RESEARCH ARTICLE

Investigation of Antimicrobial Activity and Characterization of Isolated Allyl Isothiocyanate

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ABSTRACT

Allyl isothiocyanate (AITC) is a natural compound known for its potential antimicrobial properties. In this study, we present a comprehensive investigation into the isolation, characterization, and antimicrobial activity of AITC which is obtained from mustard seeds. The isolation of AITC was achieved through an efficient extraction process, employing extraction assembly. Subsequently, AITC was subjected to a series of physicochemical tests to elucidate the presence of physicochemical constituents. Furthermore, we employed UV-visible spectroscopy and infrared (IR) spectroscopy to obtain valuable insights into the structural features of AITC. To gain a deeper understanding of the phyto-constituents present in AITC, gas chromatography-mass spectroscopy (GC-MS) was used for phytochemical profiling. The antimicrobial activity of AITC was assessed against two pathogenic microorganisms, *Staphylococcus* and *Escherichia coli*. The outcomes of our study reveal the possible potential of AITC as an antimicrobial agent, demonstrating its inhibitory effects on the growth of both microbial strains and zone diameter of inhibitions are 12, 10, and 4 mm for *Staphylococcus* and 14, 12 and 6 mm for *E. coli* and hence the AITC extracts were found to have antibacterial activity. This research provides treasured comprehensive insights into the isolation, characterization, and antimicrobial belonging properties of AITC which is derived from mustard seeds. The findings suggest that AITC has promising applications in the field of antimicrobial research, opening avenues for further investigation into its potential therapeutic uses.

Keywords: Allyl isothiocyanate, Antimicrobial, Isolation, *Staphylococcus, Escherichia coli.*

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INTRODUCTION

Mustard seeds, a widely consumed condiment, have long been recognized for their diverse culinary applications and therapeutic properties. Among the myriad of bioactive compounds present in mustard seeds, allyl isothiocyanate (AITC) stands out as a potent phytochemical with remarkable antimicrobial potential. AITC is a naturally occurring isothiocyanate known for its pungent aroma and notable antimicrobial properties, making it a compelling subject of investigation for various applications, including food preservation and pharmaceuticals.[1,2]

This manuscript presents a comprehensive study on the isolation and characterization of AITC from mustard seeds, coupled with an assessment of its antimicrobial activity against two common pathogenic bacteria, *Staphylococcus* and *Escherichia coli*. Our research endeavors to shed light on the extraction process, elucidate the physicochemical properties of AITC, and explore its potential as an antimicrobial agent.[2-4]

The methodology employed in this study involves the extraction of AITC from mustard seeds using an optimized extraction assembly. Subsequently, the isolated AITC is subjected to a battery of characterization techniques, conducting a comprehensive array of physicochemical assessments encompassing carbohydrates, alkaloids, glycosides, sterols, tannin, phenolic compounds, amino acids, and fixed oil., UV-visible spectroscopy, and infrared spectroscopy, which collectively provide valuable insights into its chemical properties. Additionally, gas chromatography-mass spectroscopy (GC-MS) is utilized to identify and quantify phytoconstituents contemporary in the AITC extract.[5,6]

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Furthermore, the manuscript delves into the assessment of the antimicrobial activity of AITC against *Staphylococcus* and *E. coli*, two bacteria of significant clinical relevance due to their association with various human infections. Understanding the antimicrobial potential of AITC is vital, as it may pave the way for the development of natural and safe alternatives to conventional antimicrobial agents, addressing the growing concerns regarding antibiotic resistance.[7,8]

This interdisciplinary investigation brings together elements of chemistry, microbiology, and pharmacology to explore the isolation, characterization, and potential applications of AITC from mustard seeds. The findings presented herein may contribute not only to the expanding knowledge base in the field of natural product chemistry but also to the development of novel antimicrobial agents with broad applications in the food and healthcare industries.

