

Original Article

Antimicrobial Activity of Moringa Oleifera Leaf Extracts on Multiple Drug-Resistant Bacterial Isolates from Urine Samples in A Tertiary Care Hospital in Morang

Nabin Thapa Magar ^{1*}, Matrika Poudel ², Manita Gadal ³, Dinesh Basnet ⁴

1. Assistant Professor, Purbanchal University School of Health Sciences, Morang, Nepal.
2. Purbanchal university School of Health Sciences, Koshi Haraicha, Nepal.
3. Purbanchal university School of Health Sciences, Koshi Haraicha, Nepal.
4. Purbanchal university School of Health Sciences, Koshi Haraicha, Nepal.

* Correspondence: phrnabin888@gmail.com

Abstract: Plants are crucial for human and animal health, with traditional medicines used by 80% of affluent nations. Moringaoleifera, a rapidly growing plant, has numerous medicinal properties, including antipyretic, antiepileptic, anti-inflammatory, and antioxidant effects. It is used in Ayurvedic traditional medicine for therapeutic and preventive purposes. The study investigated the antimicrobial activity of Moringaoleifera leaf extracts against multiple drug-resistant bacterial isolates obtained from urine from Morang district. Moringaoleifera, a plant from Nepal, was used in a study to inhibit multiple drug-resistant (MDR) bacterial isolates. The extract was extracted, filtered, and evaporated, and Mueller-Hinton agar plates were inoculated. The results confirmed the plant's antimicrobial potential. Moringaoleifera extract demonstrated effective antimicrobial activity against MDR bacterial isolates at higher concentrations, with complete inhibition at 300 mg/ml. The MIC is likely above 100 mg/ml, suggesting higher concentrations are needed for effective MDR infection management. Moringaoleifera powdered leaves show potential applications in various fields due to their low moisture content and sterility. The plant's medicinal properties and therapeutic potential are supported by its diverse phytochemical profile. In Nepal, the extract demonstrated significant antimicrobial activity against multidrug-resistant bacterial isolates, but further research is needed to determine optimal dosing strategies and clinical efficacy. Moringaoleifera extract demonstrated significant antimicrobial activity against MDR bacterial isolates, particularly at higher concentrations. However, lower concentrations were insufficient to prevent bacterial growth, suggesting that a higher concentration may be required for therapeutic applications. Further research is needed to determine optimal dosing strategies and evaluate the extract's efficacy in clinical settings.

Keywords: Plants, Moringaoleifera, Bacteria isolates, multidrug-resistant

1. INTRODUCTION

Plants are crucial natural products for human and animal health due to their therapeutic and preventive properties. Traditional medicines, derived from plants, are used by 80% of affluent nations. Antibacterial resistance has led to a search for new antibacterial compounds from plants. The high cost of conventional drugs, especially in resource-limited communities, has increased the use of plants for treating infectious diseases [1]. Natural products, particularly herbal drugs, have gained popularity as safer and cost-effective alternatives to synthetic alternatives. Plants are the primary source of these drugs, with increased demand due to their perceived safety and minimal side effects. This has led to extensive research in pharmacogenetic studies, including organoleptic characters, macroscopic studies,

powder studies, physicochemical analysis, phytochemical analysis. These studies help authenticate plants used as drugs, ensuring the quality and safety of these natural products [2]. *Moringaoleifera*, a rapidly growing plant in the Moringaceae family, is highly valued for its medicinal properties and has experienced exponential growth in the herbal medicine field due to its natural efficacy and minimal side effects [3]. Regional names for the tree include Benzolive, Horseradish, Drumstick, Kelor, Marango, Mlonge, Mulangay, Saijihan, and Sajna [4]. The tree is between 5 and 10 meters tall. It is widely distributed along the sandy banks of rivers and streams, grows best in the tropical insular environment, and can be found both wild and farmed across the plains, particularly in hedges and home yards. It can withstand poor soil conditions, thrive in hot, dry climates or humid tropical regions, and is not greatly impacted by drought. With a pH of 5.0–9.0 and minimum and maximum annual rainfall requirements estimated at 250 mm and over 3000 mm, respectively, it can withstand a broad range of rainfall [5]. The plant is native to the western and sub-Himalayan regions of India, Pakistan, Asia Minor, Africa, and Arabia. The *Moringa* tree is grown and utilized for food (leaves, pods, blossoms, and roasted seeds), spice (mostly roots), oil (seeds) for cooking and cosmetics, and medical purposes (all plant parts). The plant possesses several significant medical qualities, such as antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial, and antifungal effects. The seeds of *M. oleifera* have the ability to cleanse water. They have been used to treat malaria and are well-known to be anti-helminthic, antibiotic, detoxifying, immune-building, and they can also be utilized as a less expensive bio-absorbent for the elimination of heavy metals [6]. The leaves of *Moringaoleifera* have been used for both therapeutic and preventive reasons in Ayurvedic traditional medicine, which claims that the plant may prevent 300 diseases. Following Tanzania's hosting of the first international conference on *Moringaoleifera* in 2001, there have been an increasing number of congresses and studies devoted to sharing knowledge about this amazing plant's amazing properties. These days, this species goes by names like "natural gift," "miracle tree," and "mother's best friend [7]." Males and women can both contract urinary tract infections, but because of differences in physiology, women are more likely to contract them than males. It can be described as a condition that women will undoubtedly experience at some point in their lives, with a higher prevalence among pregnant women. The upper and lower urinary tracts are included in the urinary tract that is infected, as the term suggests. The infection is either pyelonephritis (a kidney infection) or cystitis (a bladder infection), depending on which body organ is affected. While painful and frequent urination is a symptom of cystitis, which results from a bladder infection, the symptoms of kidney infections and bladder infections are different. Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances [8]. Bacteria that colonize the urethra or periurethral region typically migrate into the bladder and trigger an inflammatory response, which is the typical mechanism of infection. The gastrointestinal system is home to a group of bacteria known as Enterobacteriales, which includes *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli*. Although it is extremely uncommon, bloodstream bacteria can also migrate to the bladder or kidneys, which can lead to UTIs. Female sex, recent sexual activity, diabetes mellitus, and structural or functional urological abnormalities are risk factors for urinary tract infections [9]. Multiplicity drug-resistant (MDR) strains are highly prevalent, according to numerous investigations. MDR infections are becoming a greater danger to public health since they are sometimes much more difficult and costly to treat. It is unknown, therefore, if each bacterial taxon has its own mechanism or mechanisms for multidrug resistance, or if diseases that are distantly related have similar processes. Traditionally, different underlying mechanisms have been utilized to explain these data for different pathogens [10]. Evaluating *M. oleifera* leaves' antibacterial qualities against certain microbes and confirming their phytochemical composition are crucial. Consequently, the goals and objectives of this study are to identify the phytochemical components of *Moringaoleifera*'s leaf extracts and their antimicrobial effects on

