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# Antimicrobial and Antiproliferative Assessment of Formulation from *Zanthoxylum armatum*

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**Medicinal plants are widely available and affordable, especially in rural areas. Investigation into medicinal plants has shown that they have significant antiproliferative (cancer prevention) and antibacterial properties, which are attributed in part to bioactive components. These compounds' mechanisms of action, potential therapeutic applications, and absence of side effects in comparison to synthetic pharmaceuticals have sparked attention in the scientific literature. Therefore, these medicinal herbs are utilised for creating formulations that may serve as supplements to improve general health, such as antioxidants and immune boosters. In view of this, we characterize formulations of *Zanthoxylum armatum* (fruit and seed) using GC-MS from various locations in Kumaun, Uttarakhand, and assess their antimicrobial properties (including MIC and MBC determination) and inhibition of the growth (anti-proliferative activity) of two human cancer cell lines (SW-480 and HeLa) and one healthy cell line (KMST-6). These research' findings demonstrated that the formulation (fruit/seed; 40:60) inhibited the growth of two different cancer cell lines (HeLa and SWS 480) and showed less inhibition in the case of the normal cell line, i.e., KMST-6. Similarly, this formulation demonstrated antimicrobial activities when measured against minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In short, this formulation has higher antiproliferative and antimicrobial properties in most of the assay when tested individually, as compared to the rest of the formulation. Thus, it is sensible to use the combination of studied herbal formulations for the development of any antimicrobial supplement, food product, or anticancer molecule in the future.**

**Keywords:** Antimicrobial; Anti-proliferative; Cell lines; Formulation; *Zanthoxylum armatum*.

The "Herbal State" or "Land of Medicinal Plants," Uttarakhand is known for its diverse range of medicinal plants and biodiversity. Its distinct physical characteristics, which include the Himalayan peaks, diverse climatic regions, and fertile valleys, make this an ideal location for the cultivation of a vast variety of medicinal plants. Uttarakhand, sometimes referred to as Devbhoomi (Land of the Gods), has an ancient past and a strong cultural association with medicinal plants

because of its abundant biodiversity. The region's rich biodiversity and involvement in traditional medicine go back thousands of years, intertwining with religious literature, historical customs, and local traditions. In view of this, we choose one of the therapeutic plants, *Zanthoxylum armatum*, which belongs to the *Rutaceae* family and is commonly known as Timur.[1-4] This medicinal plant has been used since ancient times to treat a variety of illnesses, including gum bleeding, asthma, toothaches and other dental issues. Additionally, it is said that *Zanthoxylum armatum* possesses a variety of potentials, including hepatoprotective, anti-inflammatory, anti-nociceptive, pesticidal, and anthelmintic properties.[1-5] Ayurvedic and folk medicine have long utilized portions of the plant, notably the fruits, seeds, and bark, for their digestive, analgesic, carminative, and antibacterial qualities. Recent scientific investigations have revealed its ability to heal infections, lower oxidative stress, and even function as a natural pesticide. However, due to rising demand and habitat destruction, sustainable harvesting and conservation activities are critical for the continuing use and study of this vital plant. [1-4] The silver nanoparticles derived from *Zanthoxylum armatum* showed no DNA damage, antibacterial action regarding multiple kinds of bacteria, and the potential of the ethyl acetate-extracted part of the plant to promote the mitochondrial apoptotic pathway via the generation of reactive oxygen species in cells, resulting in the death of cells. The mechanism and effects of *Zanthoxylum armatum* -induced hepatotoxicity in BRL-3A cells, which also resulted in endoplasmic reticulum stress and autophagy inhibition, were also examined. As a result, apoptosis became more prevalent, and endoplasmic reticulum stress could exacerbate autophagy suppression.[3-5]

Throughout the history of mankind, medicinal plants have served a broad spectrum of biological activities. An integrative and multidisciplinary methods to drug development known as ethnopharmacology involves the evaluation, application, and biological activities analysis of traditional medicinal products.[6] The screening approach, that draws on the standard use of traditional medicines, is informed by ethnomedical data and empirically takes into account plants as an origin of chemically active compounds.[7] In order to avoid the onset of many medical diseases for which there have been no effective remedies, drug development research is crucial. Therefore, the present study aims to examine the antibacterial efficacy of the formulated fruit and seed extracts of *Zanthoxylum armatum* from different geographical regions of Kumaun, Uttarakhand.

