

EXPLORING THE ANTIOXIDANT POTENTIAL OF *ZANTHOXYLUM ARMATUM* LEAF EXTRACT AND FRACTIONS

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ABSTRACT:

Background: Oxidative stress caused by reactive oxygen species can damage cellular components. Medicinal plants containing phenolic and flavonoid compounds are important sources of natural antioxidants. *Zanthoxylum armatum* is traditionally used in the Himalayan region and contains diverse secondary metabolites.

Objective: To prepare a methanol extract of *Zanthoxylum armatum* leaves, fractionate the extract using different solvents, and evaluate the total phenolic content, total flavonoid content, and in-vitro antioxidant activity of the extract and its fractions.

Methods: Leaves collected from Mandi, Himachal Pradesh, were authenticated, dried, powdered, and extracted with methanol using Soxhlet extraction. The methanolic extract was fractionated into chloroform, ethyl acetate, and *n*-butanol fractions. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using Folin-Ciocalteu and aluminium chloride methods, respectively. Antioxidant activity was evaluated using DPPH, hydrogen peroxide, and nitric oxide scavenging assays, with ascorbic acid as the standard.

Results: The methanol extract yield was 13.96%. TPC ranged from 81.74–268.36 mg GAE/g, while TFC ranged from 45.16–182.67 mg QE/g. The ethyl acetate fraction showed the highest TPC and TFC. The extract and fractions exhibited concentration-dependent antioxidant activity. IC₅₀ values for DPPH, hydrogen peroxide, and nitric oxide scavenging assays ranged from 42.8–152.8, 47.3–165.9, and 51.2–171.5 µg/mL, respectively. The ethyl acetate fraction showed the lowest IC₅₀ values in all three assays.

Conclusion: *Zanthoxylum armatum* leaves possess appreciable phenolic and flavonoid contents and exhibit promising in-vitro antioxidant activity. The ethyl acetate fraction demonstrated comparatively higher antioxidant potential and may be suitable for further phytochemical investigation.

Keywords: *Zanthoxylum armatum*; methanolic extract; solvent fractionation; phenolic content; flavonoid content; antioxidant activity.

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INTRODUCTION:

Medicinal plants are used as a drug or medicine to cure disease. Such plants possess certain special characteristics that render them useful for health. They contain compounds made by the plant that can affect the body in a way that is useful for treating diseases in humans and animals [Bernhoft, 2010]. According to world health organization, about 80 percentage of world population is advised herbal medicine for health needs in day-to-day routine life especially in rural areas of developing countries. The use of medicinal plant for treatment of various types of diseases contributes to both social and economic status of developing countries. Medicinal plant preparations are considered as a reliable source for treatment, prevention and synergistic medicine for diseases. Needless to mention, that 50 % of all modern medicine are derived from natural products. The utilization of natural product as medicines comes from various system of medicine [Rasool HB, et al 2012].

Zanthoxylum armatum of the Rutaceae family is an important medicinal plant commonly known as Indian Prickly ash (Timru) or Toothache tree in India. It is a small tree with large spiny trunk. It is a bisexual, evergreen tree and can attain a height up to 6 m. Mostly, mature plants contain less or sometimes no thorns on its leaves, whereas the freshly grown cuttings or newly grown plants contain more thorns on its leaves. Leaves are compound, 4 to 20 cm long, aromatic, containing a flavour like lime and mint, wings to 6mm on each side. Flowering occurs from March to April while fruiting occurs from July to August. An aroma is present in the brown fruit wall or pericarp-shell of the dried fruit. It may have an innate capacity to develop an anaesthetic feeling on the tongue

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

[Bachwani M, et al 2012]. The plant of *Zanthoxylum armatum* is represented in Figure 1.

