

Assessment of antioxidant and antifungal properties of essential oil from fruits of *Zanthoxylum armatum* DC.

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Essential oil (EO) have emerged as a potent natural antioxidants and antimicrobial agents due to their predominantly non-toxic nature suggesting their potential use as alternatives to synthetic chemical. *Z. armatum* have been known to possess many biological activities like antifungal, antibacterial, antioxidant, antiviral, pesticidal/insecticidal effects. This study describes the antifungal activity, antioxidant assay and total phenolic and flavonoid content of the EO of *Zanthoxylum armatum*. The EO was found to have significant antifungal activity against *Fusarium lateritium* and *Alternaria alternata*. The half maximal inhibitory concentration (IC₅₀) by contact assay was 1.14 μ l/ml for *F. lateritium* and 1.65 μ l/ml for *Alternaria alternata*. Through volatile assay the IC₅₀ value was 0.15 μ /cm³ and 0.65 μ /cm³ for *F. lateritium* and *Alternaria alternata* respectively. The EO also exhibit good antioxidant capacity and reduced DPPH, ABTS and Fe³⁺ to 50% at IC₅₀ value of 33.66 μ l/ml, 55.26 μ l/ml and 34.02 μ l/ml respectively. The total phenolic content (TPC) was 241.92 mg/g GAE and total flavonoid content (TFC) was 155.71 mg/g QE. The findings suggest that the essential oil from *Z. armatum* could serve as a viable option for creating a secure and environmentally friendly natural antifungal and antioxidant. The findings suggest that *Zanthoxylum armatum* essential oil exhibits significant antioxidant properties and potent antifungal activity against *Fusarium lateritium* and *Alternaria alternata*, particularly in vapour phase assays. With a high phenolic and flavonoid content, this oil serves as a viable, eco-friendly candidate for natural antioxidant and antimicrobial formulations.

Keywords : *Alternaria alternata*, antifungal, antioxidant, essential oil, *Fusarium lateritium*, *Zanthoxylum armatum*

INTRODUCTION

The increasing use of synthetic fungicides has led to environmental concerns, health risks, and the development of resistant fungal pathogens, creating a need for safer and eco-friendly alternatives. Plant essential oils have emerged as promising natural products because of their antioxidant and antimicrobial properties. Owing to their biodegradability and diverse bioactive compounds, essential oils are increasingly being explored for agricultural pharmaceutical applications (Kamau *et al.* 2026). *Zanthoxylum armatum* DC. (family Rutaceae) is an important medicinal and aromatic plant widely distributed in the Himalayan region.

The fruits are particularly rich in essential oils containing compounds such as eucalyptol, limonene, linalool, and terpinen-4-ol, which are known for their antioxidant and antimicrobial activities (Vashinath *et al.* 2021; Liu *et al.* 2023). However, the composition and biological activity of these essential oils can vary depending on parts of the plant, geographical location, climate and other environmental factors (Vashisath *et al.* 2021; Cheng *et al.* 2024; Wang *et al.* 2025).

Several studies have highlighted the antifungal potential of *Z. armatum* essential oil against important plant pathogens. Slathia *et al.* (2021) demonstrated its effectiveness in controlling *Alternaria*-induced post-harvest rot in tomato fruits. More recently, studies have shown that the fruits essential oil inhibit fungal growth by disrupting fungal cell membranes through the reduction of ergosterol levels (Pasrija *et al.* 2024). In addition,

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comparative studies have confirmed that the fruit essential oil possesses significant antioxidant activity making it a potential source of natural antioxidants (Fan *et al.* 2025). A recent review further emphasized the rich phytochemical composition and wide range of pharmacological properties of *Z. armatum*, supporting its potential for medicinal and agricultural applications (Dahiya *et al.* 2025).

Despite these promising findings, studies evaluating both the antioxidant and antifungal properties of *Z. armatum* fruits essential oil from different regions remain limited. Therefore the present study aims to assess the antioxidant and antifungal activities of essential oil extracted from the fruits of *Z. armatum*. The findings will contribute to the development of environmentally friendly alternatives to synthetic fungicides and promote the utilization of this valuable medicinal plant.

MATERIALS AND METHODS

Sample collection

Plant materials were collected from Mawsiatkham, East Khasi Hills District of Meghalaya at 25°28.030'N and 91°58.542'E, at an elevation of approximately 1027 m above sea level. The study area generally experiences temperature ranging from approximately 18°C to 28°C throughout the year. The samples were obtained from wild plant of approximately 7 years. Fruits were harvested at the mature stage (not fully ripe) from one individual plant during the month of July, 2023. The samples were labelled, transported to the laboratory and processed under appropriate conditions prior to essential oil extraction.

Extraction of essential oil

1 kg of fresh fruits were subjected to hydrodistillation using Clevenger's apparatus for 8 hours duration at 40 °C. The collected oil were then stored at 4 °C for further use.

The yield of the essential oil was determined using the formula,

Percentage yield (%) = Volume of essential oil (g)/Volume of sample (g) *100

Total phenolic content

Total phenolic content (TPC) of the essential oil was determined by Folin–Ciocalteu colorimetric method described by Singleton *et al.* (1999) with slight modification. 1 ml sample solution of different concentrations was combined with 5 ml of 10% Folin-Ciocalteu's reagent, thoroughly mixed and left to stand for 5 minutes at room temperature. Subsequently, 4 ml of 7% NaHCO₃ was slowly added and the reaction mixture was allowed to stand in the dark for 30 minutes at room temperature. Absorbance readings were then recorded at 765 nm. The same process was replicated for all standard Gallic Acid solution. Total phenolic content of the essential oil was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

Total Flavonoid Content

Total Flavonoid Content was determined according to aluminium chloride colorimetric method (Zhishe *et al.* 1999). Different concentration (10-100 µl/ml) of essential oil of 1 ml each was mixed with 4 ml distilled water following by the addition of 0.3 ml of 5% sodium nitrite. After 5 minutes 3 ml of 10% Aluminium chloride was added and allowed to stand for 6 minutes after which 2 ml of 1M sodium hydroxide was added. The total volume was made up to 10 ml with distilled water and the solution was mixed well and absorbance was measured using Spectrophotometer at 510 nm. The concentration of flavonoid compound was expressed as mg quercetin equivalents per gram of sample in dry weight (mg/g).

