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Pharmacological Evaluation of Antioxidant and Antimicrobial Activities of Hydroalcoholic Extracts from *Oroxylum Indicum* and *Pongamia Pinnata*

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ABSTRACT

Background: For centuries, plants like Pongamia pinnata and Oroxylum indicum have been recognized in Ayurveda for their medicinal value. Pharmacological studies are essential to validate their traditional therapeutic uses.

Method: This work investigates the antioxidant and antimicrobial activities of hydroalcoholic extracts from Oroxylum indicum and Pongamia pinnata. Antioxidant potential was tested using multiple assays, while antimicrobial effects against Salmonella bongori and Streptococcus mutans were assessed by agar well diffusion.

Result: The extraction yield was 3.59% for Pongamia pinnata and 4.15% for Oroxylum indicum. In the DPPH assay, Oroxylum indicum showed an IC50 of 82.67 µg/ml, indicating stronger radical scavenging compared to Pongamia pinnata (102.62 µg/ml). The nitric oxide assay also favored Oroxylum indicum (82.25 µg/ml) over Pongamia pinnata (106.17 µg/ml). Similarly, in the hydrogen peroxide assay, Oroxylum indicum (76.58 µg/ml) proved more effective than Pongamia pinnata (103.04 µg/ml). Oroxylum indicum exhibited inhibition zones of 14 mm against Salmonella bongori and 14 mm against Streptococcus mutans at 100 mg/ml, whereas Pongamia pinnata showed 12.3 mm and 11 mm, respectively. Ciprofloxacin and Ofloxacin were used as standards, with Ciprofloxacin showing a maximum inhibition zone of 25 mm against Salmonella bongori.

Conclusion: Both plants demonstrated antioxidant and antimicrobial potential, with Oroxylum indicum exhibiting greater efficacy than Pongamia pinnata.

Keywords: *Oroxylum indicum*, *Pongamia pinnata*, antioxidant activity, DPPH, nitric oxide, hydrogen peroxide, antimicrobial activity, *Salmonella bongori*, *Streptococcus mutans*, percentage yield

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INTRODUCTION

Medicinal flora has been integral to conventional healthcare method across the globe for centuries. They have provided a plethora of bioactive compounds that have served as precursors for drug development.[1] Two such plants, *Pongamia pinnata* (commonly known as Karanja) and *Oroxylum indicum* (known as Shyonaka),[2] have been extensively used in Ayurvedic medicine.[3] These plants are acclaimed for their broad range of biological actions[4].

Pongamia pinnata is a leguminous tree widely distributed in tropical and subtropical regions. Its leaves, seeds, and bark are rich in flavonoids, alkaloids, tannins, and saponins, which contribute to its medicinal properties. The herbs have been conventionally utilized to take care of a range of ailments such as skin diseases, ulcers, and inflammatory conditions. Modern pharmacological studies have corroborated these traditional uses, revealing significant antimicrobial and antioxidant activities in various extracts of *Pongamia pinnata*.[5]

Oroxylum indicum, belonging to the Bignoniaceae family, is another plant with a storied history in traditional medicine. Known as the "tree of Damocles" due to its sword-like pods, *Oroxylum indicum* is used in Ayurveda for its purported health benefits, including anti-inflammatory, antioxidant, and hepatoprotective properties. The plant contains a rich array of phytochemicals, such as baicalein, chrysin, and oroxylin A, which have been demonstrated to exhibit potent biological activities in various studies.[6]

The increasing prevalence of antibiotic-resistant pathogens and oxidative stress-related diseases underscores the need for novel therapeutic agents. Antioxidants can neutralize ROS, thereby mitigating oxidative damage and its associated risks. Additionally, with the rising threat of multidrug-resistant bacteria, there is an urgent need for effective antimicrobial agents. The investigation of plant extracts for such activities is therefore of great importance.[7]
By employing in vitro antioxidant assays antioxidant action will be assessed. Additionally, their antimicrobial efficacy will be assessed by the agar well diffusion process beside common pathogens.