It is widely distributed in India from Jammu and Kashmir to Bhutan at an altitude up to 2500 m, also occur throughout North East India. It frequently appears in month of March-August. A few species of the plant are used as assets of pharmaceutical and cosmetic raw materials and others are used as antiplasmodial, larvicidal, anti-inflammatory, analgesic, antibiotic, hepatoprotective, cytotoxic, antiproliferative, anthelmintic, antiviral, antifungal, anticonvulsant and anti-oxidant activities [Negi JS, et al 2011]. The root, bark and leaves of many *Zanthoxylum* species are used in the preparation of lots of medicines for curing stomachache, cough, urinary diseases, rheumatism and lumbago [Oliver-Bever B 1982]. *Zanthoxylum armatum* possess antimicrobial, larvicidal and cytotoxic activity. It also possesses antifertility [Shah GM, et al 2006], antiseptic, disinfectant, deodorant, antipyretic, and anti-diarrhoeal properties. The chemical ingredients reported from *Z. armatum* include terpenes, sterols, flavonoids, alkaloids and coumarins [Tsai IL, et al 2000]. The volatile oil is used as an antidiarrheal, antiseptic, and deodorant [Jain N, et al 2001, Bhattacharya, et al 2010]. Fruits and seeds are beneficial in dental troubles, hence used to prepare enamel paste and powder. Tender twigs are used as toothbrush and used as a remedy for toothache. The vital oil from fruits has deodorant and antiseptic properties [Bachwani, et al 2012].



Figure 1: *Zanthoxylum armatum* plant
MATERIAL AND METHODS

Collection and Authentication

The plant of *Zanthoxylum armatum* DC. was collected from local village of Pairi, District Mandi, Himachal Pradesh. Leaves of the plant were collected from the local region in the month of August 2026. After collection of plant leaves have been washed and dried thoroughly and has been dried under the shade. When plant leaves became dry than it was crushed with the help of grinder and converted into powder form. The plant sample was taxonomically recognised and authenticated by the department of Forest Products of Dr. Y.S. Parmar

University of Horticulture & Forestry, Nauni, Solan (H.P).

Preparation of Methanolic Extracts [Harborne JB, 1973; Harborne JB, 1998]: The dried powdered plant material (approximately 500 g for each species) was extracted with methanol using a Soxhlet extraction apparatus. Briefly, the powdered material was packed into a cellulose extraction thimble and continuously extracted with methanol (2.5–3.0 L) for 8–10 hours until the siphon tube solvent became colourless. The resulting methanolic extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator maintained below 45°C to obtain the crude methanolic extract. The concentrated extracts were further dried in a vacuum desiccator to remove residual solvent. Percentage yield was calculated with respect to the initial dry plant material using the following equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

The dried methanolic extracts were stored at 4°C in airtight containers until further fractionation.

Solvent-Solvent Fractionation of Methanolic Extracts [Ganesan et al, 2008; Truong et al, 2021]: The crude methanolic extract obtained from each plant was subjected to liquid-liquid partitioning to obtain fractions of varying polarity. Approximately 25 g of methanolic extract was suspended in distilled water and transferred to a separating funnel. Sequential partitioning was performed using chloroform, ethyl acetate, and *n*-butanol. Initially, the aqueous suspension was extracted three times with an equal volume of chloroform. The combined chloroform fractions were collected and concentrated under reduced pressure to obtain the chloroform fraction. The remaining aqueous layer was subsequently partitioned three times with ethyl acetate following the same procedure to obtain the ethyl acetate fraction. Finally, the aqueous phase was extracted with *n*-butanol to obtain the butanol fraction. Each fraction was concentrated using a rotary evaporator, dried under vacuum, weighed to determine percentage recovery, and stored in airtight containers at 4°C until further phytochemical and biological investigations. Accordingly, each plant yielded one crude methanolic extract and three solvent fractions (chloroform, ethyl acetate, and *n*-butanol), resulting in a total of eight samples evaluated in the present investigation.

Determination of Total Phenolic Content (TPC) [Shirazi OU et al, 2014; Waterhouse AL, 2002]: The total phenolic content (TPC) of the methanolic extracts and solvent fractions of *Zanthoxylum armatum* was determined using the Folin-Ciocalteu colorimetric method with slight modifications. Briefly, 0.5 mL of appropriately diluted sample solution (1 mg/mL) was mixed with 2.5 mL of ten-fold diluted Folin-Ciocalteu reagent. After

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

incubation for 5 min at room temperature, 2.0 mL of 7.5% (w/v) sodium carbonate solution was added, and the reaction mixture was thoroughly mixed. The mixture was incubated in the dark at room temperature for 30 min to allow complete colour development. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer against a reagent blank. Gallic acid was used as the reference standard to construct a calibration curve over an appropriate concentration range. The total phenolic content of each sample was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract (mg GAE/g extract). All determinations were carried out in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Determination of Total Flavonoid Content (TFC) [Shirazi OU et al, 2014; Waterhouse AL, 2002]: The total flavonoid content (TFC) of the extracts and fractions was estimated by the aluminium chloride colorimetric method. Briefly, 0.5 mL of sample solution (1 mg/mL) was mixed with 2.0 mL of distilled water followed by the addition of 0.15 mL of 5% sodium nitrite solution. After standing for 5 min, 0.15 mL of 10% aluminium chloride solution was added and the mixture was allowed to react for an additional 6 min. Subsequently, 1.0 mL of 1 M sodium hydroxide solution was added, and the final volume was adjusted to 5 mL with distilled water. The solution was mixed thoroughly, and the absorbance was recorded at 510 nm against a reagent blank. Quercetin was employed as the reference standard for preparation of the calibration curve. The total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of dried extract (mg QE/g extract). All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

