RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF RECENT RESEARCH IN PHARMACY

IJRRP

AQUEOUS EXTRACT OF *ALLIUM SATIVUM* (GARLIC) LEAVES FOR ANTI-FUNGAL ACTIVITY

Sunny Patel[*1], Prakash Chandra Tiwari[2], Rohit Kumar Bijauliya[3] and Dinesh Kumar Prajapati[1]

[1]Department of Biotechnology, Invertis University, Bareilly-243123 (Uttar Pradesh), India
[2]Rohilkhand College of Paramedical Sciences, Bareilly International University, Bareilly-243006 (Uttar Pradesh), India
[3]Department of Pharmacognosy, BIU College of Pharmacy, Bareilly International University, Bareilly-243006 (Uttar Pradesh), India

Received- 10/July/2020 **Revised-** 27/July/2020 **Accepted-** 11/August/2020 **Published-** 30/September/2020

ABSTRACT

Garlic (*Allium sativum)* the Liliaceae family, is among the oldest of all cultivated plants. It has been used as a medicinal agent for thousands of years. The crude aqueous extract of leaves of *Allium sativum* (Liliaceae) was using extraction of plant, preliminary phytochemical investigation, Chromatographic technique, and antifungal activity. An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of leaves physiochemically. TLC fingerprint profiling and antifungal activity is reported. Results show that plant rich in tannin and phenolic compounds have been shown to posse's antifungal activities against a number of microorganisms.

KEYWORDS: Garlic (*Allium sativum*), Cultivated, Extraction, Phytochemical screening, Fingerprint, Chromatographic, Antifungal activity

Corresponding Author

Sunny Patel,
Research Scholar, Department of Biotechnology, Invertis University,
Bareilly-243123 (Uttar Pradesh), India

E-mail: patelsunny0111@gmail.com

Quick Response Code

INTRODUCTION

Herbal medicines are the oldest recognized source of human healthcare. Herbal medicines, Man's presence on this Planet was made possible only because of the essential function of the kingdom of plants. It still serves as a golden symbol to reinforce the amazing symbiosis phenomenon. Medicinal plants existing even before human being made their appearance on the earth [1].

Now-a-days natural products are an important part of the human health care system since the toxicity and resistance of prescription drugs are common concerns. India is one of the 12 leading centres of biodiversity with more than 45,000 different species of plants, 15,000-18,000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines [2]. Phytochemistry or natural product chemistry research is the backbone of herbal industry. For promoting use of herbals in modern medicine, phytochemistry should be envisaged for: [3]

- Isolation, purification, and characterization of new phytoconstituents.
- Use of newly isolated phytoconstituents as "lead" compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity.
- Conservation of lead phytoconstituents into medicinally important drugs.

Plant Profile

Garlic (*Allium sativum*) is one of the oldest of all plants grown. It has been used for over 4000 years as a spice, food, and folk medicine, and is the most widely studied medicinal plant [4]. Garlic is an underground, small crop of bulbs. It is botanically known as *Allium sativum* and is a member of the family Alliaceae or Liliaceae [5].

Figure 1: *Allium sativum* (Garlic) Leaves

Garlic is a perennial bulb that was believed to be native to Central Asia, Siberia and the west of the Himalayas and was cultivated in England from before 1540. Nowadays it is widely grown around the world. The medicinally used part is the lamp. European requirements state that garlic supplements contain no less than 0.45% allicin [6].

MATERIALS AND METHODS

Collection of Plant

The Leaves of *Allium sativum* (Family: Liliaceae) were collected during the month of January 2020 from District- Bareilly, (U.P.), India. The plant voucher specimen no. is DPH/01/20.

Chemicals

The various chemicals are used such as ethanol, chloroform, benzene, n-hexane, ethyl acetate, toluene, formic acid, petroleum ether, sodium & pot. hydroxide, sulphuric acid, hydrochloric acid, nitric acid, glacial acetic acid, iodine silica gel 60-80 mesh and Silica gel 60 F254 precoated Aluminium plates 0.2 mm.

