

Pharmacological study of *Oroxylum indicum*

Abstract:

Ayurvedic medicine has used the herb *Oroxylum indicum* belonging to family of Bignoniaceae also known as Sonapatha, for many years. It is a common ingredient in popular Ayurvedic medicines such as Dasamula and Chyawanprash. The root bark of this plant has tonic and astringent properties and is effective in the treatment of rheumatism. For many years *Oroxylum indicum* (L.) Kurz, jaundice, arthritis and rheumatism, stomach ulcers, tumors, respiratory diseases, diabetes, diarrhea and dysentery, etc. The current study aims to do its qualitative analysis and check its antimutagenic and antibacterial effect.

Introduction:

The tree is common in many countries, including India, China, Bangladesh, Japan and Malaysia. Many treatments require the use of whole herbs. *O. indicum* is important in folk medicine as a carminative, blood filtering and anti-diabetic agent, as well as other problems such as stomachache, anti-ulcer, gastrointestinal and anti-sensitizer. Various Ayurvedic remedies include Amritarishta, Dantadyarista, Narayana Taila, etc. pay attention to the components of plants.

Methods for collecting and processing plant samples:

Samples were obtained from an untamed region in Rajkot, Gujarat, India. These sample was obtained in powdered form from the market. Root powder was taken as a sample of *Oroxylum indicum*. Then as test onion *Allium cipa* was obtained from nearby market of small size.

Extraction of plant sample:

Preparation of plant extract: Plant materials (250 g) was soaked in 450 mL of 95/5% hydroalcoholic solvent (Methanol: water). This solvent system was placed in orbital shaker for 48 h at room temperature. After 48h, the solvent was filtered through Whatman filter paper separately in a beaker. At 55°C the filtrate was dried in a rotary evaporator (Equitron, India) to obtain the concentrated yield of extracts. Then the solvent obtained was stored at room temperature for further experiments.

Phytochemical analysis:

Quality analyzes were carried out to determine the quality of major phytochemicals in our facilities. Different tests were performed for the presence of flavonoids, terpenoids and their analogues,

saponins, steroids, tannins, phenols, carbohydrates, amino acids, cardiac glycosides and alkaloids. Phytochemical analyzes of plant extracts were carried out by standard methods. The presence of flavonoids was confirmed by the acetyl lead test by adding 1 mL of aqueous herb to 1 mL of 10% lead acetate solution, yellow color was considered a positive result. Terpenoids were determined by the Salkowski test plus 0.5 mL of plant extract and 0.2 mL of chloroform. 0.3 mL conc. H₂SO₄, if needed. A red-brown color is observed, indicating that the test is positive. removed. To check for the presence of amino acids, the Biuret A test was performed and 0.5 mL of plant extract was added. With a few drops of 2% (w/v) copper sulfate, 1 mL of ethanol followed by potassium hydroxide pellets.

The Liebermann-Buchard test was used to determine the presence of steroids in which 2 mL of acetic anhydride was mixed with 0.5 mL of plant extract followed by 2 mL of H₂SO₄. There is no color change to indicate the presence of steroids. There is a brown ring on the interface. Foam test. For saponins, add 0.5 mL of herb extract along with a small amount of ethanol and distilled water and vortex in a graduated cylinder. The appearance of a hard foam confirmed the presence of saponins in the plant. Type: 0 Tannin Test (A) Ferric Chloride (FeCl₃) Test: Mix 0.5ml vegetable juice with a few drops of 1% FeCl₃ to determine the presence of tannins.

Phenol test: (A) Ferric chloride test: Add 3~4 drops of 5% FeCl₃ solution to 0.5ml of plant extract. Blue indicates the presence of phenol. Carbohydrate test (A) Molish test: Add 2~3 drops of 1% alcohol-naphthol solution to 0.5ml of plant extract in a test tube and mix. If a purple ring forms at the junction, it is a carbohydrate.