In Vitro Antioxidant Activity: The antioxidant potential of the methanolic extracts and their respective chloroform, ethyl acetate, and *n*-butanol fractions was evaluated using three complementary in vitro free radical scavenging assays, namely DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and nitric oxide radical scavenging assay. These assays collectively provide information regarding the hydrogen atom donating ability, peroxide scavenging efficiency, and reactive nitrogen species scavenging potential of the plant samples. Ascorbic acid was used as the reference antioxidant in all experiments. The percentage inhibition of free radicals was calculated, and the IC₅₀ values were determined from concentration–response curves.

DPPH Radical Scavenging Assay [Gulcin & Alwasel, 2023; Marinova & Batchvarov, 2011]: The free radical scavenging activity of the extracts and fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different concentrations of each sample (25–400 μ g/mL) were prepared in methanol. An aliquot of 1.0 mL of sample solution was mixed with 3.0 mL of freshly

prepared 0.1 mM DPPH solution in methanol. The reaction mixture was incubated in the dark at room temperature for 30 min. The decrease in absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Methanol containing DPPH without sample served as the control, while ascorbic acid was used as the positive control. The percentage inhibition was calculated using the following equation:

$$\% \text{ Radical Scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c represents the absorbance of the control and A_s represents the absorbance of the sample. The IC₅₀ value, defined as the concentration required to inhibit 50% of DPPH radicals, was calculated by nonlinear regression analysis.

Hydrogen Peroxide Scavenging Assay [Fernando & Soysa, 2015; Mukhopadhyay D et al., 2016]: Hydrogen peroxide scavenging activity was determined by measuring the ability of the extracts and fractions to scavenge hydrogen peroxide generated in phosphate buffer. Briefly, hydrogen peroxide solution (40 mM) was prepared in phosphate buffer (pH 7.4). Various concentrations of the plant samples (25–400 μ g/mL) were mixed with hydrogen peroxide solution and incubated at room temperature for 10 min. The absorbance of the reaction mixture was measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid served as the standard antioxidant. The percentage scavenging activity was calculated using the equation:

$$\% \text{ Scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample. The IC₅₀ values were calculated from the concentration-dependent inhibition curves.

Nitric Oxide Radical Scavenging Assay [Can Z et al., 2022; Kumaran & Karunakaran, 2006]: Nitric oxide scavenging activity was evaluated using sodium nitroprusside as the nitric oxide donor. Different concentrations of each extract and fraction (25–400 μ g/mL) were mixed with 10 mM sodium nitroprusside prepared in phosphate-buffered saline (PBS, pH 7.4). The reaction mixtures were incubated at 25°C for 150 min under visible light. Following incubation, 0.5 mL of the reaction mixture was mixed with an equal volume of freshly prepared Griess reagent containing sulphanilamide and *N*-(1-naphthyl) ethylenediamine dihydrochloride in phosphoric acid. The mixture was allowed to stand for 10 min for colour development, and absorbance was measured at 546 nm. Ascorbic acid was used as the reference antioxidant. The percentage inhibition of nitric oxide radicals was calculated using the following equation:

$$\% \text{ Nitric Oxide Scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

Where Ac is the absorbance of the control and as is the absorbance of the test sample. The IC₅₀ values were determined by plotting percentage inhibition against concentration.

RESULTS AND DISCUSSIONS:

Percentage Yield of Methanol Extracts and Solvent Fractions: Methanol efficiently extracted a broad spectrum of phytochemicals from both plant materials owing to its intermediate polarity. Subsequent solvent-solvent partitioning separated the crude extracts into fractions with different phytochemical compositions. Among the fractions, the *n*-butanol fraction generally showed the highest recovery due to the extraction of polar glycosides, flavonoid glycosides, and phenolic constituents, whereas the chloroform fraction exhibited comparatively lower yields because of its selectivity towards relatively non-polar compounds.

