"Determination of Alliums: In Alliums Sativum (L.) Using Thin Layer Chromatography and High Performance Liquid Chromatography"

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Abstract:

Background: Garlic contains approximately 33 sulfur compounds (aliin, allium, allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, and others), several enzymes (allinase, peroxidases, myrosinase, and others), 17 amino acids (arginine and others), and minerals (selenium, germanium, tellurium and other trace minerals). These compounds possess antimicrobial, anticancerous, antifungal etc

Aim and Objective: "Determination of Allium: In Allium Sativum (L.) Using Thin Layer Chromatography and High Performance Liquid Chromatography"

Material and Methods: The plant bulb of *Allium sativum* was collected from the Medicinal garden of Rama Medical College Hospital and Research Centre, Mandhana, Kanpur. The Identification of the plant specimen, *Allium sativum* was done from the "Botanical Survey of India, Central National Herbarium, Howrah." The bioactive compound was isolated from Allium sativum by using multiple steps of purification and identified by rota evaporator, silica gel column chromatography, thin layer chromatography and High Performance Liquid Chromotography (HPLC).

Results: From A. sativum , organosulfur compounds 'Allium' was isolated which was the major compound in garlic also. It shows antibacterial properties. Rt values for allium was found to be 2.93. The compound gave good resolution value.

Conclusion: The present study was successful in isolating the pure phytomolecule Allicin from the plant of Allium Sativum (L.) We have run the TLC of *Allium sativum* purified compound and obtained the Rf values with the use of suitable solvent system.

Key words: Allium, Allium Sativum, Thin Layer Chromatography and High Performance Liquid Chromatography

Introduction

Garlic is grown all over the world and is used in various forms as food, spices, and medicine. Garlic is an indigenous herb of Western Asia and Mediterranean where it has been cultivated for centuries [1]. Recent taxonomy revisions place garlic in the family Alliaceous, which is made up of approximately 700 Species [2]. It belongs to the genus Allium. Allium species are rich in sulfur containing compounds that have been identified to be responsible for their characteristic odor, flavor variation, and biological activities [2].

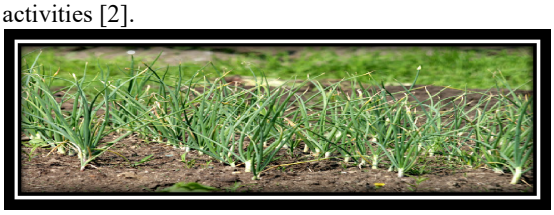

Figure 1: Garlic grown in the Medicinal Garden of Rama Medical College

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Garlic contains high levels of sulfur, zinc, phosphorus, and potassium; moderate levels of selenium, vitaminA, and vitamin C; and low levels of iron, manganese, calcium etc have been identified and isolated [3]. Consumption of large amount of garlic is associated with reduced cancer risk in humans, mostly stomach and colon cancer [4]. In addition to A. sativum, allicin, ajoene and other organo sulfides are present in A. hirtifolium and play important pharmacological roles [5]. These phytomolecules present in Garlic plays a important role in fighting against diseases caused by bacteria, virus, fungi or protozoa. This study aims in isolating Allium from A.sativum.

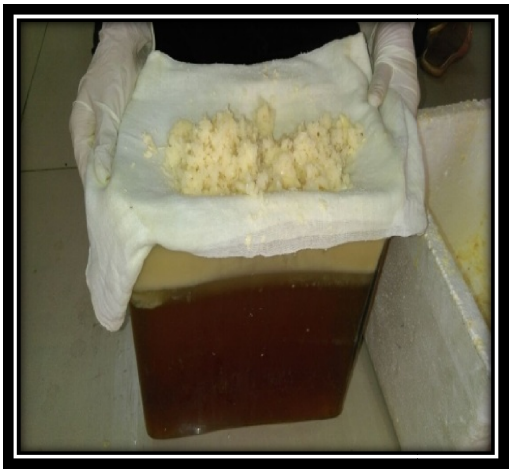
Material and Methods

The study was carried out in the Department of Microbiology and Central Research Laboratory of Rama Medical College Hospital and Research Centre, Mandhana, Kanpur. In this study plant bulb of *Allium sativum* was collected from the Medicinal garden of Rama Medical College Hospital and Research Centre, Mandhana, Kanpur [fig 1]. The Identification of the plant specimen, *Allium sativum* was done from the " Botanical Survey of India, Central National Herbarium, Howrah."

