

## RESEARCH ARTICLE

# Evaluation of Antioxidant and Anti-bacterial Activity of *Zanthoxylum armatum*: A Research

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## ABSTRACT

The genus *Zanthoxylum* of the family Rutaceae is economically important due to its nutritional, industrial and therapeutic values. *Zanthoxylum armatum* is a short tree or a shrub that grows under dry conditions. The current examination manages the full scale concentrates just as investigations of assessment of pharmacognostical, phytochemical, antioxidants and anti-bacterial movement of *Z. armatum* leaves some particular characters were noticed while contemplating their cross over areas. Physiochemical and primer phytochemical investigations of the leaves of plant *Zanthoxylum armatum* were additionally done. The antioxidants assay is performed by various methods like DPPH, nitric oxide, hydrogen peroxide. The plant extract shows various effects as antioxidants on various concentrations and showed graphically. The anti-bacterial examinations affirmed that the ethanolic removal was compelling for *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa* at 25, 50, 100, 200, 300, 500 µg/mL and tetracycline (100 µg/mL) individually. The current investigation may be helpful to enhance data concerning its distinguishing proof boundaries expected essentially in the method of the adequacy of homegrown medications in the current situation lacking administrative laws to control nature of natural medications and to discover the antioxidants and anti-bacterial action.

**Keywords:** Antioxidant, Pharmacological activities, Phytochemistry, Traditional uses, *Zanthoxylum armatum*.

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## INTRODUCTION

The medicinal property of plants came into highlights during the ancient period. During Ancient times, people were dependent on nature for the rescue from disease. At that time, they were not aware about the medicinal nature of plants. Hence we can say that healing with medicinal plants is as old as mankind itself. Human's search for medicine from plants dates from the far past.

Human has struggled for many years to learn about the therapeutic nature of many plants. Contemporary science has acknowledged their active action, and it has been included in modern pharmacotherapy, known by Ancient Civilization and used throughout millennia.

Plant medicine can be derived from different plant parts, i.e., leaves, roots, bark, fruits, seeds, and flowers. The different plant parts contain different-different ingredients within a plant. Hence, one part of the plant may be toxic while another part of the same plant is harmless. Plants are an important source of medicine and plays an important role in world health. Many countries worldwide that are two-thirds of the world population depend on herbal medicine for primary health care. In India and other developing countries herbal or medicinal plants use to cure the therapeutic curative diseases.

India is mainly the largest country worldwide, which has the highest growth of herbal plants that may have medicinal properties and are also used to prepare herbal drugs. Around 20,000 of herbal plants may exist in India, which may have been registered in Indian system of Ayurveda. Approx 7500 plants are scientifically proved as medicinal drugs.<sup>1</sup> In India, around 2500 herbal plants which used for the different formulation of herbal products. Lots of 7800 manufacturing company practitioners worked on herbal plants by using traditional system of India. India has been proved its therapies by scientific research.

Herbal medicines may have the dietary supplements which may have used as diet food for the good health. There are many ingredients included in dietary supplements such as minerals, vitamins, herbs, amino acids and other supplements, antioxidants, macronutrients, tonics, and some of herbal preparation also involves Muslipak Chyawanprash, Ashwagandha etc. They are administered as oral pills, capsules, tablets, syrups etc. Some of the botanicals found in any form such as dried food, liquid or solid extracts, tablets, capsules etc. Functional foods of herbal remedies may have flour, oil, probiotics food etc., which may reduce the risk factors of chronic diseases.<sup>2,3</sup>

*Zanthoxylum armatum* is also known as winged prickly ash or timru. It belongs to the family Rutaceae. It is an aromatic, deciduous, spiny and shrubs. Timru is an evergreen, small tree and height of 6 m. Leaves are

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around 20 cm long, aromatic, and compound. Fruits are reddish in colour and around 5 mm in diameter.<sup>4</sup> Flowers are green to yellowish colour. Seeds are shiny and bitter. Fruits, seeds and bark are used as cure of dyspepsia and fever with Fruits, seeds and bark are used as cure for dyspepsia and fever. Fruits and seeds are useful for dental trouble thus many kind of dental paste and powder are made from it. It has many antiseptic properties. Mainly found in Himalayas Warmer valley 1000 to 2100 m from the sea level and moves from Jammu Kashmir to Assam.

Synonyms of *Z. armatum* are *Z. planispinum*, Timru, Timber, Toothache tree, winged prickly ash. Found in India, Meghalaya, Mizoram and Manipur.<sup>5</sup> There are 11 species and genus of the *Z. armatum* that mainly found as medicinal plants, *Z. budrunga*, *Z. oxyphyllum*, *Z. ovalifolium*, *Z. acanthopodium*, *Z. planispinum*, *Z. armatum*, *Z. nitidum*, *Z. rhesta*, *Z. simulans*, *Z. avicennae* and *Z. limonella*. Out of these, 4 species are *Z. armatum* DC, *Z. acanthopodium* DC, *Z. oxyphyllum* Edgew, and *Z. budrunga* are present in India.<sup>4</sup> These species that may be used as medicinal plants have more effectiveness against the diseases and are more curable without any side effects.<sup>6</sup>

*Z. armatum* is used as a medicine from ancient times to cure various diseases such as toothache and problems related to tooth and asthma, used for gum bleeding, fever, dyspepsia and tonics, etc.<sup>7,8</sup> The fruit part of the plant may use to purify the water. The wood of this plant may be heavier and stronger than it is used for walking sticks. *Z. armatum* also gives and showed work against antioxidants,<sup>9</sup> antinociceptive, antifungal,<sup>10</sup> anti-inflammatory, hepatoprotective,<sup>11</sup> pesticides, anthelmintic, anti-proliferative<sup>12</sup> etc.

The phytochemical investigation shows the presence of volatile oil and resins. 1-alpha-phellandrene, linalool, carbonyl compound, methyl n-nonyl ketone. Linalyl acetate, sesquiterpene, hydrocarbon, tricosaine, dictamine, fragarine, magnoflorine, skimmianine,

xanthoplanine.<sup>13,14</sup> Essential oils known as fennel, citronella, geranium, lavender and rosemary are used in fragrance and in food and beverages.<sup>15,16</sup>

## MATERIAL AND METHODS

### Collection and Authentication

The plant of *Z. armatum* was collected from local village of Chail Chowk, District Mandi, Himachal Pradesh. Selected plants may mostly cultivate in the forest region of Mandi district, named Kamrughati jyuni valley. This experimental work was carried out at Abhilashi University Mandi in Pharmacognosy department. Leaves of the plant were collected from the forest region in August and September 2020. After collection, the plant was washed and dried thoroughly, then dried under the shade, which takes around a month. When the plant became dry, it was crushed into mortar pestle and converted into powder form. The Figure 1 shows a green intact plant of *Z. armatum*.