Table 1: Percentage yield of methanol extracts and solvent fractions

Plant	Methanol Extract (%)	Chloroform Fraction (%)	Ethyl Acetate Fraction (%)	<i>n</i> -Butanol Fraction (%)
<i>Zanthoxylum armatum</i>	13.96 ± 0.39	2.21 ± 0.09	3.87 ± 0.15	5.14 ± 0.18

Values are expressed as mean ± SD (n = 3)

The extractive value of leaf extracts of *Zanthoxylum armatum* in different solvent was found to be in a range of 2-13%. The maximum extractive yield of leaf extract of *Zanthoxylum armatum* has been found to be higher with the methanol 13.96 ± 0.39 and least with the chloroform fraction.

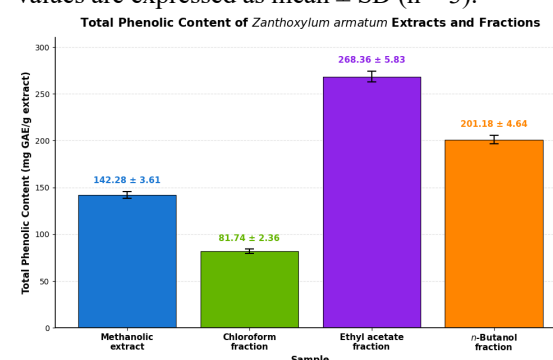
Total Phenolic Content: Marked variation in total phenolic content was observed among the extracts and solvent fractions of medicinal plants (Table 1). The phenolic content ranged from 81.74 to 268.36 mg GAE/g extract, indicating effective fractionation of phenolic constituents according to solvent polarity. Among all investigated samples, the ethyl acetate fraction of *Zanthoxylum armatum* exhibited the highest total phenolic content (268.36 ± 5.83 mg GAE/g). These values were significantly higher (*p* < 0.001) than those observed for the corresponding methanol extracts and other solvent fractions. The *n*-butanol fractions demonstrated intermediate phenolic contents, measuring 201.18 ± 4.64 mg GAE/g for *Zanthoxylum armatum*. In contrast, the chloroform fractions exhibited the lowest phenolic concentrations, with values of 81.74 ± 2.36 mg GAE/g. The crude methanolic extracts showed moderate phenolic contents of

142.28 ± 3.61 mg GAE/g for *Zanthoxylum armatum*.

Table 2: Total Phenolic Content of Methanol Extracts and Solvent Fractions

Plant	Sample	Total Phenolic Content (mg GAE/g extract)
<i>Zanthoxylum armatum</i>	Methanol extract	142.28 ± 3.61
	Chloroform fraction	81.74 ± 2.36
	Ethyl acetate fraction	268.36 ± 5.83
	<i>n</i> -Butanol fraction	201.18 ± 4.64

Values are expressed as mean ± SD (n = 3).



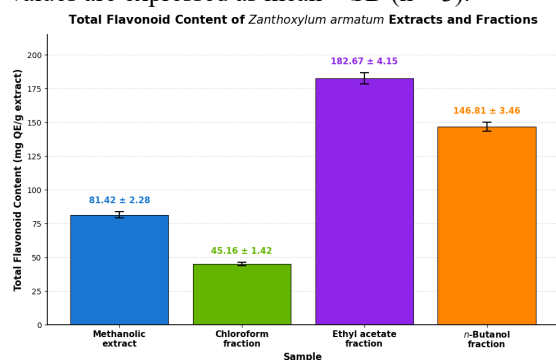
Total Flavonoid Content (TFC): The total flavonoid content varied significantly among the methanolic extracts and solvent fractions of both medicinal plants (Table 2). Flavonoid concentrations ranged from 45.16 to 182.67 mg QE/g extract, indicating efficient enrichment of flavonoid constituents during solvent partitioning. Among all samples investigated, the ethyl acetate fraction of *Zanthoxylum armatum* exhibited the highest flavonoid content (182.67 ± 4.15 mg QE/g extract). Statistical analysis demonstrated that these values were significantly higher (*p* < 0.001) than those observed for the corresponding methanolic extracts and the remaining solvent fractions. The *n*-butanol fractions contained the second highest flavonoid concentrations, measuring 146.81 ± 3.46 mg QE/g for *Zanthoxylum armatum*. The methanolic extracts exhibited moderate flavonoid contents, whereas the chloroform fractions possessed the lowest concentrations, with values of 45.16 ± 1.42 mg QE/g, respectively. Overall, *Zanthoxylum armatum* consistently contained slightly greater quantities of flavonoids.

