RESEARCH ARTICLE

©2023 The Academy of Environmental Biology, India

Journal of Ecophysiology and Occupational Health, *Vol 24(4), DOI: 10.18311/jeoh/2024/43945, 393-402, December 2024*

ISSN (Online) : 0972-4397
ISSN (Online) : 0974-0805

Preliminary Screening of Phytochemicals and Antibacterial Activity of Medicinal Plant Ber (*Ziziphus mauritiana* Lamark)

Richa Mishra[1], Neelam Pathak[1*], Vivek Kumar Mishra[1], Pankaj Singh[2], Pradeep Kumar Singh[1] and Manikant Tripathi[2]

[1]Department of Biochemistry, Dr. Rammanohar Lohia Avadh University, Ayodhya - 224001, Uttar Pradesh, India; pathak.neelam007@gmail.com
[2]Biotechnology Program, Dr. Rammanohar Lohia Avadh University, Ayodhya - 224001, Uttar Pradesh, India

Abstract

The most important genus in the Rhamnaceae family is Ber (*Ziziphus mauritiana* Lamk). Phytochemicals present in *Ziziphus* have antioxidant as well as antimicrobial properties. The present study aimed to detect various phytochemicals and evaluate the antibacterial activity in the *Z. mauritiana* leaf extracts. Leaves from 14 cultivars/germplasm/varieties of *Z. mauritiana* were used to explore the presence of various phytoconstituents such as alkaloids, flavonoids, cardiac glycosides, phenols, saponins, protein, steroids, tannins, lignins, and reducing sugar. These phytoconstituents have antioxidant, antibacterial, and hepatoprotective activities. The preliminary antimicrobial activity screening of *Ziziphus* leaf extract against bacterial strains-*Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Salmonella abony* (Gram-negative), *Escherichia coli* $DH_5\alpha$ (Gram-negative) were performed by using well diffusion method. The results of various phytochemicals like alkaloids, phenolics, flavonoids, tannins, carbohydrates, proteins, lipids, and amino acids were reported in all selected commercial varieties/cultivars/ germplasm. On the other hand, saponins were found completely absent in methanolic solvents of all varieties/cultivars. The aqueous and methanolic solvent leaf extract were tested for antibacterial activity at different concentrations (25mg/ml, 50mg/ml, and 100mg/ml) against the bacterial strains of *B. subtilis, S. aureus, S. abony,* and *E. coli* $DH_5\alpha$, respectively. This study showed that the phytochemicals extracted from *Z. mauritiana* leaves have potential antimicrobial activities.

Keywords: Antibacterial Activity, Phytochemical, Preliminary Screening, Qualitative Tests, *Ziziphus mauritiana*

1. Introduction

The ancient people of India used *Ziziphus mauritiana* for its medicinal purposes. The Yajurveda mentioned it[1]. Approximately, 25% of prescription medications in today's medicine come from medicinal plants undergoing extensive testing to determine their usefulness. Nonetheless, conventional medicine which includes plant-based therapies continues to be widely used; an estimated 80% of people worldwide still depend on these treatments[2].

Ziziphus mauritiana Lamark, also known as Ber or Indian jujube, is a tropical and subtropical plant that is grown all over the world. It belongs to the Rhamnaceae family and its cultivation is relatively low maintenance and ideal for largely restoring resources in underdeveloped areas. It's focused on everything from nutrition and food science to the credibility of medicinal and nutritional properties such as products made juice, jelly, and squash

**Author for correspondence*

Article Received on: 09.05.2024 Revised on: 07.06.2024 Accepted on: 12.06.2024

are made in different ways[3]. *Ziziphus* leaves are beneficial for the treatment of diarrhea, wounds, abscesses, swelling, fever, asthma, liver, and gonorrhoea[4]. Secondary metabolites, commonly referred to as bioactive molecules, are typically derived from plants. Plants produce primary and secondary metabolites for their growth, development, and defense, respectively[5].

It is well known that humans have been using plants as their primary source of medicine for thousands of years for decades now. Because they are safe for the population to utilize, medicinal plants are essential in research. *Ziziphus* leaves obtained from the plant, have been used in traditional medicine for centuries due to their potential health benefits. *Z. mauritiana* Lamark due to the high mineral content of leaves, certain animals, such as cattle, camels, and goats, eat them to improve their health[6]. Phytochemical screening of *Ziziphus* leaves has revealed several compounds, including alkaloids, flavonoids, cardiac glycosides, phenols, saponins, protein, steroids, tannins, lignins, and reducing sugar etc.