### Macroscopical Evaluation

Organoleptic evaluation means the study of drugs using organs of senses. It refers to the analysis methods like color, odour, taste, size, shape and special features, such as touch, texture, etc. The initial sight of the plant or extract is so specific that it tends to identify itself. If this is not enough, perhaps the plant or extract has a characteristic odour or taste. The study of form of a crude drug is morphology, while description of the form is morphography. It influences the color, odor, taste, texture, and size of the crude drug, which depends on the sense organs, also called sensory characters.<sup>17</sup>

### Study of Macroscopically Characters

#### Optical Microscopy

Free-hand sections of the lamina of the leaf of *Z. armatum* were cleared in warm chloral-hydrate solution in a flask over a boiling water bath. The cleared specimens were mounted and photomicrographs of the epidermal features were captured from the slides using an Olympus light microscope fitted with camera using AM cap software. The transverse sections (TS) of the lamina and the midrib of the fresh leaf were also cleared, mounted, observed and their features photographed.<sup>17</sup>

#### Powder Microscopy

Little quantity of leaf powder of *Z. armatum* occupied on a microscope slide and small amount of phloroglucinal and hydrochloric acid then a small drop of glycerol to the slide and observed under the microscope with 45X magnification. And the powder characters are observed like xylem, phloem, calcium oxalate crystals and starch



Figure 1: Plant of *Z. armatum*

grains by adding a drop of iodine the starch grains observed in blue color.<sup>18</sup>

### Ash Values

The determination of ash is useful for detecting low grade products, exhausted drugs, and excess of sandy or earthy matter. The residue remaining after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. Ash values help determine the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The ash remaining after complete ignition of the medicinal plant materials is determined by three different methods known as Total ash, Acid-insoluble ash and water-soluble ash.<sup>18</sup>

### Total Ash

Total ash is useful in detecting the crude drugs mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents to improve their appearance, as is done with nutmegs and ginger. About 2 g of the powdered drug was weighed accurately and spread as a fine layer at the bottom in a tared silica crucible. The crucible was incinerated at a temperature to 500-600°C until it is white, indicating the absence of Carbon. The crucible was cooled and weighed. The entire procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated with reference to the weight of the air-dried drug using the following formula.<sup>18</sup>

$$\% \text{ Total Ash} = (\text{Weight of total ash}) / (\text{Weight of crude plant material taken}) \times 100$$

### Acid-insoluble Ash

Acid insoluble ash means the ash insoluble in dilute hydrochloric acid. The majority of crude drugs contain calcium oxalate, and the quantity of calcium oxalate varies very frequently. The ash obtained in the total ash was boiled with 25 mL of hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash-less filter paper and washed with hot water. The insoluble ash was transferred to a tared silica crucible together with ash-less filter paper and ignited at a temperature not exceeding 600°C, cooled and weighed. The procedure was repeated

till a constant weight was observed. This measures the amount of silica present, especially as sand and siliceous earth. The percentage of acid-insoluble ash was calculated using the air-dried drug using the following formula.<sup>18</sup>

$$\% \text{ Acid insoluble ash} = (\text{Weight of the acid insoluble ash}) / (\text{Weight of crude plant material taken}) \times 100$$

### Water-soluble Ash

The water-soluble ash is used to detect the presence of material exhausted by water. Water-soluble ash is the difference in weight between the total ash and the residue after-treatment of the total ash with water. As described in the total ash, the ash was boiled with 25 mL of hot water for 5 minutes, filtered on an ash-less filter paper, and washed with hot water. The insoluble ash was transferred to a tarred silica crucible and ignited at 600°C. The procedure was repeated to get a constant weight. The weight of the insoluble matter was subtracted from weight of the total ash. The difference in weight was considered for water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air dried drug using the following formula.<sup>18</sup>

$$\% \text{ Water soluble ash} = (\text{Weight of the total ash} - \text{Weight of the water insoluble ash}) / (\text{Weight of crude plant material}) \times 100$$

### Sulphated Ash

Sulphated ash is done by adding sulphuric acid to get sulphate salts, and the percentage ash is calculated with reference to the air-dried drug. The silica crucible was heated to redness for 10 minutes. Allowed to cool in a desiccator and silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weighed. 1 to 2 g of the test drug substance accurately weighed into the crucible was ignited gently at first, until the substance was thoroughly charred. The residue was cooled and moistened with 1 ml of sulphuric acid, heated gently until white fumes no longer evolved, and then ignited at 800 ± 25°C until all the black particles disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool and then a few drops of sulphuric acid was added and ignited as before. It was allowed to cool and then weighed to give the Sulphated ash content. The % water soluble ash is determined by the following equation.<sup>18</sup>

$$\% \text{ Sulphated as} = (\text{Weight of the Crucible with ash} - \text{Weight of the Crucible}) / (\text{Weight of Plant material}) \times 100$$

### Moisture Content Determination

The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So, the moisture content of a drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight. Weigh 2 gm. of the crude



drug and place in the china dish and dried in the oven at 105°C for 5 hours. and weigh the drug continuously. This procedure was repeated till a constant weight was obtained. The sample's moisture content was calculated as percentage with reference to the shade-dried material.<sup>18</sup>

#### Extraction with Different Solvents

A Soxhlet extractor has three main sections: a percolator (boiler and reflux) as shown in Figure 2 which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted; and a siphon mechanism, which periodically empties the thimble. Details are given in Table 1. Mechanism: The solvent is heated to reflux. The solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days. A portion of the non-volatile compound dissolves in the solvent during each cycle.

Process: The powdered plant samples (50 g/250 mL) were extracted with petroleum ether, chloroform, methanol and water using Soxhlet apparatus at 55–85°C for 8–10 hours to extract the polar and non-polar compounds. The powdered pack material was air dried for each solvent extraction and then used. The solvents of the respective extracts were reduced under room

temperature and stored at 4°C for further use. The dried plant extracts were then re-dissolved in their respective solvent and to get the solution of 10 mg/10 mL for each extract which was subjected to analysis of *in vitro* antioxidant activities and anti-bacterial activity.<sup>19</sup>

#### In-vitro Characterization of Leaf Extract

##### Extractive Values

The term “extraction” is used, pharmaceutically to indicate “The process of separating the medicinally active portion of plant or animal tissues from the inactive or inert components by using selective solvent in standard extraction procedure”. Thus, the products are relatively impure liquids, semisolids or powders intended for oral or external use. The mode of extraction selected greatly depends on the plant material's texture and water content and the type of substance to be isolated.

