



Research Paper

**ANTI-INFLAMMATORY AND ANTI-MICROBIAL ACTIVITY OF ALLIINASE
EXTRACTED FROM *Allium sativum* AND *Allium cepa***

Lalitha, K.S., S.F. Maleeka Begum and R. Ragunathan¹

Department of Biotechnology,
Sri Krishna Arts and Science College, Coimbatore-08,
India.

¹Director, CBNR, Coimbatore, India.

Abstract

To extract allicin and alliinase from *Allium sativum* and *Allium cepa*. To study the basic phytochemical analysis. To determine anti-inflammatory study. Confirmation of allicin using TLC. To purify the compounds using affinity chromatography (DEAE cellulose). To evaluate the antibacterial activity against the bacteria. To estimate the alliinase activity using standard enzyme.

Key words: alliinase, allicin, *Allium sativum*, *Allium cepa*.

INTRODUCTION

The genus *Allium* is a very large genus (c. 750 species) and is widely spread over the holarctic region from the dry subtropics to the boreal zone. Alliinase (alliin lyase EC 4.4.1.4) has been reported to occur in many plants of the genus *Allium* such as garlic (*Allium sativum*), onion (*Allium cepa*), leek (*Allium porrum*) (Hughes *et al.*, 2005). One of the major protein fraction found in *Allium* is alliinase. The official name for this enzyme is alliin alkyl-sulphenate-lyase. (Randle W. 2000) Alliinase is a glycoprotein and shows a homodimeric organization with subunits of 448 amino acid each, corresponding to a total molecular weight of about 103kDA. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as *Escherichia coli* (Abdulzahra *et al.*, 2014). Onion have been widely reported to show their antimicrobial activities against Gram negative and Gram positive bacteria (Sridhar *et al.*, 2011). In the last few years, alliinase had found its application in agriculture, pharmaceutical and food industry.

MATERIALS AND METHODOLOGY

Collection of sample:-

Garlic and onion species were purchased from local markets. (Vadavalli and Madukarai)

Extract preparation:-

2g of the sample was dissolved in 20ml distilled water and was placed in the shaker for 30 minutes, filtered using muslin cloth. To the filtrate, 20ml acetone was added and left in the shaker for 30 minutes, filtered and added 20ml of chloroform to it, agitated and filtered. The collected extracts were used for analysis.

Identification of compounds present in the extract

Phytochemical analysis (Havsteen, B.H. 2002)

The phytochemical screening of the extract were performed by standard qualitative procedures: Mayer test for alkaloids, Harborne test for terpenoids, Shinoda test for flavonoids and Liebermann Burchard for steroids.

Thin layer chromatography (Harborne JB, 1973)

The sample was processed by placing crushed extract in 10ml of ethanol for 30 minutes, followed by addition of 5ml n-butyl and isopropanol and left undisturbed for an hour. The solvent used in the TLC chamber was isopropanol and n-butyl in the ratio 3:1. The paper was placed in the TLC chamber and allowed to stand till a clear band was observed. Then the plate was treated with iodine and incubated. Later Rf value was calculated.

Uv-characterization

The extract containing isopropanol and n-butyl were chosen for UV-visible spectrophotometer (300 to 550nm) analysis.

Anti bacterial studies [Malu *et al.*, 2009]

Anti-bacterial activity was performed using turbidity method. The nutrient broth was prepared and 10µl of *Bacillus subtilis* was inoculated in all the tubes. The compound (sample in isopropanol and n-butyl) was transferred into the test tubes with varying concentrations (25µl,50µl,75µl,100µl) except control. They were incubated at 37°C overnight and the test tubes were checked for growth and the values were measured using spectrophotometer. The growth curve was estimated at 600nm.