In summary, the pharmacological evaluation of *Pongamia pinnata* and *Oroxylum indicum* extracts aims to provide a scientific basis for their traditional medicinal uses. This study will enhance our considerate of the beneficial possible of these plants and support their use in modern medicine.

METHODOLOGY

Collection of Plant Material

Pongamia pinnata leaves were collected in June 2021 from Vindhya Herbals (MFPPARC), Bhopal. Similarly, *Oroxylum indicum leaves* were harvested from the same location. Both plant materials were chosen depending on their customary therapeutic use and identified for quality and freshness. Upon collection, the leaves were cleaned using water to eliminate any dirt or dust. They were then shade dried at room temperature to prevent the degradation of active compounds due to direct sunlight. After drying, it was powdered by a mechanical chopper to obtain a fine, uniform powder, which was stored in airtight containers until further use.[8]

Defatting of Plant Material

To remove non-polar components and impurities, the powdered leaves of both *Pongamia pinnata* and *Oroxylum indicum* were defatted using petroleum ether. In this process, the powdered leaves were soaked in petroleum ether in separate containers, and the mixtures were stirred occasionally. The maceration process continued until the powdered leaves were completely defatted, confirmed by the absence of oily residues. The petroleum ether extracts were discarded, and the defatted powdered leaves were air-dried to remove any remaining traces of the solvent.[9]

Extraction by Maceration Method

Following defatting, the extraction was performed using the maceration method with a hydroalcoholic solvent. A total of 150 grams of defatted powdered leaves from each plant were placed in separate containers, and a hydroalcoholic solvent mixture of ethanol and water in an 80:20 ratio (v/v) was added to each. The leaves were allowed to macerate in the solvent mixture, which was periodically stirred to ensure thorough mixing and optimal extraction of the phytochemicals. The maceration process was carried out for a specified period to ensure exhaustive extraction.
After the extraction period, the mixtures were filtered to separate the solvent extracts from the plant residues. The solvents were then dispersing beneath condensed force using a rotary evaporator at temperatures above the boiling points of the solvents to obtain concentrated

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extracts. The residual solvents in the concentrated extracts were removed completely by drying them under controlled conditions to yield the final hydroalcoholic extracts of *Pongamia pinnata* and *Oroxylum indicum*.[10]

Determination of Percentage Yield

The percentage yield of the hydroalcoholic extracts was considered to conclude the effectiveness of the extraction method.

For this study, the weight of the powdered leaves taken for extraction was 150 grams for each plant. After the evaporation and drying process, the weights of the hydroalcoholic extracts obtained were measured. The percentage yield of the *Pongamia pinnata* hydroalcoholic extract was calculated to be 3.59%, while the yield for *Oroxylum indicum* was similarly calculated (specific yield value to be provided based on actual measurements). These yields indicate the efficiency of the extraction process and provide insights into the quantity of extractable phytochemicals present in each plant material.[11]

In-vitro Antioxidant Activity

DPPH radical scavenging assay

The DPPH assay was utilized to assess the antioxidant activity of the extracts of hydroalcoholic of *Pongamia pinnata* and *Oroxylum indicum*. A popular test for determining a compound's capacity to neutralise the DPPH radical by acting as a hydrogen donor or free radical scavenger is the DPPH technique. A DPPH solution containing 0.1 mM was made in methanol. Ascorbic acid was utilised as an established antioxidant whilst various quantities of botanical isolates (10, 20, 40, 60, 80, and 100 µg/mL) were produced in methanol. One millilitre of the DPPH solution was mixed with one millilitre of ascorbic acid and every amount of plant extracts in a set of test tubes. To enable the reaction to happen, the mixtures used for the experiment were vortexed and allowed to sit at ambient temperature approximately 30 minutes in the dark.[12]

