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GCMS analysis and antifungal activity of leaf extracts of *Ailanthus excelsa* (Roxb), against *Fusarium oxysporum* causal agent of Fusarium wilt disease in tomato

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Abstract
Fusarium wilt disease, caused by *Fusarium oxysporum*, poses a significant threat to tomato (*Solanum lycopersicum*) production worldwide. In this study, leaf extracts of *Ailanthus excelsa* were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to identify its chemical constituents. The GC-MS analysis revealed the presence of various bioactive compounds, including alkaloids, terpenoids, phenolic compounds, and fatty acids. Notably, several compounds with known antifungal properties were identified, such as squalene, quercetin, and β-sitosterol. The antifungal activity of *Ailanthus excelsa* leaf extracts against *Fusarium oxysporum* was evaluated using *in vitro* assays. The extracts exhibited significant inhibitory effects on the mycelial growth and spore germination of *Fusarium oxysporum*. In conclusion, this study demonstrates the potential of *Ailanthus excelsa* leaf extracts as a source of natural antifungal agents against *Fusarium oxysporum*, the causal agent of Fusarium wilt disease in tomato. The GC-MS analysis identified bioactive compounds that contribute to the antifungal activity. These findings offer promising prospects for the development of eco-friendly and sustainable strategies to combat fungal diseases in agricultural settings, reducing the reliance on chemical pesticides and promoting environmentally friendly practices in tomato cultivation.

Keywords: Anti-fungal, Fusarium wilt, *Ailanthus excelsa*, Leaf extracts, GCMS analysis.

1. Introduction
Fusarium wilt disease, caused by the soil-borne fungus *Fusarium oxysporum*, stands as a formidable challenge to the global tomato industry. This devastating disease leads to severe yield losses, reduced fruit quality, and significant economic repercussions for tomato growers worldwide (Smith, 2007) [16]. Traditional management strategies often rely on chemical pesticides, which can have adverse environmental impacts and raise concerns about food safety (Fortunati, *et al.*, 2019) [8]. As a result, there is a growing need for sustainable and environmentally friendly approaches to combat Fusarium wilt disease in tomato crops.

One promising avenue of research involves exploring the potential of plant-based natural products as alternative antifungal agents. *Ailanthus excelsa* (Roxb), commonly known as the tree of heaven, has drawn attention for its rich and diverse phytochemical composition, which includes alkaloids, terpenoids, phenolic compounds, and fatty acids (Kundu & Laskar, 2010) [11]. Many of these compounds have demonstrated potent antimicrobial and antifungal properties in previous studies. Consequently, *Ailanthus excelsa* emerges as a candidate for investigation as a source of natural compounds with antifungal potential against *Fusarium oxysporum* (Bansal & Gupta, 2000) [2].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a powerful tool that can help identify the specific chemical constituents present in *Ailanthus excelsa* leaf extracts. This analytical technique can provide insights into the bioactive compounds responsible for any observed antifungal activity. By uncovering the chemical profile of these extracts, researchers can better understand their potential as natural fungicides (Suganthy & Gajendra, 2019) [17].

In addition to chemical analysis, evaluating the practical effectiveness of *Ailanthus excelsa* leaf extracts against *Fusarium oxysporum* is crucial. *In vitro* assays can assess the inhibitory effects on fungal growth, while *in vivo* experiments on tomato plants can gauge the extracts' capacity to protect against Fusarium wilt disease. Such research not only addresses the need for sustainable disease management but also contributes to a broader understanding of plant-fungal interactions and the development of eco-friendly agricultural practices.

In this study explores the GC-MS analysis of *Ailanthus excelsa* leaf extracts to identify potential antifungal compounds and investigates their inhibitory effects on *Fusarium oxysporum*, shedding light on the potential of *Ailanthus excelsa* as a natural resource for managing Fusarium wilt disease in tomatoes. This research holds the promise of reducing the environmental impact of tomato cultivation while offering growers a more sustainable means of combating this economically significant fungal pathogen.

2. Material and Methods

2.1 Collection of Plant Material

Mature plant leaf of *Ailanthus excelsa* L. were collected from forest area, Jalna Maharashtra. The Plant identification and authentication was confirmed by Department of Botany, R.G. Bagdia Arts, S.B. Lakhotia Commerce and R. Bezonji Science College, Jalna.

2.2 Extract Preparation and Phytochemical Analysis

Plant leaf extract of *Ailanthus excelsa* L. are used for estimation of GC-MS analysis. To obtain sample, the powdered plant material of leaves part was taken separately and subjected to Soxhlet extraction procedure (Redfern *et al*., 2014) [21]. Preliminary phytochemical analysis was carried out for each solvent extract according to the standard procedure (Evans *et al.*, 2009; Yadav *et al.*, 2011) [4, 19].