Isolation of Bioactive Compounds from cloves of *Allium sativum*: Fresh garlic cloves was grinded to

make it fine slurry and then subjected to Hexane extraction with 1:3 (w/w). It was mixed vigorously and kept for overnight at RT. Then Filter the mixture with double layer of Muslin cloth. Filtrate and solid residue was collected. Solid residue was again subjected for the Hexane wash 1:3 (w/w). and again repeat the procedure 2 times by folding the muslin cloth. Now, the solid residue was assorted with methanol and water 1:1 (W/W). Mixed vigorously and kept for overnight at RT. Filter the mixture with double layer of Muslin cloth, Compose filtrate and solid residue then Solid residue was again performed for the methanol and water wash 1:1 (W/W). Repeat this procedure two times by folding the muslin cloth. Filtrate was again composed and has stored the solid residue. [Fig 3]

Figure 2: Fresh Garlic collected from Medicinal Garden of Rama Medical College



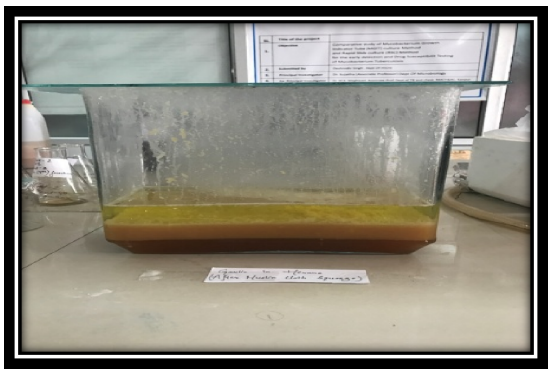


Figure 3:(a.)Fine Garlic slurry for fatty acid removal, (b) Double layer of Muslin cloth Filtration, (c) Garlic subjected to Hexane

Rota Evaporator concentration: All the filtrate was collected and concentrated with the help of Rota-evaporator.[fig 4] The crude concerted extract was lyophilized and stored at -20oC [6].

Figure 4: Rota evaporator concentrating *Allium sativum* extract

Portioning of *Allium sativum* extract [fig 5]: The solid mass of the extract was lyophilized inside lyophilizes, it was partitioned with the use of chloroform inside inverted funnel, the ratio of the concentrated solid mass and chloroform (w/w) was 1:1. The development of chloroform extraction was recurring for 4 times so that maximum compounds get transfer in the chloroform layer. After the mixing and standing the funnel, chloroform layer was together and dried entirely. From the dried compounds we equipped a column and proceeded through column chromatography [**7**]

Figure 5: Portioning of *Allium sativum* extract with chloroform

Purification of Allium sativum extract [fig 6a]*:* A silica gel 120 column was packed in hexane. The column length was 125 cm and its diameter was 3.5 cm. A step gradient (0%, 5%, and 10%, till 100% of ethyl acetate) of hexane and ethyl acetate was applied to purify the crude extract. In each step gradient 5 fractions of 100ml each were eluted [8]

Figure 6: (a) Glass column used as column chromatography for the purification of *Allium sativum (b)* Thin Layer Chromatography of Allium sativum (c) High Pressure Liquid Chromatography of *Allium sativum* extract

Thin Layer Chromatography (TLC)[fig 6b]: Thin Layer chromatography of all the inaccessible fractions were passed out by mobile phase, chloroform: acetone: formic acid (9:2:1, V/V) and sprayed by ansaldehyde: water: acetone: perchloric acid in the ratio of (0.025:4:1:0.5) [9].