The ability of nitric oxide (NO) to scavenge radicals

The Griess reagent method was utilized to ascertain the extracts' ability to scavenge nitric oxide (NO) radicals by measuring the nitrite ions generated when NO reacts with oxygen. The Griess reagent was used for assay. One millilitre of sodium nitroprusside mixture was combined with different plant extract quantities (10, 20, 40, 60, 80, and 100 µg/mL), and the mixture was incubated for 150 minutes at 25°C. After incubation the reaction blend was mix with 1 millilitre of Griess reagent and left to react for half an hour. Using a UV-visible spectrophotometer, the absorbance of the resultant chromophore was determined at 546 nm.[13]

Activity of hydrogen peroxide (H_2O_2) scavenging

To evaluate the plant extracts' potential as antioxidants, it was shown that they could scavenge hydrogen peroxide (H_2O_2). In phosphate buffer (pH 7.4), a H_2O_2solution containing 40 mM was created. In hydrogen peroxide solution, various quantities of plant extracts (10, 20, 40, 60, 80, and 100 µg/mL) were added. A UV was utilized to compute the absorbance of the reaction mixtures at 230 nm after 10 minutes of incubation.[14,15]

In vitro Antimicrobial Intensity

The process of making nutrient agar plates involved dissolving 28 grammes of nutritional agar powder in one litre of distilled water, autoclaving the mixture to sterilise it, and then letting the agar firm in petri dishes. *Salmonella bongori* as well as *Streptococcus mutans* bacterial suspensions were made and calibrated using the 0.5 McFarland standard.

Agar plates were punctured, and wells were then loaded with 100 µL of plant extracts at various doses (10, 20, 40, 60, 80, and 100 µg/mL), as well as sterile distilled water and common antibiotics (ciprofloxacin and ofloxacin) as controls. For twenty-four hours, the plates were kept warm at 37°C. To evaluate the effectiveness of the antibiotics, the zones of inhibition surrounding each well were assessed after incubation. The efficacy of botanical extracts as antimicrobial substances was assessed by comparing the diameters of the inhibition zones to those generated by conventional antibiotics.[16,17]

Results

Table 1 presents the percentage yield of hydroalcoholic extracts from *Pongamia pinnata* and *Oroxylum indicum*. The extract of *Oroxylum indicum* exhibited a higher yield of 4.12% compared to *Pongamia pinnata*, which had a yield of 3.59%. This indicates that *Oroxylum indicum* has a slightly higher concentration of extractable compounds under the given extraction conditions. This data is crucial for understanding the potential availability of bioactive compounds in these plants and their feasibility for further pharmacological evaluation.

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Table 1: Results of % Yield of Hydroalcoholic Extracts

Plant	% Yield (W/W)
Pongamia pinnata	3.59
Oroxylum indicum	4.12

Table 2: Results of Antioxidant Activity

Concentration (µg/ml)	DPPH Assay Method			Nitric Oxide Method			Hydrogen Peroxide Method		
	% Inhibition								
	AA	EOI	EPP	AA	EOI	EPP	AA	EOI	EPP
10	43.16	12.99	18.10	-	-	-	-	-	-
20	50.12	14.39	22.51	51.06	81.25	6.53	45.54	24.67	19.42
40	54.06	34.80	25.75	56.69	27.96	12.16	64.04	32.28	21.52
60	72.62	59.86	35.03	62.77	37.39	28.72	66.54	39.90	34.25
80	77.26	43.85	37.12	67.33	41.49	36.02	75.72	53.15	38.45
100	84.92	50.12	53.36	82.83	66.41	46.66	80.84	61.15	50.79
IC_{50}	**22.83**	**82.67**	**102.62**	**21.95**	**82.25**	**106.17**	**19.82**	**76.58**	**103.04**

Ascorbic acid-AA; Extract *of Oroxylum indicum*-EOI; Extract *of Pongamia pinnata*-EPP