particular microbes [6]. *M. oleifera* is a fast-growing, deciduous tree that can reach a height of 10–12 m (33–39 ft) and trunk diameter of 46 cm (18 in). The bark has a whitish-gray color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches, and the leaves build up a feathery foliage of tripinnate leaves [11]. It grows best in tropical or subtropical region with altitude of 2000m *Moringaoleifera* is a small fast-growing evergreen or deciduous tree usually grows up to 10 or 12 m in height. It has spreading, fragile branches, feathery foliage of tripinnate leaves, and whitish gray bark. The leaves are bipinnate or commonly tripinnate up to 45 cm long the leaflets are hairy, green and almost hairless on the upper surface. The twigs are hairy and green, these are compound leaves with leaflets of 1–2 cm long [12]. Fruits are tri-lobed capsules and are referred to pods it is pendulous, brown triangular, and splits into three parts lengthwise when dry 30 – 120 cm long, 1.8 cm wide fruits production mostly occurs in March and April. Fruit contains around 26 seeds during their development stage. Immature pods are green in color they turn brown on maturity [13]. *Moringaoleifera* is a plant with several traditional uses, leaves are typically the most utilized component of the plant. They are specifically utilized in traditional medicine and in the diet of humans and animals. Protein, minerals, beta-carotene, and antioxidant compounds all of which are frequently deficient in populations in developing or undeveloped nations are abundant in leaves. As dietary integrators, moringa leaves are added to food preparations. Moringa fights infections with its antibacterial and antifungal qualities. It works well against blood and urinary tract infections, stomach issues, and some fungal and bacterial strains that cause skin infections. *Moringaoleifera* roots are said to be abundant in antimicrobial compounds and to possess antibacterial properties. It has been discovered that Moringa bark extract has antifungal properties, and that the juice from the bark and stem has antibacterial properties against *Staphylococcus aureus* [13]. Antibiotic-resistant bacteria, particularly those causing urinary tract infections, pose a growing public health threat. As these bacteria withstand multiple antibiotics, treatments become less effective, complicating patient recovery and straining healthcare systems worldwide, requiring more resources and specialized care. The declining effectiveness of conventional antibiotics in treating UTIs caused by MDR bacteria is posing significant challenges in medical care, leading to longer hospital stays, complex treatment plans, and higher healthcare costs. This underscores the urgent need for new and effective treatment strategies to manage these infections. Antibiotic resistance is causing renewed interest in natural remedies, particularly plant extracts. *Moringaoleifera*, a plant rich in phytochemicals, has potential antimicrobial effects, offering hope for developing new treatments against resistant bacterial strains. It's a plant with bioactive compounds, has been discovered as a potential antimicrobial agent. Initial studies show it can inhibit certain bacterial strains, but more comprehensive studies are needed to fully understand its clinical application [14]. Urinary tract infections are a prevalent issue affecting all ages and demographics, necessitating the development of new treatments. As traditional antibiotics lose effectiveness, new alternatives are crucial to ensure effective infection management and prevent severe health consequences. The study investigated the antimicrobial activity of *Moringaoleifera* leaf extracts against multiple drug-resistant bacterial isolates obtained from urine from Morang district.

2. MATERIALS & METHOD

It is Prospective with Experimental type of research design. It is both qualitative and quantitative type of research.

Table 01: Parts used of *Moringa oleifera* [5]

Plant part	Medicinal Uses
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a

	laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation
Leave	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling
Stem bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity
Gum	Used for dental caries, and is astringent and rubefacient; Gum, mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism
Flower	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolemia rabbits and increased the excretion of fecal cholesterol.
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosides have been isolated from the acetate phase of the ethanolic extract of Moringa pods.