MATERIAL AND METHODS

Materials

The mustard crop should be sown by drilling method in own farm and obtained mustard seed; create a coarse powder by the grinder. This will increase the surface area for extraction. Methanol, anhydrous sodium sulfate, and dichloromethane were procured from the Loba Chemie Pvt. Ltd, Mumbai. All additional chemicals and reagents employed were of analytical grade and utilized without any alterations and further modification.

Isolation of Allyl Isothiocyanate from Mustard Seed

Accurately weighing 100 g of mustard seed powder was mixed with 100 mL methanol and then boiled for 24 hours at 20 to 40°C in an extraction assembly. Subsequently, the extracted product was allowed to stand for 1-hour with intermittent shaking. Allows it repeated two times in extraction assembly for 24 hours at 20°C for proper isolation. Further, the extract was filtrated through filter paper (Whatman no. 1). The filtrate underwent three extractions with dichloromethane and was then dehydrated using anhydrous sodium sulfate. The myrosinase enzyme facilitates the production of AITC in mustard seeds by catalyzing the release of volatile AITC from the glucosinolate—sinigrin. The rotary evaporator, operated at 33 ± 2°C with a vacuum of 0.096 PM, was employed to remove dichloromethane and other components. Ultimately, the resulting oily residues containing AITC were gathered and analyzed by various physicochemical tests, UV spectroscopy, and DSC.[9-11]

Physicochemical Test

For the confirmation of physicochemical constituents were different physicochemical tests are as performed test for carbohydrates, alkaloids, steroids, sterols, glycosides, saponins, tannin and phenolic compounds, protein and amino acids, fixed oil.[11-13]

Phyto-constituents detection by gas chromatography-mass spectroscopy

For the confirmation of phytoconstituents of isolated AITC and mustard seed extract were detected by gas chromatography-mass spectroscopy (GC-MS) with their retention time, %area, molecular weight, molecular formula and constituent name. High-purity solvents and reagents were employed for sample preparation and analysis.[14,15]

UV–visible spectroscopic analysis

For UV–visible spectroscopic examination, the centrifuged isolated AITC underwent filtration using filter paper after spinning at 3000 rpm for 10 minutes. The resultant filtrate was employed for spectroscopic analysis. The UV-vis spectroscopic characteristics of the isolated AITC were investigated using a Shimadzu spectrophotometer, covering a wavelength range from 200 to 300 nm.[16]

Fourier transform infrared spectroscopy

FTIR spectroscopy studies were conducted using a Shimadzu 8400s instrument from Japan to explore the composition of functional groups within AITC, covering the wavenumbers ranges of 4000 to 400 cm^{-1}. Sample pellets were prepared by combining the AITC with KBr in a 1:100 ratio and subsequently compressed using a motorized pellet press under a pressure of 10 to 12 tons, finally measured for FTIR spectra.[17,18]

Isolation of the Pathogenic Microorganism's Samples

Samples for pathogenic microorganisms, *Staphylococcus* and *E. coli* were grown from the mother culture in nutrient agar. Serial dilution was done and the 10^{-2} dilution was taken for culturing using spread plate technique with a sterile L-rod. The plate was incubated for 24 hours in the incubator for the growth of pathogenic microorganisms.[19,20]

Antimicrobial Study

Analysis of antimicrobial activity proceeds with three petri plates were prepared with potato dextrose agar after which the plates were inoculated with culture using a cotton swab by streak plate method. Four wells were punched with one as blank (distilled water) and the other three for the leaf extracts. In 10 µL of sample (AITC extract was loaded in one petri plate). Subsequently, the plates underwent a 24-hour incubation period, and the presence of zone inhibition was examined. The experimental procedure was carried out in triplicates.[20,21]

RESULT AND DISCUSSION

Physicochemical Test

The mustard seed extracts were tested for their phytochemical contents. The phytoconstituents flavonoids, carbohydrates, phenols, tannins, and alkaloids were present in mustard seed extract. Table 1 shows the qualitative analysis of phytochemicals in mustard seeds. The qualitative analysis of phytochemical AITC from mustard seeds reveals the presence of a diverse array of bioactive compounds (Table 2). These phytoconstituents play essential roles in the plant's metabolism and often contribute to the therapeutic and nutritional properties of the plant. Table 3 summarizes the results of the phytochemical analysis, which include the detection of various classes of phytoconstituents in the mustard seeds extracts. The identified phytoconstituents include carbohydrates, alkaloids, glycosides, steroids and sterols,