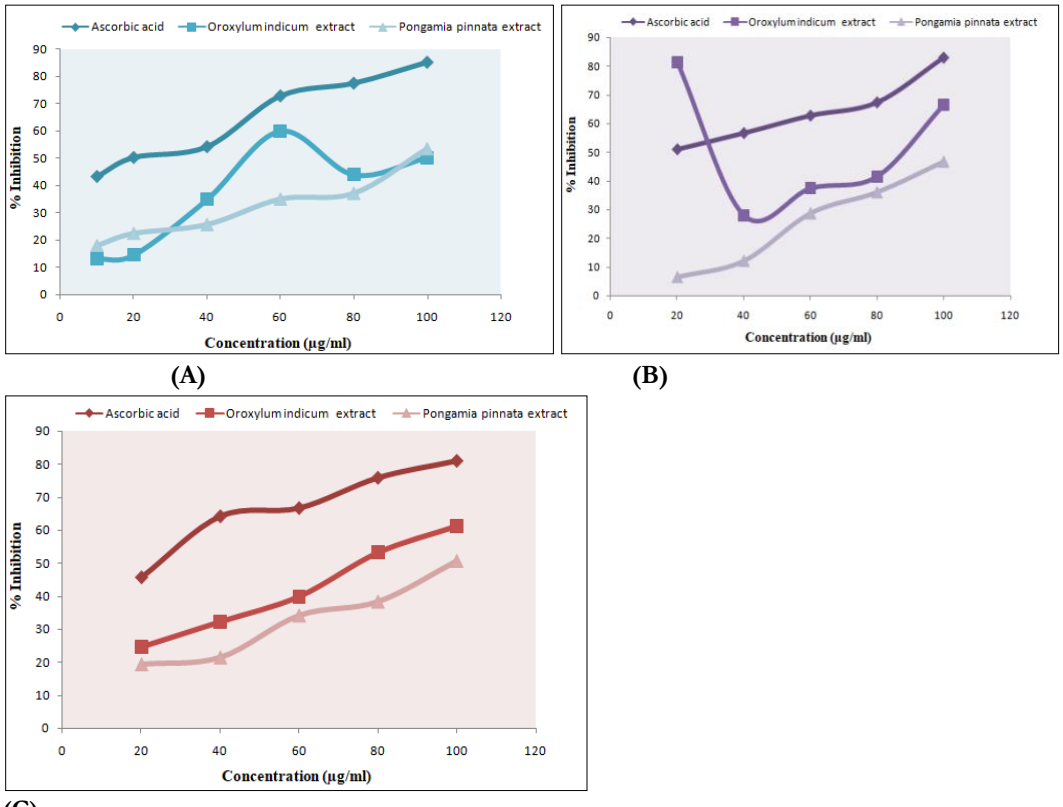

Figure 1: (A) Results of DPPH method; (B) Results of NO method; (C) Results of hydrogen peroxide method

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Table 2 and Figure 1A reveal that ascorbic acid, *Oroxylum indicum*, and *Pongamia pinnata* extracts have different radical scavenging activities according to the DPPH technique. Standard antioxidant ascorbic acid inhibited the most at all doses, demonstrating its antioxidant power. At 10 µg/ml, ascorbic acid inhibited DPPH radicals by 43.16%, increasing to 84.92% at 100 µg/ml. Ascorbic acid effectively scavenges DPPH radicals at low concentrations, with an IC_{50} value of 22.83 µg/ml.