Extraction of Drug

The leaves of Healthy plants of garlic were collected and washed thoroughly in tap water and they are surface sterilized by keeping them in 1% Mercuric Chloride for 5 minutes. They are then ground into a paste in a grinder. A crude extract is prepared from this paste with distilled water and sterilized water in equal proportion (1.0g/1ml) this extract is used as an *Aspergillus niger* and *Candida albicans* (fungi). Pure cultures of these two micro-organisms are prepared by technique of repeated Sub-culturing. These pure cultures are employed in this study [7].

Phytochemical Screening

Preliminary Phytochemical Investigation

Phytochemical tests were done in plant extracts for the detection of presence of different chemical constituents such as alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins and other substances which are responsible for the biological activity. So, the chemical tests are performed in the Aqueous extract (A.E.) of *Allium sativum* for the detection of different chemical constituents:

(A) Test for Carbohydrates

Molisch Test

In Aqueous extract add few drops of α-naphthol solution in alcohol and concentrated H_2SO_4 is added from the sides of the test tube in small amount. Violet ring is formed at the junction of two liquids shows the presence of carbo-hydrates. This is a general test for carbohydrates.

Fehling Test

Mix Fehling's A & Fehling's B solution & heat in boiling water bath to boil for one min. then add equal volume of test solution. Yellow to brick red precipitate occurs which reveals the presence of reducing sugars.

Barford's Test

Test solution and add equal volume of Barford's reagent and then boil for 1-2 min. in boiling water bath & cool. Red precipitate is formed which shows the presence of monosaccharides.

Iodine Test

In test solution add half volume of the test solution of dil. Iodine solution. Blue color is obtained which disappear on heating and appear on cooling which shows the confirmation of starch.

Tannic Acid Test

In test solution add 20% tannic acid solution. Precipitate appear which reveals the presence of starch.

(B) Test for Proteins

Biuret Test

In test solution add 4% NaOH solution and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears which shows the presence of protein. This is the general test for proteins.

Million's Test

In test solution add million's reagent gives white ppt. which on heating white ppt. turn into brick red or the ppt. dissolves giving red coloured solution. That shows the presence of proteins.

Xanthoproteic Test

In test solution add conc. H_2SO_4 gives white ppt. which on boiling gives yellow ppt. add NH_4OH solution which turn into orange ppt. that reveals the confirmation of proteins. This test is for the protein containing tyrosine or tryptophan.

Test for Protein Containing Sulphur

In test solution add 40% NaOH solution and 2 drops of Lead acetate solution. On boiling solution becomes black or brownish due to PbS formation. That shows the presence of protein containing sulphur.

Precipitation Test

In test solution add 5% $HgCl_2$ solution, 5% $CuSO_4$ solution and 5% lead acetate solution. White colloidal ppt. is obtained which shows the presence of proteins.

(C) Test for Amino Acids

Ninhydrin Test

In test solution add equal volume of ninhydrin solution in boiling water bath. Purple or bluish colour appears which reveals the presence of amino acids.

Test for Tyrosine

In test solution add 3 drops of million's reagent gives dark red color which shows the presence of tyrosine.

Test for Cysteine

In test solution add few drops of 40% NaOH and 10% lead acetate then boil. Black ppt. of lead sulphate is formed which shows the confirmation of cysteine.

(D) Test for Alkaloids

Evaporate the aqueous extract & in residue add dil. HCl, shake well & filter. With filtrate perform following tests:

Mayer's Test

To the filtrate add few drops Mayer's reagent, orange brown ppt. obtained which shows the presence of alkaloids.

Hager's Test

To the filtrate add few drops Hager's reagent (Saturated solution of picric acid), ppt. obtained which confirm the presence of alkaloids.

Wagner's Test

To the filtrate add Wagner's reagent (Iodine in potassium iodide), gives yellow ppt. which confirms the alkaloids.

(E) Test for Tannins & Phenolic Compounds

1. To the test solution add 5% $FeCl_3$ solution, deep blue-black colour appear which shows the presence of phenolic compounds.
2. To the test solution add lead acetate solution, white ppt. is obtained which confirms the phenolic compounds.
3. To the test solution add acetic acid. Red color is formed which confirm the phenolic compounds.
4. To the test solution add dil. Iodine solution. Transient red color is obtained which confirms the phenolic compounds.
5. To the test solution add dil. HNO_3, gives reddish to yellow colour which confirm the phenolic compounds.