Antioxidant Activities

DPPH radical scavenging activity

DPPH scavenging ability of the essential was determined following the protocol described by Bozin *et al.* (2006). 1 ml of different concentration (10-100 µg/ml) of essential oil was mixed with 1 ml of 90 µM DPPH solution. The total volume was made to 4 ml with methanol and the mixture was shaken well and kept in the dark at room temperature for 1 hour. The absorbance was recorded by using spectrophotometer at 517 nm. Methanol was used to prepare the control

solution. The percentage of Radical Scavenging Activity (RSA) was calculated using the formula,

$$\%RSA = \left(\frac{A_o - A_s}{A_o} \right) * 100$$

A_o is the absorbance of the control, A_s is the absorbance of sample. Ascorbic acid was used as a reference and EC50 (50% inhibition of free radical) value was calculated from the graph by plotting % RSA against oil concentration.

ABTS radical scavenging activity

The ABTS⁺ free radical scavenging activity of the essential oil was determined following the protocol given by Re *et al.* (1999). Production of ABTS cations (ABTS⁺) was done by mixing ABTS (2, 20-azinobis-(3-ethylbenzothiazoneline-6-sulphonic acid) (7 Mm) with potassium persulphate (2.45 Mm) in an amber coloured bottle. The solution was kept in the dark for 16 hours at room temperature. The ABTS working solution was diluted with methanol till an absorbance of 0.70 was obtained at 734 nm. 3 ml of this working solution was added to 1 ml of each concentration of essential oil (10, 20, 40, 60, 80 and 100 µl/ml) and then allowed to stand for 6 minutes in the dark after which the absorbance was recorded at 734 nm. Methanol served as the control and ascorbic acid was used as a reference compound and the capability of the EO to scavenged the ABTS⁺ was calculated using the equation,

$$\%RSA = \left(\frac{A_1 - A_2}{A_1} \right) * 100$$

A_1 is the absorbance of the control, A_2 is the absorbance of the sample. EC50 value was determined by plotting % RSA against oil concentration. The experiment was performed in tri-replicate.

FRAP reducing assay

Reduction of Fe³⁺ to Fe²⁺ was measured by the method described by Oyaizu (1986). EO of different concentration (10,20,40,60,80 and 100 µl/ml) were mixed with 2.5 ml of phosphate buffer

(0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 %) and were then incubated for 20 minutes at 50 °C. After incubation 2.5 ml of 10% Trichloroacetic acid was added and centrifuge for 10 minutes at 3000 rpm. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and then 2.5 ml of ferric chloride (1%) was added and the absorbance was measured at 700 nm using UV-VIS spectrophotometer. Increasing colour intensity indicated increasing reducing power. A control solution containing distilled water was also prepared. Reducing power of the EO was calculated using the formula,

$$RP\% = (A_o - A_s) * 100$$

Where A_o and A_s is the absorbance of the control and sample respectively. Percentage of Inhibition was plotted against the oil concentration and the equation of the line was used to obtain the RP₅₀ value and compared to that the standard ascorbic acid.

Antifungal activity

Isolation and Identification of fungal pathogens

Fungal pathogens from infected leaves of *Z. armatum* were isolated using Agar plate method (Muskett and Malone, 1941) and incubated at 28°C for 5 days duration. Pathogens were identified morphologically and Koch's postulate was performed to confirm the pathogenicity. The fungal pathogens were molecularly identified by PCR amplification of the internal transcribed spacer (ITS) region of ribosomal DNA, followed by sequencing services provided by Eurofins Genomics, Bengaluru, India. The sequences were deposited in NBRI Genbank database to obtain the accession number.

Contact assay

Antifungal activity of the EO was conducted by contact assay described by Guleria *et al.* (2013). Various concentration of EO (0.1, 0.15, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, and 2.5 µl/ml) were dissolved in 0.5% v/v DMSO and incorporated into molten Potato Dextrose Agar (PDA). Subsequently, 5 mm of the test fungus was

inoculated at the centre of the plates, and the incubation was carried out at 28°C for duration of 10 days. Each treatment was performed in triplicates. DMSO was used as the negative control to determine the specific effect of essential oil.

Volatile assay

Volatile activity of the EO was done following the protocol given by Basim *et al.* (2000), where different concentration of EO (0.1, 0.15, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, and 2.5 µl/ml) were impregnated into sterile filter papers (Whatman No. 1). These impregnated filter papers were then mounted onto the inner surface of the inverted lids of petri plates. Following this, a 5 mm sample of the test fungus was inoculated at the centre of the plates and the entire setup was incubated at 28°C for a period of 10 days. Three replicates were maintained for each concentration. Distilled water was used as the negative control to evaluate the effect produced specifically by the essential oil.

The antifungal activity of the EO for contact and volatile assay was calculated using the formula:

$$\% = \frac{1 - Da}{Db} * 100$$

Where, Da is the diameter of treated fungi and Db is the diameter of control.

Scanning Electron Microscopy (SEM) Analysis

The effect of the essential oil on the pathogens was examined using Scanning Electron Microscopy (JEOL JSM-6360 model) at the Sophisticated Analytical Instrument Facility, North-Eastern Hill University, Shillong. Samples were prepared following the protocol given by NEHU-SAIF.