The antioxidant activity of *Oroxylum indicum* extracts was lower but remained considerable. Percentage inhibition ranged from 12.99% at 10 µg/ml to 50.12% at 100 µg/ml. The IC_{50} for *Oroxylum indicum* was 82.67 µg/ml. This higher IC_{50} result shows that *Oroxylum indicum* extract must be at a greater concentration to match ascorbic acid's antioxidant activity. *Pongamia pinnata* extracts were intermediate antioxidants. The inhibition percentage ranged from 18.10% at 10 µg/ml to 53.36% at 100 µg/ml. The IC_{50} value for *Pongamia pinnata* was 102.62 µg/ml, the highest among the investigated samples. This suggests that *Pongamia pinnata* has less antioxidant activity than ascorbic acid and *Oroxylum indicum*.
While ascorbic acid is the most efficient antioxidant, *Oroxylum indicum* and *Pongamia pinnata* also have antioxidant capability, albeit to a lesser extent. *Oroxylum indicum* has a moderate antioxidant IC_{50} value greater than ascorbic acid but lower than *Pongamia pinnata*. As the least antioxidant, *Pongamia pinnata* had the greatest IC_{50} value, indicating weaker radical scavenging. These findings show that plant extracts have various antioxidant potentials and require more investigation to fully understand their bioactive capabilities. The comparison shows that ascorbic acid is a benchmark antioxidant and that *Oroxylum indicum* and *Pongamia pinnata* may be useful in producing natural antioxidant therapies or supplements.
Table 2 shows that ascorbic acid, *Oroxylum indicum*, and *Pongamia pinnata* extracts neutralize NO radicals to varied degrees. Figure 1B indicates strong NO scavenging capacity of ascorbic acid, with high percentage inhibition values at all doses. At 20 µg/ml, ascorbic acid had a 51.06% inhibition rate, rising to 82.83% at 100 µg/ml. The IC_{50} value for ascorbic acid is 21.95 µg/ml, suggesting its considerable effectiveness in scavenging NO radicals at low concentrations. *Oroxylum indicum* extracts also inhibited NO radicals, but not as significantly as ascorbic acid. Percentage inhibition ranged from 81.25% at 20 µg/ml to 66.41% at 100 µg/ml. The IC_{50} value for *Oroxylum indicum* is 82.25 µg/ml, indicating that it effectively scavenges NO radicals but requires a larger concentration than ascorbic acid to achieve comparable inhibition. *Pongamia pinnata* extracts had the lowest NO scavenging activity of the three, tested compounds. Percentage inhibition ranged from 6.53% at 20 µg/ml to 46.66% at 100 µg/ml. With an IC_{50} value of 106.17 µg/ml, *Pongamia pinnata* has the lowest NO radical scavenging activity, requiring a larger concentration to match *Oroxylum indicum* and ascorbic acid.
The NO technique shows that ascorbic acid is the most powerful nitric oxide radical scavenger, with a low IC_{50} value and high % inhibition at all doses. *Oroxylum indicum* is less effective than ascorbic acid but still has antioxidant characteristics due to its higher IC_{50} value. These results show that ascorbic acid is the most effective NO scavenger, followed by *Oroxylum indicum* and *Pongamia pinnata*. This emphasizes the need of choosing antioxidants with specific radical scavenging capabilities for therapeutic use.

Table 1 shows substantial variations in hydrogen peroxide neutralization by ascorbic acid, *Oroxylum indicum* and *Pongamia pinnata* extracts. Ascorbic acid scavenged hydrogen peroxide best. Ascorbic acid inhibited at 45.54% at 20 µg/ml and 80.84% at 100 µg/ml. Ascorbic acid, with an IC_{50} value of 19.82 µg/ml, effectively neutralises hydrogen peroxide, demonstrating its antioxidant capabilities. *Oroxylum indicum* moderately scavenged hydrogen peroxide. Percentage inhibition ranged from 24.67% at 20 µg/ml to 61.15% at 100 µg/ml. The IC_{50} value of 76.58 µg/ml suggests that *Oroxylum indicum* needs a greater concentration than ascorbic acid to achieve comparable inhibition. It has decent antioxidant activity despite having much lower scavenging ability then ascorbic acid. *Pongamia pinnata* has the lowest hydrogen peroxide scavenging activity of the three. The inhibition percentage in figure 1C ranged from 19.42% at 20 µg/ml to 50.79% at 100 µg/ml. *Pongamia pinnata* had the lowest antioxidant ability among investigated samples, with an IC_{50} value of 103.04 µg/ml, indicating less effective hydrogen peroxide scavenging.

