indicates presence of glycosides.

Test for cardiac glycosides

Keller-Killani test- To 1 ml of a test solution, 1.5 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Carefully few drops of concentrated sulphuric acid were added to the sides of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Tests for Tannin and Phenolic compounds

Ferric chloride test 5%- A small amount of the extract was dissolved in distilled water. To this solution 500 µl of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates the presence of phenolic compounds.

Lead Acetate Test- One ml of the extract was dissolved in distilled water. To this solution, few drops of lead acetate solution were added. Formation of white precipitate indicates the presence of phenolic compounds.

Dilute iodine solution test- One ml of extract, few drops of dilute iodine solution was added. Formation of transient red color indicates the presence of phenolic compounds.

Ferric chloride test 10% or ferric chloride test- Three gm of the powdered sample was boiled in 50 ml distilled water for 3 minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drops of 10% ferric chloride solution added. A blue-green colour indicates the presence of tannins.

Hydrolysable tannin- 400 µl of the plant extract was taken in a test tube and 4 ml of 10% NaOH solution was added. Formation of an emulsion on shaking indicated the presence of hydrolysable tannin.

Test for Saponins

Froth test- One ml of the extract was diluted with 2ml of distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Protein and Amino acids

Ninhydrin test- One ml of the test solution was heated with 1 drop of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of the blue color indicates the presence of amino acids.

Biuret test- The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of the violet or pink color indicates the presence of proteins.

Tests for Triterpenoids and Steroids

Salkowski's test- One ml of the extract was treated with 1ml of chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, a steroid is present. Presence of the golden yellow layer at the bottom indicated the presence of triterpenoids.

Carbohydrate Estimation- The total carbohydrate estimation was done by the method of Hedge and Hofreiter, 1962 ^[19] with some of the modification. Glucose was used as standard and absorbance was taken at 630 nm.

Protein Estimation- BSA was used to as standard protein. Bradford assay ^[20] was performed for quantification of protein. Absorbance was taken at 595 nm.

Thin layer chromatography- Each of the extracts was to begin with, checked by thin layer chromatography (TLC) on analytical plates over silica gel-G of 0.2 mm thickness. These plates were developed in Butanol: Acidic acid: Water having a ratio of 2:1:1. The developed TLC plates were air dried followed by hot air oven for 20 minutes. Freshly prepared 0.2% ninhydrin solution was used to detect the bands on the TLC plates.

The movements of the spots were expressed by its retention factor (Rf):

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Antioxidant activity- The total antioxidant capacity of the methanol extract of different parts of *A. sativum* was evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto *et al.* ^[21]. The assay is based on the reduction of Mo (VI) to Mo (V) by the methanol extract of different part of garlic and subsequent formation of green phosphate/Mo (V) complex at acid pH. One mL of various concentrations (3-21 µg/mL) of the extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. BHT was used as a standard. A typical blank solution contained 3 ml of the reaction mixture and the appropriate volume of the same solvent used for the samples/standard. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer.

RESULTS

A. sativum leaves, roots, undeveloped bulbs and fully developed bulbs were screened for the presence of phytochemical components. Different phytochemicals tests were performed by distinctive reagent, for example; mayer's test, wagner's test & hager's test, all were performed for the detection of alkaloids. Alkaloids are a class of nitrogenous organic compounds of plant origin which have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. All tests for alkaloids shown positive results for both extracts except Mayer's test which shown negative results for 5 gm and 10 gm of garlic leaves. Molish's and Barfoed's tests were done in the presence of carbohydrate. Molish's test shown positive results mostly for aqueous extraction. Whereas Barfoed's test shown negative results for both aqueous and methanolic extraction. Presence of reducing sugar was monitored by Fehling's and Benedict test. Mostly methanolic extract except for undeveloped bulb shows positive results with

both the tests (Fehling & Benedict). While both 5 & 10 gm aqueous extraction shown variable results.

Flavonoids are hydroxylated polyphenolic compounds that carry out important functions in plants, including attracting pollinating insects; combating environmental stresses, such as microbial infection; and regulating cell growth. Six major subclasses of flavonoids, namely anthocyanidins, flavan-3-ols, flavonols, flavanones, flavones, and isoflavones; flavonols are the most widespread in the human diet. Tests for flavonoids show positive results for both aqueous and methanolic extraction while undeveloped bulbs do not show the presence of flavonoids. Test for glycosides are not very impressive and it varies from test to test and from different parts of the plant. Cardiac glycosides are a class of organic compounds that increase the output force of the heart and decrease its rate of contraction by acting on the cellular Na-K ATPase pump. Keller Killani test for cardiac glycosides showed positive results except for garlic undeveloped bulbs.