Anti-inflammatory studies [Sakat *et al.*, 2010]

Anti-inflammatory was studied under in-vitro conditions using Anti-proteinase action. To 0.06mg of trypsin, 1ml of 20mM Tris HCL was added. The solution was mixed well and 1ml of the sample was added, incubated at 37°C for 10 minutes. After incubation, 1ml of 0.8% casein solution was added and was incubated for 20 minutes. After 20 minutes of incubation, 2ml of 7% perchloric acid was added and mixed well. Now these were centrifuged at 5000rpm for 5 minutes. Then the OD values were taken at 210nm.

Purification of alliinase:-

The sample in isopropanol and n-butyl were purified. 10ml of isopropanol was added and this was allowed to evaporate for few days. The compound was scrapped using phosphate buffer and was stored.

DEAE cellulose chromatography [Sagar *et al.*, 2016]

The crude sample was further purified using DEAE cellulose column and various fractions were collected and OD values were read at 280nm.

SDS page [Sagar *et al.*, 2016]

Alliinase during the course of purification was analysed using 10% SDS-PAGE. Broad range protein marker was used as a standard marker. After separation, the proteins were detected using CBB staining.

GC-MS [THERMO GC- TRACE ULTRA VER: 5.0, THERMO MS DSQ II]

The chromatographic column used here is DB 35- MS CAPILLARY STANDARD and which is NON-POLAR of internal diameter 0.25mm. The stationary phase coated over the chromatographic column is 0.25µm. Helium is used as carrier gas and the flow rate was 1.0 ML/Min. Initially the temperature was 70 C and was raised to 260 C at an interval of 6 C /MIN. The volume of sample injected is 1 micro liter.

Enzyme assay [Jianquin Zhou, 2009]

1g of the standard alliinase was dissolved in 5ml phosphate buffer. To 1ml of the samples (crude extract and the fraction obtained from affinity chromatography) 20µl

standard with phosphate buffer was added. Then the solutions were incubated at room temperature for 5 minutes. To the solutions TCA was added and checked for precipitation. Then 1ml of 1N sodium hydroxide was added and incubated at room temperature for 5 minutes. The OD values were measured at 445nm.

RESULTS

The data pertaining to phytochemical analysis of the selected sample *Allium sativum* and *Allium cepa* are presented in Table 4.1. Qualitative analysis were performed using thin layer chromatography for both Allium species. Rf value for garlic and onion is 0.71 and 0.56 respectively. Rf factor is calculated using the formula:

$$\text{Retention factor} = \text{solute} / \text{solvent}$$

Organic molecules present in the extract were identified using UV-visible spectrophotometer from minimum range of 300nm to maximum range of 550nm. (Fig. 4a) Anti bacterial activity was performed using turbidity method and the growth curve was estimated at 600 nm. (Fig. 4b) The percentage of inhibition (Fig. 4c) was calculated using the formula:

$$\% \text{ percentage of inhibition} = (C - T/C) \times 100$$

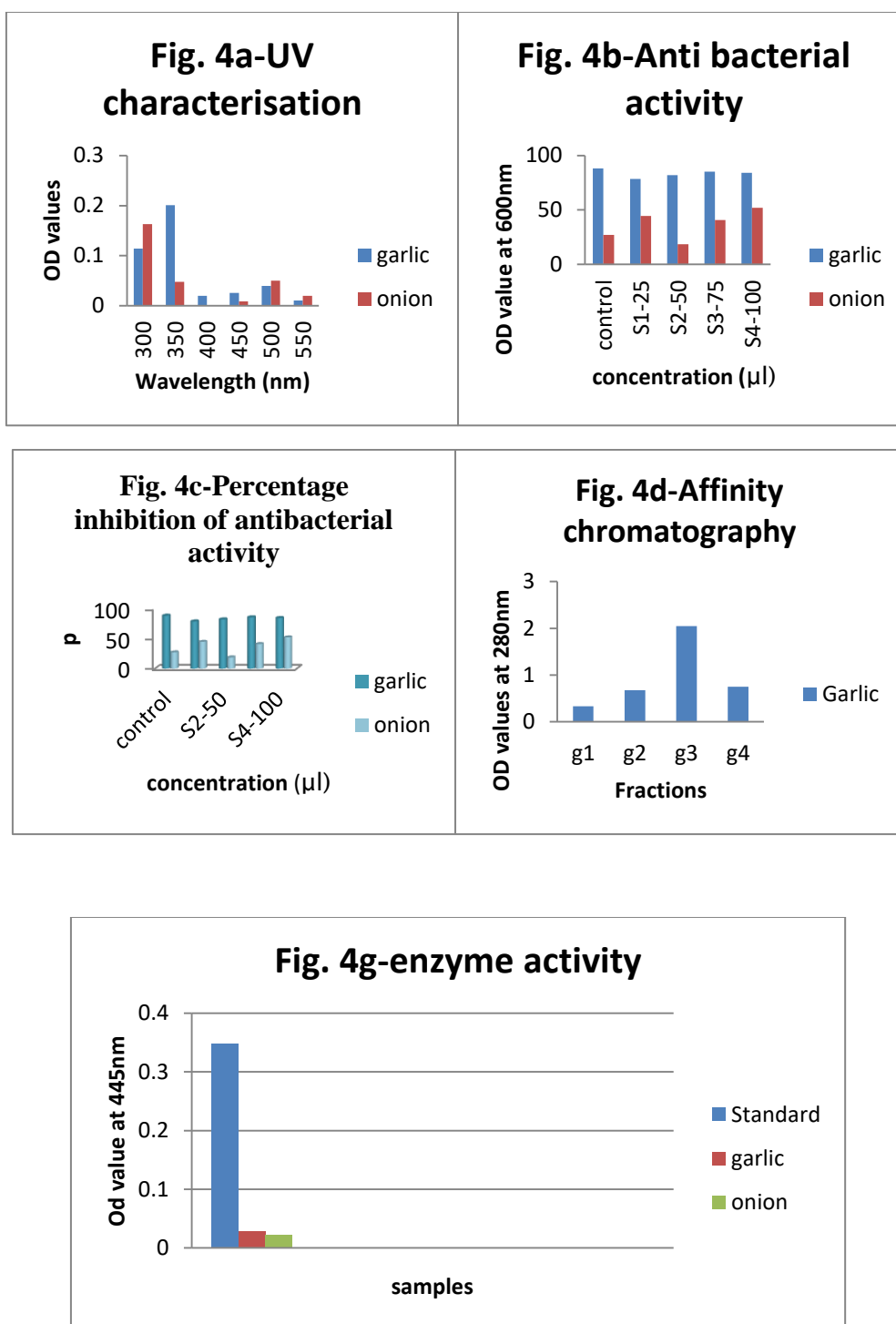
Anti-inflammatory were studied under in-vitro condition using Anti-proteinase action. The OD values for garlic and onion were estimated at a range of 210 nm and were observed to be 0.434 and 0.109 respectively. Percentage efficiency of anti-inflammatory activity (Fig.4c) was calculated using the formula:

$$\% \text{ efficiency of anti-inflammatory} = (\text{control of absorbance} - \text{sample absorbance} / \text{control}) \times 100$$

The column chromatography was performed by DEAE cellulose. The OD values were scanned at 280nm. (Fig.4d) To analyze the molecular weight of the enzyme, SDS-page was performed. The molecular weight of the enzyme was found to be 66kDa. (Fig.4e) The enzyme activity was performed using standard Allinase enzyme. The OD values were scanned at 445nm. (Fig. 4g)

Table 4.1- Phytochemical analysis of *Allium sativum* and *Allium cepa*.