Ascorbic acid neutralises hydrogen peroxide best, as seen by its low IC_{50} value and high % inhibition across all concentrations. *Oroxylum indicum* has moderate scavenging action and a higher IC_{50} value, indicating greater doses are needed to neutralise hydrogen

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peroxide. With the highest IC_{50} value, *Pongamia pinnata* is the least effective of the three extracts for scavenging hydrogen peroxide and mitigating oxidative stress generated by it.

The investigated compounds' antioxidant efficiency varied greatly, with ascorbic acid being the most effective, followed by *Oroxylum indicum* and *Pongamia pinnata*. The varying efficacy of various extracts emphasises the necessity to select antioxidant drugs based on their radical scavenging capacities for therapeutic usage. Future research could explain these variations and find new ways to use these extracts to treat oxidative stress.

Antimicrobial Activity

Table 3: Results of Antimicrobial activity

Drug	Microorganism	Zone of Inhibition (mm)		
		10 µg/ml	**20 µg/ml**	**30 µg/ml**
Ciprofloxacin	*Salmonella bongori*	17±0.15	23±0.86	25±0.5
Ofloxacin	*Streptococcus mutans*	16±0	13±0	11±0
		25 mg/ml	**50 mg/ml**	**100mg/ml**
Oroxylum indicum	*Salmonella bongori*	9±0.86	12±0.5	14±0
extract	*Streptococcus mutans*	10±0.57	12±0.74	14±0
Pongamia pinnata	*Salmonella bongori*	10±0.5	11±0	12.3±2.5
extract	*Streptococcus mutans*	6±0	9±0.74	11±0

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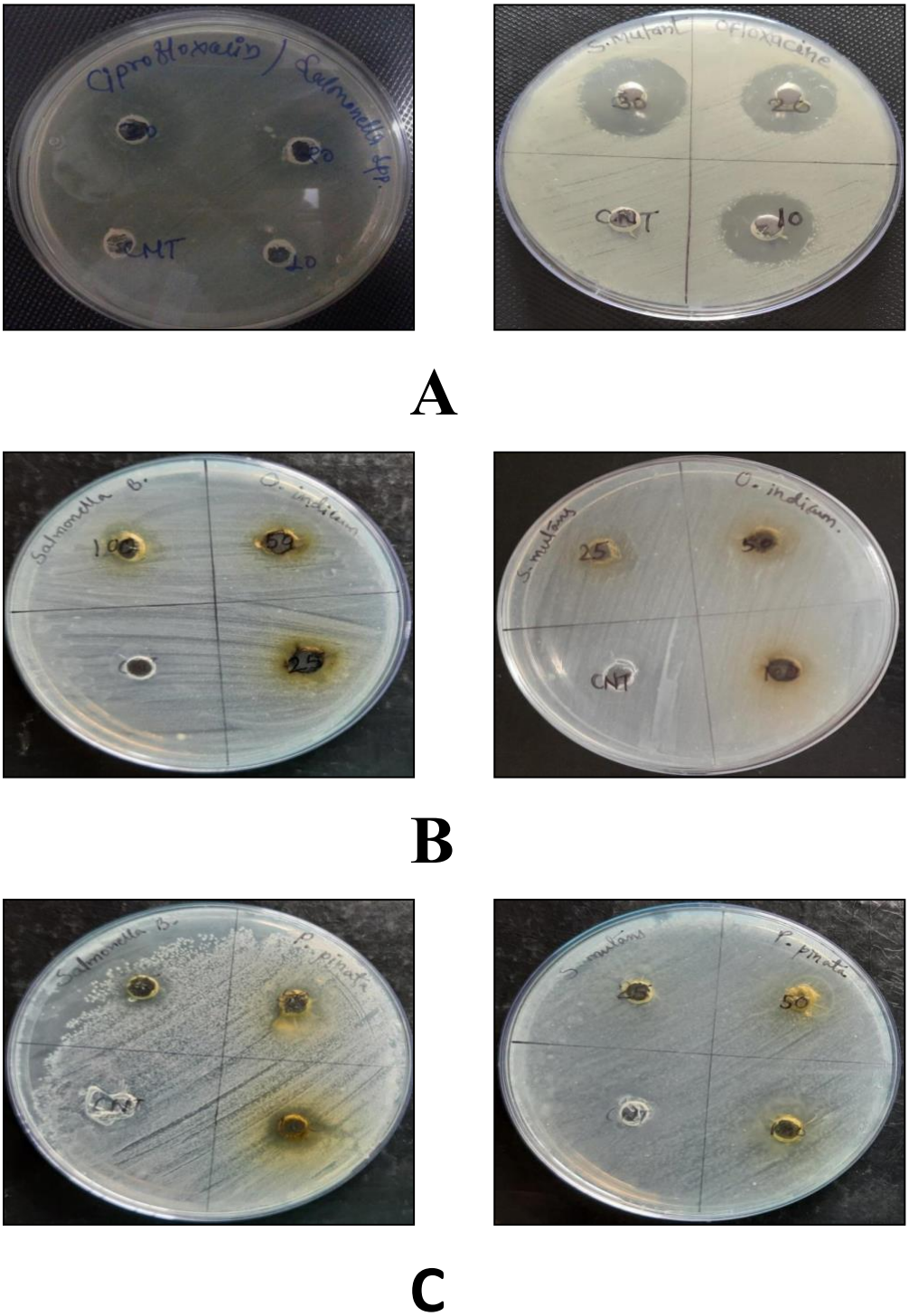