High Pressure Liquid Chromatography (HPLC)[fig 6c]: HPLC of the inaccessible extract was conceded out by Nova Pak C-18 (4µm) column (3.9X150mm) using a HPLC (Agilent Technologies) system, with a mobile phase retaining methanol and water (HPLC grade) with a linear gradient, flow rate of 0.75 µl/min and eluted compound were observed at 286-400 nm

Results

The purified compound was analyzed with the use of TLC and HPLC chromatograms. In the extract of A. *sativum* there was mainly one compound obtained. The compound was *Allium* which was one of the major organosulfur compounds in garlic measured to be biologically dynamic. The compounds was identified on the basis of its Rf values from the available literature. The Rf value of Allium was 0.9.

High pressure liquid chromatography (HPLC) was performed for the separation of Allicin. HPLC fitted with C_{18} columns was applied in this work. The compound was separated well under HPLC conditions. HPLC of *A. sativum* was recorded to check the purity of compound got their Rt values with the use of proper solvent system. Rt values for allium was found to be 2.93

The compound gave good resolution value. The following chromatogram was obtained; [fig 7]

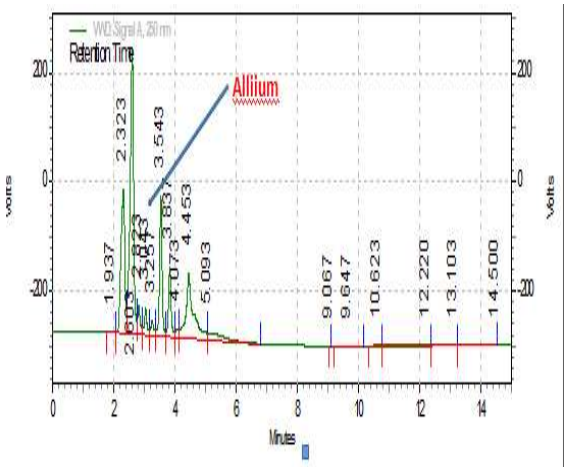


Figure 7: HPLC Chromatogram of purified compounds from Allium

Discussion

In this research TLC was run of *A. sativum* purified compound and obtained the Rf value with the use of appropriate solvent system. Comparable type of work also has been done by several co-worker of the same ground on the same plant and recorded in [Table 1].

Table 1: TLC of *A. sativum* extract

Sr. No.	Author	Compounds name	Rf value	Solvent system	Year
1	Anitha Vedikeetil et al.[10]	Allium	0.80	ethyl acetate	2015
2	Veena Sharma et al., [11]	Allium	0.79	Ethyle Acetate: Methanol, (7:2)	2018
3	Present study	Allium	0.9	Chloroform: acetone: formic acid (9:2:1, V/V)	2018-19

During the study we have checked the purity of the compound Allicin by HPLC of A. sativum purified compound and got the Rt values with the use of proper solvent system. The parallel type of work was also conducted by many other co-worker of the same field on the same plant as recorded below.

Table 2: HPLC of A. sativum extract

Sr. No.	Author	Compounds name	Rt value	Solvent system	Year
1	Eric Block et al., [12]	Allium	3.05	Methanol water Formic acid	1992
2	NISHU SEKAR et al., [13]	Allium	2.96	Methanol water	2015
3	Present study	Allium	2.93	Methanol water gradient	2018-19

Conclusion

Most of plants contain many of active compounds such as flavonoids, tannis, saponins, alkaloids, terpenes, heavy metals. Plants can be used as potent agent for the treatment of infectious diseases. The present study was successful in isolating the pure phytomolecule Allicin from the plant of Allium Sativum (L.) We have run the TLC of *Allium sativum* purified compound and obtained the Rf values with the use of suitable solvent system.

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