Statistical Analysis

Data analysis was conducted using SPSS software, version 16 (Chicago, IL, USA), and MS-Excel, v.7 (Microsoft, Washington, DC, USA).

RESULTS AND DISCUSSION

Essential oil

Based on the weight of the plant sample the yield of essential oil from fruits was 2.3% (w/w). The yield of essential oil was much higher compared to that recorded by Jain *et al.* (2001), Tiwary *et al.* (2007), Prakash *et al.* (2012) with 1.5, 1.3 and 1.8% respectively. Various factors such as environmental conditions, genetic variations, collection timing, and seasonal variations can influence the yield of the essential oil (Rahimmalek *et al.* 2009; Peng *et al.* 2025). Phuyal *et al.* (2019) demonstrated that changes in elevation, cultivation conditions, and soil-related factors have a substantial impact on essential oil production.

Total Phenolic and Flavonoid Content

The total phenolic and flavonoid content of the essential oil of *Z. armatum* is given in Table 1. The total phenolic content was 241.92 mg/g GAE and the total flavonoid content was 155.71 mg/g QE. The result was similar with the study of Phuyal *et al.* (2020) where they reported that the phenolic content of cultivated fruits extract was 226.3 mg GAE/g and for wild fruits was 185.02 mg GAE/g and the flavonoid content for cultivated fruits extract was 135.17 mg QE/g and 103.7 mg QE/g for wild fruit extract. However, the TPC and TFC of the ethanolic fruit extract reported by Barkatullah *et al.* (2011) was much lower with 21.68 mg/g and 22.80 mg/g respectively. Dai and Mumper (2010) and Jing *et al.* (2015) suggested that extraction temperature, duration, pH, and polarity of the extraction solvent could affect the concentration of phenolic and flavonoid content of the extract. Phenolic compounds exhibit redox properties, serving as reducing agents, suppliers of hydrogen and scavengers of singlet oxygen (Chang *et al.* 2001).

Antioxidant activities

DPPH and ABTS radical scavenging activity:

The EO exhibit scavenging activity in proportion to their concentrations, causing a 50% inhibition of DPPH and ABTS radical, as indicated by their IC50 values (Table 1). A substance qualifies as

Table 1 : Scavenging activity and total phenolic and flavonoid content of essential oil from fruits of *Z. armatum*

Conc. $\mu\text{l/ml}$	DPPH RSA %	ABTS RSA %	FRAP RP %	Total phenolic content (mg/g GAE)	Total flavonoid content (mg/g QE)
0 (control)	0.02 $\pm$ 0.0 ^g	0.0 $\pm$ 0.00 ^g	0.02 $\pm$ 0.00 ^g		
10	24.09 $\pm$ 0.17 ^f	17.11 $\pm$ 0.16 ^f	39.05 $\pm$ 0.14 ^f		
20	41.81 $\pm$ 0.15 ^e	29.30 $\pm$ 0.14 ^e	48.00 $\pm$ 0.12 ^e		
40	62.05 $\pm$ 0.15 ^d	40.05 $\pm$ 0.17 ^d	54.00 $\pm$ 0.13 ^d		
60	78.18 $\pm$ 0.14 ^c	59.44 $\pm$ 0.14 ^c	61.30 $\pm$ 0.16 ^c		
80	89.09 $\pm$ 0.13 ^b	66.49 $\pm$ 0.13 ^b	63.15 $\pm$ 0.15 ^b	241.92 $\pm$ 0.21	155.71 $\pm$ 0.19
100	95.23 $\pm$ 0.12 ^a	73.55 $\pm$ 0.09 ^a	68.65 $\pm$ 0.15 ^a		
IC50 $\mu\text{l/ml}$	33.66	55.26	34.02		
Ascorbic acid	28.82 $\mu\text{g/ml}$	-	27.65 $\mu\text{g/ml}$		
Gallic acid	-	29.61 $\mu\text{g/ml}$	-		

*Values are means of three replicates $\pm$ Standard Deviation. Within column values followed by different letter(s) are significantly different by Tukey test at $p \leq 0.05$. RSA-Radical scavenging activity; RP-Reducing power; GAE-Gallic acid Equivalent; QE- Quercitin equivalent.

Table 2 : Pearson correlation coefficients for concentrations and antioxidant activity parameters of *Z. armatum* essential oil

Variable	r value	P- value	Interpretation
Conc. vs DPPH	0.960	< 0.001	Very strong positive correlation
Conc. vs ABTS	0.974	< 0.001	Very strong positive correlation
Conc. vs FRAP	0.826	< 0.001	Very strong positive correlation
DPPH vs ABTS	0.995	< 0.001	Very strong positive correlation
DPPH vs FRAP	0.933	< 0.001	Very strong positive correlation
ABTS vs FRAP	0.915	< 0.001	Very strong positive correlation

*Pearson correlation coefficients (r) were calculated using the mean values of antioxidant activity at concentration ranging from 0-100 $\mu\text{l/ml}$.

an antioxidant if its IC₅₀ is below 5 mg/ml (Abdillah *et al.*, 2015). In this study, the EO of *Z. armatum* has proven its ability to scavenge free radical with IC₅₀ of 33.66 $\mu\text{l/ml}$ for DPPH and 55.26 $\mu\text{l/ml}$ for ABTS. The bark essential oil of *Z. armatum* from different altitudinal range as reported by Dhami *et al.* (2018) have IC₅₀ value of 12.58, 17.8 and 17 $\mu\text{l/ml}$. However, according to Phuyal *et al.* (2020) the IC₅₀ values of fruits extract for cultivated and wild fruits was 40.62 and 45.62 $\mu\text{g/ml}$ respectively. Several studies of EO and extract from different plant parts have also been documented demonstrating significant antioxidant activity *Z. armatum* (Singh *et al.* 2013; Guleria *et*

al. 2013; Karmakar *et al.* 2015; Mukhija *et al.* 2015; Dhami *et al.* 2018, Punniyamoorthy *et al.* 2025). The antioxidant properties of medicinal plants are attributed to their phenolics and flavonoids (Diaz *et al.* 2012), which help mitigate oxidative stress-related diseases (Dragland *et al.* 2003) by minimizing the production of free radicals (Ravipati *et al.* 2012). While phenolic compounds have traditionally been acknowledged for their role in conferring antioxidant properties, recent research has indicated that volatile compounds, either independently or in combination may also play a significant part in overall antioxidant capabilities (Sarikurkcu *et al.* 2015; Martucci *et al.* 2015, Chen *et al.* 2023).