The medicinal plants are more resistant to bacterial and fungal attacks due to the presence of biologically active substances. The plant's secondary metabolites are responsible for antibacterial activity, these medicinal plant varieties can be used as a natural drug. *Ziziphus* leaves have been intensively examined for their antibacterial activities and demonstrated significant effects against various bacterial strains[7]. These compounds have various health benefits, such as anti-inflammatory, antioxidant, antimicrobial, and anticancerous effects[8]. Hence, these medicinal plants have been utilized in conventional and many modern medications that are derived partially or entirely from leaves *Ziziphus mauritiana* Lamark is another name for *Ziziphus jujuba* mill[9].

This has led to greater importance on phytochemical investigations by researchers and the pharmaceutical industries[10]. In addition, the indigenous people utilize these phytochemicals found in the *Ziziphus jujuba* mill. leaves to treat certain diseases. In pharmaceuticals, several medications that come from secondary metabolites play an important role that essentially just reproduces or simple synthetic modifications of these naturally occurring compounds. Now, modern scientific research has revealed that the biologically active substances in jujube have cancer prevention, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective, and antiviral properties, and other healthcare effects including improving the immune system[11]. This study is focused on the qualitative evaluation of various phytochemicals and evaluates the antibacterial activity of different leaf varieties/cultivars/germplasm of *Z. mauritiana* Lamark. Indian jujube (*Z. mauritiana* Lamark) has great commercial importance due to its helpfulness all over plant parts.

2. Materials and Methods

2.1 Collection and Identification of Plant Materials

Fourteen commercial cultivars/germplasm/varieties of *Z. mauritiana* Lamark namely (ZU, ZS-3, ZCIAH-1, ZK, ZS-5, ZNBS new-10, ZNBS NEW-1, ZCH, ZNBS new-2, ZG, ZGK, ZCHES-2, ZCHES NEW, ZNBS-9) leaf samples were collected from the orchard of NDUAT (Narendra Deva University of Agriculture and Technology) Kumarganj, Ayodhya, Uttar Pradesh, India UP. The taxonomy of the plants was authenticated.

The bacterial strains of *B. subtilis* (Gram-positive), *S. aureus* (Gram-positive), *S. abony* (Gram-negative), *and E. coli* $DH_5\alpha$ (Gram-negative) were collected from the microbiology laboratory of Integral University, Lucknow, UP, India. The extracts were tested on two groups of bacteria *i.e.* Gram-positive and Gram-negative bacteria.

2.2 Plant's Extract Preparation

To get rid of dirt and other dust particles, the fresh plant samples (leaves) were collected and cleaned under the running tap water. Leaves were allowed to air dry for fifteen days at room temperature in a laboratory. The samples of dry leaves were ground using a mixer grinder, thoroughly into a fine powder. Further, 20g air-dried fine powder was immersed in 100 ml of solvents such as aqueous and methanol were each kept separately in an orbital shaker at room temperature for 24 h. Using Whatman No. 1 filter paper, the extracts were filtered. After that extract was used for its preliminary screening process such as Alkaloid (Iodine, Wagner and Dragendroff's test), glycosides (Keller-killing, conc.H_2SO_4, Molisch test), Flavonoids (Pew's, Shinoda, NaOH test), Saponins (Foam test), Tannins (Gelatine test and Lead Acetate tests), Sterol (Libermann-Burchard test, Salkowaski test), Lignin (Labat test, Lignin test), Phenol (Ellagic acid test, phenol test) estimation[12]. And resulting *Ziziphus* leaf extract was collected in sterile bottles for further analysis at 4-8°C.

Screening of antibacterial activity of leaf extract with the Soxhlet apparatus with two distinct solvents, aqueous and methanol. The extraction from leaves was performed at Dr. Rammanohar Lohia Avadh University, Ayodhya. *Ziziphus* leaf extract was stored in an airtight bottle for further analysis at 5°C.

2.3 Phytochemicals Screening

The various phytoconstituents found in 14 cultivars/ germplasm/varieties of *Z. mauritiana* Lamark leaves have been examined during the analysis of each prepared sample. Numerous chemical reagents were used for the screening of phytochemicals. There are several qualitative tests available for phytochemical screening. The tests were conducted using standard operating procedures.

2.3.1 Alkaloids

1-2 ml of Dragendroff's reagent was added to 2-3 ml of leaf extract, and the formation of an orange-brown color precipitate took place which confirms the presence of alkaloids[13]. Other hand;2 ml of the leaf extract was mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_3$& conc. H_2SO_4. The reddish-brown color appears at the junction of the two liquid layers and the upper layer appears blue color confirming the presence of glycosides[14].