S.no	Allium species	Alkaloids	Terpenoids	Flavonoids	Steriods
i)	Onion	+	+	+	+
ii)	Garlic	+	+	+	+



DISCUSSION

Phytochemical studies were performed to analyse the presence of various constituents. Alkaloids involve in relaxation of muscles and nasal congestion. Terpenes are psychoactive chemicals that are found similar to cannabis which aids in further investigation on anti-inflammatory activity. Flavonoids possess both bacteriostatic and bacteriocidal effects on some strains of bacteria that helps in investigation of antibacterial studies and furthermore it inhibits the activity of reverse transcriptase and protease.(Gazuwa et.al., 2013) Thin layer chromatography was performed and the R_f value for garlic and onion is 0.71 and 0.56 respectively. Thus, this indicates the presence

of purity of extract. The extract was taken for further analysis such as UV absorption assay which reveals the presence of following substance at a varied range of wavelength. Antibacterial studies were performed and the maximum inhibition was found in *Allium sativum* at a concentration of 75% and the percentage efficiency was 85.22 compared to *Allium cepa*. Similarly anti inflammatory studies were performed and the efficiency was found to be high in *Allium sativum* and the percentage efficiency was 40.95. DEAE cellulose chromatography was performed and the purified compound was maximum at the third fraction when compared with other fractions. Sagar *et al.*, 2016 performed DEAE cellulose chromatography on *Cupriavidus necator* to purify alliinase enzyme and its elution profile was studied. On comparison of SDS-page results with the work of Sagar *et al.*, 2016, it was observed that the alliinase enzyme extracted from *Cupriavidus necator* has 55kDa whereas the extraction of alliinase from our study for *Allium sativum* is 60kDa. Hans *et al.*, 2014 has illustrated that the enzymes can be detected either qualitatively or quantitatively. The enzymatic assay was spectrometrical scanned at 445nm and qualitative results were obtained. Thus our study reveals that alliinase extracted from *Allium sativum* stands as a potent antibacterial and anti-inflammatory agent.

REFERENCE

1. AbdulzahraDM,MohammedHF. (2014). The Antibacterial Effect of Ginger and Garlic Extracts on Some Pathogenic Bacteria Isolated from Patients with Otitis Media.*Int. Res. J. Medical Sci.*;2:(5).
2. Hans Bisswanger 2014, Enzyme assays. Perspectives in Science 1, 41-55.
3. Harborne JB.(1973). Phytochemical methods, London, Chapman and Hall, Ltd.; 49-188.
4. Havsteen, B.H. (2002). The Biochemistry and Medicinal significance of flavonoids, *Pharmaco Ther*; 96(2):67-68.
5. Hughes J, Tregova A, Tomsett AB, Jones MG, Cosstick R, Collin HA (2005). Synthesis of the flavour precursor, alliin, in garlic tissue cultures. *Phytochem*.66: 187-194.
6. Jianqin Zhou, (2009) Immobilization of alliinase and its application: Flow injection enzymatic analysis for alliin. *African Journal of Biotechnology* Vol. 8 (7), pp. 1337-1342, 6 April.
7. Randle W. M, Lancaster J.E. (2000) Chapter 14 „Sulphur compounds in *Alliums*“ In: H.D. Rabinowitch and L. Currah (eds.) *Allium Crop Science-Recent Advantages*, CABI Publishing, Wallingford UK, page 332 – 339.
8. Sagar Chhabria, Krutika Desai, (2016) purification and characterisation of alliinase produced by *Cupriavidus necator* and its application for generation of cytotoxic agent: Allicin. *Saudi Journal of Biological Sciences*, Elsevier (01) 003.
9. Sakat S, Juvekar AR, Gambhire MN. (2010) In vitro antioxidant and anti-inflammatory activity of methanol extract of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Pharmacological Sciences* 2(1):146-155.
10. Sridhar TM,Josthna P, Naidu CV. (2011) *In Vitro*Antibacterial Activity and Phytochemical Analysis of *Solanumnigrum* Linn.- An Important Antiulcer Medicinal Plant. *J of Experimental Sciences*.2(8): 24-29.