Table 02: List of material

S. No	Name of Equipment's	Source/company
1.	Grinder	FZ102
2.	Rotary vacuum evaporator	EYELAN-1300
3.	Soxhlet apparatus	ReliGls India, JSGW
4.	Digital Balance	HANDK
5.	Hot air oven	101-OAB Faithful
6.	Muffle furnace	Huanghua Faithful
7.	Biological safety cabinet, Class II, Type A ₂	Digi lab
8.	Autoclave	Faithful
9.	Refrigerator	Digi lab
10.	Heating mantle	Digi lab
11.	Water bath	Digi lab
12.	Scale	Natraj
13.	Separating funnel, Test tube, Measuring cylinder, Petri dish, other Glass wears	Borosil

Table 03: List of chemicals

S. No	Name of Chemicals	Source/company
-------	-------------------	----------------

1.	Methanol	SD Fine chem., Mumbai
2.	Distilled Water	Varun Aqua Industries
3.	Water for injection	A R Corporation
4.	Sodium hypochlorite solution	Marach Pvt. Ltd.
5.	Peptone Water	Oxford lab fine chem. LLP
6.	Mueller Hinton Agar	HI Media laboratories Pvt. Ltd.
7.	Nutrient Agar	HI Media laboratories Pvt. Ltd.

The fresh leaf is collected from local geographical area of Ratuwamai, Morang, Koshi Province, Nepal as per traditional method. The plant was identified and authenticated by “National Herbarium and Plant Laboratories” Godavari, Lalitpur, Nepal. The leaf of *Moringaoleifera* was washed with tap water, dried at room temperature for 15 days, and then ground into powder using a mechanical grinder. The powder was stored in a labeled airtight container. The powdered plant material was subjected to a Soxhlet apparatus and extracted using methanol for the analysis of preliminary phytochemicals and antimicrobial activity. The extract was filtered using sterile Whatman No. 1 filter paper, and the obtained filtrate was later evaporated using a rotary vacuum evaporator to remove excess solvents. The final extract was stored in a cool, dry place [15]. About 2 grams of the drug were weighed in a petri plate, placed in a hot air oven at 105°C, and dried until a constant weight was obtained. The weight loss during drying was recorded, and the difference in weight indicated the loss on drying of the powdered drug.(59)(60) The total percentage of loss on drying was calculated using the formula:

$$\% \text{ of loss on drying} = (\text{Weight loss} / \text{Weight of sample}) * 10$$

About 2 g of the ground, air-dried material was accurately weighed in a previously ignited and tarred crucible. The material was spread in an even layer and ignited by gradually increasing the heat to 600°C at 2 hours until it turned white, indicating the absence of carbon. It was then cooled in a desiccator and weighed [16]. Finally, the content of total ash in milligrams per gram of air-dried material was calculated using the formula:

$$\% \text{ Total Ash} = (\text{Ash weight} / \text{Weight of sample}) * 100$$

The Methanolic extract were tested for growth or contamination. This was carried out by inoculating 1ml each of them on Nutrient agar and incubated at 37°C for 48 hours. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile. The extracts were then accessed for antimicrobial activity. Chemical tests were carried out on the extract using standard procedures to identify the phytoconstituents.

Table 04: Qualitative test for Phytochemical screening

Test		Procedure	Observation (indicating positive tests)
Detection of Alkaloid			
1	Dragendroff's test	2ml of extract + 1ml of dragendroff's reagent	A reddish-brown precipitate
Detection of Glycosides			
1	Borntrager's test	2ml filtrated hydrolysate + 3ml chloroform + shaken well + chloroform layer is separated + 10% ammonia solution	A pink color solution

Detection of Protein			
1	Millon's test	2ml extract + few drops of Millon's reagent	A white precipitate
Detection of saponin			
1	Foam test	5ml of test solution + shaken well for 5minutes	Formation of stable foam
2	Olive oil test	2ml of extract + few drops of olive oil	Formation of soluble emulsion
Detection of flavonoid			
1	Lead acetate test	1ml of test solution + 10% of lead acetate	Formation of yellow precipitate

Samples are collected from “Microbiology departments of Koshi Hospital, Biratnagar, Morang” and Microbiology department of Madan Bhandari Hospital & Trauma Center Mangal bare, Morang”. The study specifically targeted bacterial colonies that were isolated from urine sample and identified as multiple drug-resistant. These samples were obtained from two tertiary care hospitals: Koshi Hospital in Biratnagar and Madan Bhandari Hospital & Trauma Center in Urlabari, Morang. The study included participants of both male and female genders across all age groups. Samples that were sensitive to routine antibiotics during the antibiotic susceptibility tests were excluded. Sterile samples, which showed no bacterial growth, were also excluded. Furthermore, isolates from sources other than urine were not considered for inclusion in the study. The experimental protocol for the antimicrobial investigation was designed to assess the growth inhibition of multiple drug-resistant (MDR) bacterial isolates using a dilution method. Plant extract solutions were prepared at various concentrations: 300 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, each dissolved in water for injection to maintain sterility. A control solution consisting of only water for injection was also prepared to serve as a baseline comparison. The next step involved preparing a slightly modified Mueller-Hinton agar (MHA), which is widely used for antimicrobial susceptibility testing. The MHA was poured into petri dishes and allowed to solidify. Each concentration of the plant extract was then incorporated into separate MHA plates to create a series of agar plates containing different concentrations of the extract. MDR bacterial isolates were inoculated onto the surface of the agar plates. The plates were incubated at 37°C for 24-48 hours to allow sufficient time for bacterial growth or inhibition to occur. The focus of this method was to observe the presence or absence of bacterial growth on the agar plates containing different concentrations of the extract. After the incubation period, the plates were examined for growth or no growth of the bacterial isolates. The presence of bacterial colonies indicated that the extract concentration was not effective in inhibiting the growth of the MDR isolates, while the absence of growth suggested that the extract was effective at that particular concentration. This dilution method allowed for a straightforward assessment of the antimicrobial potential of the plant extracts by directly observing the impact of different concentrations on bacterial growth. Our investigation was conducted in a Pharmacognosy and Microbiology laboratory at Purbanchal University School of Health Sciences because it offered a controlled setting where different parameters could be accurately controlled and modified.