## MATERIALS AND METHODS

### Gathering of plant specimen

The fruits and seeds of *Zanthoxylum armatum* were gathered in February-March 2024 from three distinct sites in the geographically diverse area. The research areas i.e. latitude, longitude, temperature, and average rainfall of Shama Dhura, Bageshwar; Lohaghat, Champawat and Dharchula, Pithoragarh, are all noted.

### Fruit and seed extracting from plant specimens

The formulation employed in the current research, containing metabolites (primary as well as secondary), was created using a maceration process. Specimen were disinfected using 70% alcohol in order to eliminate dust, as well as their surfaces were permitted to dry in the shady area. Each fruit and seed specimen were weighed to the closest 30 g, and then the solvent used to dissolve them was methanol. After that, the samples spent a minimum of seven days on a revolving platform. For evaporation, place the solvents in a water bath set to 60 degrees Celsius. The formulations were then filtered repeatedly with syringe filters made of Whatman No. 1 filter paper and tracked at 4°C.

### Preparation of formulation

After being separately extracted, the fruits and seeds were lyophilised and crushed into a powder. To prepare the formulation, 2 g of the lyophilised powdered components were extracted individually and placed in 100 mL of an 80/20 ethanol/water solution. After churning the mixture for 2-3 h at the ambient temperature, it was sonicated for 10-15 min to accomplish this. For every sample, the pellet has been eliminated once more following a centrifugation at 4800 rpm. Following the collection of the supernatants, the solvents being used were forced to evaporate at 40°C under vacuum on a revolving evaporator. Ultimately, the dry residues were dissolved with DMSO (1%) to produce new PBS formulations. Fruits and seeds were made in varying proportions

by soaking in the saline and regulating the pH levels.

**GC-MS**

The samples (formulations) were analysed qualitatively and quantitatively using a Shimadzu GC-MS model (QP2010-Ultra; Rtx-5 ms-30 m × 0.25 mm × 0.25 ìm, capillary column). Utilizing the split mode, formulations were inserted (1.61 ml/minute) while helium serving as the carrier gas. Beginning at 60°C, the temperature program increased by 10°C every minute until it reached its final temperature of 300°C. Using scan mode, each sample was examined in the m/z 40–500 charges to ratio range.[8]

**Antimicrobial Activity (Disc Diffusion Method)**

MHA was adjusted into sterile Petri dishes in 20 ml aliquots. The strains of bacteria and standardised bacterial stock suspension had been adjusted and streaked onto MHA medium plates using a sterile cotton swab. After soaking in formulations of varying strengths (fruit and seed), sterile filter paper discs were put on the agar surface. Plates were incubated for 24 hours at 37°C, and inhibition zones were measured in millimetres.[8, 9] The lowest concentration that stopped bacterial strains from growing was used to calculate the MIC.

**Susceptibility test of formulations using Antimicrobial Discs**

The disc diffusion technique had been employed to test the antibiotic susceptibility of bacterial strains. After the isolates were suspended in sterile normal saline, the turbidity was brought down to 0.5 McFarland solutions, which is the usual concentration. Following that, the isolates were inoculated onto MHA. Ampicillin (25 µg) and ciprofloxacin (50 µg) were the antibiotic discs implemented in this investigation. Each disk has antibiotics in the ideal amounts, positioned one centimeter apart from the wall. The plates were subsequently kept warm for 24 hours at 37 degrees Celsius. The zones of inhibition were tracked in millimeters and evaluated using the Clinical Laboratory Standard Institute (CLSI) technique.[10]

**Broth Dilution Technique for Detecting MIC and MBC**

The formulations' MBC and MIC were ascertained by using established protocols. Fruit and seed formulations with various concentrations were developed. The bacterial concentration was calibrated (0.5, McFarland standard) and the formulations were diluted with the microorganisms. A 24-hour observation of the turbidity of the fluid in each tube was made to determine whether any bacterial growth had occurred. The turbidity-free tubes were reported as having MIC values. The MBC value was assumed to be the least extract dilution concentration, which did not exhibit any discernible growth.[11]