Alkaloid test: (A) Dragendorff reagent (potassium bismuth iodide solution): Put 0.5 g of bismuth nitrate into an empty beaker. Add 10 ml concentrated hydrochloric acid. Add 4 g of KI and a little water to another beaker and stir until all of the KI is dissolved. Notice the formation of thick orange juice. (B) Meyer's reagent (potassium iodide-mercury solution): Dissolve potassium iodide (5.00g) and mercury chloride (1.36g) in 100ml of water. (C) Wagner reagent (iodine-potassium iodide solution): Dissolve 6 g of potassium iodide and 2 g of iodine in 100 ml of water. (D) Hager reagent (picric acid saturated solution): Dissolve 1 g of picric acid and plant extract in 100 ml of water. (A) Ferric chloride (FeCl₃) test: Measure tannin by mixing a few drops of 1% FeCl₃ with 0.5ml of plant extract. Test for phenol by adding 0.5 ml of plant extract.

Antimitotic Assay:

Allium cipa was planted in tap water at room temperature for 72 hours in the dark to obtain long roots. The center of the bulb used in this study is at least 2-3 cm long, and the seeds are divided into 3 groups with 3 bulbs. The first group was given a dip in Water. Place a test (*Allium Cipa*) sample

into each container containing plant sample and third set was dipped in colchicine which was taken as standard. Then it was allowed to dip for 24 hours and next day it was observed under microscope. To calculate Mitotic Index following formula was used:

$$\text{Mitotic Index (\%)} = \frac{\text{Number of dividing Cells}}{\text{Total number of cells}} \times 100$$

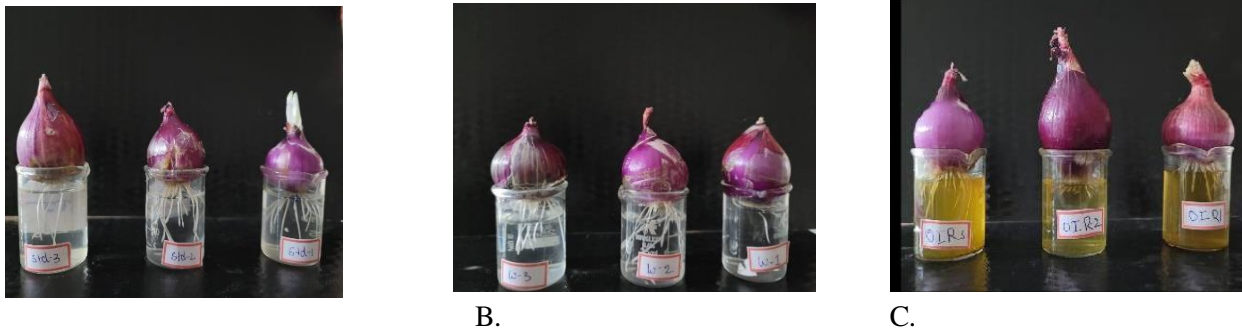


Figure 1. (A) *Allium cepa* bulbs with water (B) *Allium cepa* bulbs with drug (Colchicine)(0.1mg/L) (C) *Allium*

Antibacterial Assay:

Model: 0 Antibacterial test: *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, and *Shigella flexneri* are four types of bacteria used to test plant samples for their antibacterial properties. Sterilize the medium with pipettes, Petri dishes, and metal staples in an autoclave at 121 °C and 15 psi for 15 min. Add the medium to the Petri dish under sterile conditions. These four strains were obtained from the Department of Biotechnology, Atmiya University, India. *cepa* with plant sam

The formation of bacterial colonies on the surface is carried out using nutrient agar. As part of the agar spreading process, bacteria are plated onto the solidified agar media and a 7 mm diameter hole is drilled into the agar media using a sterile wire drill. Plates were incubated at 37° C. for 24 hours and stimulation was monitored using control wells containing antibiotics. Plot diameters were measured after 24 hours of incubation and compared to control plots to determine bacterial resistance. Plant extracts were assayed for antibiotic resistance using rifampicin test strips. Plant extracts were added at different concentrations (10 mg, 25 mg, 50 mg, 100 mg) and stored in an oven for 24 h to evaluate the desired results. *Salmonella typhi* and *Shigella flexneri*. Plates were incubated for 24 hours.