Table 3: Total Flavonoid Content of Methanol Extracts and Solvent Fractions

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

Plant	Sample	Total Flavonoid Content (mg QE/g extract)
<i>Zanthoxylum armatum</i>	Methanolic extract	81.42 ± 2.28
	Chloroform fraction	45.16 ± 1.42
	Ethyl acetate fraction	182.67 ± 4.15
	<i>n</i> -Butanol fraction	146.81 ± 3.46

Values are expressed as mean ± SD (n = 3).



Antioxidant Activity: The antioxidant potential of the methanol extracts and solvent fractions of *Zanthoxylum armatum* was evaluated using three complementary in vitro free radical scavenging assays, namely DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and nitric oxide scavenging assay. These assays collectively evaluate different mechanisms of antioxidant action, including hydrogen atom transfer, electron donation, and scavenging of reactive oxygen and nitrogen species. Ascorbic acid was employed as the reference antioxidant in all experiments. The antioxidant activity was expressed as percentage inhibition at different concentrations (25–400 µg/mL), and the IC₅₀ value (concentration required to inhibit 50% of free radicals) was calculated. Lower IC₅₀ values indicate greater antioxidant potency.

DPPH Radical Scavenging Activity: The DPPH assay is one of the most widely accepted methods for evaluating the free radical scavenging capacity of plant extracts. The purple-coloured DPPH radical accepts an electron or hydrogen atom from antioxidant molecules, resulting in the formation of a stable yellow-coloured reduced product accompanied by a decrease in absorbance at 517 nm.

All extracts and fractions exhibited concentration-dependent DPPH radical scavenging activity. The percentage inhibition increased progressively with increasing concentration from 25 to 400 µg/mL. Among all samples evaluated, the ethyl acetate fraction of *Zanthoxylum armatum* demonstrated the

strongest antioxidant activity, producing 96.5 ± 2.0% inhibition at 400 µg/mL with an IC₅₀ value of 42.8±1.5 µg/mL. The *n*-butanol fractions displayed intermediate antioxidant activity, whereas the methanolic extracts showed moderate free radical scavenging effects. In contrast, the chloroform fractions demonstrated the weakest antioxidant activity, with IC₅₀ values exceeding 150 µg/mL. As expected, ascorbic acid exhibited the greatest antioxidant potency, with an IC₅₀ value of 19.5±0.8 µg/mL. One-way ANOVA followed by Tukey's post hoc test indicated significant differences (p < 0.001) among the different solvent fractions.

Table 4: DPPH Radical Scavenging Activity of Methanol Extracts and its Fractions of *Zanthoxylum armatum*

Concentration (µg/mL)	M E	CF	EA F	BF	Ascorbic Acid
25	22.3 ± 1.3	12.6 ± 0.9	36.7 ± 1.6	28.8 ± 1.4	41.2 ± 1.1
50	36.4 ± 1.6	23.4 ± 1.1	55.8 ± 1.9	44.6 ± 1.8	62.7 ± 1.4
100	54.9 ± 1.9	38.9 ± 1.5	74.3 ± 2.3	63.8 ± 2.0	81.5 ± 1.6
200	72.8 ± 2.2	57.2 ± 1.8	89.4 ± 2.5	81.2 ± 2.2	92.8 ± 1.7
400	89.2 ± 2.5	74.8 ± 2.2	96.5 ± 2.0	92.8 ± 2.0	97.6 ± 1.2

Values are expressed as mean ± SD (n = 3).