##### Petroleum Ether Soluble Extractive Value

The powdered plant samples (50 g/250 mL) were extracted with petroleum ether using Soxhlet apparatus at 55–85 °C for 8–10 hours in order to extract non-polar compounds. The solvent with extracted material filtered through Whatman filter paper no. (41) to attain a clear filtrate. The filtrate was reduced, evaporated and dried under reduced pressure using rotatory vacuum evaporator. The extract was stored in a small bottle in fridge at 5°C. The percentage yield of petroleum ether extract was calculated with using the following formula.<sup>19</sup>

$$\% \text{ Extractive value} = (\text{Weight of dried extract}) / (\text{Weighted of plant material}) \times 100$$

##### Ethyl Acetate Soluble Extractive Value

The powdered plant samples (50 g/250 mL) were extracted with Ethyl acetate using Soxhlet apparatus at 55–85°C for 8–10 hours in order to extract non-polar compounds. The solvent with extracted material filtered through Whatman filter paper no. (41) to attain a clear filtrate. The filtrate was reduced, evaporated and dried under reduced pressure using rotatory vacuum evaporator. The extract was stored in a small bottle in fridge at 5°C. The percentage yield of chloroform extract was calculated with using the following formula.<sup>19</sup>

$$\% \text{ Extractive value} = (\text{Weight of dried extract}) / (\text{Weight of plant material}) \times 100$$



Figure 2: Assembly of Soxhlet extraction apparatus

Table 1: Extraction of plant parts of *Z. armatum* in different solvents

S. No.	Code	Parts of plant	Solvent for Extraction	Amount of plant material (gm.)	Volume of solvent
1	ZAP	Leaves	Petroleum ether	50	250
2	ZAC	Leaves	Chloroform	50	250
3	ZAM	Leaves	Methanol	50	250
4	ZAW	Leaves	Water	50	250
5	ZAE	Leaves	Ethyl acetate	50	250

**Chloroform Soluble Extractive Value**

The powdered plant samples (50 g/250 mL) were extracted with chloroform using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract non-polar compounds. The solvent with extracted material filtered through Whatman filter paper no. (41) to attain a clear filtrate. The filtrate was reduced, evaporated and dried under reduced pressure using rotatory vacuum evaporator. The extract was stored in a small bottle in fridge at 5°C. The percentage yield of chloroform extract was calculated with using the following formula.<sup>19</sup>

$$\% \text{ Extractive value} = (\text{Weight of dried extract}) / (\text{Weight of plant material}) \times 100$$

**Alcohol Soluble Extractive Value**

The powdered plant samples (50 g/250 mL) were extracted with methanol using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract non-polar and polar compounds. The solvent with extracted material filtered through Whatman filter paper no (41) to attain a clear filtrate. The filtrate was reduced, evaporated and dried under reduced pressure using rotatory vacuum evaporator. The extract was stored in a small bottle in fridge at 5°C. The percentage yield of methanol extract was calculated with using the following formula.<sup>19</sup>

$$\% \text{ Extractive value} = (\text{Weight of dried extract}) / (\text{Weighted of plant material}) \times 100$$

**Water Soluble Extractive Value**

The powdered plant samples (50 g/250 mL) were extracted with water using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract polar compounds. The solvent with extracted material filtered through Whatman filter paper no. (41) to attain a clear filtrate. The filtrate was reduced, evaporated and dried under reduced pressure using rotatory vacuum evaporator. The extract was stored in a small bottle in fridge at 5°C. The percentage yield of water extract was calculated with using the following formula.<sup>19</sup>

$$\% \text{ Extractive value} = (\text{Weight of dried extract}) / (\text{Weighted of plant material}) \times 100$$

**Preliminary Phytochemical Analysis**

The extract was tested for the presence of bioactive compounds by using following standard methods.<sup>20</sup>

**Detection of Carbohydrates**

100 mg of extract was dissolved in 10 ml of water and filtered. The filtrate prepared was used to test the presence of proteins and amino acids.

**a) Molisch's Test**

To the 1 ml of filtrate add 2 drops of Molisch's reagent in a test tube and add 2 ml of concentrated sulphuric acid

carefully along the sides of the test tube. Formation of violet colour at the interface of two liquids indicates the presence of carbohydrates.

**b) Fehling's Test**

To the 1 mL of filtrate add 4 mL of Fehling's reagent (2 ml Fehling A and 2 mL Fehling B solutions) in a test tube and heated for about 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

**c) Barfoed's Test**

1 mL of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on water bath. Formation of a brick-red precipitate within 5 minutes indicates the presence of mono saccharides. Disaccharides generally don't give any reaction even for ten minutes.

**Detection of Proteins and Amino Acid**

100 mg of extracts were dissolved in water (10 mL) and then it was filtered. The filtrate was used to test the presence of proteins and amino acids.

**a) Millon's Test**

2 ml of filtrate was treated with 2 mL of Millon's reagent in a test tube and it was heated in a water bath for about 5 min, cooled and few drops of NaNO<sub>2</sub> solution were added to the test tube. Formation of white precipitate and it turns to red upon heating indicates the presence of proteins and amino acids.

**b) Ninhydrin Test**

To the 2 mL of filtrate add 2-3 drops of Ninhydrin reagent in a Test tube and boiled for about 2 min. Formation of deep blue colour indicates the presence of amino acids.

**Detection of Glycosides**

0.5 g of extract was hydrolysed with 20 mL of dilute 0.1 N HCL and then filtered. The filtrate obtained was used to test the presence of glycosides.

**a) Borntrager Test**

To the 1 mL of filtrate add 2 mL of 1% ferric chloride solution in a test tube and heated for about 10 min in boiling water bath. The mixture was cooled and was shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Appearance of pink colour in the ammonical layer indicates the presence of glycosides.

**Detection of Flavonoids**

Shinoda Test To the extract (100 mg) in a test tube adds few fragments of magnesium metal. To the test tube add 3 to 4 drops of conc. HCL. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

### Detection of Alkaloids

0.5 g. of extract was taken and it was dissolved in 10 ml of dilute 0.1 N HCL and then filtered. The filtrate was used to test the presence of alkaloids.

#### a) Dragendorff's Test

To the 2 mL of filtrate, Dragendorff's reagent (2-3 drops) was added. Appearance of reddish brown coloured precipitate indicates the presence of alkaloids.

#### b) Mayer's Test

To the 2 mL of filtrate, 2-3 drops of Mayer's reagent were added, this leads to formation of cream coloured precipitate indicates the presence of alkaloids.

#### c) Wagner's Test

To the 1 mL of the extract, add 2 ml of Wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

### Detection of Phenolic Compounds

100 mg of extract mixed with 1 ml of water and then it was boiled and filtered. The filtrate was used for the following test.

#### a) Ferric Chloride Test

Take 2 mL of filtrate in a test tube to that add 2 mL of ferric chloride solution (1%). Formation of bluish to black colour indicates the presence of phenolic nucleus.

#### b) Lead Acetate Test

To the 2 mL of filtrate in a test tube add 2 to 3 drops of lead acetate solution. Appearance of yellowish precipitate indicates the presence of tannins.

#### c) Alkaline Reagent Test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

## Antioxidant Activity

### Determination of DPPH Free Radical Scavenging Activity

**Principle:** This method is based on an antioxidant compounds' hydrogen donating or radical scavenging ability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical to 2,2-diphenyl-1-picrylhydrazine resulting in a pale-yellow solution. The decrease in the absorbance as the colour of the solution fades (from deep violet to light yellow) is monitored at 517 nm.