A

B

C

Figure 2: (A) Photo plates of antimicrobial activity of standard; (B) Photo plates of antimicrobial activity of *Oroxylum indicum* extract; (C) Photo plates of antimicrobial activity of *Pongamia pinnata* extract

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Standard medicines Ciprofloxacin and Ofloxacin (table 3 and figure 2A) were tested against *Salmonella bongori* and *Streptococcus mutans*. Ciprofloxacin displayed significant activity against *Salmonella bongori*, with inhibition zones rising from 17 ± 0.15 mm at 10 µg/ml to 25 ± 0.50 mm at 30 µg/ml. The dose-dependent inhibitory effect of Ciprofloxacin against this bacterium is considerable. In contrast, Ofloxacin had varying levels of activity against *Streptococcus mutans*, with inhibition zones of 16 ± 0 mm, 13 ± 0 mm, and 11 ± 0 mm at dosages of 10 µg/ml, 20 µg/ml, and 30 µg/ml.

Oroxylum indicum extract inhibited *Salmonella bongori* and *Streptococcus mutans*. Table 3 and figure 2B shows inhibition zones for *Salmonella bongori* were 9 ± 0.86 mm at 25 mg/ml and 14 ± 0 mm at 100 mg/ml. This reveals that antibacterial activity increases with concentration. The extract showed inhibition against *Streptococcus mutans*, with zones of inhibition ranging from 10 ± 0.57 mm at 25 mg/ml to 14 ± 0 mm at 100 mg/ml. *Oroxylum indicum* appears to be antibacterial, especially at higher concentrations.

The *Pongamia pinnata* extract in table 3 and figure 2C had antibacterial action but was weaker than the conventional medicines and *Oroxylum indicum* extract. The inhibition zones for *Salmonella bongori* were 10 ± 0.5 mm, 11 ± 0 mm, and 12.3 ± 2.5 mm at doses of 25 mg/ml, 50 mg/ml, and 100 mg/ml. This shows moderate concentration-induced action. The extract inhibited *Streptococcus mutans* with zones of 6 ± 0 mm, 9 ± 0.74 mm, and 11 ± 0 mm. Figure 2C shows that higher concentrations enhance inhibition but lower activity than *Oroxylum indicum*.