**Antiproliferative activity assay**

Using the MTT test, the antiproliferative ability of formulations that varied fruit and seed amounts was examined. Exponentially after washing in 96 well plate, developing cells were planted at 15000 cells/well (growth media, 200 µl). Following a full day of incubation, a partial monolayer formed. The media was withdrawn, and 200 µl of the formulation-containing medium was added, followed by 48 hours of incubation. After aspirating 100 µl of the media, 15 µl of the MTT solution was added to each well's remaining 100 µl of medium. After 4 hours of interaction with the MTT solution, blue crystals developed. One hour was spent incubating after adding 100 µl of the stop solution. At 550 nm, reduced MTT was measured with a microplate reader. The identical amount of ethanol (0.1%) was given to the control groups. Vincristine sulphate-treated cells had served to act as positive controls, whereas cells with no treatment were serving as a negative control.[12]

The MTT assay was undertaken in three stages. In the first and second stage, variable concentration of the fruit and seed formulation separately was tested against the cancer cell lines (SW-480, HeLa) and normal cell line i.e. KMST-6. All these selected formulations (fruit and seed) that cause more than 50% inhibition of proliferation on any cell line were selected for further investigations in stage 3 (where fruit and seed were mixed together in different proportions). The selected formulation that showed more than 50% inhibition were selected for IC50 determination in stage 3 and tested against the three cell lines. IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line. The average of three replicates was used to report the data. The optical density (OD) of the treated cells had been contrasted to that of the control (untreated cells) in order to assess the antiproliferative effect of the tested formulations.[13]

**Table 1.** GC-MS analysis of formulation of *Zanthoxylum armatum* from three different regions of Kuman region, Uttarakhand

| S. No. | (40/60) Bageshwar | (40/60) Champawat | (40/60) Pithoragarh |
|---|---|---|---|
| 1 | Tricyclo [4.2.1.0(2,5)] non-7-en-3-one | 4,5-Dihydro-2(1H)-pentalenone | N-(2-Propynyl)aziridine |
| 2 | 2-Butenal, (Z) | 4-(Methylamino)-1,2,5-oxadiazole-3-carbonitril | Methanamine-butylidene-, N-oxide |
| 3 | Ethanamine, N,N-difluoro | 1-Ethyl-2-propyl-pyrazolium bromide | diazoadamantane |
| 4 | 7-Azanorbornene | 7-Azanorbornene | 7-Azanorbornene |
| 5 | 4-Propanoylbenzonitrile | 2,3-Pyridinedicarbonitrile | 4-Nonene, 2,3,3-trimethyl-, (E) |
| 6 | Hexanenitrile, 5-methyl- | cis-1,2-Cyclohexanedicarbonitrile | 7-Azanorbornene |
| 7 | 1,6-Naphthyridin-4-amine | (E)-9,11-Dodecadien-1-ol | 2,3-Pyridinedicarbonitrile |
| 8 | Isopropyl phosphine | 1,1(Diethylcarbamoyl)succinimide | 5-Cyano-quinoxaline |
| 9 | 1,2-Nonadiene | (E)-9,11-Dodecadien-1-ol | 2-Hydroxyskatole |
| 10 | 1-Propen-2-ol, acetate | Cyclopropane,(R,R)-1-((Z)-hex-1'-enyl)-2-ethenyl | Propanoic acid, 3-(trimethylsilyl)-, methyl ester |
| 11 | 3-Hexyne, 2-methyl- | 4H-1,3-Benzodioxin, 2-phenyl- | Aminoacetonitrile |
| 12 | 11-Dodecadien-1-o | 9-Thiabicyclo [3.3.1]nonan-3-one 9,9-dioxide | 9-Thiabicyclo[3.3.1]nonan-3-one 9,9-dioxide |
| 13 | Manganese, tetracarbonyl (N, N-dimethyl-á-alanyl) | Cyclobutane,3-ethenyl-1,1,2-trifluoro- | (E)-9,11-Dodecadien-1-ol |
| 14 | 1-3Hexyl-2-nitrocyclohexane | 2-Furancarbothioamide | Cyclobutanone, 2-methyl-2-oxiranyl- |
| 15 | 1,3-Methanopentalene, octahydro- | Oxirane,3-[5-(4-azidophenoxy)-3-methyl-3-pentenyl]-2,2-dimethyl-, (E)-ñ | cis-1,3-Cyclohexanedicarbonitrile |
| 16 | Phenol, 4-[[(4,5-dimethyl-1H-1, 2,3-triazol-1-yl) imino]methyl] | Dimethyl selenide | 1,1-(Diethylcarbamoyl)succinimide |
| 17 | 4H-1,3-Benzodioxin, 2-phenyl- | N-(Dimethylthiophosphinyl)allylamine | Ethanone, 2-(formyloxy)-1-phenyl- |
| 18 | 2-(3-methyl-2-cyclopenten-1-yl) -2-methylpropionaldehyde | Cyclohexanol, 2-amino-, cis- | Thiophene-2-ol, benzoate |
| 19 | trans-1,4-Cyclohexanedicarbonitrile | . (E)-1,4-Dichloro-2-trimethylsilyl-2-butene | trans-1,4-Cyclohexanedicarbonitrile |
| 20 | Oxirane, 3-[5-(4-azidophenoxy) -3-methyl-3-pentenyl]-2,2-dimethyl-(E)-ñ- | 2(3H)-Furanone,3-(15-hexadecynylidene) dihydro-4-hydroxy-5-methyl-,[4R(3Z,4à,5á)] | 5-Acetoxymethyl-2,6,10-trimethyl-2,9-undecadien-6-ol |
| 21 | 3-Pentenoic acid, 2,2-diethyl- | N-(Dimethylthiophosphinyl)allylamine | 5,10-Pentadecadienal, (Z,E)- |
| 22 | Azabicyclo [6.1.0]nonan-9-amine, cis | 3-Methyl-2-(2-oxopropyl)furan | Propylamine |
| 23 | 3-Hexyne, 2-methyl- | cis-1,3-Cyclohexanedicarbonitrile | 4-Heptenal, (E) |
| 24 | Cyclobutaneacetonitrile, 1-methyl-2-(1-methylethenyl)- | 1H-Imidazole, 2-propyl- | Acetonitrile, (hydroxyimino)-, (E) |
| 25 | Ethanamine | 2,5-Dimethylpyrimidine | 1,4-Dioxane-2,5-dione, 3,6-diethyl |