**Procedure:** The plant extract was prepared at various concentrations ranging from 100 µg/mL to 700 µg/mL in methanol. The reaction mixture consisted of 1 mL of sample, 3 mL of methanol and 0.5 mL of 1 mM methanolic

solution of DPPH. The reaction mixture was then vortexed and left to stand for 5 minutes. The absorbance of the resulting solution was measured at 517 nm. A mixture of methanol and DPPH solution served as a blank while a reaction mixture of methanol, DPPH, and standard (vitamin C) served as the positive control. All tests were run in triplicates. The percentage radical scavenging activity was calculated according to the following formula.<sup>21</sup>

$$\% \text{ DPPH Radical Scavenging activity} = (A_0 - A_1) / A_1 \times 100$$

Where, control A<sub>0</sub> = Absorbance of the control, A<sub>1</sub> = Absorbance of extract. The percentage radical scavenging activity was then plotted against various concentrations and the IC<sub>50</sub> (half maximal inhibitory concentration) was determined graphically.

### Determination of Nitric Oxide Radical Scavenging Activity

**Principle:** This assay is based on the theory that sodium nitroprusside (SNP) spontaneously generates nitric oxide which interacts with molecular oxygen to form nitrite ions that may be estimated using Griess reagent. Scavengers of nitric oxide in the extract compete with molecular oxygen resulting in reduced production of nitrite ions.

**Procedure:** Nitric oxide radical scavenging activity of methanolic extract of leaf of *Zanthoxylum armatum* was determined. The reaction mixture constituting a solution of SNP (5 mole/l) in phosphate-buffered saline pH 7.4 and different concentrations of the extract ranging from 100 µg/mL to 700 µg/mL, prepared in methanol, was incubated for 30 minutes at 25°C. After incubation, an aliquot of the incubated solution (1.5 mL) was diluted with 1.5 mL of Griess reagent (0.1% N-1-naphthyl ethylene diamine di-hydrochloride [NED], 1% sulphanilamide, and 2% phosphoric acid). Quercetin was used as a standard drug. Diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED generated a pink chromosphere, whose absorbance was measured spectrophotometrically at 546 nm against a blank. The blank contained all the reactants except the extract. All the tests were performed in triplicate. The percentage of radical scavenging activity was computed using the formula below.<sup>21</sup>

$$\% \text{ Nitric oxide radical scavenging activity} = (A_0 - A_1) / A_1 \times 100$$

Where, A<sub>0</sub> = control reaction absorbance (blank) and A<sub>1</sub> = extract or quercetin absorbance.

### Determination of Hydrogen Peroxide Radical Scavenging Activity

**Principle:** This method is based on the decrease in



absorbance of  $H_2O_2$  following reduction of  $H_2O_2$  by the antioxidant compound.

**Procedure:** A solution of 40 mm hydrogen peroxide  $H_2O_2$  was prepared in phosphate buffer Ph. 7.4. The plant extract (at different concentrations of 100  $\mu$ g/mL to 700  $\mu$ g/mL) was added to hydrogen peroxide solution, incubated for 10 minutes, and absorbance measured at 230 nm against a blank solution containing phosphate buffer without the hydrogen peroxide. Ascorbic acid was used as a positive control. All tests were run in triplicate and hydrogen peroxide radical scavenging activity was calculated using the following formula.<sup>21</sup>

$$\% \text{ Hydrogen peroxide radical scavenging activity} = \frac{(A_0 - A_1)}{A_1} \times 100$$

Where,  $A_0$  = control absorbance (blank) and  $A_1$   $\frac{1}{4}$  extract or ascorbic acid absorbance.

## Anti-bacterial Activity

### In-vitro Anti-Bacterial Evaluation

2 gram-negative *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and 2 gram-positive *S. aureus* (ATCC: 25923), and *B. subtilis* (ATCC: 6633) were used to check the effectiveness of methanolic extract of the *Z. armatum* medicinal plants. These bacterial strains were procured from the IMTECH.

### Preparation of Inoculums

The inoculum of each bacterium was developed by growing the organism overnight in Mueller Hinton medium at 37°C and then subcultures in Mueller Hinton agar at 37°C overnight. Four or five bacteria colonies were selected using a sterile inoculating loop and suspended in 2 mL of sterile saline. The turbidity of the bacterial suspensions was adjusted to the 0.5 McFarland standards by diluting with sterile saline. Sterile swabs were dipped into the inoculum tubes. Mueller Hinton agar plates were inoculated with bacteria by streaking the swabs.<sup>22</sup>

### Preparation of Stock Solution and Dilutions

The plant extract was stored in the refrigerator at 4°C until required for use. Stock solution (1000 mg/mL) of methanolic plant extract of leaves was initially prepared by solubilizing 10 gm of plant extract powder with 10 mL of the 1% DMSO. Further filtration for each stock solution by using Millipore unit and Whatman filter paper no. (1). the serial dilutions were prepared for stock solution with sterile distilled water to give seven dilutions 500, 300, 200, 100, 50, 25, 10  $\mu$ g/mL.

### Anti-bacterial Activity of Plant Extract

The antimicrobial susceptibility was initially assayed by the agar disk diffusion method. Base plates were prepared by pouring 15 mL Mueller Hinton (MH) agar (Bio lab, UK) into sterile Petri dishes (14 cm diameter) and allowed to set. The sterilized standard discs (6.4 mm)

were impregnated with three seven concentrations 500, 300, 200, 100, 50, 25, 10  $\mu$ g/mL of methanolic extract of the *Z. armatum* and Tetracycline as positive control and then incubated at 55°C for 1-hour. All discs were utilized to assess the anti-bacterial activity against four different bacterial strains on Mueller Hinton agar plates. The impregnated discs were placed on the Mueller Hinton agar plates were inoculated with bacteria. Petri dishes were incubated for 24 hours at 37°C. Finally, the anti-bacterial capacity of the methanolic extract of the *Z. armatum* was measured by the zone of inhibition around discs as bacterial growth inhibition, measured in mm using a Venire calliper. The disc diffusion test was performed in triplicate.<sup>22</sup>

### Minimum Inhibitory Concentration (MIC)

MIC was the lowest concentration of the methanolic extract with no visible bacterial growth. Plant extracts that gave a positive result for the disk diffusion assay were used to determine MIC using the microplate dilution method. Serial dilutions of the plant extracts were prepared in the water from the stock solution, yielding five serial dilutions of the original extract. Inoculum of organism was prepared in Mueller-Hinton broth, and the turbidity was adjusted to approximately 0.5 McFarland turbidity standard to prepare  $1 \times 10^8$  bacterial/mL. 150  $\mu$ L of plant extract was added to each well of the 96-well microplate. 50  $\mu$ L of bacterial suspension was added to each well. Tetracycline drug was used as the positive control. Microtiter plates were incubated at  $35 \pm 2^\circ\text{C}$  for 24 hours. The lowest concentration that inhibits the growth of bacteria was noted and considered as the MIC value for each bacteria strain.<sup>22</sup>

## RESULTS AND DISCUSSION:

### Collection of Plant Material

The plant of *Z. armatum* was collected in village of Chail Chowk, District Mandi, Himachal Pradesh.