2.3.2 Flavonoid

2-3 ml of extract was added to zinc powder and a red color appeared upon addition of dropwise conc. HCl which indicates the presence of flavonoids[13]. Other hand; 2-3ml of the extract was treated with a few drops of conc—HCl and magnesium in the test tube. The formation of a reddish/magenta color indicated the presence of flavonoids[13].

2.3.3 Glycosides

To the extract, 5 ml of Molisch's reagent and concentrated H_2SO_4 were added. The Violet color indicated glycosides[15]. Other hand; 0.5 mg of leaf extract was properly dissolved in 1 ml of Distilled water after that an aqueous NaOH solution was added to the test tube. The formation of a yellow color indicates the presence of glycosides[16].

2.3.4 Phenol

1 ml of extract dissolved in 5ml distilled water. Further 1% of (w/v) glacial acetic and 10% of (w/v) NaCl solution was added to it and then the precipitate produced a white color which confirms the presence of phenolics[17]. On the other hand; 2 ml of diluted leaf extract was added within sufficient droplets of glacial acetic acid 5% (w/v) and $NaNO_2$ solution of 5% (w/v) was mixed properly with a brown or cloudy color appeared indicating the presence of phenols[18].

2.3.5 Saponins

0.2gm of leaf extract was diluted with 5 ml of distilled water and suspension was shaken properly in a graduated measuring cylinder for 15 min. 1-2 cm layer of foam indicated the presence of saponins by their creamy appearance[19].

2.3.6 Protein

1 ml of diluted extract was mixed with a few drops of concentrated nitric acid in the test tube. The formation of a yellow color indicated the presence of protein[13].

2.3.7 Sterol

2 ml of a diluted extract with 2 ml of conc. H_2SO_4 and 2ml chloroform. The presence of sterol showed the first color red, then blue color, and finally greencolor[15].

2.3.8 Tannins

1 ml of filtered leaf extract was dissolved in 2 ml of Gelatin solution, which was dissolved in warm water immediately. The color was revealed by the formation of a white precipitate as well as tannin was present[20]. On the other hand, 5 ml of a diluted extract with a few drops of 10% lead acetate solution was added. When a yellow or red precipitate is formed, tannins are present[18].

2.3.9 Lignin

1 ml of diluted extract treated with a few drops of gallic acid after that it was mixed properly with the vortex mixer. The development of an olive-green color shows an action for lignins[17]. Another hand; 1 ml of the diluted extract with 2 ml of 2% (w/v) furfuraldehyde solution was added to the test tube. After that, a red color was developed which indicates the occurrence of lignin[12].

2.3.10 Reducing Sugar

0.5ml of diluted extract was treated with 1ml of boiled water for 2min and then 0.5ml of Benedict's reagent

solution was added in the test tube. The precipitate was observed as green color, yellow color, or red color[15]. Another hand;1 ml of diluted filter extract along with 1 ml of Fehling's solution A&B was added to the test tube and placed in the water bath. The red precipitate confirms the presence of reducing sugar[19].

2.3.11 Carbohydrate

2 ml of extract was mixed with 2 drops of alcoholic α naphthol, 1 ml of conc. H_2SO_4 and a few drops of Molisch's regent were added to the test tube carefully. The formation of the radish-yellow color indicated the occurrence of carbohydrates[21].

2.4 Preparation of Ziziphus Leaves Extract Sample for Antibacterial Activity

The test sample of leaf extracts for the antibacterial activity was prepared by dissolving different concentrations (25, 50, and 100 mg/ml) of extracted powder in DMSO (Dimethyl sulfoxide). The solution of extract and DMSO must be transparent for the authenticated results. The samples were incubated at 37°C for 30 minutes after adding DMSO and then vortexed for further applications.

2.4.1 Cleaning of Laminar airflow (LAF) and Maintenance of Bacterial Culture

Before starting work in LAF, it is necessary to clean it properly by using 70% ethanol. The floor and walls of LAF are wiped with cotton dipped in the alcohol. After cleaning of LAF with alcohol the UV lamps of LAF are turned on for 20 min and after that turn off the UV lamps and start work under the spirit lamp flame.

Nutrient broth was prepared and autoclaved. Under aseptic conditions in laminar air flow 5 ml nutrient broth was transferred in the sterilized glass tubes and bacterial inoculums transferred to the broth from slants. Before the test was performed these tubes of broth were incubated at 37°C for 24 hours.

2.4.2 Slant Preparation

All the work such as media pouring, inoculation, and streaking was performed under aseptic conditions in laminar airflow. The nutrient agar media was prepared and autoclaved. 5 ml of the nutrient agar media were poured into the glass tubes and left for the solidification. After solidifying the media, bacterial inoculums were transferred to the tubes from the master plates. These tubes were incubated at 37°C for 24 hours.