Table 3: Inhibition of two fungi by essential oil from fruits of *Z. armatum*

EO Conc. ($\mu\text{l}/\text{cm}^3$ and $\mu\text{l}/\text{ml}$)	Inhibition Percentage (%) $\pm$ SD (Volatile assay)		Inhibition Percentage (%) $\pm$ SD (Direct contact assay)	
	<i>F. lateritium</i>	<i>A. alternata</i>	<i>F. lateritium</i>	<i>A. alternata</i>
0 (Control)	0.42 $\pm$ 0.13 ^j	0.53 $\pm$ 0.08 ^k	0.61 $\pm$ 0.11 ^l	0.32 $\pm$ 0.04 ^k
0.1	38.67 $\pm$ 0.06 ⁱ	9.38 $\pm$ 0.10 ^j	6.60 $\pm$ 0.10 ^k	4.61 $\pm$ 0.10 ^j
0.15	50.94 $\pm$ 0.10 ^h	31.25 $\pm$ 0.10 ⁱ	14.15 $\pm$ 0.06 ^j	11.79 $\pm$ 0.06 ⁱ
0.25	66.98 $\pm$ 0.10 ^g	44.79 $\pm$ 0.06 ^h	19.81 $\pm$ 0.06 ⁱ	15.38 $\pm$ 0.10 ^j
0.5	72.64 $\pm$ 0.13 ^f	58.33 $\pm$ 0.15 ^g	32.07 $\pm$ 0.10 ^h	23.08 $\pm$ 0.10 ^h
0.75	79.25 $\pm$ 0.10 ^e	64.58 $\pm$ 0.15 ^f	41.51 $\pm$ 0.06 ^g	27.69 $\pm$ 0.10 ^g
1.0	83.96 $\pm$ 0.15 ^d	72.39 $\pm$ 0.15 ^e	48.11 $\pm$ 0.06 ^f	35.38 $\pm$ 0.10 ^f
1.25	87.94 $\pm$ 0.06 ^{cd}	75.00 $\pm$ 0.10 ^e	54.72 $\pm$ 0.10 ^e	37.94 $\pm$ 0.06 ^f
1.5	90.57 $\pm$ 0.06 ^c	81.77 $\pm$ 0.15 ^d	60.38 $\pm$ 0.10 ^d	43.07 $\pm$ 0.10 ^e
1.75	95.28 $\pm$ 0.06 ^b	87.50 $\pm$ 0.10 ^c	68.87 $\pm$ 0.10 ^c	49.74 $\pm$ 0.15 ^d
2.0	100 $\pm$ 0.00 ^a	90.63 $\pm$ 0.10 ^{bc}	87.74 $\pm$ 0.06 ^b	58.46 $\pm$ 0.10 ^c
2.25	100 $\pm$ 0.00 ^a	95.31 $\pm$ 0.10 ^{ab}	100 $\pm$ 0.00 ^a	66.15 $\pm$ 0.10 ^b
2.5	100 $\pm$ 0.00 ^a	100 $\pm$ 0.00 ^a	100 $\pm$ 0.00 ^a	73.84 $\pm$ 0.10 ^a
IC50	0.15 $\mu\text{l}/\text{cm}^3$	0.65 $\mu\text{l}/\text{cm}^3$	1.14 $\mu\text{l}/\text{ml}$	1.65 $\mu\text{l}/\text{ml}$

*Values are means of three replicates + standard Deviation, Within column values followed by different letter(s) are significantly different by Tukey test at $p \leq 0.05$. RSA Redical scavenging activity; RP-reducing power; GAE-Gallic acid Equivalent; QE-Quercitin equivalent.

FRAP reducing assay

The result of FRAP assay for the EO are shown in Table 1. The reducing activity of the essential oil are concentration dependent i.e., it increases with increasing concentration. This finding aligns with Punniyamoorthy *et al.* (2025) who also observed the increasing activity of the EO with the increase in concentration. The reducing power of the EO with 50% inhibition (IC50) was 34.02 $\mu\text{l}/\text{ml}$. The existence of reducing agents in the solution induces the transformation of the Fe^{3+} complex into its ferrous form through reduction (Guleria *et al.*, 2013). Bark EO from three different altitudes have a much higher reducing activity with 17.91 $\mu\text{l}/\text{ml}$, 18.59 $\mu\text{l}/\text{ml}$ and 21.49 $\mu\text{l}/\text{ml}$ IC50 values as compared to the result observed in this study (Dhami *et al.*, 2018). Similarly, EO from seeds of *Z. armatum* from two altitudes of Uttarakhand have higher reducing capability with IC50 of 18.49

$\mu\text{l}/\text{ml}$ and 19.09 $\mu\text{l}/\text{ml}$ (Dhami *et al.*, 2019). Guleria *et al.* (2013) reported that the IC50 value of leaf EO of *Z. armatum* was 11.9 mg/ml. The established order of reducing activity in essential oils is evidently linked to the presence of phenolic compounds and terpenes containing conjugated hydrogen bonds (Dorman *et al.*, 2003, Wojtunik-Kulesza and Oniszczyk, 2024). According to literature findings, the assessment of antioxidant properties is markedly affected by the hydrogen ion concentration within the measured solution (Dawidowicz and Olszowy, 2010).