### Assessment of Quality of Plant Materials

#### Macroscopic Evaluation

Macroscopic evaluation was conducted to evaluate the morphological parameters of the leaves of *Z. armatum*. An image of *Z. armatum* leaves is shown in Figure 3. The morphological parameters include size, color, shape, texture, fracture, taste, and odour in Table 2.

#### Microscopically Characters

Transverse Section (T.S.) of leaf lamina of *Z. armatum* showed upper epidermis followed by palisade mesophyll, spongy mesophylls and then lower epidermis. Collateral

Vascular bundle was also present (Figure 4) Upper epidermis was non-stomatiferous and covered with thin cuticle, composed of rectangular-shaped, compactly arranged cells. The spongy mesophyll cells (some of which were idioblast, i.e., containing Cu-oxalate crystals) were rounded to somewhat elongated in shape and were arranged loosely with large intercellular spaces.

Adaxial surface of *Z. armatum* leaf midrib was planoconvex, while the adaxial surface was semicircular. There was a complete absence of any kind of appendage on it. T.S of midrib showed upper epidermis, hypodermis, vascular bundle, cortex and lower epidermis. Upper



Figure 3: Leaves of the *Z. armatum*

Table 2: Macroscopic evaluation parameters of *Z. armatum* leaves

S. No	Parameters	Observations
1.	Colour	Dark green upper surface, light green lower surface
2.	Size	Lanceolate, acuminate, imparipinnate
3.	Shape	Small leaves 2.3–3 cm, large leaves 12–14 cm
4.	Taste	Spice
5.	Odour	Aromatic, spicy
6.	Fracture	Brittle when dry

Table 3: % Moisture content of leaves of the *Z. armatum*

Plant material	%Moisture content
Leaves	0.53 ± 0.16

Table 4: Total ash value, acid-insoluble ash, water-soluble ash, sulfated ash of leaves of the *Z. armatum*

S. No.	Plant material	Total Ash value (%w/w)	Acid-insoluble ash (%w/w)	Water-soluble ash (%w/w)	Sulphated ash (%w/w)
1	Leaves	2.60 ± 0.28	0.97 ± 0.49	1.47 ± 0.50	1.14 ± 0.23

epidermal was single-layered with oval to rectangular-shaped cells. Phloem was characterized by rounded cells with almost the same dimensions as that of xylem. Vascular bundle was surrounded by idioblast cells having calcium oxalate crystals either present solitary or aggregated in clusters. The lower epidermis of the midrib region was composed of rectangular cells.

### Moisture Content Determination

Moisture is one of the major factors responsible for the deterioration of drugs and herbal formulations. The moisture promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. The moisture content was dried leaves of the *Z. armatum* was shown in Table 3.

This study recorded a moisture range of 0.3–0.7%, which is deemed good as water content in vegetable drugs should not be greater than 14%.

### Ash Values

Ash is an inorganic substance leftover from the combustion of organic material. An image of Muffle furnace used for determining Ash values is shown in Figure 5. Ignition process is that all organic substances will burn into black charcoal. With continuous heating, all organic substances (charcoal) will be burned out and ash will be obtained in the form of the remaining substances consisting of inorganic substances in the form of metal oxides. The growth process in nature requires nutrients, including those from minerals and other organic compounds. Total ash values for the leaves of the *Z. armatum* were observed  $2.60 \pm 0.28\%$  (Table 4) lied, within the standard range of % moisture content recommended by the Indian pharmacopoeia. A high ash value indicates contamination, substitution or adulteration by minerals.

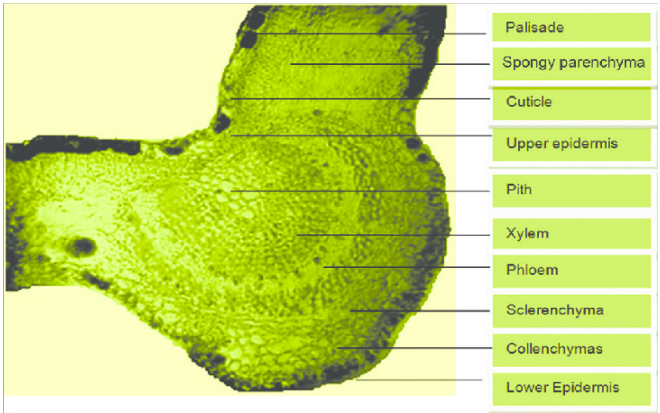


Figure 4: T. S. of *Z. armatum* leaf.



Furthermore, the value of water-soluble ash, a part of the total ash content, got easily soluble in the water for the leaves of the *Z. armatum* was observed to be  $1.47 \pm 0.50\%$ . Similarly, the value of insoluble acid ash and sulfated ash value was found to be  $0.97 \pm 0.49\%$  and  $1.14 \pm 0.23$ , respectively. The value of all parameters for ash value was found to be in the range of pharmacopoeia (Figure 6).

#### Extraction with Different Solvents using Soxhlet Process

The dried leaves of *Z. armatum* were reduced to fine powder (40 size mesh) and around 50 gm of crude dried powder was subjected to successive extraction with different solvent-based on the polarity. The dried extract was first subject to the non-polar solvent petroleum ether to remove the fatty material and further the plant residue material in each step material solvent was subjected to the extraction with solvent of increasing polarity and finally, the dried material was subjected to the extract with water to extract the water-soluble phytoconstituent. The effective extraction process yielded that the liquid solution was further concentrated and dried using a rota evaporator. The residue obtained after evaporation was weighed and the percentage yield was calculated. The above residue was subjected to successive washed with petroleum ether, Ethyl acetate, Chloroform, Methanol and water. Before extracting with the next solvent, the extracted material (Residue) was dried at room temperature.

#### In-vitro Characterization of Extract of *Z. armatum* Leaves

##### Physical Observation of Extract of *Z. armatum* Leaves

All prepared extracts demonstrated the light greenish-brown color on visual observation as shown in Table 5.

##### Extractive Value

It is employed for material to which no suitable chemical or biological assays exist. The extracts obtained by exhausting crude drugs with different solvents are approximate measures of their chemical constituents. Extractive values determine the amount of active constituents present in given plant material in a given solvent. Extracts were prepared with various solvents by Soxhlet methods as described. The percentage of the dry extract was calculated in terms of air-dried crude drug powder. Various solvents are used according to the type of the constituents to be analyzed. Water-soluble extractives is used for crude drugs containing water-soluble constituents like glycosides, tannins, mucilage etc. alcohol-soluble extractive is used for crude drugs containing tannins, glycosides, resins, etc. and ether-soluble extractives are used for drugs containing volatile

constituents and fats. The extracted value of all prepared extracts of leaves of *Z. armatum* is shown in Table 6.