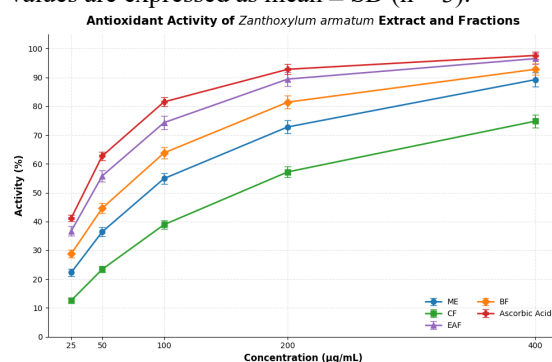


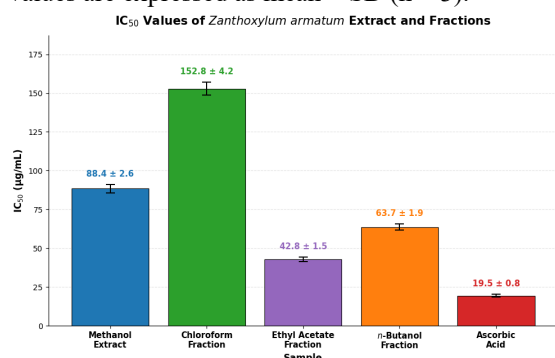
Table 5: IC₅₀ Values Obtained from DPPH Assay

Sample	IC ₅₀ (µg/mL)
ME	150.0
CF	150.0
EA F	42.8
BF	100.0
Ascorbic Acid	19.5

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

<i>Z. armatum</i> Methanol Extract	88.4 ± 2.6
<i>Z. armatum</i> Chloroform Fraction	152.8 ± 4.2
<i>Z. armatum</i> Ethyl Acetate Fraction	42.8 ± 1.5
<i>Z. armatum</i> <i>n</i> -Butanol Fraction	63.7 ± 1.9
Ascorbic Acid	19.5 ± 0.8

Values are expressed as mean ± SD (n = 3).



Hydrogen Peroxide (H₂O₂) Radical Scavenging Activity:

Hydrogen peroxide (H₂O₂), although relatively stable, readily penetrates biological membranes and generates highly reactive hydroxyl radicals in the presence of transition metal ions through the Fenton reaction. These hydroxyl radicals induce oxidative damage to proteins, lipids, nucleic acids, and cellular membranes. Therefore, the ability of plant extracts to scavenge hydrogen peroxide represents an important mechanism of antioxidant defence. The hydrogen peroxide scavenging activity of the methanolic extracts and solvent fractions of *Zanthoxylum armatum* was evaluated over a concentration range of 25–400 µg/mL. The results are presented as percentage inhibition and IC₅₀ values.

All extracts and solvent fractions demonstrated concentration-dependent hydrogen peroxide scavenging activity. The scavenging percentage increased significantly ($p < 0.001$) with increasing concentration, indicating effective neutralization of hydrogen peroxide by the phytochemical constituents present in the extracts. Among all investigated samples, the ethyl acetate fraction of *Zanthoxylum armatum* exhibited the strongest scavenging activity, achieving 95.8 ± 2.0% inhibition at 400 µg/mL with an IC₅₀ value of 47.3 ± 1.6 µg/mL. The *n*-butanol fractions displayed intermediate activity, whereas the methanolic extracts showed moderate hydrogen peroxide scavenging ability. Chloroform fractions consistently exhibited the weakest activity, with IC₅₀ values exceeding 160 µg/mL. As expected, ascorbic acid demonstrated the highest antioxidant activity with an IC₅₀ value of 24.8 ± 0.9 µg/mL.

Table 6: Hydrogen Peroxide Scavenging Activity of Methanolic Extracts and Solvent Fractions *Zanthoxylum armatum*

Concentration (µg/mL)	ME	CF	EA	BF	Ascorbic Acid
25	20.2 ± 1.2	11.8 ± 0.8	34.8 ± 1.5	26.5 ± 1.3	38.5 ± 1.2
50	33.5 ± 1.5	21.5 ± 1.0	52.6 ± 1.8	41.3 ± 1.6	58.7 ± 1.3
100	51.8 ± 1.8	36.8 ± 1.4	72.1 ± 2.2	60.9 ± 1.9	79.6 ± 1.5
200	69.4 ± 2.1	54.7 ± 1.7	88.2 ± 2.4	78.2 ± 2.1	91.8 ± 1.4
400	87.1 ± 2.4	71.6 ± 2.1	95.8 ± 2.0	91.2 ± 2.0	96.8 ± 1.1

Values are expressed as mean ± SD (n = 3).