2.3 The Isolation and Identification of Pathogen

Infected parts of the tomato plant were collected and sealed in airtight polythene bags, properly labeled, and brought to the Laboratory at the Department of Botany, JES College Jalna. The pathogen was isolated on potato dextrose agar (PDA) medium (Agrios, 2005) [1]. Infected tomato plant parts were cut into small pieces, washed in running tap water, sterilized in a 0.1% mercuric chloride solution, and thoroughly rinsed with sterilized distilled water to remove the mercuric chloride solution. Three of these sterilized pieces were transferred to PDA plates and then incubated at 25 °C±2 °C for 15 days to facilitate the recovery of the pathogen. *Fusarium oxysporum* was purified using the single spore method, and identification was carried out based on its morphological characteristics with the assistance of standard keys (Booth, 1971; Mukadam, *et al.*, 2006) [3, 13]. Pathogenicity tests were conducted on potted tomato plants in accordance with Koch's postulate.

2.4 Determination of antifungal activity

The impact of varying extract concentrations on Fusarium strain's radial growth was assessed using an agar dilution technique. The extract concentration (ranging from 5%, 10% and 15%) was mixed with molten PDA in a 1:19 ratio. The resulting mixtures were solidified, and a 1 cm mycelia block from a 7-day-old *F. oxysporum* colony was inoculated at the center of each Petri plate. Incubation occurred at approximately 25 °C for seven days. The colony diameter of *F. oxysporum* was measured after this incubation period, with three replicates following a randomized design for each treatment and fungicide (Carbendazim) amended media served as the positive control. The effectiveness of medicinal plant products was determined by calculating the percent inhibition of radial mycelial growth compared to the control using the formula (Dissanayake, 2014) [7].

Inhibition (%) = [(C − T)/C] × 100

Where, C and T represent the diameters of the control and treated colonies, respectively. Mycelial growth data were recorded at 3, 5 and 7th days after inoculation (DAI), and to prevent bacterial contamination, 0.5 g of the antibacterial agent streptomycin was added to PDA medium.

2.5 Gas Chromatography-Mass Spectrometry (GCMS) analysis

Gas Chromatography-Mass Spectrometry (GCMS) analysis GC-MS analysis of plant leaf extract (Ethanol) was done at the Sophisticated Analytical Instrument Facility (SAIF) labs, MIT CARS, Department of agriculture Engineering College, Aurangabad, using standard GCMS model as explained below. The procedure followed was of Dandekar *et al.*, 2015 [6].

2.6 Instrument details

The Shimadzu GC-MS analyzer (GC 2010 Plus, GCMSOP2020) used was equipped with an automated gas valve with helium (He) as the carrier gas, quadruple detector, capillary column, a flame ionization detector (FID), and a thermal conductivity detector (TCD). The analyses were performed using a GC-MS system (GC-2010 plus) Shimadzu, Agilent Technologies Inc.,) equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 mm, Agilent Technologies Inc). The injection volume of each sample was 1 µL. Helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the injection port was 250 °C, and the column temperature program was as follows: 50 °C for 2 min, followed by an increase to 180 °C at a rate of 5 °C/min, an increase to 270 °C at a rate of 20 °C/min, and maintenance at 270 ˚C for 5 min. The MS (QP-2020) conditions included an EI ion source temperature of 230 °C, ionization energy of 70 eV, and a mass scan range of 40–500 amu.

3. Results

3.1 Effects of plant extracts on *Fusarium oxysporum*

The mycelium growth of *Fusarium oxysporum* on *Ailanthus excelsa* extracts at different concentrations displayed significant variation (Table 01). After 7 days of incubation, the percentage inhibition for 5%, 10%, and 15% concentrations was 62.37%, 66.75% and 64.87% respectively, compared to the control. Notably, the 10% concentration proved to be the most effective in inhibiting *F. oxysporum* growth, while the 5% concentration was the least effective. In a comparative study, the efficacy of plant extracts was assessed alongside the fungicide Carbendazim to control *F. oxysporum*. Various concentrations of Carbendazim (ranging from 0.0 to 8.0 ppm) were prepared. It was observed that Carbendazim effectively suppressed mycelial growth of *F. oxysporum*, with the most significant inhibition (79.39%) occurring at the 4 ppm concentration. In line with similar studies, researchers such as Dahar & Rai (2019) [15] and Bansal & Gupta (2000) [2] investigated the influence of plant extracts on Fusarium. Their findings indicated that different concentrations of extracts derived from plants such as *Adhatoda vasica*, *Lantana camara*, and *Ailanthus excelsa* demonstrated similar inhibitory effects on Fusarium growth. This underscores the comparable efficacy of these plant extracts in suppressing Fusarium, underscoring their promise for disease control.