Table 1: Qualitative analysis of phytochemicals in mustard seeds

Physicochemical test	*Results*
Test for carbohydrate:	
a) Molisch's test	+++
b) Fehlings test	+++
Test for alkaloids:	
a) Wagners test	---
b) Hagers test	+++
Test for Glycosides:	
a) Legal test	++
b) Baljet test	+
Test for steroids and sterols:	
a) Libermann - Burchard test	++
Test for saponins:	+
Saponin test	
Test for tannin and phenolic compounds:	
Ferric chloride test	+++
Potassium dichromate test	++
Test for protein and amino acids	
a) Biuret test	++
b) Ninhydrin test Amino acids	+++
Test for fixed oil :	
a) Copper sulphate test	+++

+++ - Strong, ++ Moderate, + -low

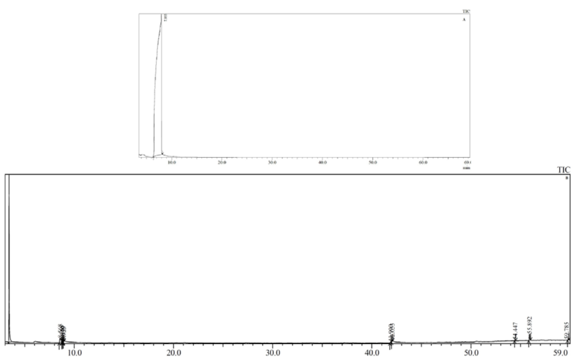

Figure 1: GC-MS spectra of A: isolated AITC and B: mustard seed extract

saponins, tannins, phenolic compounds, proteins and amino acids, and fixed oil. Each of these classes of phytoconstituents can have unique biochemical and biological activities.[18,22]

Carbohydrates

Carbohydrates serve as a primary source of energy in plants and are integral to various metabolic processes. Their presence in the extract highlights the nutritive value of *Brassica nigra* seeds.

Alkaloids

Alkaloids are organic compounds with potential pharmacological activities. Their presence in the mustard seed extract may contribute to its bioactive properties.

Glycosides

Glycosides are secondary metabolites known for their diverse biological activities. They are often implicated in plant defense mechanisms.

Steroids and sterols

Steroids and sterols are essential components of cell membranes and can have physiological significance.

Saponins

Saponins are glycosides with surfactant properties. They are known for their foaming and emulsifying properties and may have health benefits.

Tannins and phenolic compounds

Tannins and phenolic compounds are antioxidants that play a crucial role in protecting plant cells from oxidative stress. They are also known for their potential health benefits.

Proteins and amino acids

Proteins are essential macromolecules in plant physiology and can serve as a source of dietary protein for humans and animals.

Fixed oil

Fixed oils are lipid compounds that can have various uses, including as a source of essential fatty acids or in cosmetic and industrial applications.

The presence of these phytochemicals in *Brassica nigra* seeds underscores their potential nutritional and therapeutic value. AITC, the target compound of this study, is just one of the bioactive constituents found in these seeds. A comprehensive understanding of the phytochemical profile of *B. nigra* seeds is essential for exploring their diverse applications in nutrition, medicine, and industry.[23,24]

The qualitative analysis presented in Table 3 confirms the existence of flavonoids, carbohydrates, phenols, tannins, and alkaloids in the mustard seeds extract. This chemical diversity adds depth to our understanding of the phytochemical composition of mustard seeds and hints at the potential health-promoting properties associated with these compounds. Further quantitative analyses and investigations into the synergistic effects of these phytochemicals with AITC are warranted for a more comprehensive exploration of their biological activities and potential applications.