Phenolic compounds are any compounds derived from the phenol group and include acid, ester, glycoside, and aglycone forms. Phenolics contribute to the colour, structure, astringency, etc. Tannins are large molecular weight compounds resulting from polymerization reaction of smaller phenolic compounds. Test for tannin and phenolics by different methods shown in the presence of their compounds in all the plant parts we used except undeveloped bulbs. Saponins are glycosides with foaming characteristics and have many health benefits. Saponins are present in all the plant parts used in this study. Ninhydrin tests showed mostly positive results while Biuret tests are opposite to the ninhydrin test. Plant terpenoids are used extensively for their aromatic quality and play a role in traditional herbal remedies. There are different terpenoids found in different plants and one of the most studied is curcuminoids found in turmeric and mustard seeds. Similar to saponins, terpenoids are present in all the parts of garlic we used in this study.

TLC analysis was also shown the different types of bioactive compounds in different parts of garlic extracts. Fig. 1 shown the photographs of the studied TLC slides of the different plant parts in both aqueous and methanolic extracts, which shows different spots for various phytochemicals and the Table 1 reports Rf values for various extracts. The reported spots are separated with enough space and having various Rf values showing the presence of at least four phytochemicals in both aqueous and methanolic extracts of fully developed bulb and aqueous 5 gm extracts of leaves. Methanolic extracts of leaves and undeveloped bulb shows six spots. Aqueous extraction of 10 gm of leaves shown five spots while methanolic extraction of roots shown nine spots.

Table 1: Comprative screening of phytochemicals of aqueous and methanolic extracts of *A. sativum*

S. No.	Phytochemical Tests	Garlic Leaves		Garlic fully developed bulb		Garlic undeveloped bulb	Garlic Root
		Aq. Ex.	Met. Ex.	Aq. Ex.	Me. Ex.	Met. Ex.	Met. Ex.
		5 g	10 g	5 g	10 g		
1.	Test for alkaloids						
	(A) Mayer's test	-ve	-ve	+ve	+ve	+ve	+ve
	(B) Wagner's test	+ve	+ve	+ve	+ve	+ve	+ve
	(C) Hager's test	+ve	+ve	+ve	+ve	+ve	+ve
2.	Tests for carbohydrate						
	(A) Molisch test	+ve	+ve	-ve	+ve	+ve	-ve
	(B) Barfoed's test	-ve	-ve	-ve	-ve	-ve	-ve
3.	Test for reducing sugar						
	(A) Fehling's test	-ve	-ve	+ve	-ve	-ve	+ve
	(B) Benedict's test	+ve	+ve	+ve	-ve	-ve	+ve
4.	Tests for flavonoids						
	(A) Alkaline reagent	+ve	+ve	-ve	+ve	+ve	-ve
	(B) Lead acetate	+ve	+ve	+ve	+ve	+ve	-ve
	(c) Ammonia test	+ve	+ve	+ve	+ve	+ve	-ve
5.	Test for glycosides						
	(A) Borntrager's test	-ve	-ve	-ve	+ve	+ve	+ve
	(B) Legal's test	-ve	-ve	+ve	+ve	+ve	-ve
	(C) 10% NaOH test	+ve	+ve	+ve	-ve	-ve	-ve
6.	Test of cardiac glycosides						
	(A) Keller Killani test	+ve	+ve	+ve	+ve	+ve	-ve
7.	Test for tannin and phenolic						
	(A) Ferric chloride test (5%)	+ve	+ve	+ve	-ve	-ve	-ve
	(B) Lead acetate test	-ve	-ve	-ve	+ve	-ve	+ve
	(C) Dilute iodine test	+ve	+ve	+ve	+ve	+ve	+ve
	(D) Ferric chloride test	+ve	+ve	+ve	-ve	-ve	-ve
8.	Tests for saponin						
	(A) Forth test	+ve	+ve	+ve	+ve	+ve	+ve
9.	Tests for amino and protein						
	(A) Ninhydrin test	+ve	+ve	+ve	+ve	+ve	+ve
	(B) Biuret test	-ve	-ve	-ve	-ve	-ve	+ve
10.	Test for terpenoids						
	(A) Salkowski's test	+ve	+ve	+ve	+ve	+ve	+ve

“+” = Positive; “-” = Negative; Aq. Ex.= Aqueous Extract; Met. Ex. = Methanolic Extract

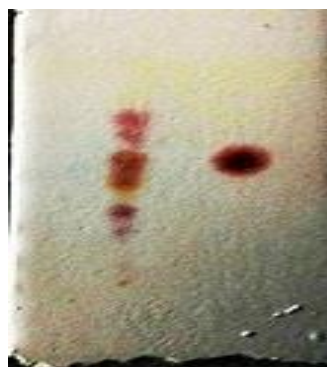
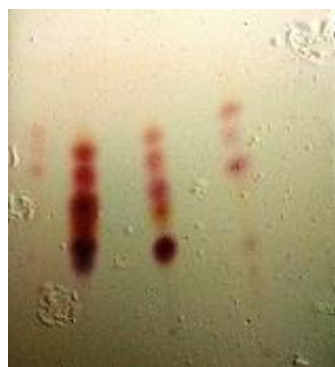


Fig. 1 (a) (b) (c)

Fig. 2 (a) (b) (c)

Fig. 3

Fig. 4

Fig. 1: Garlic Fully Developed Bulb; **1a.** Aq. 10 gm; **1b.** Aq. 5 gm; **1c.** Met.