Result:

Oroxylum indicum contains various alkaloids, terpenoids, phenols and flavonoids which are used to treat gastric problems, cancer, asthma and various skin problems

Phytochemical analysis:

The plant extract underwent phytochemical analysis, which revealed the presence of secondary metabolites as flavonoids, terpenoids, carbohydrates, and alkaloids as listed in Table 1.

| <i>No.</i> | <i>Phyto constitute</i> | <i>Name of test</i> | <i>Plant</i> |
|------------|-------------------------|--------------------------------|--------------|
| <i>1</i> | <i>Flavonoids</i> | Alkaline test | + + |
| | | Lead-acetate test | + + |
| <i>2</i> | <i>Terpenoids</i> | <i>Salkowski test</i> | + + |
| <i>3</i> | <i>Saponins</i> | <i>Foam test</i> | + + |
| <i>4</i> | <i>Steroids</i> | <i>Libermann-burchard test</i> | -- |
| <i>5</i> | <i>Tannins</i> | <i>Ferric chloride test</i> | + + |
| <i>6</i> | <i>Phenol</i> | <i>Ferric chloride test</i> | + |
| <i>7</i> | <i>Carbohydrates</i> | <i>Molisch's test</i> | + |
| <i>8</i> | <i>Amino acid</i> | <i>Biuret test</i> | + |
| <i>9</i> | <i>Cardic glycoside</i> | <i>Killer killani test</i> | -- |
| <i>10</i> | <i>Alkaloids</i> | Dragendroff's test | -- |
| | | Mayer's test | + |
| | | Wagner's test | + + |

Table 1. Phytochemical constituents present in *Oroxylum Indicum* where + slightly present ++ moderately present, +++ highly present, - absent,

Antimitotic Assay:

After staining the root tip cells with colchicine dye they were observed under light microscope. Figure 3 show the root tip in microscope.

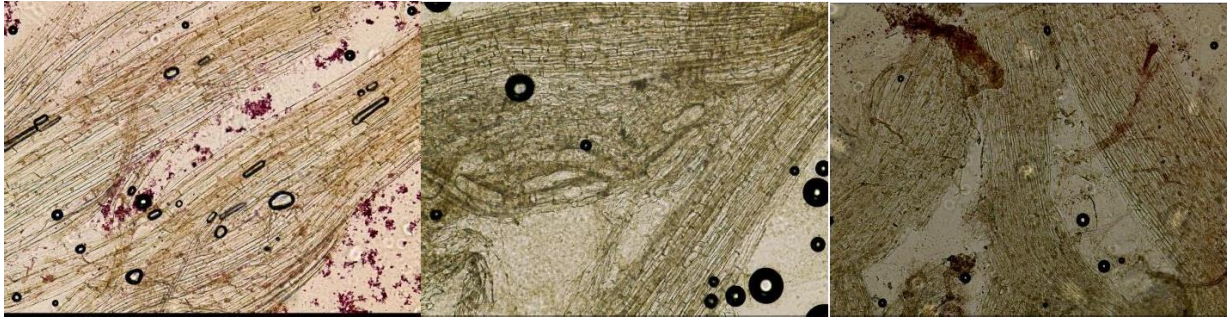


Fig 3. Microscopic observation at 10X magnification of *Allium cepa* meristematic cells treated with Water.



Fig4. Microscopic observation at 10X magnification of *Allium cepa* meristematic cells treated with colchicine.

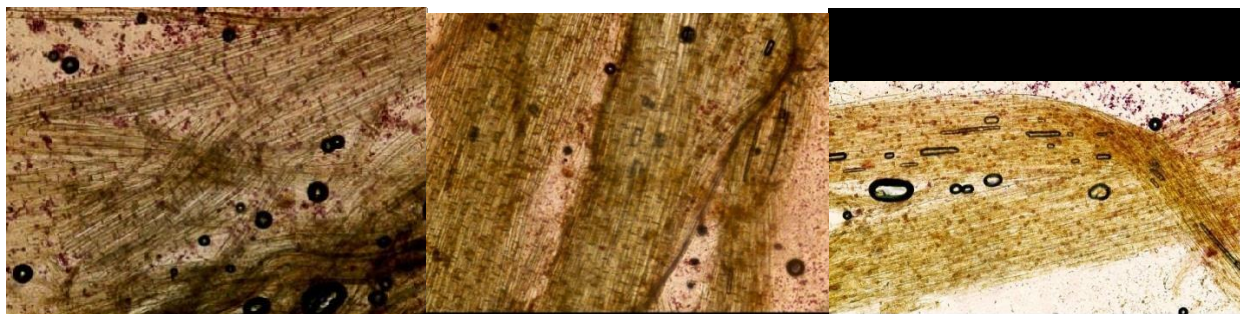


Fig5. Microscopic observation at 10X magnification of *Allium cepa* meristematic cells treated with plant

Analytical statistics:

Three replicates of the data were used to get the mean, and the standard deviation was also calculated.