3. RESULTS AND DISCUSSION

Table 05: loss on drying of powdered leaves of *Moringa oleifera*

Initial weight (gm)	Final constant weight (gm)	Loss on drying (gm)	%LOD
2.005	1.895	0.110	5.49%

Table 06: Ash Value of powered leaves of Moringaoleifera

Fresh weight (gm)	Ash weight(gm)	Total Ash (%w/w)
2.002	0.220	10.98%

Table 07: Sterility test of the Moringa oleifera leaves extracts

Procedure	Observation
Nutrient agar + 1ml of extract	No growth at 37 ⁰ c after 48 hours

Table 08: Phytochemical screening of Moringa oleifera leaves in Methanolic extract

S. N	Secondary metabolites	Phytochemical test	Methanolic extract
1	Alkaloid	Dragendroff's test	+
2	Glycoside	Borntrager's test	+
3	Protein	Millon's test	-
4	Flavonoid	Lead acetate test	+
5	Saponin	Foam test	+
		Olive oil test	+

(+) represent positive and (-) represent negative.

Table 09: Sources of data gathering vs patients arrived in institutes.**Table 10:** Gender distribution of patients.

Gender Distribution		
Gender	Frequency	Percent (%)
Male	135	35.9
Female	241	64.1
Total	376	100.0

Table 11: Age distribution of patients.

Age Distribution		
Age Groups	Frequency	Percent (%)
Less than 10 years	41	10.9
10-20 years	57	15.2
21-45 years	157	41.8
46-60 years	259	68.9
More than 60 Years	376	100.0
Total	376	100.0
Sources of data Gathering		
Name of Institution	Frequency	Percent (%)
Madan Bhandari Hospital &trauma Center	117	31.1
Koshi Hospital, Biratnagar	259	68.9
Total	376	100.0

A total of 376 patients arrived at the microbiology departments of Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar, for urine culture sensitivity tests. The majority of these patients (68.9%) were treated at Koshi Hospital, indicating a higher patient load there. Female patients made up 64.1% of those tested, reflecting a higher prevalence or reporting rate of urinary tract issues among women. The largest age group was 16-30 years, accounting for 25.8% of the total, followed by those aged 31-45 years (22.6%). This relatively even age distribution suggests that urinary tract issues are common across different age groups, though slightly more prevalent in younger adults. This data is valuable for guiding resource allocation and patient care strategies in the hospitals' microbiology departments.

Table 12: Sources of data Gathering vs Urine culture sensitivity of the patients.

Sources of data Gathering vs Urine culture sensitivity			
Name of Institutes	Urine culture sensitivity		Total
	Sterile	Growth of Bacteria	
Madan Bhandari Hospital & trauma Center	96 (82.1%)	21 (17.9%)	117 (100.0%)
Koshi Hospital, Biratnagar	218 (84.2%)	41 (15.8%)	259 (100.0%)
Total	314 (83.5%)	62 (16.5%)	376 (100.0%)

Table 13: Age vs Urine culture sensitivity of the patients.

Age vs Urine culture sensitivity			
Age Groups	Urine culture sensitivity		Total
	Sterile	Growth of bacteria	
Less than 15 years	36 (87.8%)	5 (12.2%)	41 (100.0%)
16-30 Years	82 (84.5%)	15 (15.5%)	97 (100.0%)
31-45 Years	77 (90.6%)	8 (9.4%)	85 (100.0%)
46-60 Years	61 (79.2%)	16 (20.8%)	77 100.0%
More than 60 Years	58 (76.3%)	18 (23.7%)	76 (100.0%)
Total	314 (83.5%)	62 (16.5%)	376 (100.0%)

376 patients underwent urine culture sensitivity tests at Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar, with the majority (83.5%) showing sterile results and 16.5% indicating bacterial growth. Koshi Hospital handled a larger patient load and had a slightly higher rate of sterile results (84.2%) compared to Madan Bhandari Hospital (82.1%). The incidence of bacterial growth was similar between males (16.3%) and females (16.6%), with younger patients, particularly those under 15 and those aged 31-45 years, having a higher rate of sterile results. In contrast, older patients, especially those over 60, had the highest proportion of bacterial growth (23.7%), suggesting a greater susceptibility to urinary tract infections in this age group.

Table 14: Identification of bacterial isolates from total arrived patients

Identification of bacterial isolates			
Name of Bacteria	Frequency	Percent from total	Percent from only isolates
Escherichia coli	31	8.2%	50.0%
Klebsiella pneumonia	17	4.5%	27.4%

Enterobacter species	4	1.1%	6.5%
Enterococcus species	3	.8%	4.8%
Others isolate Bacteria	7	1.9%	11.3%
Total	62	16.5%	100.0%

62 positive bacterial isolates were identified from urine culture sensitivity tests conducted in the microbiology departments of Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar. *Escherichia coli* was the most frequently identified bacterium, responsible for 50% of the positive isolates, underscoring its role as a leading cause of urinary tract infections (UTIs). *Klebsiella pneumoniae* was the second most common isolate, accounting for 27.4% of the cases, followed by *Enterobacter* species (6.5%) and *Enterococcus* species (4.8%), both of which were less frequently detected but still clinically significant. Other bacterial isolates made up 11.3% of the cases, representing a variety of less common species involved in UTIs. This distribution highlights the prevalent pathogens associated with UTIs during this period.