## RESULTS

### GC–MS analysis

When the bioactive ingredients have been determined through comparison of their peak retention time, peak area (%), height (%), and mass spectral fragmentation patterns to those of the previously identified compounds listed in the National Institute of Standards and Technology (NIST) library, the GC–MS chromatogram of the formulation from *Zanthoxylum armatum* revealed a total of four major peaks. Four chemicals were found in the formulation, according to the results, as shown in Table 1 and Fig. 1.

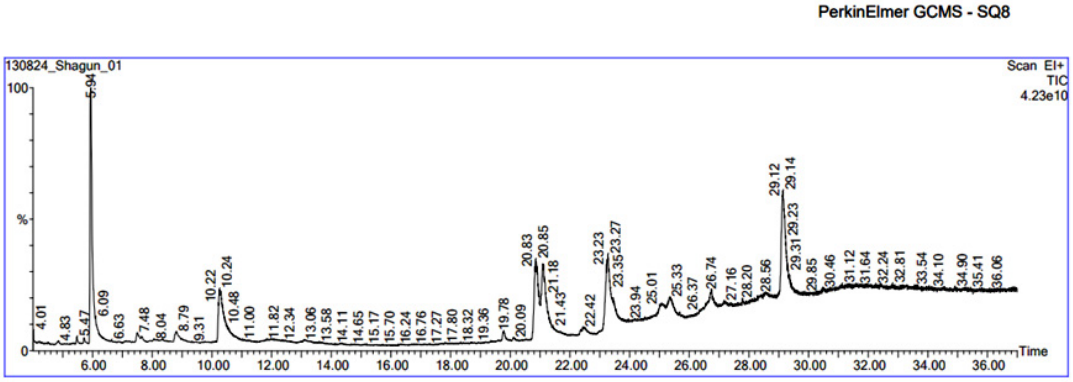
### Antimicrobial activity and MIC determination using the broth dilution method