2.4.3 Antimicrobial Assay

Autoclaved and wrapped plates were opened in the laminar airflow and 30 ml of autoclaved nutrient agar media was poured into sterilized Petri plates. These Petri plates were allowed to solidify at room temperature in laminar airflow. After the solidification of plates, the freshly prepared microbial broth culture suspension (about 0.1 ml) was spread over the nutrient agar media using an L-shaped sterilized glass spreader separately under aseptic conditions using laminar airflow. The wells were created in Petri plates with the help of a borer of 8mm diameter. About 30 microliters of each leaf extract were loaded in these wells. The leaf extract was poured into wells using a micropipette with a concentration of 25, 50, and 100 mg/ml. The plates were then incubated at 37°C for 24 hours[22].

3. Results and Discussion

The preliminary phytochemical detection tests were conducted on leaf extracts by using methanol and aqueous solvents. To analyze the results of the leaves of *Z. mauritiana* Lamark, the data was summarized in Table 1. The results were compared based on the color tests. In the table, each row represents a different leaf species extract, and each column represents a solvent used in the phytochemical test. The observed phytochemicals are recorded during the tests present/absent in the respective cells.

Preliminary tests indicated the presence or absence of various secondary metabolites present in *Z. mauritiana* Lamark leaves. Phytochemicals such as alkaloids, flavonoids, cardiac glycosides, phenolic compounds, saponins, protein, steroids, tannins, lignin, reducing sugar, and carbohydrates were present in aqueous and methanolic solvents. While saponins were completely absent in methanolic solvent. These phytochemical analyses of plants are needed to discover and extend to novel therapeutic agents with improved efficiency.

A preliminary screening of antibacterial activity was carried out on leaf extracts using methanol and aqueous solvents to examine the results of the *Z. mauritiana* Lamark leaves the data is summarized in Table 2. The table helps to understand whether antibacterial activity is

present or absent. In this table, each row represents an extract of a different leaf variety/cultivar, and each column represents the concentration of samples, standard, and control. The screening of antibacterial activity was observed and recorded during the experiment based on the presence and absence of bacterial growth in the respective cells. *Z. mauritiana* Lamark. leaf extracts of aqueous and methanols were screened for antibacterial activity at different concentrations (25mg/ml, 50mg/ml, and 100mg/ml) against the bacterial strains. The different cultivars/varieties of leaf extracts showed antibacterial activity at all three concentrations against all bacterial strains used in experimentations. Additional screening results obtained during preliminary screening were summarized in Table 2.

Phytochemicals found in medicinal plants, such as alkaloids, flavonoids, quinones, coumarins, and others, have demonstrated potent antimicrobial activity against many pathogenic bacteria, including multidrug-resistant strains. The antimicrobial properties of medicinal plants are attributed to the various phytochemicals they contain, which have the potential as alternative treatments against drug-resistant microbial infections[23-25]. Various medicinal plants contain different phytochemicals, including alkaloids, glycosides, phenols, tannins, quinine, steroids, cardiac glycosides, volatile oils, etc these phytoconstituents are found in plant parts. According to the literature survey results obtained, curcumin found in turmeric has been observed to boost blood pressure, glucose absorption, insulin resistance, and inflammation reduction[26]. Flavonoids reduce risk primarily from cancer and cardiovascular illnesses[27]. Saponin is attributed with cytotoxic, antibacterial, and antiviral effects in addition to decreasing cholesterol[15]. Tannin exhibits a growth-inhibiting effect that suggests anticancer properties[28]. Medicinal Plants containing a high amount of flavonoids could be useful as anti-bacterial[27]. Antioxidants may benefit from plants that contain phenolic substances. Plants such as Ocimum, Nyctanthes, Mentha, and others that contain quinine have the potential to lower fever because of their antipyretic properties. Additionally, Mentha is utilized as an antibacterial and anti-helmintic agent, as well as a calming agent for toothaches[29]. The phenolic compound, tannin, terpenoid, and flavonoids possess an anti-helmintic property. So, the herb, Zanthoxylum, Acorus could be used to treatment for stomach issues[27]. Preliminary phytoconstituent screening of medicinal plant *Cayratia auriculata* extracts revealed several phytoconstituents. The methanolic extract had high flavonoids, saponins, tannins, and phenolic chemicals, moderate proteins, triterpenoids, and steroids, and little alkaloid carbohydrates and glycosides[14]. Jujube leaves are a rich source of these bioactive compounds, which have been found to have various physiological and pharmacological functions, such as antioxidant and anti-inflammatory properties[30].