(F) Test for Glycosides

i) Test for Flavonoids

Hydrolyse the extract with mineral acid (dil. HCl/dil. H_2SO_4):

1. To the test solution or dry powder add lead acetate solution, yellow colour ppt. which confirms the presence of flavonoids.
2. To the test solution or dry powder add increasing amount of NaOH solution, yellow colour appear which becomes decolourises after addition of acid. That reveals the presence of flavonoids.

ii) Test for Cardiac Glycosides

Legal's Test

To the test solution add 1ml. pyridine and sodium nitroprusside, pink to red colour appear which shows the presence of cardiac glycosides.

Keller-Killani Test

To the test solution add few ml of glacial acetic acid and add 1 drop of 5% $FeCl_3$ and Conc. H_2SO_4, reddish - brown colour appear at junction of two liquid layer & upper layer bluish green which reveals that the presence of deoxy sugar.

iii) Test for Anthraquinone Glycosides

Brontrager's Test

To the test solution add dil. H_2SO_4. Then boil and filter, cool the filtrate adds benzene or chloroform shake well & separate the organic solvent. Then add ammonia, ammonical layer shows pink to red colour which confirms the presence of anthraquinone glycosides

Modified Brontrager's Test for C-glycosides

To the test solution add 5% $FeCl_3$ solution and equal amount of dil. HCl. Heat in boiling water bath for 5 min., cool and then add benzene or any other organic solvent shake well properly, separate organic layer and add ammonia solution. Ammonical layer shows pinkish red colour which shows the presence of anthraquinone glycosides.

iv) Test for Saponin Glycosides

Foam Test

To the test solution or dry powder add sufficient water and shake well properly. Persistent foam observed which shows the presence of saponin glycosides.

v) Test for Cyanogenetic Glycosides

Sodium picrate test: Soak a filter paper strip in 10% picric acid then in Na_2CO_3. Place this filter paper in conical flask place moistened powdered drug. Filter paper turns into brick red or maroon which shows the presence the presence of cyanogenetic glycosides.

(G) Test for Steroids

Salkowski Reaction

In test solution add equal volume of chloroform and conc. H_2SO_4, shake properly. Chloroform layer becomes red & acid layer greenish yellow fluorencenses which shows the presence of steroids [8, 9].

Thin Layer Chromatography

Introduction

Thin Layer spread as a thin layer on a rigid supporting plate; & the mobile phase, a liquid is allowed to migrate across the surface of the plate [10]. The Aqueous extract of powdered leaves of *Allium sativum* Linn. was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test.

Experimental Procedure

The procedure for the Thin Layer Chromatography is as follows:

1) Selection of Coating Materials

A large number of coating materials are known. Few important adsorbents used in TLC are silica gel, alumina, Kiesleguhr, cellulose powder. Adsorbent do not adhere to the glass plates, so for the sticking, some binders like gypsum (Calcium Sulphate), Starch are added to the adsorbents.

2) Preparation of Thin Layer on Glass Plates

The adsorbent used for TLC is Silica Gel G. take sufficient quantity of Silica gel G in pestle & mortar and then add sufficient amount of distilled water. Stir continuously with glass rod until it become homogenous & left for about 15 min. if required water, add sufficient amount of water & silica gel slurry transferred in beaker.

The TLC plates should be flat, free from any greasy matter & pour the slurry uniformly so that the layer of slurry on the glass plates should be uniform.

3) Storage & Activation of the Plate

The coated plates were dried in air about 30 min. for removal of water vapour& then placed in hot air oven for activation about 110-120 °C for 1 hour.

4) Application of Samples

Sample volume of 1 to 5µl are applied point wise in TLC & the sample concentration lies in the range .01% to 1.00% & the spot diameter in TLC should not exceed from 5 mm. The sample should be spotted by the capillary tube from the bottom edge of the plate at a distance of 1.5 cm. Spots should be completely dried before the development into the solvent.