The extractive value of leaf extracts of *Z. armatum* in different solvent was found to be in a range of 13–36%.



Figure 5: Ash value determination of crude drug

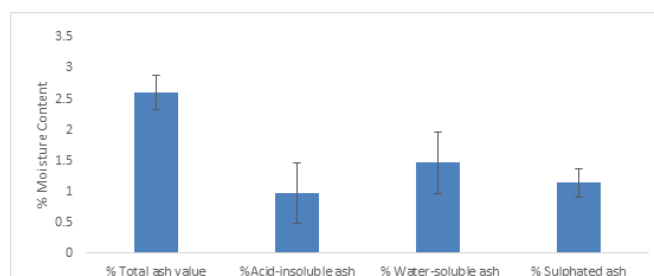


Figure 6: Bar graph of the total ash value, acid-insoluble ash, water-soluble ash, sulfated ash of leaf of the *Z. armatum*

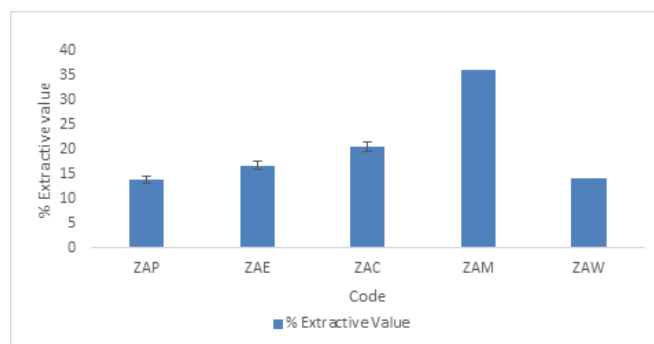


Figure 7: %Extractive value of different extract of leaves of *Z. armatum*

Table 5: Physical appearance of all prepared extract

S. No.	Code	Physical appearance
1	ZAP	Light greenish-brown color
2	ZAE	Light greenish-brown color
3	ZAC	Light greenish-brown color
4	ZAM	Light greenish-brown color
5	ZAW	Light greenish-brown color

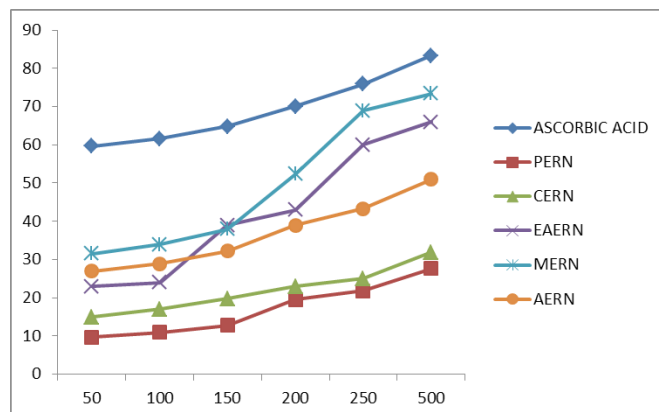
Table 6: Extractive value of different extract of leaves of *Z. armatum*

S. No.	Code	%Extractive value
1	ZAP	$13.81 \pm 0.25$
2	ZAE	$16.70 \pm 0.89$
3	ZAC	$20.60 \pm 0.69$
4	ZAM	$36.32 \pm 0.86$
5	ZAW	$14.18 \pm 0.99$

**Table 7:** Phytochemical test of different leaves extract of *Z. armatum*

Phytochemical tests		Methanolic extract	Aqueous extract	Chloroform extract	Ethyl acetate extract	Petroleum ether extract
Carbohydrates	Molisch's test	+	+	-	-	-
	Barfoed's Test	+	+	-	-	-
	Fehling's test	+	+	+	+	-
Proteins and amino acids	Millon's Test	-	+	-	-	+
	Ninhydrin Test	-	+	-	-	+
Phytosterols	Lieberman -Burchard test	+	+	+		+
Glycosides	Brontranger's test	+	+	+	+	+
Flavonoids	Shinoda test	+	-	+	+	+
Alkaloids	Wagner's	+	-	+	+	+
	Mayer's	+	-	+	+	+
	Draggendorf's test	+	+	+	+	+
Phenolic Compounds	Ferric chloride test	+	+	+	-	+
	Alkaline reagent test	+	+	+	-	+
	Lead Acetate test	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	-	+
Saponin	Foam test	+	+	+	+	+
Triterpenoids	Salkowsky test	+	+	+	+	+
	Hishron test	+	+	+	+	+
Gum and Mucilage	Alcohol test	+	+	-	-	-

+ = Present; - = Absent

**Figure 7:** Bar graph of the percentage inhibition of the extract in DPPH assay

The maximum extractive yield of leaf extract of *Z. armatum* is higher with the methanol  $36.32 \pm 0.86\%$  and least with the petroleum ether solvent, respectively as indicated in Table 6 and Figure 7.

#### Preliminary Phytochemical Analysis

Qualitative analysis of leaves, *Z. armatum* showed the presence of various phytochemicals. More than one test was employed in the case of alkaloids, flavonoids, tannins, saponins and steroids. Alkaloids contents are detected in leaves. Analysis showed the presence of flavonoids, saponins, phenolic compounds, and terpenoids in all five extracts, including petroleum ether extract, ethyl acetate extract, methanolic extract, aqueous extract, and chloroform extract, with their respective tests.

#### Optimization of the Extract

Based on the above, *in-vitro* characterization parameters, including the %extract value of the leaf's methanolic, were selected for further *In vitro* anti-bacterial and antioxidant activity.

#### In-vitro Antioxidant Activity of Methanolic Extract of Leaves of *Z. armatum*

There is no universal method by which antioxidant activity can be measured quantitatively and precisely. Thus, in this study, we have opted to evaluate the antioxidant activities *in vitro* via the three most commonly used tests and combine the results of these complementary tests to indicate the antioxidant capacities of methanolic leaf extract *Z. armatum*.

#### Measurement of Antioxidant Activity by DPPH Test

The chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is one of the first free radicals used to study antiradical activity. It has an unpaired electron on an atom of the nitrogen bridge; this relocation is responsible for the violet colour characteristic of the DPPH radical. The efficacy of an antioxidant is measured by measuring the decrease in violet coloration due to the recombination of DPPH radicals measurable at 517 nm. The trapping of radicals by the antioxidant (hydrophilic or lipophilic molecules) is attributed principally to the liberation of the electron which is unpaired. In this study, we realized

**Table 8:** Percentage inhibition of the Extract in DPPH assay

Concentration ( $\mu\text{g/mL}$ )	% Inhibition
Control	$0.40 \pm 0.35$
100	$15.35 \pm 0.463$
200	$35.67 \pm 0.606$
300	$52.53 \pm 0.763$
400	$62.12 \pm 0.606$
500	$73.64 \pm 0.303$
600	$84.04 \pm 0.631$
700	$83.48 \pm 0.214$

**Table 9:** Percentage inhibition of the extract in Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) scavenging activity