Phyto-constituents Detection by gas Chromatography-Mass Spectroscopy

The GC-MS analysis has provided valuable data on the phytoconstituents existing in the mustard seed extract and isolated AITC, shedding light on its chemical complexity and potential bioactive compounds Figure 1. Tables 2 and 3 data represent the chemical composition of the isolated AITC and mustard seed extract with data of RT, Area (%), molecular formula, RI^C (calculated) and RI^R (reported) can serve as a basis for further studies exploring the nutritional, medicinal, and industrial applications of mustard seeds and their constituents.[25,26]

UV-visible spectral analysis

Figure 2 depicts the UV spectrum of AITC, isolated from mustard seed extract. The spectrum reveals a prominent absorption band at 240 nm, which is recognized as the characteristic peak associated with AITC. This distinct peak

Table 2: Chemical composition of the isolated AITC

Peak no	*Compound name*	*RT*	*Area (%)*	*Molecular formula*	*RI^C calculated*	*RI^R reported*
	Allyl Isothiocyanate	7.955	91.84	C_4H_5NS	930	--

Table 3: Chemical composition of the mustard seed extract

Peak no	*Compound name*	*RT*	*Area (%)*	*Molecular formula*	*RI^C calculated*	*RI^R reported*
	Isothiocyanic acid, 3-butenyl ester	8.568	10.28	C_5H_7NS	950	--
	Isothiocyanic acid, 3-butenyl ester	8.760	1.65	C_5H_7NS	956	--
	No hit compound	8.840	1.46	--	--	--
	No hit compound	8.925	0.61	--	--	--
	1,E-11,Z-13-Octadecatriene	41.900	0.57	$C_{18}H_{32}$	1777	1817
	(11Z)-11-Pentadecen-1-ol	42.053	0.75	$C_{15}H_{30}O$	1781	1763
	Tetraprenol	54.447	0.59	$C_{20}H_{34}O$	2251	2192
	Pentacosane, 2-methyl-	55.892	5.16	$C_{26}H_{54}$	2293	2542
	Lauron	59.785	0.59	$C_{23}H_{46}O$	2429	2443

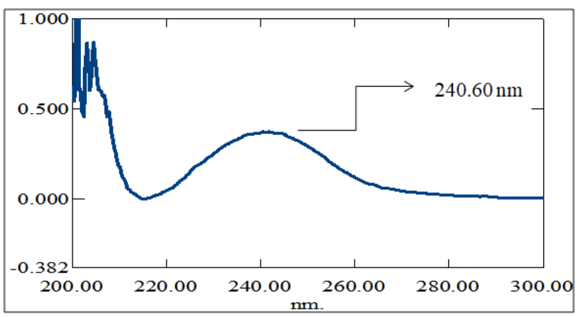

Figure 2: UV spectra of AITC

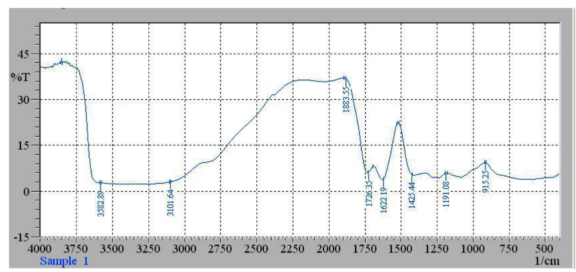

Figure 3: FTIR spectra of isolated AITC

provides strong evidence of the successful presence of AITC within the mustard seed extract. The observation of a strong absorption band at 240 nm aligns with the well-established spectroscopic properties of AITC. This result is consistent with previous studies that have identified the 240 nm absorption peak as a hallmark of AITC presence. The successful detection of AITC in the mustard seed oil extract is of great significance. In conclusion, the UV spectrum presented in Figure 2 unequivocally demonstrates the successful detection of AITC in the mustard seed oil extract, as indicated by the characteristic absorption peak at 240 nm.[27,28]

FTIR analysis

Figure 3 represents the FTIR spectrum of isolated AITC, offering valuable insights into the molecular structure and functional groups present within the compound. The spectrum reveals several prominent peaks, each corresponding to specific vibrational modes of chemical bonds within AITC.