5) Developing Solvent System

The choice of the solvent system depends upon the nature of the components of the mixtures to be separated. A number of developing solvents from low polarity to high polarity were tried but the satisfactory resolution was obtained in the solvent system which mentioned in the **Table 3**.

6) Development of Chromatogram

Chromatographic rectangular chamber is used for the TLC development. There are many types of developments possible in TLC but ascending development is more preferable. The suitable solvent system is put to the bottom of the chamber. For the saturation of the chamber, a filter paper is placed three side of the chamber for 1 hour. After saturation, the spotted TLC plate is put along the chamber at an angle of 45°C for the successful development. After the solvent has transferred one half to two-thirds of the length of the plate, then removed from chamber, dried & position of the components are determined by the several ways.

7) Detection of the Components

After removing the plates from the development chamber, dried in air & kept it in the iodine chamber for the detection of spots. The spots which not displayed by the iodine chamber then spray with the 0.5% vanillin solution in dil. H_2SO_4Isabelle [11]. Then the R_f value of the different spots can be determined by the following formula:

$$R_f \text{ value} = \frac{\text{Distance travelled by solute front from origin line}}{\text{Distance travelled by solvent front from origin line}}$$

Where R_f = Retention factor

Antifungal Activity

Microorganisms and Growth Conditions

Investigate fungal strains were prepared in laboratory of Department of Medicinal Microbiology, Bareilly International University, Bareilly, India. The investigate microorganisms were tested against standard antifungal. The standard antifungal used for fungal strains was Griseofulvin and Nystatin.

Determination of Zone of Inhibition Method

In-vitro antifungal activities were examined for aqueous extracts. Antifungal activities of plant part extracts against two pathogenic fungi were investigated by the agar disk diffusion method [12, 13, 14]. Each purified extract were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C.

For the determination of zone of inhibition, fungal strains were taken as a standard antifungal for comparison of the results. All the extracts were screened for their antifungal activities against the fungi *Candida albicans*, and *Aspergillus niger*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Allium sativum* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Control experiments were carried out under similar condition by using nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

RESULTS AND DISCUSSION

Extraction of Plant Materials

Aqueous extract of *Allium sativum* leaves showed in **Figure 2**. The percentage yield of the aqueous extract is given below in **Table 1**.