The results of these formulations (40:60) from *Zanthoxylum armatum* are displayed in Table 2 and Table 3 and showed antimicrobial activity as compared to control. The most active formulation in this investigation was found to be the 40:60 (fruit and seed), which had MICs on the three microbiological strains tested ranging from 40:60 to 80:20. The MIC of 40:60 for *Staphylococcus aureus, Salmonella typhi*, and *Pseudomonas aeruginosa* was similarly shown to be greatest in the fruit and seed formulation from Bageshwar region against these three bacteria. Strong microbial inhibitors possessed MIC values of equal or higher than 60:40, which indicates that the MIC value of 80:20 obtained for formulation against *Bacillus subtilis, Salmonella typhi*, and *Pseudomonas aeruginosa* shows remarkable antibacterial activity. Additionally, the MIC of the formulation from champawat and Pithoragarh samples against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was found to be 80:20 mg/ml, while the MIC of the formulation from champawat and Pithoragarh samples against *Salmonella typhi* and *Staphylococcus aureus* was found to be 40:60. Ampillcin and tetracycline (50 and 20 µg/ml) were sensitive against these bacterial strains, with an MIC of 0.5 mg/ml reported.

### Antiproliferative activity of formulations using MTT assay

The antiproliferative effect of these formulations from *Zanthoxylum armatum* was evaluated on HeLa, SW-480, and KMST-6 cells through an MTT assay. The findings demonstrated that every cell line reacted differently with respect

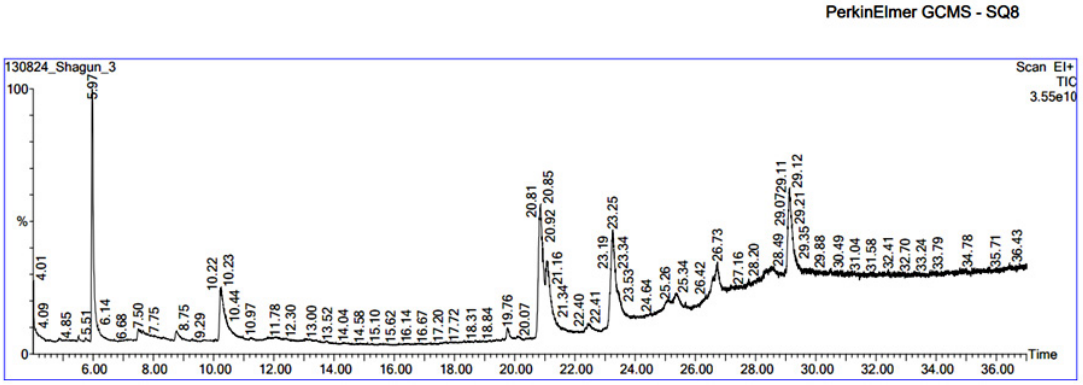
A) Bageshwar




The retention time (RT), name of the compounds is shown in the chromatogram. A total of 25 compounds were identified in *Z. armatum* methanolic fruit and seed extract (Bageshwar). There is specific 4 compounds which have shown greater % in terms of peak area mentioned below.

| S. No. | RT | Height | Area | Area % | Compound |
|---|---|---|---|---|---|
| 1. | 5.94 | 41,173,938,176 | 4,104,156,416.0 | 12.82 | Tricyclo[4.2.1(2,5)]non-7-en-one |
| 2. | 8.82 | 1,603,009,152 | 343,568,640.0 | 1.07 | 7-Azanorbornene |
| 3. | 10.24 | 8,708,254,720 | 2,327,229,952.0 | 7.27 | 4-Propanoxylbenzonitrile |
| 4. | 12.08 | 872,036,352 | 204,215,280.0 | 0.64 | 1,2-Nonadiene |

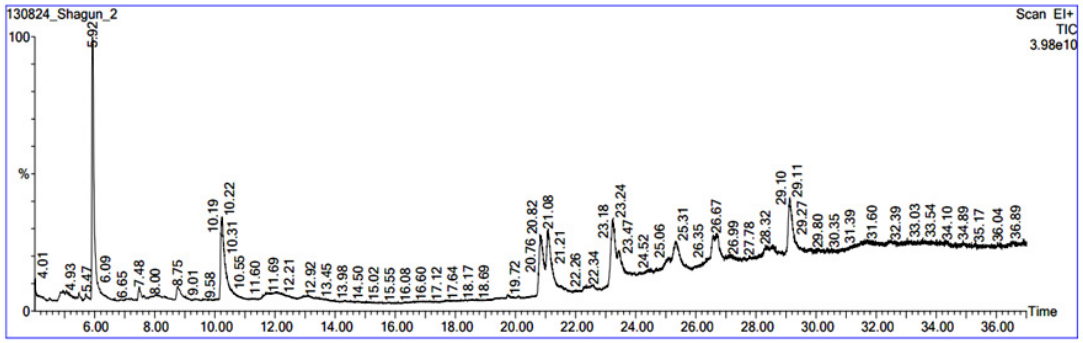
B) Champawat




The retention time (RT), name of the compounds is shown in the chromatogram. A total of 25 compounds were identified in *Z. armatum* methanolic fruit and seed extract (Champawat). There is specific 4 compounds which have shown greater % in terms of peak area mentioned below.