Pearson correlation coefficients for concentrations and antioxidant activity parameters of *Z. armatum* essential oil revealed very strong correlation between concentrations and activities, as well as regression plots for the same have been shown in Table 2, Fig.1.

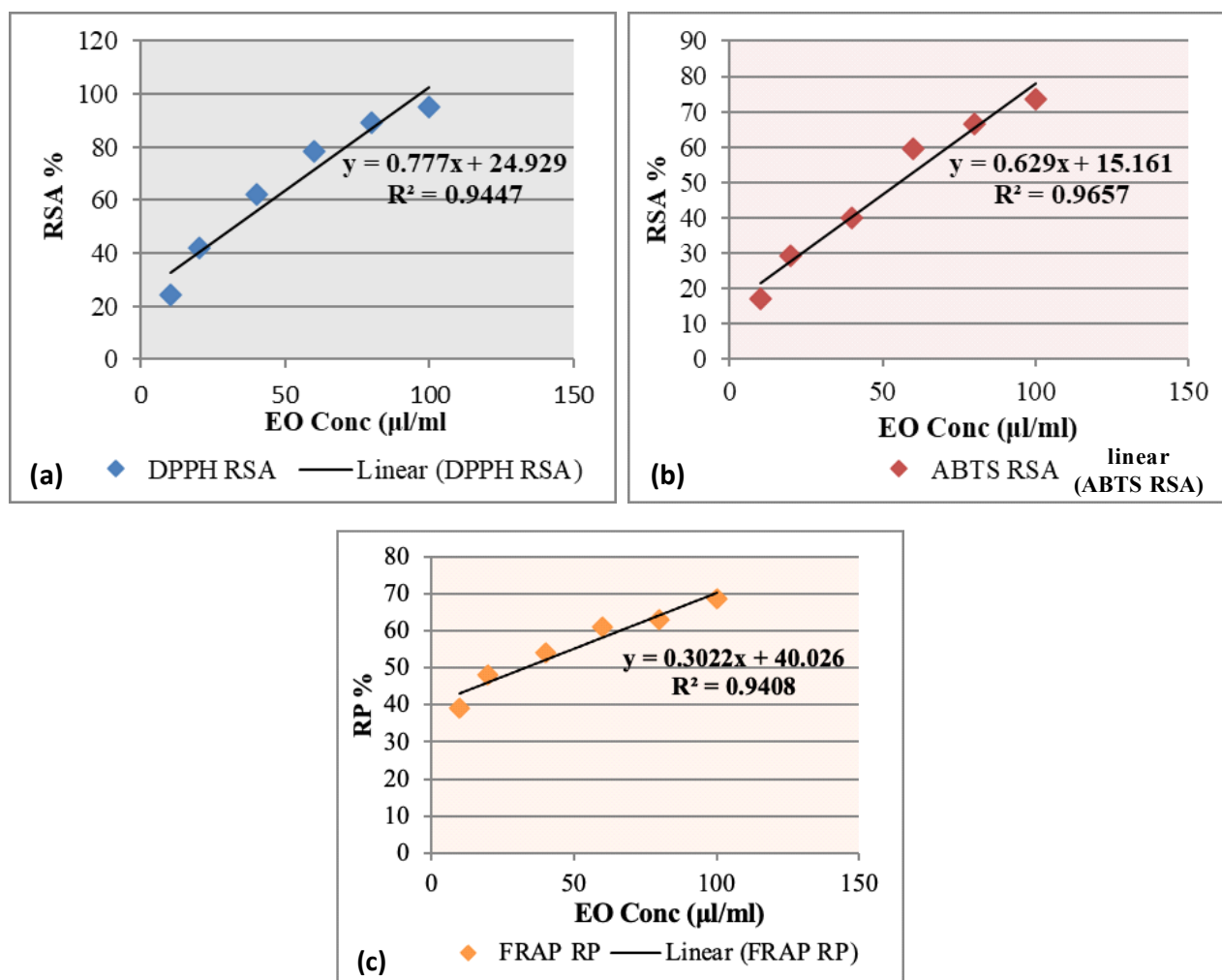


Fig. 1: Scatter plot showing the fitted linear regression equation and coefficient of determination (R^2) for the relationship between concentration and antioxidant activity (%): (a) DPPH activity (b) ABTS activity (c) FRAP activity.

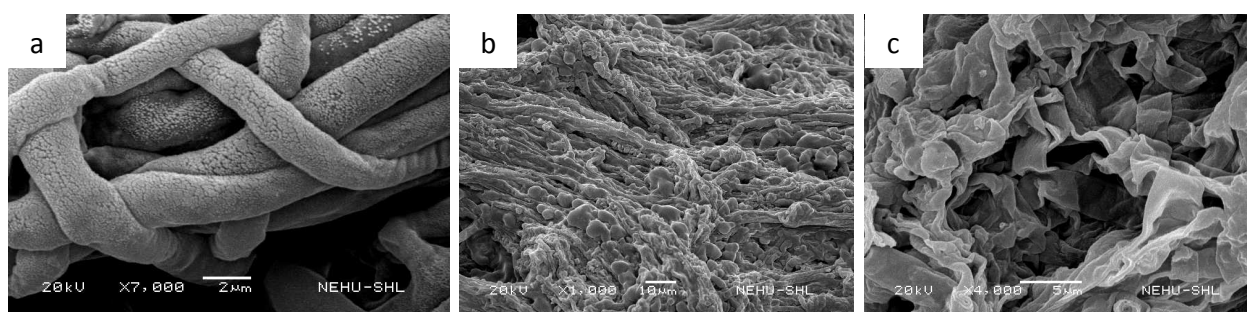


Fig.2: Scanning Electron micrographs showing the effect of essential oil on hyphae of a pathogen (*F. lateritium*) (a) Control/untreated fungi (b) and (c) Treated fungi

Anti-fungal activity

Identification of fungal pathogen

Using morphological and molecular identification, isolated fungal pathogens were identified as *Alternaria alternata* and *Fusarium lateritium* having accession number OR921619 and

OR934537 respectively. BLAST program showed 99.82% similarity for *Alternaria alternata* and 100% similarity for *Fusarium lateritium* when compared with the available sequences.