Table 1: Percentage Yield of Aqueous Extract Garlic Leaves

S. No.	Weight of Drug	% Yield
1	50 gm	7.2

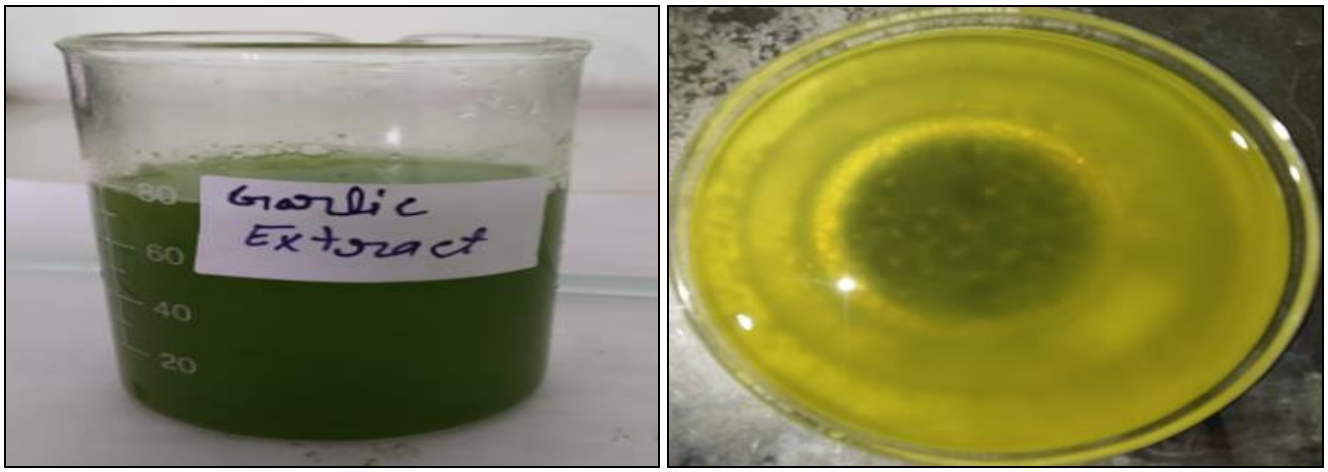

Figure 2: Extraction of *Allium sativum* Leaves

Phytochemistry

Photochemical tests were done in plant extracts for the detection of presence of different chemical constituents such as alkaloids, glycosides, flavonoids, essential oils, carbohydrates, proteins, tannins, and other substances which are responsible for the biological activity. So, the chemical tests are performed in the aqueous extract of *Allium sativum*. For the detection of different chemical constituents are observed in the **Figure 3** and **Table 2** given below respectively.

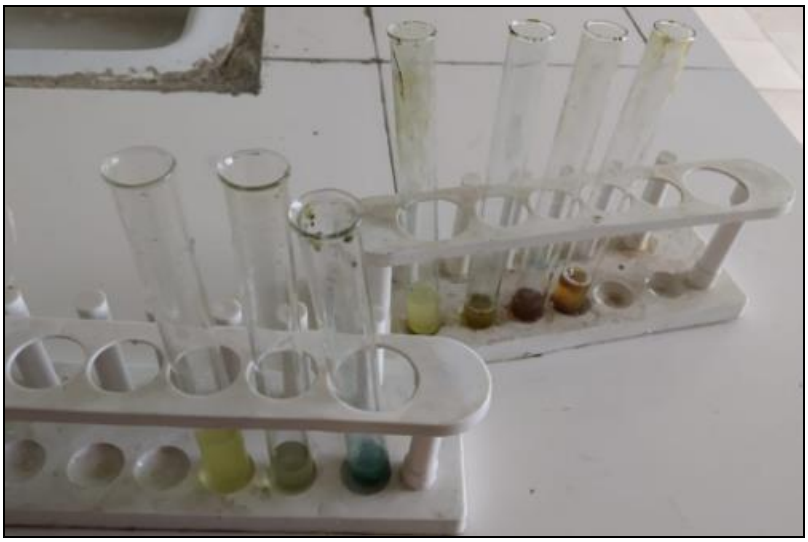

Figure 3: Phytochemistry of *A. sativum* Leaves

Table 2: Data for the Phytochemical Screening of Powdered Leaves of Aqueous extract of *Allium sativum*

S. No.	Test to be performed	Result
	Test for Carbohydrate	
(a)	Molisch test	+ve
(b)	Fehling test	+ve
(c)	Barford's test	-ve
(d)	Iodine test	-ve
(e)	Tannic Acid test	+ve
	Test for Proteins	
(a)	Biuret test	+ve
(b)	Million's test	+ve
(c)	Xanthoproteic test	-ve
(d)	Test for protein containing sulphur	-ve
(e)	Precipitation test	+ve
	Test for Amino acids	
(a)	Ninhydrin test	-ve
(b)	Test for tyrosine	-ve
(c)	Test for cysteine	+ve
	Test for Steroids	
(a)	Salkowski Reaction	+ve
	Test for Alkaloids	
(a)	Mayer's test	-ve
(b)	Hager's test	-ve
(c)	Wagner's test	-ve
	Test for tannins & phenolic compounds	
(a)	Tannins & phenolic compounds	+ve
	Test for Glycosides	
	(a) Test for flavonoids	
(a)i	Lead acetate test	+ve
(a)ii	Sodium hydroxide test	-ve
	(b) Test for Cardiac Glycosides	
(b)i	Legal's test	-ve
(b)ii	Keller Killiani test	-ve
	(c) Test for Anthraquinone glycosides	

(c)i	Bontrager test	-ve
(c)ii	Modified Bontrager test	-ve
	(d) Test for Saponin glycosides	
(d)i	Foam test	+ve
	(e) Test for Cyanogenetic glycosides	
(e)i	Sodium picrate test	-ve

Chromatographic Studies

TLC plate showing the 8 spots with different colour with different R_f value in 0.5% vanillin in dil. H_2SO_4 in solvent system (Chloroform: Benzene: few drops of formic acid) in a ratio of 7: 3: few drops of formic acid.