Table 1: Efficacy *Ailanthus excelsa* leaf extracts on *Fusarium oxysporum*

Concentration (%)	Inhibition after 3rd Day		Inhibition after 5th day		Inhibition after 7th day	
	Radial Growth of Pathogen (mm)	% Inhibition	Radial Growth of Pathogen (mm)	% Inhibition	Radial Growth of Pathogen (mm)	% Inhibition
5	12.6	37	20.9	47.75	30	62.37
10	11.6	42	18.9	52.75	26.6	66.75
15	10.2	49	18.6	53.5	28.1	64.87
Control	20	-	40	-	80	-

3.2 Preliminary Phytochemical Analysis

The outcomes of extracted contents of plant leaf extract tested for presence or absences of various phytochemicals (in qualitative form) are noted in Table 2. The results show that *Ailanthus excelsa* L. plant contains a maximum ten types of phytochemical groups, such as Alkaloids, Flavonoids, Phenols, Steroids, Glycosides, Saponins and Terpenoids. The findings were consistent with those of Malviya and Dwivedi in their 2019 study, which conducted both qualitative and quantitative phytochemical of *Ailanthus excelsa* L. to ascertain the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, and saponins in the extracts.

Table 2: Phytochemical screening of *Ailanthus excelsa* leaf extracts

Sr. No.	Test	95% Ethanol	Distilled Water
1	**Alkaloids**		
	Wagner's Test	+ ve	- ve
	Dragendorff Test	+ ve	- ve
2	**Flavonoids**		
	Shinoda Test	+ ve	- ve
	NaOH Test	+ ve	- ve
3	**Glycosides**		
	Conc. H2 SO 4	+ ve	- ve
	Molisch's Test	+ ve	- ve
4	**Phenol**		
	Ellagic Test	+ ve	+ ve
	Phenol Test	+ ve	+ ve
5	**Lignin**		
	Lignin Test	+ ve	- ve
	Labat Test	+ ve	- ve
6	**Saponins**		
	Foam Test	+ ve	+ ve
	Haemolysis Test	+ ve	+ ve
7	**Sterols**		
	Liberman- Burchard Test	- ve	- ve
	Salkowski Test	- ve	- ve
8	**Tannins**		
	Gelatin Test	+ ve	+ ve
	Lead Acetate Test	+ ve	+ ve

Where (+) sign indicates presence of corresponding phytocomponents

3.3 GC-MS Analysis

Gas chromatography and mass spectroscopy analysis of compounds was carried out in ethanolic leaves extract of *Ailanthus excelsa*. Chromatogram GC-MS analysis showed the presence of 18 major peaks and the components corresponding to the peaks were determined is shown in Figures 1. In this observation, the active principle with their Retention time (RT), Molecular formula, Molecular weight (MW) and structure of compound of these identified bio-compounds are presented in Table 3.

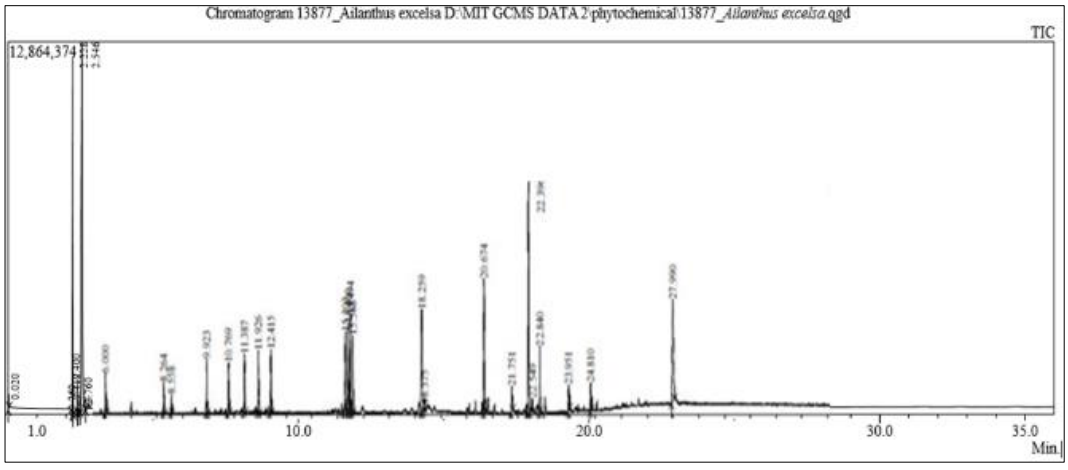
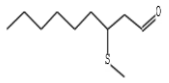
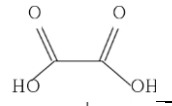
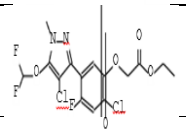
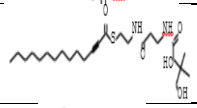
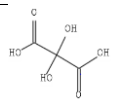
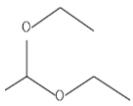
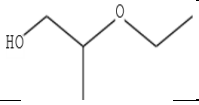
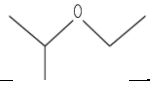
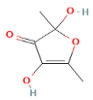
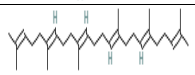
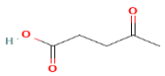
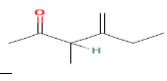
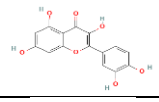
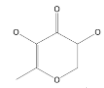
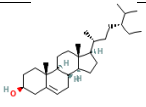
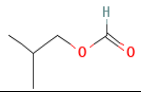
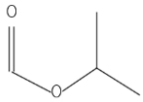