| S. No. | RT | Height | Area | Area % | Compound |
|---|---|---|---|---|---|
| 1. | 5.97 | 33,943,316,480 | 2,826,308,352.0 | 8.02 | 4,5-Dihydro-2(1H)-pentalenone |
| 2. | 20.85 | 17,828,235,264 | 3,139,627,520.0 | 8.91 | (E)-9,11-Dodecadien-1-ol |
| 3. | 21.06 | 10,209,372,160 | 2,262,830,592.0 | 6.42 | 1,1-(Diethylcarbamoyl)succinimide |
| 4. | 23.25 | 13,092,749,312 | 3,023,435,776.0 | 8.58 | (E)-9,11-Dodecadien-1-ol |

c) Pithoragarh




The retention time (RT), name of the compounds is shown in the chromatogram. A total of 25 compounds were identified in *Z. armatum* methanolic fruit and seed extract (Pithoragarh). There is specific 4 compounds which have shown greater % in terms of peak area mentioned below.

| S. No. | RT | Height | Area | Area % | Compound |
|---|---|---|---|---|---|
| 1. | 4.96 | 1,462,791,296 | 254,704,720.0 | 0.83 | N-(2-Propynyl)aziridine |
| 2. | 5.92 | 38,218,895,360 | 3,403,834,112.0 | 11.10 | Diazoadamantane |
| 3. | 8.75 | 1,962,461,824 | 335,722,144.0 | 1.09 | 7-Azanorbornene |
| 4. | 10.22 | 12,061,136,896 | 2,177,545,984.0 | 7.10 | 2,3-Pyridinedicarbonitrile |

**Table 2.** Antimicrobial activity

| Formulation (100 µl) | Bageshwar, Zone of inhibition (mm) | | Champawat, Zone of inhibition (mm) | | Pithoragarh, Zone of inhibition (mm) | |
|---|---|---|---|---|---|---|
| | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* |
| Formulations | | | | | | |
| 20 µl (F):80 µl (S) | 8.1 | 7.4 | 6.8 | 5.6 | 7.3 | 5.9 |
| 40 µl (F):60 µl (S) | 11.38 | 11.2 | 8.9 | 7.6 | 8.1 | 8.1 |
| 60 µl (F):40 µl (S) | 9.46 | 10.8 | 7.8 | 7.3 | 9.4 | 7.8 |
| 80 µl (F):20 µl (S) | 8.6 | 9.6 | 7.2 | 6.8 | 7.8 | 7.4 |
| 1 ml (F) | 9.16 | 8.4 | 9.16 | 8.4 | 9.16 | 8.4 |
| 1 ml (S) | 7.98 | 6.7 | 7.98 | 6.7 | 7.98 | 6.7 |
| Standard | 15.4 | 13.6 | 15.4 | 13.6 | 15.4 | 13.6 |
| Control | 2.1 | 1.8 | 1.9 | 1.8 | 2.2 | 1.6 |

to both the dosage and duration and the cytotoxic nature of the chloroform fraction. In addition, 20:80 formulations of fruit and seed do not show any antiproliferative effects on these cell lines (data not shown). The IC50 values for the 24, 48, and 72-hour treatments are shown in Table 4 against three different cell lines. In this study, however, the HeLa cancer cells responded more strongly to the 40:60, as demonstrated by its IC50 values for 24 (191.4 µg/mL), 48 (154.1 µg/mL), and 72 (89.2 µg/mL) hours. Similarly, IC50 values of SW-480 for 24, 48, and 72 h (302.2, 181.5, and 104.2 µg/mL). Additionally, the formulation 40:60 from Bageshwar region of Kumaun demonstrated preferential cytotoxicity in KMST-6, a normal fibroblast cell, exhibiting greater CC50 values in 24, 48, and 72 hours, respectively, of 359.6, 331.7, and 322.3 µg/mL.