Table 15: Sources of data Gathering vs. Isolates Bacteria with MDR (Sample of study)

Sources of data Gathering vs. Isolates Bacteria with MDR			
Name of Institutes	Selection of MDR sample		Total
	Isolates with MDR (sample)	Isolates without MDR (No include sample)	
Madan Bhandari Hospital & trauma Center	11 (52.4%)	10 (47.6%)	21 (100.0%)
Koshi Hospital, Biratnagar	21 (51.2%)	20 (48.8%)	41 (100.0%)
Total	32 (51.6%)	30 (48.4%)	62 (100.0%)

Table 16: Gender vs Isolates Bacteria with MDR of arrived patients.

Gender vs Isolates Bacteria with MDR			
Gender	Selection of MDR sample		Total
	Isolates with MDR (sample)	Isolates without MDR (No include sample)	
Male	13 (59.1%)	9 (40.9%)	22 (100.0%)
Female	19 (47.5%)	21 (52.5%)	40 (100.0%)
Total	32 (51.6%)	30 (48.4%)	62 (100.0%)

Table 17: Age vs Isolates Bacteria with MDR of arrived patients.

Age vs Isolates Bacteria with MDR			
Age Groups	Selection of MDR sample		Total
	Isolates with MDR (sample)	Isolates without MDR (No include sample)	
Less than 15 years	2 (40.0%)	3 (60.0%)	5 (100.0%)
16-30 Years	6 (40.0%)	9 (60.0%)	15 (100.0%)
31-45 Years	4 (50.0%)	4 (50.0%)	8 (100.0%)
46-60 Years	12 (75.0%)	4 (25.0%)	16 (100.0%)
More than 60 Years	8 (44.4%)	10 (55.6%)	18 (100.0%)

62 bacterial isolates were identified from urine culture sensitivity tests conducted in the microbiology departments of Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar. Of these, 51.6% were multidrug-resistant (MDR). The occurrence of MDR isolates was consistent across the two hospitals. The highest incidence of MDR bacteria was found in patients aged 46-60 years (75%), indicating a higher vulnerability to drug-resistant infections in this age group. Gender-wise, a higher proportion of MDR isolates was found in male patients (59.1%) compared to females (47.5%), suggesting potential differences in the prevalence of MDR infections between genders. This data highlights the significant presence of MDR bacteria among urinary tract infections and underscores the need for targeted interventions to manage these resistant strains.

Table 18: Observation of antimicrobial activity with different concentration of *M. oleifera*.

Concentration of extract of <i>M. oleifera</i>		Name of Bacterial isolates with MDR					Total
		<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Enterobacter species</i>	<i>Enterococcus species</i>	Others isolate	
300mg/ml	Growth	0	0	0	0	0	0
	No growth	14	11	3	1	3	32
200mg/ml	Growth	2	1	0	1	0	4
	No growth	12	10	3	0	3	28
150mg/ml	Growth	3	8	3	1	1	16
	No growth	11	3	0	0	2	16
100mg/ml	Growth	8	11	3	1	3	26
	No growth	6	0	0	0	0	6
50mg/ml	Growth	13	11	3	1	3	31
	No growth	1	0	0	0	0	1
25mg/ml	Growth	14	11	3	1	3	32
	No growth	0	0	0	0	0	0
12.5mg/ml	Growth	14	11	3	1	3	32
	No growth	0	0	0	0	0	0
Water for injection	Growth	14	11	3	1	3	32
	No growth	0	0	0	0	0	0

Moringaoleifera extract showed effective antimicrobial activity against MDR bacterial isolates at higher concentrations (≥ 150 mg/ml), with complete inhibition at 300 mg/ml. The MIC for the extract is likely above 100 mg/ml for most bacteria, indicating that higher concentrations are needed to achieve effective inhibition of MDR strains. Lower concentrations (≤ 50 mg/ml) were insufficient to prevent bacterial growth. This suggests that for therapeutic purposes, a higher concentration of *Moringaoleifera* extract may be required to manage infections caused by MDR bacteria effectively.

Serving as a control, this solution had no antimicrobial effect, confirming that observed results are due to the *Moringaoleifera* extract.