Reports of fungal diseases in *Zanthoxylum armatum* are limited, with *Alternaria alternata* being previously identified as a pathogen of

certain *Zanthoxylum* species (Yang *et al.* 2013). In contrast, no published reports of *Fusarium lateritium* infecting *Z. armatum* are currently available. Nevertheless, *F. lateritium* has been widely reported as pathogen of several other crop and woody plant species, indicating its broad pathogenic potential (Santori *et al.* 2010; Jing *et al.* 2016; Zhao *et al.* 2019).

Contact assay and Volatile assay

Antifungal activity of different concentration of essential oil from the fruits of *Z. armatum* against *F. lateritium* and *A. alternata* are shown in Table 3. Inhibitory activity of the EO by contact and volatile method are both concentration dependent. By contact assay 100% inhibition of *F. lateritium* was achieved at 2.25 $\mu\text{l/ml}$, however for *A. alternata* inhibition was only 66.15%. The IC₅₀ (50% inhibition) for *F. lateritium* by contact assay was 1.14 $\mu\text{l/ml}$ and for *A. alternata* it was 1.65 $\mu\text{l/ml}$. Through volatile assay it was observed that 100% inhibition against *F. lateritium* was achieved at concentration 2.0 $\mu\text{l/cm}^3$ and IC₅₀ at 0.15 $\mu\text{l/cm}^3$ and for *A. alternata* 100% suppression was achieved at 2.5 $\mu\text{l/cm}^3$ with IC₅₀ value of 0.65 $\mu\text{l/cm}^3$. The results revealed that volatile assay was more effective in inhibiting the mycelial growth of both the phytopathogens. Similar findings were reported by Feng *et al.* (2011), who observed that the volatile assay exhibited greater antifungal activity than the contact assay under *in vitro* conditions. 50% mycelial inhibition of fruit EO of *Z. armatum* against *A. alternata* was observed at 0.5 $\mu\text{l/ml}$ as reported by Slathia *et al.* (2021). Mycelial growth of *P. capsici*, *Py. aphanidermatum* and *Py. ultimum* was completely inhibited by fruit essential oil of *Z. armatum* at a concentration of 10 $\mu\text{l/ml}$, while against *P. sojae* and *P. parasitica* inhibition rate was 92.83% and 95.75% respectively (Yang *et al.* 2022). In their 2013 study, Guleria *et al.* (2013) demonstrated the significant antifungal potential of *Z. alatum* leaf essential oil against *A. alternata*, *A. brassicae* and *Curvularia lunata*. Moderate antifungal activity of fruit extract of *Z. armatum* against *Aspergillus flavus* and *Fusarium solani* was also reported by Pathak *et al.* (2021) and Li *et al.* (2021) as well illustrates the antifungal properties of *Z. armatum* EO, particularly its effectiveness as an antifungal agent against *A. flavus*.

Effect of Essential oil on fungal hyphae

The SEM observation (Fig.2) in the present study revealed the significant morphological alterations in the fungal hyphae when treated with essential oil, including swelling, collapse, thinning, and rupture of the cell wall, indicating damage to the cell membrane and wall structure. Similar observations have been reported in earlier studies. Essential oil from *Citrus sinensis* epicarp, rich in limonene, was found to inhibit the growth of *Aspergillus niger* and caused irreversible structural damage such as cytoplasmic loss and budding at the hyphal tips (Gogoi *et al.* 2008; Tian *et al.*, 2011). Likewise, Iscan *et al.* (2016) reported extensive damage to fungal cell wall and cytoplasmic membrane after treatment with thymoquinone, the major constituent of black cumin seed essential oil. According to Maurya *et al.* (2022), the bioactive constituents of essential oil can disrupt the lipid bilayer of the cell membrane, thereby increasing membrane permeability and causing the release of vital intracellular components. These findings collectively support the antifungal potential of essential oils through disruption of fungal cell membrane integrity and hyphal morphology.

CONCLUSION

This current study shows that *Z. armatum* essential oils can be used as an effective antifungal and antioxidant agents having the potential in tackling the health and agricultural issues caused by fungal pathogens. The dual capability of these oils to hinder fungal proliferation and combat oxidative stress not only emphasizes their versatility but also establishes them as valuable resources in protecting human health and agricultural output. The continuous research and increasing acceptance of essential oils underscore their promising contribution to bridging traditional knowledge with contemporary wellness practices, promising a harmonious synergy between the two.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