Concentration ( $\mu\text{g/mL}$ )	%Inhibition
Control	$1.04 \pm 1.248$
100	$12.46 \pm 0.692$
200	$30.68 \pm 0.720$
300	$48.44 \pm 0.34$
400	$55.71 \pm 0.69$
500	$70.13 \pm 0.87$
600	$77.85 \pm 1.03$
700	$76.99 \pm 0.24$

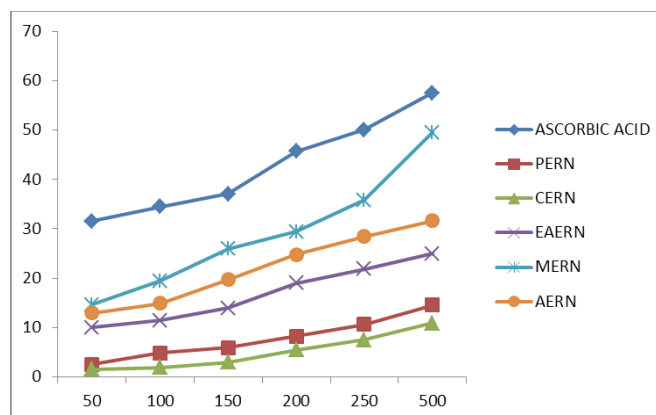
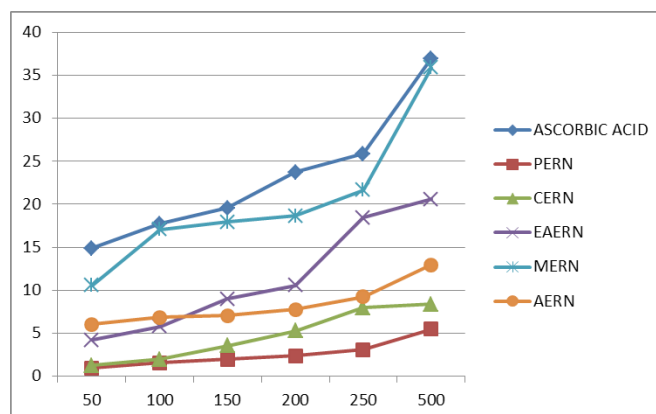
**Table 10:** Percentage inhibition of the extract in nitric oxide scavenging activity

Concentration ( $\mu\text{g/mL}$ )	%Inhibition
Control	$0.89 \pm 0.88$
100	$11.70 \pm 0.67$
200	$30.52 \pm 0.68$
300	$49.48 \pm 0.92$
400	$60.59 \pm 0.25$
500	$72.15 \pm 0.93$
600	$75.56 \pm 0.44$
700	$73.78 \pm 0.40$

the DPPH trapping test to measure the antiradical activity of Methanolic leaf extract of *Z. armatum* extracts and we expressed our results in  $\text{IC}_{50}$  ( $\mu\text{g/mL}$ ). We noted that the percentage of inhibition increases as the concentration of the extracts is increased. Comparing the %inhibition of methanolic extracts  $84.04 \pm 0.63\%$  and reference antioxidants ascorbic acid  $0.40 \pm 0.35\%$  showed that Methanolic leaf extract of *Z. armatum* extracts has a good anti-free radical activity. The Value of  $\text{IC}_{50}$  for the methanolic extract of the leaves of the *Z. armatum* was found to  $301 \pm 0.12$  (Table 8).

#### Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) Scavenging Activity

$\text{H}_2\text{O}_2$  is not a free radical, but it can generate in the presence of transition metals (iron and copper) highly reactive radicals such as hydroxyl-radical  $\text{OH}\cdot$ . It can be formed by the dis-stimulation of  $\text{O}_2^-$  by the superoxide dismutase, or produced by the bivalent reduction of oxygen through a high number of dehydrogenase. The percentage of

**Figure 8:** bar graph of the percentage inhibition of the extract in Hydrogen peroxide ( $\text{h}_2\text{O}_2$ ) scavenging activity**Figure 9:** Bar graph of the percentage inhibition of the extract in nitric oxide scavenging activity

trapping of hydrogen peroxide by methanolic extract of the leaves of the *Z. armatum* extracts is illustrated in Table 9 and Figure 8. The extracts at the concentration of ( $600 \mu\text{g/mL}$ ) can trap the radical  $\text{H}_2\text{O}_2$  with an advantage for methanolic extract  $77.85 \pm 1.308\%$ , which has a higher activity than the positive control (ascorbic acid)  $1.04 \pm 1.248\%$ . The value of  $\text{IC}_{50}$  was found to be  $312 \pm 0.99\%$ .

#### Nitric Oxide Scavenging Activity

The study showed that the methanolic leaf extract of *Z. armatum* caused a concentration-dependent increase in nitric oxide radical scavenging activity as shown in Table 10 and Figure 9.

We noted that the percentage of inhibition increases as the concentration of the extracts is increased. Comparing the %inhibition of methanolic extracts  $75.56 \pm 0.44\%$  and reference compound control  $0.89 \pm 0.88\%$  showed that the methanolic leaf extract of *Z. armatum* extracts has a good anti-free radical activity. The figure demonstrated that the  $\text{IC}_{50}$  value of methanolic extract of the leaves of *Z. armatum* was observed to be  $318 \pm 0.56$ .

#### Anti-bacterial Activity of Methanolic Extract of Leaves of *Z. armatum*

The methanolic extract of leaves of *Z. armatum* was tested for their putative anti-bacterial activity against 4 different