DISCUSSION

The study on the powdered leaves of *Moringaoleifera* reveals significant findings that highlight its potential applications in various fields. The loss on drying (LOD) was found to be 5.49%, indicating a relatively low moisture content, which is beneficial for the stability and shelf life of the powdered leaves. The ash value, calculated at 10.98%, provides insight into the total mineral content, suggesting that *Moringaoleifera* leaves are rich in essential minerals. The sterility test results demonstrate that the methanolic extract of *Moringaoleifera* leaves is sterile, showing no microbial growth after 48 hours of incubation at 37°C, which ensures the safety of the extract for potential therapeutic applications. Phytochemical screening of the methanolic extract revealed the presence of several important secondary metabolites, including alkaloids, glycosides, flavonoids, and saponins, all of which contribute to the plant's medicinal properties. The absence of proteins in the methanolic extract is noteworthy, as it suggests that the active compounds are likely concentrated in the other classes of metabolites. These findings support the traditional use of *Moringaoleifera* in herbal medicine and suggest further exploration of its bioactive compounds for pharmaceutical development. The results are consistent with existing literature, which also emphasizes the plant's diverse phytochemical profile and therapeutic potential. This study, conducted over a ten-day period, provides valuable insights into the incidence of bacterial growth in urine culture sensitivity tests among a sample of 376 patients from Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar. The results indicate that a significant majority of the patients (83.5%) had sterile urine cultures, while 16.5% showed bacterial growth [17]. These findings are in line with previous research conducted in similar populations, where sterile cultures typically dominate, reflecting the overall lower prevalence of urinary tract infections (UTIs) in the general population [18]. In terms of patient demographics, female patients accounted for 64.1% of those tested, reflecting a higher prevalence or reporting rate of urinary tract issues among women [19]. This aligns with other studies that have shown a higher incidence of UTIs in females due to anatomical and physiological differences [20]. The age distribution among patients showed that those aged 16-30 years were the largest group (25.8%), followed by those aged 31-45 years (22.6%). This relatively even distribution of age groups indicates that urinary tract issues are prevalent across various age brackets, though they appear slightly more common in younger adults, a finding consistent with previous research [21]. The findings from this study, provide critical insights into the microbial landscape of urinary tract infections (UTIs) in two prominent Nepali healthcare institutions: Madan Bhandari Hospital & Trauma Center and Koshi Hospital, Biratnagar. The identification of 62 positive bacterial isolates, with *Escherichia coli* (*E. coli*) being the predominant pathogen, aligns with existing literature, both globally and within Nepal, confirming *E. coli* as the most common causative agent of UTIs. The high prevalence of *E. coli* (50%) in this study is consistent with other research conducted in Nepal, [22] which also identified *E. coli* as the leading uro pathogen in similar clinical settings. This consistency underscores the persistent public health challenge posed by *E. coli* in the context of UTIs and highlights the need for targeted interventions, including the development of effective antibiotic stewardship programs to combat increasing resistance patterns observed in this bacterium. *Klebsiella pneumoniae*, identified in 27.4% of cases, was the second most common isolate. This bacterium's significant presence is notable and aligns with findings from [23] which reported *Klebsiella* species as a major contributor to UTIs in hospitalized patients across Nepal. The rise of *Klebsiella* infections, particularly in hospital environments, is concerning due to the bacterium's ability to acquire resistance to multiple antibiotics, including carbapenems, which are often considered drugs of last resort. The detection of *Enterobacter* species (6.5%) and *Enterococcus* species (4.8%), although less frequent, is still clinically significant. These pathogens have been increasingly recognized for their role in

complicated UTIs and their association with antimicrobial resistance. Studies such as those by [24] in Nepali hospitals have documented the emergence of these bacteria, particularly in patients with underlying health conditions, emphasizing the need for vigilant monitoring and tailored therapeutic approaches in managing these infections [25]. The remaining 11.3% of isolates consisted of various other bacterial species, reflecting the diverse etiological agents that can cause UTIs. This diversity highlights the complexity of diagnosing and treating UTIs, especially in a setting where empirical treatment is often guided by local antimicrobial resistance patterns. The consistency in the occurrence of MDR isolates across both hospitals suggests a widespread issue, not confined to a single institution, which is indicative of a broader, systemic challenge in managing UTIs. This is consistent with findings from a study by [26], which reported similar MDR patterns in UTIs across various hospitals in Nepal, emphasizing the pervasive nature of antibiotic resistance in the country. The age distribution of MDR cases, with the highest incidence (75%) found in patients aged 46-60 years, highlights a particular vulnerability in this age group. This could be attributed to several factors, including comorbid conditions, more frequent use of antibiotics, and a potentially weakened immune system in older adults. A study by [27] also reported higher rates of MDR infections in middle-aged and older adults in Nepal, suggesting that age is a significant risk factor for the development of drug-resistant UTIs. Gender-wise, the higher proportion of MDR isolates in male patients (59.1%) compared to females (47.5%) points to potential differences in susceptibility or healthcare-seeking behavior between genders. While UTIs are generally more common in females, the data suggests that when men do acquire UTIs, these infections are more likely to be drug-resistant. This finding is in line with research by [28], which noted that males, particularly those with underlying health issues, are at a higher risk of developing complicated, and often drug-resistant, UTIs. The findings of this study indicate that *Moringaoleifera* extract demonstrates significant antimicrobial activity against multidrug-resistant (MDR) bacterial isolates, particularly at higher concentrations (≥ 150 mg/ml). Complete bacterial inhibition was observed at a concentration of 300 mg/ml, highlighting the potential of *Moringaoleifera* as an effective natural antimicrobial agent. The minimum inhibitory concentration (MIC) for the extract appears to be above 100 mg/ml for most bacteria, which underscores the necessity of using higher concentrations to achieve effective inhibition of MDR strains. The study also found that lower concentrations of *Moringaoleifera* extract (≤ 50 mg/ml) were insufficient to prevent bacterial growth. This suggests that for therapeutic applications, particularly in treating infections caused by MDR bacteria, a higher concentration of the extract may be required to achieve the desired antimicrobial effects. The control experiments, which showed no antimicrobial activity, confirm that the observed inhibitory effects are due to the *Moringaoleifera* extract itself, rather than any other factors. These results align with existing research on the antimicrobial properties of *Moringaoleifera*. For instance, a study by [29] found that *Moringaoleifera* extracts exhibited substantial antimicrobial activity against various pathogenic bacteria, including MDR strains. Similarly, a study by [30] reported that the bioactive compounds in *Moringaoleifera* are effective against a broad spectrum of bacteria, reinforcing the potential use of this plant in combating antibiotic-resistant infections [5]. In conclusion, *Moringaoleifera* holds promise as a natural alternative for managing MDR bacterial infections. However, further research is needed to determine the optimal dosing strategies and to evaluate the extract's efficacy in clinical settings.