- *OH Stretching Vibration (3582 cm⁻¹)*

The peak observed at 3582 cm^{-1} is attributed to the stretching vibration of the hydroxyl (OH) group. This suggests the presence of a hydroxyl group, which is an important functional group often associated with the reactivity and chemical properties of organic compounds.

Table 4: Zone of inhibitions against *Staphylococcus* and *E. coli*

S. No.	*Name of pathogenic microorganism*	*Zone of inhibition (mm)*		
		I	*II*	*II*
1	*Staphylococcus*	12	10	04
2	*E. coli.*	14	12	06

- *C-H Stretching (3101 cm^{-1})*

The peak at approximately 3101 cm^{-1} corresponds to the stretching vibration of carbon-hydrogen (C-H) bonds, C-H stretching vibrations are common in organic molecules and are indicative of aliphatic hydrocarbons, reinforcing the organic nature of AITC.

- *C=O Group (1726 cm^{-1})*

The broad peak observed around 1726 cm^{-1} is characteristic of the carbonyl (C=O) functional group. This suggests the presence of a carbonyl group in AITC, which is often found in compounds with key chemical reactivity.

- *C=C Deformation Vibration (1622 cm^{-1}) and C-H Deformation Vibration (1191 cm^{-1})*

The peaks at 1622 and 1191 cm^{-1} correspond to the deformation vibrations of carbon-carbon double bonds (C=C) and carbon-hydrogen (C-H) bonds, respectively. These peaks further

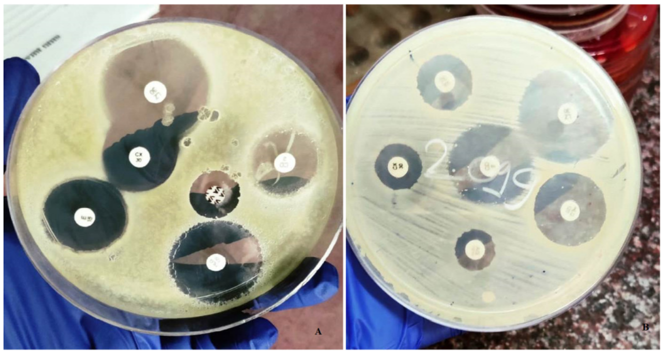

Figure 4: AITC antimicrobial activity against A: *Staphylococcus* and B: *E. coli*

confirm the presence of these structural elements within AITC. These findings are consistent with the known chemical structure of AITC, which contains a hydroxyl group, a carbonyl group, and multiple C-H and C=C bonds. The presence of these functional groups is in line with the expected chemical properties of AITC and its role as a bioactive compound found in mustard plants.[29]

Antimicrobial Study

The antimicrobial activity was analyzed by performing the zone of inhibition using the streak plate and well punching method. Developments of clear zones were observed (Figure 4) for the AITC extracts in Figure 4. The zone of inhibition was clearly seen with the diameter of 12, 10, and 4 mm for *Staphylococcus* and 14, 12, and 6 mm for *E. coli* and hence the AITC extracts were found to have antibacterial activity. Table 4 represents the zone of inhibitions against *Staphylococcus* and *E. coli*.[20,21]]

CONCLUSION

In conclusion, this study delves into the isolation, characterization, and antimicrobial potential of a natural compound, AITC, obtained from mustard seeds. The efficient extraction process facilitated the isolation of AITC, which underwent thorough physicochemical tests, UV-visible spectroscopy, infrared spectroscopy, and gas chromatography-mass spectroscopy for comprehensive characterization. The results revealed the presence of phytoconstituents and provided structural insights into AITC. Notably, the antimicrobial activity of AITC was evaluated against Staphylococcus and Escherichia coli, demonstrating inhibitory effects on the growth of both microbial strains. The outcomes of this investigation pave the way for further exploration and highlight the significance of AITC in the development of novel antimicrobial agents.

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