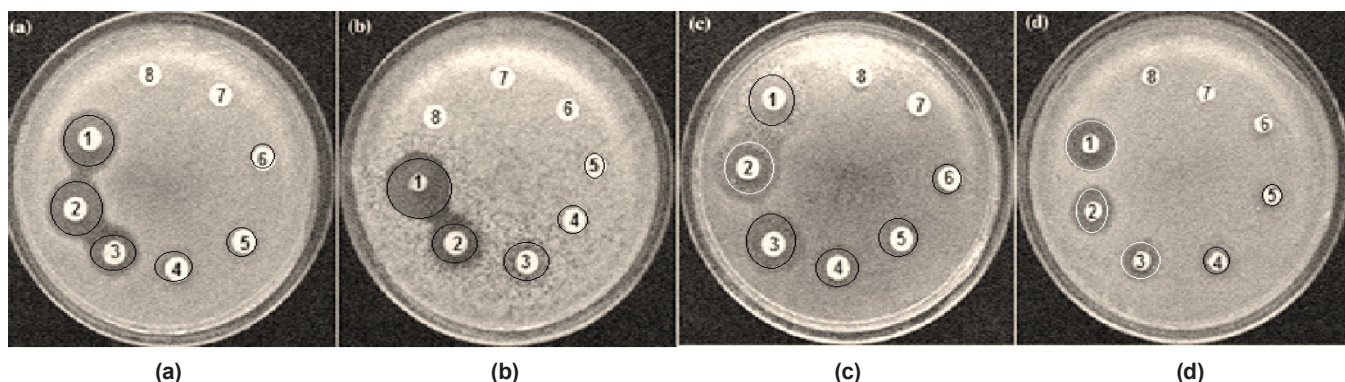


**Table 12:** Zone of inhibition of the methanolic extract of leaves of *Z. armatum*

Concentration ( $\mu\text{g/mL}$ )	Zone of inhibition (mm)			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Tetracycline (100 $\mu\text{g/mL}$ )	19.33 $\pm$ 1.52	17 $\pm$ 1	19.33 $\pm$ 1.55	16 $\pm$ 2.0
500	17.33 $\pm$ 0.57	15.33 $\pm$ 1.14	17 $\pm$ 1.73	12.66 $\pm$ 1.89
300	11.3 $\pm$ 1.15	12.66 $\pm$ 1.52	13.66 $\pm$ 0.58	7.33 $\pm$ 1.02
200	9 $\pm$ 1	8 $\pm$ 1	11 $\pm$ 1	5.66 $\pm$ 1.48
100	4.33 $\pm$ 1.16	4.66 $\pm$ 2	7 $\pm$ 1.9	1.33 $\pm$ 1.51
50	1.33 $\pm$ 1.56	1.33 $\pm$ 1.32	3.33 $\pm$ 1.51	0 $\pm$ 0
25	0.66 $\pm$ 0.58	0.33 $\pm$ 1.50	1.66 $\pm$ 1.38	0 $\pm$ 0
10	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

**Table 13:** MIC value of the methanolic extract of leaves at different concentrations against four different bacteria.

Parameter	Bacteria			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
MIC value	19.34 $\pm$ 1.32	32.67 $\pm$ 1.15	17.11 $\pm$ 0.67	75.36 $\pm$ 0.54

**Figure 10:** Images of zones of inhibition of methanolic extract of leaves against the pathogenic strains *E. coli* (a), *P. aeruginosa* (b), *S. aureus* (c) and *B. subtilis* (d) in antimicrobial susceptibility disk diffusion method.  
1-Tetracycline, 2-500, 3-300, 4-200, 5-100, 6-50, 7-25, 8-10  $\mu\text{g/mL}$ 

kinds of bacteria. The zone of inhibition value extract against each bacteria was presented in Table 13 and Figure 10. The results showed that the extract had certain anti-bacterial activity on all tested bacteria, including gram-positive and gram-negative bacteria. A narrow-spectrum antibiotic (tetracycline) was used as positive control to determine the antagonistic spectrum of the extract. The results also indicated that the methanolic extract of leaves of *Z. armatum* had an anti-bacterial function like tetracycline.

The zone of inhibition value of methanolic extract of leaves of *Z. armatum* against four different bacteria varied from to 18 mm (Table 11). The largest zone of inhibition value was obtained for *E. coli*, followed by the *S. aureus*, *P. aeruginosa*, and *B. subtilis*. We could see that the methanolic extract had a better anti-bacterial activity at 500 ( $\mu\text{g/mL}$ ) on these bacteria because the zone of inhibition value was comparable with control (12–17 mm).

#### MIC Value

MIC was the minimum essential oil concentration that could prevent the bacteria from obvious growth. The MIC value of extract against different bacteria was

determined by measuring the zone of inhibition against two gram-positive bacteria and two gram-negative bacteria. The MIC value of the methanolic extract of leaves of *Z. armatum* against different gram-positive and gram-negative bacteria was presented in Tables 12 and 13. The results showed on increasing the concentration of the extract, the area of the zone of inhibition was increased as displayed that the methanolic extract had certain anti-bacterial activity on all of the tested bacteria. According to the report above, the study about the methanolic extract of leaves has important implications for traditional medicine.

#### CONCLUSION

The present study evaluates phytochemical screening, *in vitro* antioxidant, anti-bacterial activities on *Z. armatum* leaves.

T.S. of leaf lamina of *Z. armatum* showed upper epidermis followed by palisade mesophyll, spongy mesophyll and then lower epidermis. The spongy mesophyll cells (some of which were idioblast, i.e., containing Cu-oxalate crystals) were rounded to

somewhat elongated shape and arranged loosely with large intercellular spaces. Phloem was characterized by rounded cells with almost the same dimensions as that of xylum. Percentage moisture range leaves of 0.3–0.7% which is deemed good as the water content in vegetable drugs should not be greater than 14%.

Total ash values for the leaves of the *Zanthoxylum armatum* were observed  $2.60 \pm 0.28\%$ , acid insoluble ash and sulphated ash value was found to be  $0.97 \pm 0.49\%$  and  $1.14 \pm 0.23$  respectively. dried leaves of *Zanthoxylum armatum* was reduced to fine powder (40 size mesh) and around 50 gm. of crude dried powder was subjected to successive extraction with different solvent petroleum ether, Ethyl acetate, Chloroform, Methanol and water based on the polarity using Soxhlet apparatus.

The extractive value of leaf extracts of *Zanthoxylum armatum* in different solvent was found to be in a range of 13–36%. The maximum extractive yield of leaf extract of *Zanthoxylum armatum* has been found to be higher with the methanol  $36.32 \pm 0.86\%$  and least with the petroleum ether solvent.

Preliminary phytochemical analysis showed the presence of flavonoids, saponins, phenolic compound and terpenoids in all five extract including petroleum ether extract, ethyl acetate extract, methanolic extract, aqueous extract and chloroform extract with their respective tests as shown in Table 7.

In DPPH antioxidant assay, The comparison of the % inhibition of methanolic extracts  $84.04 \pm 0.63\%$  and that of reference antioxidants ascorbic acid  $0.40 \pm 0.35\%$  showed that Methanolic leaf extract of *Zanthoxylum armatum* extracts has a good anti-free radical activity. The Value of IC<sub>50</sub> for the methanolic extract of the leaves of the *Zanthoxylum armatum* was found to  $301 \pm 0.12$ ,

In Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity, the extracts at the concentration of (600 µg/ml) have the capacity to trap the radical H<sub>2</sub>O<sub>2</sub> with an advantage for methanolic extract  $77.85 \pm 1.308\%$  which has a higher activity than the positive control (ascorbic acid)  $1.04 \pm 1.248\%$ . The value of IC<sub>50</sub> was found to be  $312 \pm 0.99\%$ .

Nitric Oxide Scavenging Activity, the comparison of the % inhibition of methanolic extracts  $75.56 \pm 0.44\%$  and that of reference compound control  $0.89 \pm 0.88\%$  showed that Methanolic leaf extract of *Zanthoxylum armatum* extracts has a good anti-free radical activity. The figure demonstrated that the IC<sub>50</sub> value of methanolic extract of the leaves of *Zanthoxylum armatum* was observed to be  $318 \pm 0.56$ .

In anti-bacterial activity, the zone of inhibition value of the methanolic extract of leaves of *Zanthoxylum armatum* against four different bacteria varied from to 18 mm. The largest zone of inhibition value was obtained for *E. coli*,

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