REFERENCES

- Abdillah, S., Tambunan, R.M., Farida Y., Sandhiutami, N.M.D., Dewi R.M. 2015. Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia. *Asian Pac. J. Trop. Dis.* **5**: 454–457.
- Barkatullah, B., Muhammad Ibrar, M.I., Naveed Muhammad, N.M. 2011. Evaluation of *Zanthoxylum armatum* DC for in-vitro and in-vivo pharmacological screening. *Afr. J. Pharm. Pharmacol.* **5**: 1718–1723.
- Bozin, B., Mimica-Dukic, N., Simin, N., Anackov, G. 2006. Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J. Agri. Food Chem.* **54**: 1822–1828.
- Chang, S.T., Wu, J.H., Wang, S.Y., Kang, P.L., Yang, N.S., Shyur, L.F. 2001. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *J. Agri. Food Chem.* **49**: 3420–3424.
- Chen, X., Shang, S., Yan, F., Jiang, H., Zhao, G., Tian, S., Chen, R., Chen, D., Dang, Y. 2023. Antioxidant activities of essential oils and their major components in scavenging free radicals, inhibiting lipid oxidation and reducing cellular oxidative stress. *Molecules* **28**: 4559.
- Cheng, Y., Fu, Y., Gu, D., Huang, Y., Lu, Y., Liu, Y., ... Li, Y. 2024. Seasonal variation in chemical composition and antioxidant and antibacterial activity of essential oil from *Cinnamomum cassia* leaves. *Plants* **14**: 81.
- Dai, J., Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* **15**: 7313–7352.
- Dawidowicz, A.L., Olszowy, M. 2010. Influence of some experimental variables and matrix components in the determination of antioxidant properties by α -carotene bleaching assay: Experiments with BHT used as standard antioxidant. *Eur. Food Res. Technol.* **231**: 835–840.
- Dhami, A., Palariya, D., Singh, A., Kumar, R., Prakash, O., Kumar, R., Pant, A.K. 2018. Chemical composition, antioxidant, in vitro anti-inflammatory and antibacterial activity of seeds essential oil of *Zanthoxylum armatum* DC. collected from two different altitudes of Kumaun region, Uttarakhand. *Int. J. Chem. Stud.* **6**: 363–370.
- Dhami, A., Singh, A., Palariya, D., Kumar, R., Prakash, O., Rawat, D.S., Pant, A.K. 2019. α -Pinene rich bark essential oils of *Zanthoxylum armatum* DC. from three different altitudes of Uttarakhand, India and their antioxidant, in vitro anti-inflammatory and antibacterial activity. *J. Essent. Oil Bear Plants* **22**: 660–674.
- Diaz, P., Jeong, S.C., Lee, S., Khoo, C., Koyyalamudi, S.R. 2012. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. *Chin. Med.* **7**: 1–9.
- Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J. 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chem.* **83**: 255–262.
- Dragland, S., Senoo, H., Wake, K., Holte, K., Blomhoff, R. 2003. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J.Nutr.* **133**: 1286–1290.
- Feng, W., Chen, J., Zheng, X., Liu, Q. 2011. Thyme oil to control *Alternaria alternata* in vitro and in vivo as fumigant and contact treatments. *Food Contr.* **22**: 78–81.
- Gogoi, P., Baruah, P., Nath, S.C. 2008. Microbiological Research. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiol. Res.* **163**: 337–344.
- Guleria, S., Tiku, A.K., Koul, A., Gupta, S., Singh, G., Razdan, V.K. 2013. Antioxidant and antimicrobial properties of the essential oil and extracts of *Zanthoxylum alatum* grown in North-Western Himalaya. *Sci. World J.* **2013**: 790580.
- Iscan, G., Iscan, A., Demirci, F. 2016. Anticandidal effects of thymoquinone: Mode of action determined by transmission electron microscopy (TEM) *Nat. Prod. Commun.* **11**: 977–978.
- Jain, N., Srivastava, S.K., Aggarwal, K.K., Ramesh, S., Kumar, S. 2001. Essential oil composition of *Zanthoxylum alatum* seeds from northern India. *Flavour. Fragr. J.* **16**: 408–410.
- Jing, G.A.O., Yuan-Yuan, Z., Kai, W., Jian, Z., Gui, Z., Jun, Z. 2016. Identification of sunflower wilt pathogen and its biological characteristics. *Chin. J. Oil Crop Sci.* **38**: 214.
- Jing, L., Ma, H., Fan, P., Gao, R., Jia, Z. 2015. Antioxidant potential, total phenolic and total flavonoid contents of *Rhododendron anthopogonoides* and its protective effect on hypoxia-induced injury in PC12 cells. *BMC Complement Altern. Med.* **15**: 1–12.
- Kamau, P.G., Cruz Romero M.C., Alzate P.C., Morris M.A. Kerry, J.P. (2026). Synergistic activity of natural antimicrobials against common fish spoilage and pathogenic bacteria. *Food Sci.Nutr.* **14**: e72025.
- Karmakar, I., Haldar, S., Chakrabo, M., Dewanjee, S., Haldar, P.K. 2015. Antioxidant and cytotoxic activity of different extracts of *Zanthoxylum alatum*. *Free Radic.Antiox.* **5**: 21–28.
- Li, T., Chen, M., Ren, G., Hua, G., Mi, J., Jiang, D., Liu, C. 2021. Antifungal activity of essential oil from *Zanthoxylum armatum* DC. on *Aspergillus flavus* and aflatoxins in stored platycladi semen. *Front.Microbiol.* **12**: 633714.
- Liu, F., Kan, Q., Feng, K., Chen, Y., Wen, L., He, B., ... Liu, G. 2023. Process of *Zanthoxylum armatum* DC. oil by a novel low-temperature continuous phase transition extraction: Evaluation of aroma, pungent compounds and quality. *Lwt Food Sci. Technol.* **176**: 114523.
- Martucci, J.F., Gende, L.B., Neira, L.M., Ruseckaite, R.A. 2015. Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. *Ind. Crops Prod.* **71**: 205–213.
- Maurya, A.K., Sharma, A., Mukhia, S., Rani, D., Kumar, A., Kumar, D., Kumar, R., Padwad, Y.S., Chand, G., Agnithotri, V.K. 2022. Essential oil composition, in vitro biological activities and safety evaluation of cultivated *Hedychium spicatum* seeds. *Indian J. Pharm. Sci.* **84**: 783–90.
- Mukhija, M., Singh, M.P., Dhar, K.L., Kalia, A.N. 2015. Cytotoxic and antioxidant activity of *Zanthoxylum alatum* stem bark and its flavonoid constituents. *J.PharmacognPhytochem.* **4**: 86–92.
- Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucose amine. *The J.Nutr.* **44**: 307–315.
- Pathak, I., Rokaha, S., Bajracharya, K.B. 2021. Phytoconstituents and biological activities of *Zanthoxylum armatum* fruit extract. *J. Nepal Chem. Soc.* **42**: 125–131.
- Peng, Y., Xu, D., Liao, W., Qian, Q., Wu, J., Gan, T., Zhuo, Z. 2025. Dynamic analysis of composition, insecticidal, and antifungal activities of *Zanthoxylum armatum* DC. at different harvesting periods. *Front. Plant Sci.* **16**: 1603963.