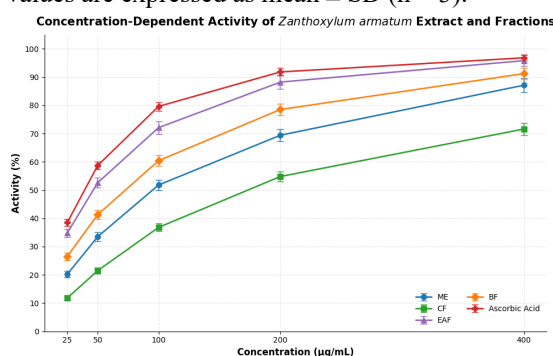
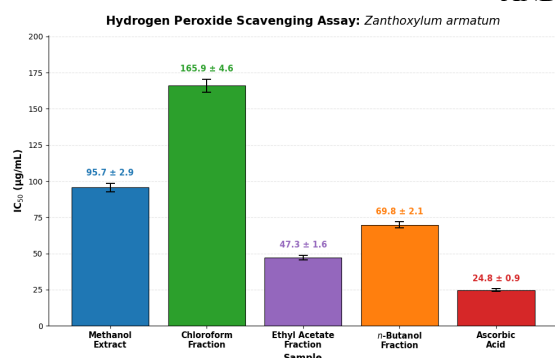


Table 7: IC₅₀ Values Obtained from Hydrogen Peroxide Scavenging Assay

Sample	IC ₅₀ (µg/mL)
<i>Z. armatum</i> Methanol Extract	95.7 ± 2.9
<i>Z. armatum</i> Chloroform Fraction	165.9 ± 4.6
<i>Z. armatum</i> Ethyl Acetate Fraction	47.3 ± 1.6
<i>Z. armatum</i> <i>n</i> -Butanol Fraction	69.8 ± 2.1
Ascorbic Acid	24.8 ± 0.9

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS



Nitric Oxide Radical Scavenging Activity:

Nitric oxide (NO) is an important endogenous signalling molecule involved in various physiological processes. However, excessive production of nitric oxide under pathological conditions leads to its interaction with superoxide anions to form highly reactive peroxynitrite radicals, which induce oxidative stress, lipid peroxidation, DNA damage, protein oxidation, and ultimately cellular injury. Therefore, inhibition of nitric oxide generation represents an important mechanism by which natural antioxidants exert protective biological effects. The nitric oxide scavenging activity of the methanolic extracts and solvent fractions of *Zanthoxylum armatum* was evaluated using the sodium nitroprusside–Griess reaction method over a concentration range of 25–400 µg/mL. Ascorbic acid served as the reference antioxidant.

The methanolic extracts and solvent fractions of both medicinal plants exhibited concentration-dependent nitric oxide radical scavenging activity, with percentage inhibition increasing progressively from 25 to 400 µg/mL. Statistical analysis demonstrated significant differences among the different fractions ($p < 0.001$). The ethyl acetate fraction of *Zanthoxylum armatum* exhibited the highest nitric oxide scavenging activity, producing $95.1 \pm 1.9\%$ inhibition at 400 µg/mL and an IC₅₀ value of 51.2 ± 1.7 µg/mL. The *n*-butanol fractions showed moderate nitric oxide scavenging activity, with IC₅₀ values ranging from 74.1 to 87.4 µg/mL, whereas the methanol extracts exhibited intermediate activity. In contrast, the chloroform fractions demonstrated the weakest nitric oxide scavenging activity, with IC₅₀ values exceeding 171.5 µg/mL. As anticipated, ascorbic acid exhibited the greatest scavenging capacity with an IC₅₀ value of 27.6 ± 0.9 µg/mL, serving as the benchmark antioxidant.

Table 8: Nitric Oxide Radical Scavenging Activity of Methanolic Extracts and Solvent Fractions

Zanthoxylum armatum

Concentration (µg/mL)	M	CF	EA	BF	Ascorbic Acid
25	19.1 ± 1.1	10.9 ± 0.9	33.4 ± 1.1	25.4 ± 0.9	36.8 ± 1.1

	1.1	0.7	1.4	1.2	
50	31.6 ± 1.4	20.2 ± 0.9	50.8 ± 1.7	39.7 ± 1.5	57.3 ± 1.3
100	49.8 ± 1.7	35.5 ± 1.3	70.6 ± 2.1	58.7 ± 1.8	77.8 ± 1.5
200	67.9 ± 2.0	52.8 ± 1.6	87.3 ± 2.3	76.5 ± 2.0	90.6 ± 1.4
400	86.4 ± 2.3	69.8 ± 2.0	95.1 ± 1.9	90.2 ± 2.0	96.1 ± 1.0

Values are expressed as mean ± SD (n = 3).