The programmed GraphPad Prism was used to analyse p values. The significant difference between the standard, methanolic, and *Oroxylum indicum* was examined using one-way analysis of variance (ANOVA). $p = 0.05$ was regarded as being significant.

| | Sample 1 | | | Sample 2 | | | Sample 3 | | |
|----------------------|----------------|--------------------|-------------|----------------|--------------------|-------------|----------------|--------------------|-------------|
| | dividing cells | Non Dividing Cells | Total Cells | dividing cells | Non Dividing Cells | Total Cells | dividing cells | Non Dividing Cells | Total Cells |
| Water | 321 | 200 | 521 | 237 | 200 | 437 | 120 | 105 | 225 |
| Colchicine | 84 | 287 | 371 | 110 | 297 | 407 | 125 | 300 | 425 |
| Plant Extract | 142 | 244 | 386 | 150 | 268 | 418 | 175 | 298 | 463 |

Table 2. No. of cells observed in the root tip cells of *Allium cepa* when observed under microscope.

The result of effect of on *Oroxylum indicum* mitotic index of *Allium cepa* root tip cells is given in Table3.

| | Water | Colchicine | Plant extract |
|---------|-------|------------|---------------|
| Sample1 | 61% | 22.6% | 36.7% |
| Sample2 | 54% | 27% | 33.8% |
| Sample3 | 53% | 29.4% | 35.6% |

Table3. Mitotic Index of comparison between water, colchicine and plant extract

Statistical Analysis:

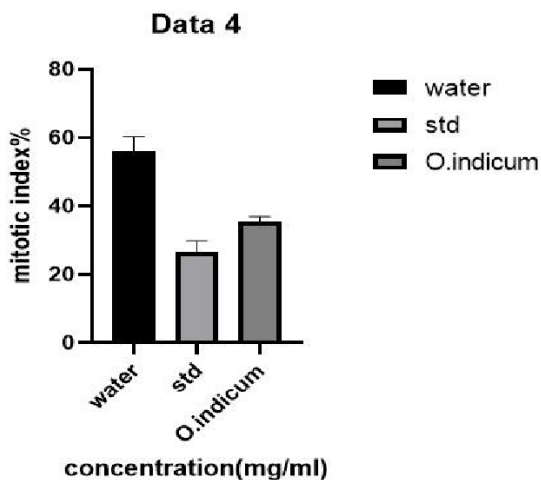


Fig6. Mitotic index (MI) comparison between water, colchicine and plant extract

| ANOVA summary | |
|--|--------|
| F | 12.17 |
| P value | 0.0077 |
| P value summary | ** |
| Significant diff. among means (P < 0.05) | Yes |
| R squared | 0.8022 |

Table4. The statistical table comparing the p value

Antibacterial Analysis:

Zones of inhibition were clearly visible when *Oroxylum indium*'s antibacterial effectiveness was tested in vitro against *Staphylococcus aureus*, *Bacillus subtilis*, *Sheighella flexneri*, and *Salmonella typhi*. In our study it was observed and concluded that my plant sample has an inhibitory effect against all the four test samples as zone of inhibition was confirmed.