- Phuyal, N., Jhaa, P.K., Raturi, P.P., Gurung, S., Rajbhandary, S. 2019. Essential oil composition of *Zanthoxylum armatum* leaves as a function of growing conditions. *Int. J. Food Prop.* **22**: 1873–1885.
- Prakash, B., Singh, P., Mishra, P.K., Dubey, N.K. 2012. Safety assessment of *Zanthoxylum alatum* Roxb. essential oil, its antifungal, antiaflatoxin, antioxidant activity and efficacy as antimicrobial in preservation of *Piper nigrum* L. fruits. *Int. J. Food Microbiol.* **153**: 183-191.
- Punniyamoorthy, S., Adhikari, A., Baral, J., Singh, G., Pokharel, Y.R. 2025. Essential oils from fruits of *Zanthoxylum armatum* exhibit antioxidant, anti-inflammatory, and anticancer properties by inhibiting p38-MAPK pathway. *Results Chem.* **18**: 102618.
- Rahimmalek, M., Tabatabaei, B.E.S., Etemadi, N., Goli, S.A.H., Arzani, A., Zeinali, H. 2009. Essential oil variation among and within six *Achillea* species transferred from different ecological regions in Iran to the field conditions. *Ind. Crops Prod.* **29**: 348-355.
- Ravipati, A.S., Zhang, L., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Bartlett, J., Smith, P.T., Shanmugam, K., Münch, G., Wu, M.J., Satyanarayanan, M. 2012. Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. *BMC Complement. Altern. Med.* **12**:1-14.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **26**: 1231-1237.
- Santori, A., Vitale, S., Luongo, L., Belisario, A. 2010. First report of *Fusarium lateritium* as the agent of nut gray necrosis on hazelnut in Italy. *Plant Dis.* **94**: 484-484.
- Sarikurkcu, C., Zengin, G., Oskay, M., Uysal, S., Ceylan, R., Aktumsek, A. 2015. Composition, antioxidant, antimicrobial and enzyme inhibition activities of two *Origanum vulgare* subspecies (subsp. *vulgare* and subsp. *hirtum*) essential oils. *Ind. Crops Prod.* **70**: 178-184.
- Singh, G., Kapoor, I.P.S., Singh, P., de Heluani, C.S., de Lampasona, M.P., Catalan, C.A. 2013. Chemistry and antioxidant properties of essential oil and oleoresins extracted from the seeds of tomer (*Zanthoxylum armatum* DC). *Int. J. Food Prop.* **16**: 288-300.
- Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Oxidants and Antioxidants Part A* **299**: 152–178.
- Slathia, S., Sharma, Y.P., Hakla, H.R., Urfan, M., Yadav, N.S., Pal, S. 2021. Post-harvest management of *Alternaria* induced rot in tomato fruits with essential oil of *Zanthoxylum armatum* DC. *Front. Sustain. Food Syst.* **5**: 679830.
- Tian, J., Ban, X., Zeng, H., Huang, B., He, J., Wang, Y. 2011. *In vitro* and *in vivo* activity of essential oil from dill (*Anethum graveolens* L.) against fungal spoilage of cherry tomatoes. *Food Contr.* **22**: 1992-1999.
- Vashisath, S., Maurya, A.K., Agnihotri, V.K. 2021. Comparative chemical profiling of *Zanthoxylum armatum* DC. from Western Himalayan bioresource. *J. Essent. Oil Res.* **33**: 592-600.
- Wang, X., Chan, S.W., Singaram, N., Teoh, M.L., Mah, S.H., Looi, C.Y. 2025. Essential oil from *Aquilaria* spp. (agarwood): a comprehensive review on the impact of extraction methods on yield, chemical composition, and biological activities. *J. Essent. Oil Res.* **37**: 110-144.
- Wojtunik-Kulesza, K.A., Oniszczuk, A. 2024. Ability of selected monoterpenes to reduce Fe (III) ions being pro-neurodegenerative factors: tests based on a FRAP reaction extended to 48 hours. *Int. J. Mol. Sci.* **25**: 2191.
- Yang, J., Wang, Q., Li, L., Li, P., Yin, M., Xu, S., Chen, Y., Feng, X., Wang B. 2022. Chemical composition and antifungal activity of *Zanthoxylum armatum* fruit essential oil against *Phytophthora capsici*. *Molecules* **27**(23): 8636.
- Yang, W.X., Liu, F., Zhang, N., Ren, X.D., Liu, D.Q. 2013. First report of *Alternaria alternata* causing blight on *Zanthoxylum piperitum* in China. *Plant Dis.* **97**: 840-840.
- Zhao, Z.Y., Liu, Y.Y., Yang, J.H., Yang, X.L., Wang, J.H. 2019. First report of *Fusarium lateritium* causing fruit rot of yellow peach (*Amygdalus persica*) in China. *New Dis. Rep.* **39**: 2044-0588.
- Zhishen, J., Mengcheng, T., Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **64**: 555-559.