The bacterial strain *B. subtilis* was examined for antibacterial activity in *Z. jujuba* used methanolic and aqueous solvents. It was reported that the antimicrobial properties of *Ziziphus jujuba* fruits found that crude extracts from the fruits had effective antibacterial impacts[7]. The antibacterial activity of *B. ciliate*, *J. officinale*, and *S. album* was recorded against *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *S. aureus*. Plant extracts dissolved in cold and hot water showed variable antibacterial activity against selected bacterial pathogens[31].

The antibacterial activity of *E. coli* $DH_5\alpha$ in *Ziziphus* using methanol and aqueous solvents. The extracts of methanol and aqueous of *Z. Jujube* confirmed antibacterial activity against *E. coli* $DH_5\alpha$. methanol extract showed higher antibacterial activity while the aqueous extract showed moderate antibacterial activity in comparison to the methanol. Additionally, the methanol extracts of *Ziziphus* species were effective against *E. coli* $DH_5\alpha$, highlighting the potential of these extracts for antibacterial purposes.

Plant crude methanolic extracts were tested against gram-positive and negative bacteria for bioactive compounds[32]. Health benefits of the sample extracted from *Z. jujube* using methanol and aqueous solvents include antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties, importance of the therapeutic potential of this plant[33]. The antibacterial activity between methanol and aqueous extracts of *Z. jujube* leaves. The study results indicated that both methanol and aqueous extracts of Z. *jujube* leaves showed antibacterial activity against bacterial strain with the methanol extracts generally showing higher potency[32]. In a study, the standard antibiotics used as control showed better activity on the organisms than the extracts which was not surprising because standard antibiotics are well-refined industrial products, ensuring higher activity compared to crude extracts[34]. In this study, also highlighted that the methanol extracts of certain plant species performed better than the aqueous extracts, which is attributed to the nature of the active components in the plant materials.

Table 1. Screening of phytochemicals in methanol and aqueous solvent of leaves different *Ziziphus mauritiana* Lamark varieties

Different *Ziziphus* cultivar/Variety/ genotype	Phytochemical analysis in Aqueous =(a) and Methanol = (b)																					
	ALK[a]	ALK[b]	FLA[a]	FLA[b]	GCS[a]	GCS[b]	PHE[a]	PHE[b]	SAP[a]	SAP[b]	PRO[a]	PRO[a]	STR[a]	STR[b]	TAN[a]	TAN[a]	Lig[a]	Lig[b]	RS[a]	RS[b]	Carbo[a]	Carbo[b]
ZU	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZS-3	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZCIAH-1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZK	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZS-5	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZNBS NEW-10	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZNBS NEW-1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZCH	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZNBS NEW-2	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZG	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZGK	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZCHES-2	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZCHES NEW	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ZNBS-9	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+

ZU- *Ziziphus Umran*, **ZS-3**- *Ziziphus* sanaur-3, **ZCIAH-1**- *Ziziphus*CIAH-1, **ZK**- *Ziziphus kaithali*, **ZS-5**- Ziziphus sanaur-5, **ZNBS new-10**- Ziziphus Narendra ber selection new-10, **ZNBS NEW-1**- *Ziziphus Narendra* ber selection new-1, **ZCH**- *Ziziphus chhuhara,* **ZNBS new-2**- *Ziziphus Narendra* ber selection new-2, **ZG**- *Ziziphus Gola*, **ZGK**- *Ziziphus gumakirti*, **ZCHES-2**- *Ziziphus* CHES-2, **ZCHES NEW**- *Ziziphus* CHES new, **ZNBS-9**- *Ziziphus Narendra* ber selection-9.

ALK- Alkaloids**; FLA**- Flavonoids**; GCS**- Glycosides**; PHE**- Phenol**; SAP**- Saponin**; PRO**- Protein**; STR**- Steroids**; TAN**- Tannin**; Lig**- Lignin**; RS** - Reducing Sugar**; Carb**- Carbohydrate**. +** = Presence**, -** = Absent**, a=** Aqueous**, b=** Methanol**.**

Table 2. Screening of antimicrobial activity of leaves of different varieties of *Z. mauritiana* Lamark in aqueous and methanol