4. CONCLUSIONS

Plants are crucial for human and animal health, with traditional medicines used by 80% of affluent nations. *Moringaoleifera*, a rapidly growing plant, has numerous medicinal properties, including antipyretic, antiepileptic, anti-inflammatory, and antioxidant effects. It is used in Ayurvedic traditional medicine for therapeutic and preventive purposes. The study investigated the antimicrobial activity of *Moringaoleifera* leaf extracts against multiple drug-resistant bacterial isolates obtained from urine from Morang district. *Moringaoleifera*, a plant from Nepal, was used in a study to inhibit multiple drug-

resistant (MDR) bacterial isolates. The extract was extracted, filtered, and evaporated, and Mueller-Hinton agar plates were inoculated. The results confirmed the plant's antimicrobial potential. Moringaoleifera extract demonstrated effective antimicrobial activity against MDR bacterial isolates at higher concentrations, with complete inhibition at 300 mg/ml. The MIC is likely above 100 mg/ml, suggesting higher concentrations are needed for effective MDR infection management. Moringaoleifera powdered leaves show potential applications in various fields due to their low moisture content and sterility. The plant's medicinal properties and therapeutic potential are supported by its diverse phytochemical profile. In Nepal, the extract demonstrated significant antimicrobial activity against multidrug-resistant bacterial isolates, but further research is needed to determine optimal dosing strategies and clinical efficacy. A study on Moringaoleifera powdered leaves found its potential applications in various fields. The powdered leaves had a low moisture content, indicating stability and shelf life. The methanolic extract of Moringaoleifera leaves was sterile, showing no microbial growth after 48 hours. The plant's medicinal properties were supported by its diverse phytochemical profile and therapeutic potential. A study conducted in Nepal found that a significant majority of patients (83.5%) had sterile urine cultures, with female patients reporting higher urinary tract issues. Multidrug-resistant (MDR) isolates were found in patients aged 46-60 years, with the highest incidence in male patients. Moringaoleifera extract demonstrated significant antimicrobial activity against MDR bacterial isolates, particularly at higher concentrations. However, lower concentrations were insufficient to prevent bacterial growth, suggesting that a higher concentration may be required for therapeutic applications. Further research is needed to determine optimal dosing strategies and evaluate the extract's efficacy in clinical settings.

REFERENCES

- [1] Moyo. Antimicrobial Activities Of Moringa Oleifera Lam Leaf Extracts. *African J Biotechnol.* 2012;11(11):2797–802.
- [2] Singh M, Singh S, Verma D. Morphological And Pharmacognostical Evaluation Of Moringa Oleifera Lam. (Moringaceae): A Plant With High Medicinal Value In Tropical And Subtropical Parts Of The World. *Pharmacogn Rev.* 2020;14(28):138–45.
- [3] Chitrasen Yadav, Prof. Pradeep Vaidya, Dr. Laligen Awale, Dr. Geha Raj Dahal, Sanjeeb Jha & Krishna Yadav (2024). Evaluation of Surgical Site Infections and Patterns of Microorganisms involved in Emergency Laparotomy in TUTH . *Dinkum Journal of Medical Innovations*, 3(08):557-569.
- [4] Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional Characterization Of Moringa (Moringa Oleifera Lam.) Leaves. *African J Biotechnol.* 2011;10(60):12925–33.
- [5] Jang MH, Piao XL, Kim JM, Kwon SW, Park JH. Inhibition Of Cholinesterase And Amyloid- β Aggregation By Resveratrol Oligomers From Vitis Amurensis. *Phyther Res* [Internet]. 2008;22(4):544–549. Available From: [Http://Www3.Interscience.Wiley.Com/Journal/117934759/Abstract](http://Www3.Interscience.Wiley.Com/Journal/117934759/Abstract)
- [6] Ayesha Nasir (2023). Common Bacteria Involved in Fascial Space Infection and Antibiotics Sensitivity. *Dinkum Journal of Medical Innovations*, 2(03):97-110.
- [7] Paikra BK, Dhongade HKJ, Gidwani B. Phytochemistry And Pharmacology Of Moringa Oleifera Lam. *J Pharmacopuncture.* 2017;20(3):194–200.
- [8] Thapa K, Poudel M, Adhikari P. Moringa Oleifera: A Review Article On Nutritional Properties And Its Prospect In The Context Of Nepal. *Acta Sci Agric.* 2019;3(11):47–54.
- [9] Bartlett JG. Laboratory Diagnosis Of Urinary Tract Infections In Adult Patients. *Infect Dis Clin Pract.* 2004;12(6):360–1.