Fig. 2: Garlic Leaves; **2a.** Met.; **2b.** Aq. 10 gm; **2c.** Aq. 5 gm;

Fig. 3: Garlic Undeveloped Bulb (Met.)

Fig. 4: Garlic Root (Met.)

Table 2: Rf values of both extracts of different parts of Garlic (*A. sativum*)

S No.	Plant Extract	No. of Spots	Rf Values
			Butanol: Acidic acid: Water (2: 1: 1)
Fully developed bulb			
1.	Met. Ex.	4	0.36,0.52,0.6,0.76
	Aq. Ex. 5g	4	0.25,0.42,0.45,0.53
	Aq. Ex.10g	4	0.28,0.42,0.47,0.6
Leaves			
2.	Met. Ex.	6	0.36,0.47,0.57,0.63, 0.68,0.73
	Aq. Ex. 5g	4	0.36,0.65,0.78,0.82
	Aq.Ex.10g	5	0.36,0.52,0.63,0.68,0.75
3.	Undeveloped bulb		
	Met. Ex.	6	0.25,0.31,0.41,0.45,0.54,0.58
4.	Root		
	Met. Ex.	9	0.24,0.28,0.36,0.4 ,0.48,0.52 ,0.56,0.64 ,0.68

The total antioxidant capacity test (TAC) is based on the reduction of Mo (VI) to Mo (V) by the extract and formation of green phosphate/Mo (V) complex at acidic pH. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity. Extracts were subjected to the evaluation of antioxidant activity. All the plant parts were shown antioxidant activity.

DISCUSSION

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibers to protect human against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour and colour. Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example, alkyl sulfide (found in onions and garlic),

carotenoids (from carrots), and flavonoids (present in fruits and vegetables) [22]. Reactive oxygen species (ROS) have been implicated in many diseases and in the aging process. These free radicals, which cause tissue damage via oxidative stress, are usually generated by aerobic respiration, inflammation, and lipid peroxidation. Antioxidant systems minimize or prevent the deleterious effects of the ROS [23]. Due to the medicinal values of garlic, it is important to determine some of the phytochemicals presents. The medicinal value of the plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [24]. In our present study of *A. sativum*, the phytochemical investigation on the aqueous & methanolic extract of *A. sativum* leaves; developed bulb and methanolic extract of undeveloped bulb and roots which indicates the presence of the rich amount of secondary metabolites such as

alkaloids, flavonoids, glycosides, cardiac glycosides tannin, phenolic compounds, saponins, terpenoids and steroids. This is in agreement with the work done by Idowu *et al.* [25].

Secondary metabolites especially alkaloids, flavonoids, saponins, and tannins are known to have curative activity against several pathogens [26]. These compounds are known primarily for their role in reducing the permeability of blood capillaries and strengthening their resistance [27]. The presence of alkaloids in *A. sativum* extract in this study shows the potential of the extract to have an analgesic, anti-inflammatory and adaptogenic effects, which may help the host (man and animal) to develop resistance against disease and endurance against stress [28].

Flavonoids are water soluble polyphenolic molecules and therefore belong to the polyphenol family. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and herbs. Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Flavonoids detected in *A. sativum* could be used in the treatment of various disease conditions like edema, toothache, fever, common cold, diarrhea and dental caries. These could be possible as the root extracts contain some antibacterial activities. The flavonoids are acting on bacteria by inhibiting its protein synthesis [29].

Saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties and their hemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities [30]. Saponins cause a reduction of blood cholesterol by preventing its reabsorption [31]. Plants produce saponins to fight infections by parasites and in humans saponins help the immune system and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity, which may result in reduced risk of cancer and heart diseases [31].

Tannins may elicit antibacterial activities via cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions [32]. They are also reported to have physiological effects like anti-irritant, anti-secretolytic and anti-parasitic effects. Plants containing tannins are used to treat non specific diarrhea and inflammation of the mouth [33,34]. The rich tannin content present in garlic leaves, undeveloped, developed and roots of methanolic and aqueous extracts. Our results shown phenolic components present in leaves, root undeveloped bulb, developed bulb of garlic in both aqueous and methanolic extracts. Phenolic compounds are secondary metabolites normally synthesized by plants during development or in response to stress conditions [35,36].

There were different bands and spots observed in the aqueous and methanolic extract. Aqueous extraction of

garlic leaves shows five spots whereas methanolic extraction shows six spots. Therefore, it may be concluded that solvent used for extraction influences the presence or absence of bioactive components. Further, besides this concentration of plant used for extraction also influences the results.

CONCLUSIONS

This study shows that aqueous and methanolic extract of *A. sativum* leaves, bulb, and roots contain important and active phytochemical compounds, which justify the various therapeutic uses attributed to it in folklore medicine. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, and tannins. Phenolic compounds in general and flavonoids in particular have the ability to provide protection against oxidative stress. Thus in this study, the presence of flavonoids and phenolic compounds in the extract could be considered responsible for conferring antioxidant ability.

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