Conc. (mg/ml)	**25**		**50**		**100**		**Standard**	**Control**
Name of bacteria and leaf extract	**Antibacterial activity**						**Ampicillin (10µg/disc)**	**DMSO**
B. subtilis								
***Ziziphus* germplasm/cultivar/varieties**	**a**	**b**	**a**	**b**	**a**	**b**		
ZU	-	-	-	-	+	+	+	**0**
ZS-3	-	-	+	+	+	+	+	**0**
ZCIAH-1	-	-	-	-	-	-	+	**0**
ZK	-	-	-	-	+	+	+	**0**
ZS-5	+	+	+	+	+	+	+	**0**
ZNBS NEW-10	-	+	-	+	+	+	+	**0**
ZNBS NEW-1	-	-	-	-	+	+	+	**0**
ZCH	+	+	+	+	+	+	+	**0**
ZNBS NEW-2	-	-	-	-	+	+	+	**0**
ZG	-	-	-	-	+	+	+	**0**
ZGK	-	-	-	+	-	+	+	**0**

ZCHES-2	-	-	-	-	+	+	+	**0**
ZCHES NEW	-	-	-	-	+	+	+	**0**
ZNBS-9	-	-	-	+	+	+	+	**0**
S. abony								
ZU	-	-	-	-	+	+	+	**0**
ZS-3	-	-	-	+	+	+	+	**0**
ZCIAH-1	-	-	-	-	-	-	+	**0**
ZK	-	+	+	+	+	+	+	**0**
ZS-5	+	+	+	+	+	+	+	**0**
ZNBS NEW-10	-	-	+	+	+	+	+	**0**
ZNBS NEW-1	-	-	-	+	+	+	+	**0**
ZCH	-	-	-	+	+	+	+	**0**
ZNBS NEW-2	-	-	-	-	+	+	+	**0**
ZG	+	+	+	+	+	+	+	**0**
ZGK	-	-	-	-	+	+	+	**0**
ZCHES-2	-	-	-	+	+	+	+	**0**
ZCHES NEW	-	-	-	-	+	+	+	**0**
ZNBS-9	-	-	-	+	+	+	+	**0**
E. coli DH$_5$α								
ZU	-	-	-	+	+	+	+	**0**
ZS-3	+	+	+	+	+	+	+	**0**
ZCIAH-1	-	-	-	-	+	+	+	**0**
ZK	-	-	-	-	+	+	+	**0**
ZS-5	+	+	+	+	+	+	+	**0**
ZNBS NEW-10	+	+	+	+	+	+	+	**0**
ZNBS NEW-1	-	-	+	+	+	+	+	**0**
ZCH	-	-	-	+	+	+	+	**0**
ZNBS NEW-2	-	-	-	+	+	+	+	**0**
ZG	-	+	-	+	+	+	+	**0**
ZGK	-	-	-	+	+	+	+	**0**
ZCHES-2	+	-	+	+	+	-	+	**0**
ZCHES NEW	-	-	-	+	+	+	+	**0**
ZNBS-9	-	-	-	-	+	+	+	**0**

				S. aureus				
ZU	-	-	-	+	+	+	+	**0**
ZS-3	+	+	+	+	+	+	+	**0**
ZCIAH-1	-	-	+	+	+	+	+	**0**
ZK	-	-	-	+	+	+	+	**0**
ZS-5	+	+	+	+	+	+	+	**0**
ZNBS NEW-10	-	-	-	+	+	+	+	**0**
ZNBS NEW-1	-	-	-	+	+	+	+	0
ZCH	-	-	-	+	+	+	+	0
ZNBS NEW-2	-	-	+	+	+	+	+	0
ZG	-	+	+	+	+	+	+	0
ZGK	-	-	-	+	+	+	+	0
ZCHES-2	+	+	+	+	+	+	+	0
ZCHES NEW	-	-	-	-	+	+	+	0
ZNBS-9	-	+	-	+	+	+	+	0

+ = Antibacterial activity present, -=Antibacterial activity absent, **a**= *Aqueous*, **b**= *Methanol*, **DMSO**=*Dimethyl sulfoxide*, **Standard**= *Ampicillin*.

4. Conclusion

The phytochemical screening of the selected plant samples was conducted. This study indicates that Indian jujube/Ber plant (*Z. mauritiana* Lamark) leaves contain various phytochemicals that can be used to treat numerous disorders. Plant leaves include various phytoconstituents such as alkaloids, flavonoids, cardiac glycosides, phenolic compounds, saponins, protein, steroids, tannins, lignin, reducing sugar, carbohydrates, etc. The phytochemical screening technique identified several phytoconstituents in leaf extracts. Preliminary screening of phytochemical and antimicrobial analysis is crucial for evaluating the potential medicinal properties of plants, as well as determining the active components responsible for their known biological activities. The extraction of phytochemicals from *Z. mauritiana* Lamark leaves extract depends on the aqueous and methanol, and the preliminary screening test applied for phytochemical analysis can determine the presence or absence of specific phytochemicals in the leaves sample. In this study, it was found that the *Z. mauritiana* Lamark leaves have antimicrobial activities against *B. subtilis, S. aureus, S. Abony,* and *E. coli* DH5α. High antimicrobial activity at the concentration of 100mg/ml was observed on all tested bacterial strains and less antimicrobial effect, at the concentration of 25mg/ml. The results indicated that both the aqueous and methanol extracts of *Z. mauritiana* Lamark leaf have a phytochemical and antimicrobial activity that is promising for the possible treatment of many human diseases.