- [10]Shaltout AA, Hassan FAS. Seasonal Variability Of Elemental Composition And Phytochemical Analysis Of Moringa Oleifera Leaves Using Energy-Dispersive X-Ray Fluorescence And Other Related Methods. *Biol Trace Elem Res*. 2021;199(11):4319–29.
- [11]Fidelis KR, Alves RR De V, Patriota LL De S, Coelho LCBB, Ferreira MRA, Soares LAL, Et Al. Saline Extract From Moringa Oleifera Leaves Has Antidepressant And Anxiolytic Effects In Mouse Models. *Nutraceuticals*. 2024;4(1):65–81.
- [12]Bhavana Dnyandeo Tambe, Pranjali Dnyanshwar Gadhave. Phytochemical Screening And In Vitro Antibacterial Activity Of Moringa Oleifera (Lam.) Leaf Extract. *Int J Sci Res Arch*. 2024;12(1):125–30.
- [13]Dr. Md. Hasan Moshir Shawon & Prof. Dr. Shanjoy Kumar Paul (2024). Risk Factors of Urinary Tract Infection Caused by Extended-Spectrum Beta-Lactamases-Producing Bacteria in Children . *Dinkum Journal of Medical Innovations*, 3(02):102-117.
- [14]Rockwood JL, Anderson BG, Casamatta D A. Potential Uses Of Moringa Oleifera And An Examination Of Antibiotic Efficacy Conferred By M. Oleifera Seed And Leaf Extracts Using Crude Extraction Techniques Available To Underserved Indigenous Populations. *Int J Phytotherapy Res*. 2013;3(2):61–71.
- [15]Mehta LK, Balaraman R, Amin AH, Bafna PA, Gulati OD. Effect Of Fruits Of Moringa Oleifera On The Lipid Profile Of Normal And Hypercholesterolaemic Rabbits. *J Ethnopharmacol*. 2003;86(2–3):191–5.
- [16]Rocchetti G, Blasi F, Montesano D, Ghisoni S, Marcotullio MC, Sabatini S, Et Al. Impact Of Conventional/Non-Conventional Extraction Methods On The Untargeted Phenolic Profile Of Moringa Oleifera Leaves. *Food Res Int* [Internet]. 2019;115:319–27. Available From: <https://doi.org/10.1016/j.foodres.2018.11.046>
- [17]Gidamis AB, Panga JT, Sarwatt S V., Chove BE, Shayo NB. Nutrient And Antinutrient Contents In Raw And Cooked Young Leaves And Immature Pods Of Moringa Oleifera, Lam. *Ecol Food Nutr*. 2003;42(6):399–411.
- [18]Verma AR, Vijayakumar M, Mathela CS, Rao C V. In Vitro And In Vivo Antioxidant Properties Of Different Fractions Of Moringa Oleifera Leaves. *Food Chem Toxicol* [Internet]. 2009;47(9):2196–201. Available From: <http://dx.doi.org/10.1016/j.fct.2009.06.005>
- [19]Pakade V, Cukrowska E, Lindahl S, Turner C, Chimuka L. Molecular Imprinted Polymer For Solid-Phase Extraction Of Flavonol Aglycones From Moringa Oleifera Extracts. *J Sep Sci*. 2013;36(3):548–55.
- [20]González-Burgos E, Ureña-Vacas I, Sánchez M, Gómez-Serranillos MP. Nutritional Value Of Moringa Oleifera Lam. Leaf Powder Extracts And Their Neuroprotective Effects Via Antioxidative And Mitochondrial Regulation. *Nutrients*. 2021;13(7).
- [21]Sultana S. Nutritional And Functional Properties Of Moringa Oleifera. *Metab Open* [Internet]. 2020;8:100061. Available From: <https://doi.org/10.1016/j.metop.2020.100061>
- [22]Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Et Al. International Clinical Practice Guidelines For The Treatment Of Acute Uncomplicated Cystitis And Pyelonephritis In Women: A 2010 Update By The Infectious Diseases Society Of America And The European Society For Microbiology And Infectious Diseases. *Clin Infect Dis An Off Publ Infect Dis Soc Am*. 2011 Mar;52(5):E103-20.
- [23]Foxman B. Epidemiology Of Urinary Tract Infections: Incidence, Morbidity, And Economic Costs. *Am J Med*. 2002 Jul;113 Suppl:5S-13S.
- [24]Panchanadeswaran S, Johnson SC, Mayer KH, Srikrishnan AK, Sivarani S, Zelaya CE, Et Al. Gender Differences In The Prevalence Of Sexually Transmitted Infections And Genital Symptoms In An Urban Setting In Southern India. *Sex Transm Infect*. 2006 Dec;82(6):491–5.

- [25] Nicolle LE. Urinary Tract Infections In The Older Adult. Clin Geriatr Med. 2016 Aug;32(3):523–38.
- [26] Kaur R, Kaur R. Symptoms, Risk Factors, Diagnosis And Treatment Of Urinary Tract Infections. Postgrad Med J. 2021 Dec;97(1154):803–12.
- [27] Rijal KR, Banjara MR, Dhungel B, Kafle S, Gautam K, Ghimire B, Et Al. Use Of Antimicrobials And Antimicrobial Resistance In Nepal: A Nationwide Survey. Sci Rep [Internet]. 2021;11(1):1–14. Available From: <https://doi.org/10.1038/S41598-021-90812-4>
- [28] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary Tract Infections: Epidemiology, Mechanisms Of Infection And Treatment Options. Nat Rev Microbiol. 2015 May;13(5):269–84.
- [29] Goyal BR, Agrawal BB, Goyal RK, Mehta AA. Phyto-Pharmacology Of Moringa Oleifera Lam. - An Overview. Nat Prod Radiance. 2007;6(4):347–53.
- [30] Pokhrel, R. Et Al. (2022). "Multidrug Resistance Patterns In Urinary Tract Infections: A Study From Hospitals In Eastern Nepal." Journal Of Nepal Medical Association, 60(240), 123-128.