Ofloxacin has limited action against *Streptococcus mutans*, however Ciprofloxacin is particularly efficient against *Salmonella bongori*. *Oroxylum indicum*, a plant extract, inhibits *Salmonella bongori* and *Streptococcus mutans* at greater concentrations. *Pongamia pinnata* has antibacterial action, but less than the conventional medicines and *Oroxylum indicum* extract.[18, 19.]

DISCUSSION

This study assessed the antioxidant and antimicrobial potential of hydroalcoholic extracts from *Oroxylum indicum* and *Pongamia pinnata*. The extraction process yielded 3.59% from *Pongamia pinnata* and 4.12% from *Oroxylum indicum,* indicating reasonable extraction efficiency.

The antioxidant activity was evaluated using three different assays: DPPH, nitric oxide (NO), and hydrogen peroxide (H_2O_2) scavenging methods. The DPPH assay revealed that *Oroxylum indicum* had an IC_{50} value of 82.67 µg/ml, showing considerable antioxidant potential, while *Pongamia pinnata* had a higher IC_{50} value of 102.62 µg/ml, indicating lower potency in scavenging DPPH radicals compared to *Oroxylum indicum*. In the NO scavenging assay, *Oroxylum indicum* exhibited an IC_{50} of 82.25 µg/ml, while *Pongamia pinnata* had a higher IC_{50} of 106.17 µg/ml. This suggests that *Oroxylum indicum* is more effective in neutralizing nitric oxide. The hydrogen peroxide assay showed *Oroxylum indicum* with an IC_{50} value of 76.58 µg/ml and *Pongamia pinnata* with 103.04 µg/ml, reinforcing that *Oroxylum indicum* has superior antioxidant activity.

The antimicrobial activity was assessed using the agar well diffusion method. *Oroxylum indicum* demonstrated significant inhibition against *Salmonella bongori* with inhibition zones of 9 mm, 12 mm, and 14 mm at 25 mg/ml, 50 mg/ml, and 100 mg/ml, respectively. For *Streptococcus mutans*, the inhibition zones ranged from 10 mm to 14 mm at the same concentrations. In contrast, *Pongamia pinnata* showed lower antimicrobial activity, with maximum inhibition zones of 12.3 mm for *Salmonella bongori* and 11 mm for *Streptococcus mutans* at 100 mg/ml. This suggests that *Oroxylum indicum* possesses more potent antimicrobial properties compared to *Pongamia pinnata*.

In summary, *Oroxylum indicum* exhibits strong antioxidant and antimicrobial activities, making it a promising candidate for further exploration in therapeutic applications. While *Pongamia pinnata* also shows some antioxidant and antimicrobial potential, its efficacy is relatively lower compared to *Oroxylum indicum*. These findings underscore the importance of *Oroxylum indicum* in potential therapeutic and medicinal applications, while suggesting that further research is needed to enhance the activity of *Pongamia pinnata*.

CONCLUSION

The study evaluated the antioxidant and antimicrobial properties of hydroalcoholic extracts from *Oroxylum indicum* and *Pongamia pinnata*. The extraction process yielded 3.59% from *Pongamia pinnata* and 4.12% from *Oroxylum indicum*. *Oroxylum indicum* showed significant antioxidant potential, with a DPPH scavenging IC_{50} value of 82.67 µg/ml, while Pongamia pinnata had a higher IC_{50} value of 102.62 µg/ml. *Oroxylum indicum* was more effective in neutralizing nitric oxide and hydrogen peroxide. It also showed significant inhibition against *Salmonella bongori* and *Streptococcus mutans*. The findings highlight *Oroxylum indicum's* potential for therapeutic applications, while *Pongamia pinnata's* efficacy is lower.

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