Fig 1: Chromatogram of GCMS analysis of leaf extract of *Ailanthus excelsa*

In this study, the leaf extracts of A. *excelsa* were found to contain specific compounds associated with fungicidal properties, such as Squalene (Senthilkumar *et al.*, 2011) [15], Quercetin (Yang *et al.*, 2020) [18] and Beta-Sitosterol (Saeidnia *et al.*, 2014) [14], as indicated in Table 2. These compounds are likely to possess antifungal potential and contribute to the suppression of Fusarium growth. These results align with previous findings from studies involving various leaf extracts in ethanolic solvents. In a study by Zenita Devi *et al.* in 2015 [20], the antifungal activity and phytochemical analysis of *Zanthoxylum acanthopodium* were conducted to validate its traditional use. They identified 43 organic compounds through GC-MS analysis, including β-sitosterol, Hexadecanoic acid (palmitic acid), and Phytol, which exhibited antifungal properties. Similarly, Kapil Bansode *et al.* in 2022 [9] investigated the GC-MS analysis and efficacy of *Lawsonia inermis* L. against post-harvest decaying fungi in *Psidium guajava*. Their study identified 11 major bioactive compounds, with Phenol, Benzoic acid, Phytol, and Squalene known for their antifungal activities.

Table 2: The presence of different bioactive compounds in the ethanol extract of *Ailanthus excelsa* detected through GC-MS

Peak	R. Time	Name	Molecular Formula	M. Weight	Area%	Structure of Compound
1	0.02	Nonanal, 3-(methylthio)-	C10H20OS	188	0.4	
2	2.228	Oxalic acid	C2H2O4	144	22.9	
3	2.26	PYRAFLUFEN-ETHYL	C15H13Cl2F3N2O4	412	1.58	
4	2.4	2-Myristynoyl pantetheine	C25H44N2O5S	484	1.66	
5	2.419	Propanedioic acid, dihydroxy-	C3H4O6	136	2.65	
6	2.546	Ethane, 1,1-diethoxy-	C6H14O2	118	69.5	
7	2.76	1-Propanol, 2-ethoxy-	C5H12O2	104	1.23	
8	4.585	1,2,3-Propanetriol	C3H10O4	92	2.01	
9	4.802	2,4-Dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one	$C_6H_8O_4$	144	0.25	
10	5.635	Squalene	C30H50	410.7	0.22	
11	5.81	4-Oxo-Pentanoic acid	C5H8O3	116	0.19	
12	6.243	4-Ethyl-3-methyl-4-penten-2-one	C8H14O	126	1.23	
13	6.53	Quercetin	C15H10O7	302.23	0.49	
14	7.157	2-Acetyl-2-Hydroxy-.Gamma.-Butyrolactone	C6H8O4	144	0.44	
15	7.317	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	2.25	
16	7.431	Beta-Sitosterol	C29H50O	414.7	0.08	

17	7.875	Isobutyl formate	C5H10O2	102	0.04	
19	8.167	Formic acid, 1-methylethyl ester	C4H8O2	88	0.29	

Conclusion

In this study, the analysis of leaf extracts from *Ailanthus excelsa* revealed the presence of specific compounds known for their fungicidal properties, including Squalene, Quercetin, and Beta-Sitosterol. These compounds are likely contributors to the observed antifungal potential, which led to the suppression of *Fusarium* growth. This outcome not only supports the growing body of evidence regarding the effectiveness of various plant extracts against fungal pathogens but also highlights the potential of *Ailanthus excelsa* as a valuable natural resource in the field of disease control.

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