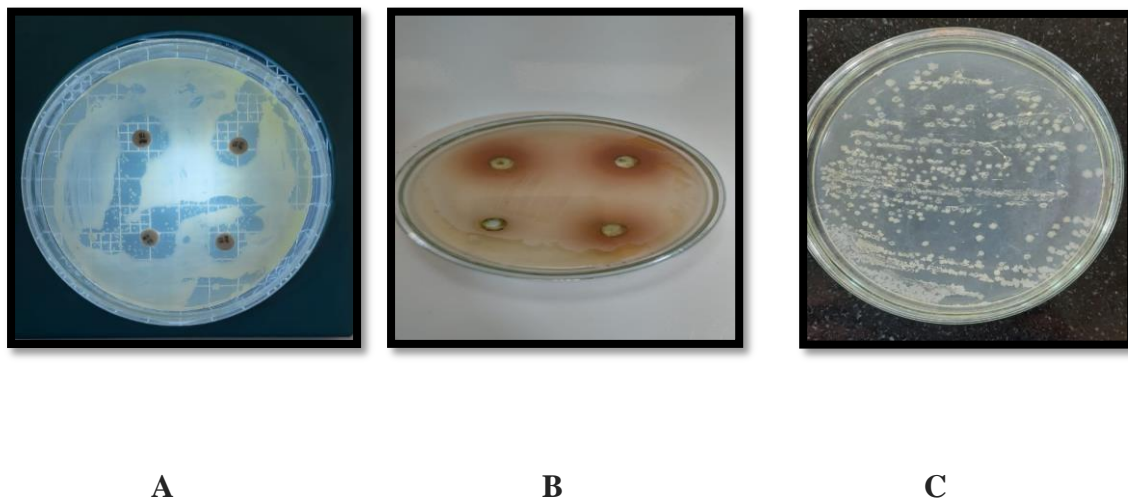


Fig7. Well diffusion assay of *Bacillus subtilis* with (A) Standard Rifampicin (Showing zone of inhibition) (Positive Control) (B) N-Agar plate with plant extract (Test) (C) Negative Control

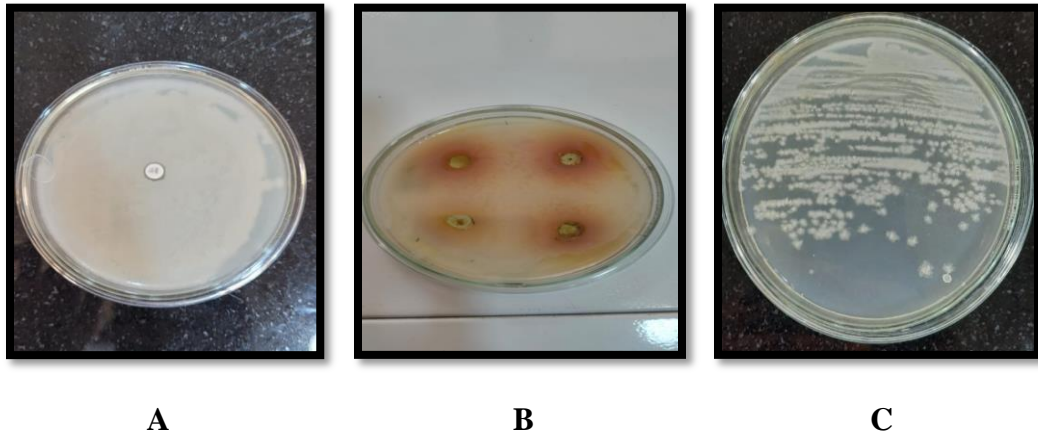


Fig 8. Well diffusion assay of *Salmonella typhi* with (A) Standard Rifampicin (Showing zone of inhibition) (Positive Control) (B) N-Agar plate with plant extract (Test) (C) Negative Control