Detecting Reagents

0.5% Solution of Vanillin in dil. H_2SO_4 and Ethanol (4:1). The seven spots with different R_f value have been obtained in TLC of Aqueous extract of *Allium sativum* Linn. Leaves.

The best solvent system for TLC of AEAS is Chloroform: Benzene: Formic acid (7: 3: few drops). TLC of AEAS shows the presence of seven compounds with different R_f values in different colour using the 0.5% Vanillin in dil. H_2SO_4 as detecting reagent that suggests the presence of seven compounds in the extract.

Figure 4: Thin Layer Chromatography of *Allium sativum* leaves

Table 3: TLC of Aqueous Extract of *Allium sativum* Leaves

Extract	Solvent System	No. of Spots	Colour of Spots	R_f value
Aqueous Extract	Chloroform: Benzene: Formic acid (7: 3: few drops)	8	Yellowish green	0.09
			Yellowish green	0.10
			Light Green	0.42
			Dark Green	0.52
			Dark Green	0.53
			Light Yellow	0.82
			Dark Green	0.83
			Dark Green	0.84

Antifungal Activity

The Antifungal activity of the extracts of *Allium sativum* were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against two fungal strains (*Aspergillus niger* MTCC 282, *Candida albicans* MTCC 227). These strains have been selected for the basis of its application purpose of further formulation study. Antifungal potential of extracts was assessed in terms of zone of inhibition of fungal growth. The results of the antifungal activities are presented in **Tables 4** to **5**. The antifungal activities of the extracts increased linearly with increase in concentration of extracts (µg/ml).

Table 4: Antifungal Activities of Aqueous Extracts of Leaves of *Allium Sativum* against Fungal Test Organism

Antifungal activity (Zone of inhibition)						
S. No.	Fungal Strain	*Allium sativum* - Zone of inhibition in mm Concentration in µg/ml Aqueous extracts (µg/ml)				
		5	25	50	100	250
1	*Aspergillus niger*	-	14	16	18	20
2	*Candida albicans*	-	15	17	19	20

Values are mean ± SD of three parallel measurements: - = No zone of inhibition

Table 5: Antifungal Activity of Standard Drugs against Fungal Test Organism

Antifungal activity (Zone of inhibition)				
S. No.	**Drug**	**Concentration in (µg/ml)**	**Zone of inhibition in mm**	
1	Griseofulvin	5	*A. niger*	*C. albicans*
		25	15	15
		50	17	18
		100	18	19
		250	20	20
2	Nystatin	5	13	14
		25	15	15
		50	18	17
		100	19	18
		250	20	20

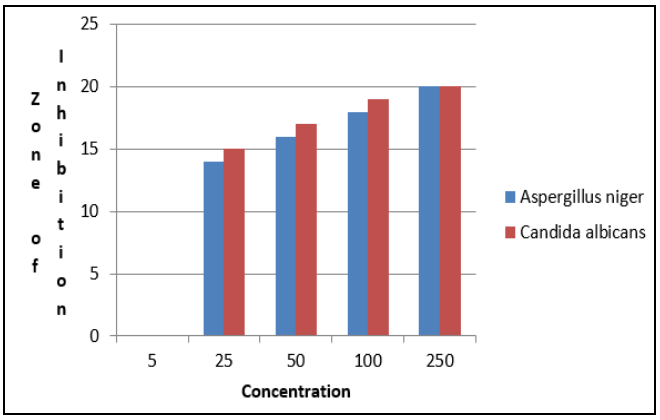

Figure 5: Antifungal Activities of Aqueous Extracts of Leaves of *Allium Sativum* Against Fungal Test Organism

As compared with standard drugs, the results revealed that in the extracts for fungal activity, *C. albicans* shows good result as compare with *A. niger*. The growth inhibition zone measured ranged from 14 to 20 mm for fungal strains **Figures 5**. The results show that the extracts of *Allium sativum* were found to be more effective against all the fungus tested.