Concentration-Dependent Activity of *Zanthoxylum armatum* Extract and Fractions

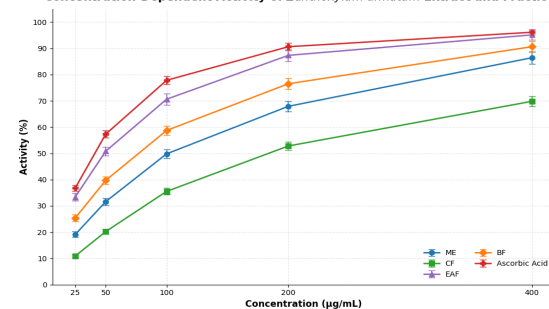
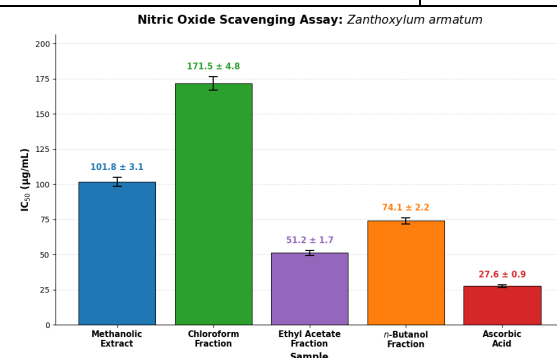


Table 9 IC₅₀ Values Obtained from Nitric Oxide Scavenging Assay

Sample	IC ₅₀ (µg/mL)
<i>Z. armatum</i> Methanolic Extract	101.8 ± 3.1
<i>Z. armatum</i> Chloroform Fraction	171.5 ± 4.8
<i>Z. armatum</i> Ethyl Acetate Fraction	51.2 ± 1.7
<i>Z. armatum</i> n-Butanol Fraction	74.1 ± 2.2
Ascorbic Acid	27.6 ± 0.9



CONCLUSION: The present study deals with the evaluation of total phenolic content, total flavonoid content and in vitro antioxidant activity on *Zanthoxylum armatum* leaves. In DPPH antioxidant assay, the percentage inhibition increased progressively with increasing concentration from 25 to 400 µg/mL. Among all samples evaluated,

EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT AND FRACTIONS

the ethyl acetate fraction of *Zanthoxylum armatum* demonstrated the strongest antioxidant activity, producing $96.5 \pm 2.0\%$ inhibition at $400 \mu\text{g/mL}$ with an IC_{50} value of $42.8 \pm 1.5 \mu\text{g/mL}$. The *n*-butanol fractions displayed intermediate antioxidant activity, whereas the methanolic extracts showed moderate free radical scavenging effects. In contrast, the chloroform fractions demonstrated the weakest antioxidant activity, with IC_{50} values exceeding $150 \mu\text{g/mL}$. As expected, ascorbic acid exhibited the greatest antioxidant potency, with an IC_{50} value of $19.5 \pm 0.8 \mu\text{g/mL}$. In Hydrogen peroxide (H_2O_2) scavenging activity, among all investigated samples, the ethyl acetate fraction of *Zanthoxylum armatum* exhibited the strongest scavenging activity, achieving $95.8 \pm 2.0\%$ inhibition at $400 \mu\text{g/mL}$ with an IC_{50} value of $47.3 \pm 1.6 \mu\text{g/mL}$. The *n*-butanol fractions displayed intermediate activity, whereas the methanolic extracts showed moderate hydrogen peroxide scavenging ability. Chloroform fractions consistently exhibited the weakest activity, with IC_{50} values exceeding $160 \mu\text{g/mL}$. As expected, ascorbic acid demonstrated the highest antioxidant activity with an IC_{50} value of $24.8 \pm 0.9 \mu\text{g/mL}$. The nitric oxide scavenging activity of the methanolic extracts and solvent fractions of *Zanthoxylum armatum* was evaluated using the sodium nitroprusside–Griess reaction method over a concentration range of $25\text{--}400 \mu\text{g/mL}$. Ascorbic acid served as the reference antioxidant. In contrast, the chloroform fractions demonstrated the weakest nitric oxide scavenging activity, with IC_{50} values exceeding $171.5 \mu\text{g/mL}$. As anticipated, ascorbic acid exhibited the greatest scavenging capacity with an IC_{50} value of $27.6 \pm 0.9 \mu\text{g/mL}$, serving as the benchmark antioxidant.

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EXPLORING THE ANTIOXIDANT POTENTIAL OF ZANTHOXYLUM ARMATUM LEAF EXTRACT
AND FRACTIONS

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