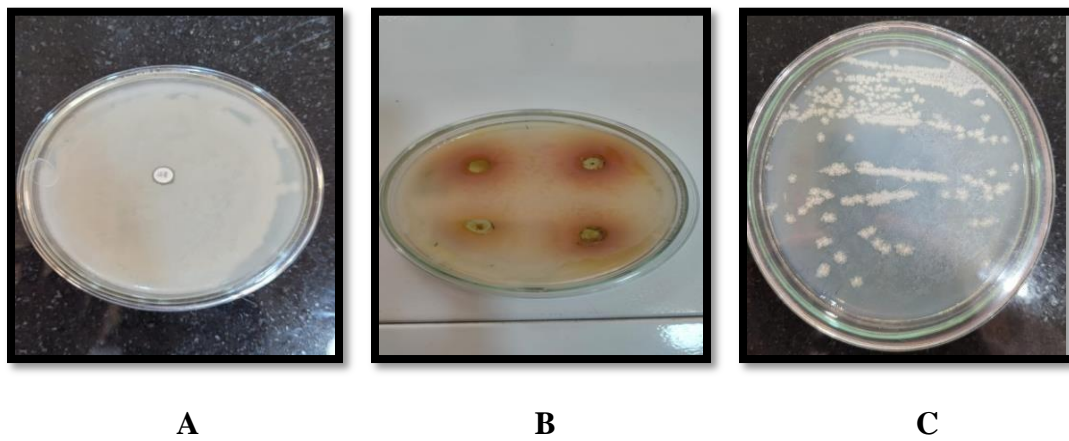


Fig 9. Well diffusion assay of *Staphylococcus aureus* with (A) Standard Rifampicin (Showing zone of inhibition) (Positive Control) (B) N-Agar plate with plant extract (Test) (C) Negative Control

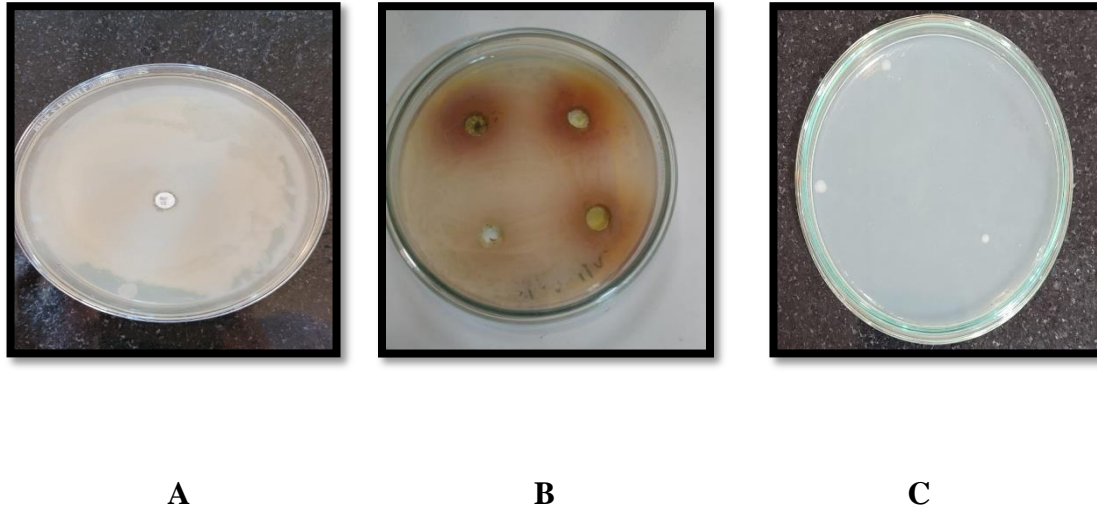


Fig 9. Well diffusion assay of *Shigella flexneri* with (A) Standard Rifampicin (Showing zone of inhibition) (Positive Control) (B) N-Agar plate with plant extract (Test) (C) Negative Control

Conclusion:

This study examined the antibacterial and antimutic properties of the *Oroxylum indicum* and found that they exist. *Oroxylum indicum* can be used as an anti-inflammatory and antibacterial agent because these applications demonstrate the presence of important bioactive substances and biochemical properties. Understanding the beneficial effects of bioactive substances requires further research. LCMS data is analyzed for identifying various phytochemicals presence.

Discussion:

Although plant parts of *Oroxylum indicum* have been extensively studied, their roots have received less attention, as evidenced by areas found true for inhibition, despite their excellent antimutic and antimicrobial properties. More research can be done to refer to the use of antibiotics and antibiotics. Inhibitory effects were also observed on pathogenic *Shigella flexneri* and *Salmonella typhi* strains.

Acknowledgement:

I am very happy to share with you the report on the treatment of Plant *Oroxylum Indicum* in the last year of the Master's degree. Special Thanks to Dr. Nutan Prakash Vishvakarma Head of The Department of Biotechnology for allowing me to use All The facilities and thanks and thanks and to my project consultant, Dr. Praveen Gupta, Assistant Professor, Atmiya University, Rajkot, made careful suggestions and organized my material in many ways. I also do not want to miss this opportunity and would like to thank all the teachers and staff in the department for their help and cooperation during the development of my project. Finally, I would like to thank my friends for their successful cooperation.

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