CONCLUSION

The leaves of the plant were extracted with Water and then aqueous extract of leaves extract of *Allium sativum* was subjected for phytochemical screening for the detection of various plant constituents it is found that Steroids and flavonoids compounds are present as major active principle.

The best solvent system for TLC of *Allium sativum* is Chloroform: Benzene: few drops of formic acid TLC of *Allium sativum* shows the presence of eight compounds with different R_f values in Different colour using the 0.5% Vanillin in Dil. H_2SO_4 detecting reagent which suggests that the presence of eight compounds in the extract. Overall study reveals that the aqueous extract of leaves of *Allium sativum* shows the mild to moderate antifungal activity with comparison to nystatin and Griseofulvin shows good antifungal activity.

ACKNOWLEDGEMENT

I would like to thank my mentor and supervisor Mr. Prakash Chand Tiwari (External Guide), Mr. Rohit Kumar Bijauliya (External Co-guide) and Dr. Dinesh Kumar Prajapati (Internal Guide) for his good supervision, advice and guidance from the very early stage of this research as well as giving me extraordinary experiences throughout the work.

Above all and the most needed, he provided me unflinching encouragement and support in various ways. His truly scientist intuition has made him as a constant oasis of ideas and passions in science, which exceptionally inspire and enrich my growth as a student, a researcher and a scientist want to be. I am indebted to him more than he knows.

CONFLICT OF INTEREST

None

REFERENCES

1. Kokate CK, Purohit AP and Gokhale SB. "Textbook of pharmacognosy". Nirali Prakashan, Pune, Edition IVth, 1996.
2. Mukherjee PK, Sahu M and Suresh B. "Indian herbal medicines". The Eastern Pharmacist, XLI, 1998: 490, pp. 21-23.
3. Bhanu PSS and Zafar R. "Herbal drugs". The Indian Pharmacist (TIP), 2003; II (12), pp. 13-6.
4. Milner JA. "Garlic: its Anticarcinogenic and Antitumor Properties". Nutr. Rev. (NV), 1996; 54, pp. S82-86.
5. Parle M and Kaura S. "A hot way leading to healthy stay". Int Res J Pharm (IRJP), 2012; 3(6), pp. 21-5.
6. http://www.globalherbalsupplies.com/herbinformation/garlic.htm
7. Gunda MM. "Studies on antimicrobial activity of garlic (*Allium sativum* L.) Extract against *Bacillus subtilis* and *aspergillus niger*". International Journal of Current Advanced Research (IJCAR), 2018; 7(1G), pp. 9169-9171.
8. Khandelwal KR. "Practical Pharmacognosy Techniques and Experiments". Nirali Prakashan 2007; 18, pp. 149-153.

9. Kokate CK, Purohit AP and Gokhale SB: "Pharmacognosy". Nirali Prakashan 2003; 23, pp. 115-116.
10. Bailey LC. "Remington The Science & Practice of pharmacy". Indian edition: Edition 20th, 2000: 1, pp. 606-609.
11. Kagan IA and Flythe MD. "Thin-layer Chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds". J Vis Exp. (JVE), 2014; 85, pp. 51411.
12. Alzoreky NS and Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int J Food Microbiol. (IJFM), 2003; 80, pp. 223–30.
13. Bauer AW, Kirby WMM, Sherris JC and Turck M. "Antibiotic susceptibility testing by standardized single disc method". Am J Clin Patrol (AJM) 1966; 36, pp. 493–6.
14. Rios JL Recio MC and Villar A. "Screening methods for natural products with antimicrobial activity: A review of the literature". J Ethnopharmacol. (JE) 1988; 23, pp. 127–49.

How to cite this article:

Patel S, Tiwari PC, Bijauliya RK and Prajapati DK. "Aqueous extract of *Allium sativum* (Garlic) leaves for anti-fungal activity". International Journal of Recent Research in Pharmacy (IJRRP), 2020; 1(1A), pp. 131-139.