## DISCUSSION

Plant-based chemicals are naturally biologically active compounds that seem to possess substantial physiological impact in the human body. They include an assortment of chemical entities such as flavonoids, polyphenols, saponins, steroids, and vitamins, among others. Approximately 40% of current medications are of natural origin. The manufacturing of plant-derived crude pharmaceuticals that are employed with herbal treatments or raw materials in the pharmaceutical industry has expanded significantly as new technologies have advanced. When it comes to contemporary medicine, natural pharmaceuticals are unaltered, well-defined molecules that are either utilised directly or transformed using appropriate chemical or microbiological techniques before being employed as medications.[14] Even though chemical engineering has recently taken centre stage, as a means of discovering and producing pharmaceuticals, there is still enormous scope for psychoactive botanicals or their extracts for developing novel and groundbreaking goods for both the avoidance and mitigation of disease. Biologically active compounds are thought to be mostly found in plants. An estimated 50% of prescription medications in the US as well as Europe are thought to be made from natural ingredients or their derivatives. Because of the poor prognosis, high expense of traditional medications,

**Table 3.** MIC, and MBC of formulation from *Zanthoxylum armatum* (50 µl) against 4 ATCC bacterial strains

| Plant name | Formulation (100 µl) | MIC (µl or mg/ml, 50 µl) Gram positive bacteria | | MIC (µl or mg/ml, 50 µl) Gram negative bacteria | |
|---|---|---|---|---|---|
| | | *Staphylococcus aureus* | *Bacillus subtilis* | *Salmonella typhi* | *Pseudomonas aeruginosa* |
| *Zanthoxylum armatum* | Bageshwar | 40 µl (F):60 µl (S) | 40 µl (F):60 µl (S) | 40 µl (F):60 µl (S) | 60 µl (F):40 µl (S) |
| | Champawat | 80 µl (F):20 µl (S) | NA | 80 (F):20 µl (S) | NA |
| | Pithoragarh | 80 µl (F):20 µl (S) | NA | NA | 80 µl (F):20 µl (S) |
| | Ampicillin, 0.05 mg/ml | 1.56 | 0.78 | 3.12 | 3.12-6.24 |
| | Tetracycline, 0.02 mg/ml | 0.312 | 1.25 | 0.625 | NA |

| Plant name | Formulation (1 ml) | MBC (µl or mg/ml, 50 µl) Gram positive bacteria | | MBC (µl or mg/ml, 50 µl) Gram negative bacteria | |
|---|---|---|---|---|---|
| | | *Staphylococcus aureus* | *Bacillus subtilis* | *Salmonella typhi* | *Pseudomonas aeruginosa* |
| *Zanthoxylum armatum* | Bageshwar | 80 µl (F):20 µl (S) | 1000 µl (F) | 1000 µl (F) | 80 µl (F):20 µl (S) |
| | Champawat | 1000 µl (S) | NA | 1000 µl (S) | NA |
| | Pithoragarh | 1000 µl (F) | NA | NA | 1000 µl (S) |
| | Ampicillin, 0.05 mg/ml | 3.12 | NA | NA | 12.5 |
| | Tetracycline, 0.02 mg/ml | 1.25 | 5 | 5 | NA |

**Table 4.** IC50 values of formulation from *Zanthoxylum armatum* on HeLa, and SW-480 cancer cell lines and CC50 values on KMST-6 cell lines at different time intervals treatment

| Plant (*Zanthoxylum armatum*) formulation | Time | HeLa (µg/ml), IC50 values | SW-480 (µg/ml), IC50 values | KMST-6 (µg/ml), CC50 values |
|---|---|---|---|---|
| 40:60 (Bageshwar) | 24h | 181.2 | 253.18 | 437.6 |
| | 48h | 127.7 | 181.5 | 393.1 |
| | 72h | 98.4 | 112.3 | 377.8 |
| 40:60 (Champawat) | 24h | 269.4 | 331.7 | 401.2 |
| | 48h | 183.7 | 261.8 | 366.4 |
| | 72h | 132.1 | 155.7 | 291.3 |
| 40:60 (Pithoragarh) | 24h | 315.4 | 452.6 | 537.1 |
| | 48h | 298.7 | 381.3 | 498.4 |
| | 72h | 165.1 | 277.9 | 353.6 |

and negative side effects associated with orthodox cancer and antimicrobial therapy, phytochemicals are becoming an increasingly popular avenue for alternative treatments in underdeveloped nations.[15] *Zanthoxylum armatum* is one of the plants whose methanolic extract indicated the greatest antibacterial activity. Furthermore, it boasted the highest biofilm inhibition performance when compared with alternative extracts at different solvent concentrations.[1] In view of this, we developed combinations for determining the biological value of *Zanthoxylum armatum* (fruits and seeds) in different ratios. The formulations were tested during antiproliferative investigations to see whether they could stop cellular growth and through antimicrobial assays for assessing how efficient they were against a variety of harmful pathogens. These studies offer information about *Zanthoxylum armatum's* possible uses in the pharmaceutical and medical industries.