5. Acknowledgment

Sponsoring Organization: The "Research and Development of State Universities of Uttar Pradesh" project was supported by the Department of Higher Education, Government of Uttar Pradesh, Lucknow, India (Letter no. (80/2021/1543(1)/सत्तर-4-2021-4 (28)/2021). Prof. Neelam Pathak would like to express her gratitude to this organization. She would especially like to express her gratitude to the "Research and Development of State Universities of Uttar Pradesh" initiative.

6. References

1. Alsayari A, Wahab S. Genus Ziziphus for the treatment of chronic inflammatory diseases. Saudi J Biol Sci. 2021. https://doi.org/10.1016/j.sjbs.2021.07.076. PMid:34866990 PMCid:PMC8626254
2. Miller JS. The global importance of plants as sources of medicines and the future potential of Chinese plants. Drug Discovery and Traditional Chinese Medicine; 2001. https://doi.org/10.1007/978-1-4615-1455-8_4
3. Walunj AR, Papade PE. Evaluation of promising genotypes of ber (*Zizyphus mauritiana* Lamark) Against its pest complex. An International E Journal. 2019.
4. Hovaneț MV, Ozon EA, Moroșan E, Șeremet OC, Oprea E, Geană E-I, Anghel AI, Bădiceanu C, Duțu LE, Stoicescu CS. et al. Wound healing and anti-Inflammatory effects of a newly developed ointment containing Jujube leaves extract life .2022; 12:1947. https://doi.org/10.3390/life12121947 PMid:36556312 PMCid:PMC9785415
5. Hussein RA, El Anssary AA. Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. Herb Med. 69412; 2019. https://doi.org/10.5772/intechopen.76139
6. Ghasham AA, Muzaini MA, Qureshi KA, Elhassan GO, Khan RA, Farhana SA, Hashmi S, Agamy EE, Abdallah WE. Phytochemical screening, antioxidant and antimicrobial activities of methanolic extract of *Ziziphus mauritiana* Lam. leaves collected from Unaizah, Saudi Arabia. Int J Pharm Res Allied Sci. 2017.
7. Daneshmand F, Zare-Zardini H, Tolueinia B, Hasani Z, Ghanbari T. Crude extract from Ziziphus Jujuba Fruits, a Weapon against Pediatric Infectious Disease. Iran J Ped Hematol Oncol. 2013.
8. Swamy MK, editors. Plant-derived Bioactives. Springer Nature; 2020. https://doi.org/10.1007/978-981-15-1761-7
9. Ghobadi A, Behbahani F A, Yousefi A, Shirazi M T, Behnoud N. Medicinal and nutritional properties of *Ziziphus jujuba* Mill. in traditional Persian medicine and modern phytotherapy. Crescent J Med Biol Sci. 2019; 6(2).
10. Ugochukwu SC, Arukwe UI, Onuoha I. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian J Plant Sci Res. 2013; 3(3):10-13.
11. Yang LU, Tao BAO, Jianling MO, Jingdan NI, Wei CHEN. Research advances in bioactive components and health benefits of jujube (*Ziziphus jujuba* Mill.) fruit. J Zhejiang Univ Sci B. 2021. https://doi.org/10.1631/jzus.B2000594. PMid:34128368 PMCid:PMC8214949
12. Bhatt S, Dhyani S. Preliminary phytochemical screening of *Ailanthus excelsa* Roxb. Int J Curr Pharm Res. 2011; 4(1): 87-89. ISSN- 0975-7066.
13. Kancherla N, Dhakshinamoothi A, Chitra K, Komram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of *Cayratia auriculata* (*In Vitro*). Maedica-a Journal of Clin Medici. 2019. https://doi.org/10.26574/maedica.2019.14.4.350
14. Singh V, Kumar R. Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand Region. Int J Life Sci Scienti. Res.2017. https://doi.org/10.21276/IJLSSR.2017.3.6.4
15. Sharma T, Pandey B, Shrestha BK, Koju GM, Thusa R, Karki N. Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. Trib University Journal. 2020. https://doi.org/10.3126/tuj.v35i2.36183
16. Deshmukh MA, Theng MA. Phytochemical screening quantitative analysis of primary and secondary metabolites of Acacia arabica bark. Int J Curr Pharm Res. 2018; 10(2):35. https://doi.org/10.22159/ijcpr.2018v10i2.2588
17. Marka R, Talari S, Penchala S, Rudroju S, Nanna RS. Preliminary phytochemical analysis of leaf, stem, root and seed extracts of *Arachis hypogaea* L. Int J Pharm Sci Rev Res. 2013.
18. Sheel R, Nisha K, Kumar J. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR-JAC. 2014. https://doi.org 10.9790/5736-07121013
19. Padmapriya KR, Barathi SD, Pandeeswaran M. Phytochemical screening test for eleven different medicinal plants in and around Dindigul City. IJARESM. 2020.
20. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica (Thorn mimosa*). 2014. PMID: 25568701, PMCID: PMC4279630.
21. Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products (AJEONP). 2017.
22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493-496. https://doi.org/10.1093/ajcp/45.4_ts.493 PMid:5325707
23. Wintola OA, Afolayan J. The antibacterial, phytochemicals, and antioxidants evaluation of the root extracts of *Hydnoraafricana thunb*. used as antidysenteric in Eastern Cape Province, South Africa. BMC Complement Altern Med. 2015; https://doi.org/10.1186/s12906-015-0835-9 PMid:26335685 PMCid:PMC4558922
24. Kebede T, Gadisa E, Tufa A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment

of multidrug-resistant microbes. PLoS One. 2021. https://doi.org/10.1371/journal.pone.0249253 PMid:33770121 PMCid:PMC7997013

25. Ashraf MV, Pant S, Khan HMA, Shah AA, Siddiqui S, Jeridi M, Alhamdi SHW, Ahmad S. Phytochemicals as antimicrobials: Prospecting Himalayan medicinal plants as source of alternate medicine to combat antimicrobial resistance. Pharmaceuticals. 2023. https://doi.org/10.3390/ph16060881. PMid:37375828 PMCid:PMC10302623
26. Azhdari M, Karandish M, Mansoori A. Metabolic benefits of curcumin supplementation in patients with metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. Phytother Res. 2019. https://doi.org/10.1002/ptr.6323 PMid:30941814
28. Ballard CR, Maróstica MR. Health benefits of flavonoids. Bioactive Compounds. 2019. https://doi.org/10.1016/b978-0-12-814774-0.00010-4
29. Zarin MA, Wan HY, Isha A, Nurdin A. Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. Food Sci Hum Wellness. 2016. doi: 10.1016/j.fshw.2016.02.001.
30. Patil SR, Patil RS, Godghate AG. Mentha piperita Linn: Phytochemical, Antibacterial and Diptherian Adulticidal Approach. Int J of Phar and Pharma Scien. 2016.
31. Song L, Zheng J, Li Zhang, Yan S, Jun H, Liu P. Phytochemical profiling and fingerprint analysis of Chinese Jujube (*Ziziphus jujuba* Mill.) leaves of 66 cultivars from Xinjiang Province. Molecules. 2019. https://doi.org/10.3390/molecules24244528 PMid:31835666 PMCid:PMC6943635
32. Khan UA, Rahman H, Niaz Z, Qasim M, Khan J, Tayyaba, Rehman B. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. Eur J Microbiol Immunol. 2013. https://doi.org/10.1556/EuJMI.3.2013.4.6. PMid:24294497 PMCid:PMC3838543
33. Ahmad B, Khan I, Bashir SH, Azam S, Hussain F. Screening of *Zizyphus jujuba* for antibacterial, phytotoxic and haemagglutination activities. Biotech. 2011. https://doi.org/10.5897/AJB10.768.
34. Azize A, Souleymane. Potential benefits of Jujube (*Zizyphus lotus* L.) Bioactive Compounds for Nutrition and Health. Journal of Nutrition and Metabolism. 2016; 1-13. https://doi.org/10.1155/2016/2867470 PMid:28053781 PMCid:PMC5174181
35. Njateng GSS, Du Z, Gatsing D, Mouokeu RS, Liu Y, Zang HX, Jianlong G, Luo X, Kuiate JR. Antibacterial and antioxidant properties of crude extract, fractions and compounds from the stem bark of *Polyscias fulva* Hiern (Araliaceae). BMC Complement Altern Med. 2017. https://doi.org/10.1186/s12906-017-1572-z PMid:28173794 PMCid:PMC5297048