The terms antiproliferative and antimicrobial are central to research in medicinal plants, focusing on combating cancer and microbial infections, respectively. These antiproliferative agents inhibit the growth and division of cells, primarily targeting cancer cells.[13] Medicinal plants have been extensively studied for their antiproliferative potential due to their bioactive compounds e.g. Terpenoids (e.g., taxol from *Taxus brevifolia*): Phenolics (e.g., resveratrol from grapes) etc. According to an *in silico* investigation, compounds (nevadensin, asarinin, and kaempferol) identified in *Zanthoxylum armatum* could possess substantial anti-cancer effects due to their enhanced binding affinity for pyruvate kinase M2 and their inhibitory impact. In summary, these chemicals have a high likelihood of being successful against cancer due to their antineoplastic abilities and their capacity for inhibiting a protein.[16] In view of this, we evaluate the antiproliferative potential of formulations derived from the fruit and seed of *Zanthoxylum armatum* in varying proportions. Among the tested formulations, the 40:60 fruit-to-seed ratio exhibited superior antiproliferative activity against three cancer cell lines: KMST-60 (fibroblast-like cells), HeLa (cervical cancer cells), and one other normal cell line (KMST-60 (fibroblast-like cells). In the current investigation, SW-480, KMST-6, and HeLa have been examined to evaluate the cancer-fighting potential of these formulations from *Zanthoxylum armatum*. According to the study's findings, all cancer cell lines were subjected to cytotoxic effects by the chloroform fraction in a way that was dependent on time and dosage. HeLa cells are more susceptible to the plant's cytotoxic action, as indicated by the IC50 values obtained for the different treatment procedures, but KMST-6, was less sensitive to the fraction. The formulations ability to distinguish between cancerous and healthy cells through its anticancer effects is a crucial concept in the development of novel chemotherapeutic drugs. The findings demonstrate the potential of *Zanthoxylum armatum* formulations as a promising candidate for developing plant-based therapeutic agents against cancer.

Antimicrobial agents derived from plants combat bacterial, fungal, and viral infections, addressing the growing issue of antibiotic resistance. The exploration of antimicrobial activities has become even more significant with the rise of antimicrobial resistance.[17-21] In this regard, the antibacterial effectiveness of formulations made from *Zanthoxylum armatum* fruit and seed in various combinations has been investigated in this work. Among the evaluated formulations, the 40:60 fruit-to-seed ratio had the highest antibacterial efficacy towards an assortment of pathogenic microorganisms. The antibacterial potential was quantitatively tested using MIC and MBC values, which showed that the 40/60 formulation efficiently suppressed microbial growth and had bactericidal capabilities. These results highlight the possibility of using *Z. armatum* formulations as natural substitutes for antibiotic treatments.

## CONCLUSION

The main outcome of this research investigation is that both the fruit and seed formulation (40:60) had greater effectiveness towards microbial pathogens and established antiproliferative potential towards several cell lines. Further investigation is underway to figure out the antimicrobial impact of this formulation against various pathogenic microbes, maybe using it as a paste. In addition, future studies looking at the individual compounds present in the formulation of fruit and seed which is present in a large amount also analysed the formulation with different solvent systems for analysing its antimicrobial activity against bacterial pathogens and antiproliferative activity against cancer cell lines.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

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**Clinical Trial Registration**

This research does not involve any clinical trials

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Not Applicable.

**Author contributions**

Kunal Sharma performed the experiments. Dr. Neha Pandey and Dr. Amit Gupta supervised the work, provided guidance, and reviewed the manuscript. Dr. Atal Bajpai identified the plant species. Dr. Kunal Sharma and Dr. Madhulika Ether Prasad conducted the antimicrobial studies.

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