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Research Article

Antibacterial Activity of *Mirabilis jalapa* Leaves (Karifuma) Extract Against Bacterial Wound Infection from Patients Attending Rango Health Center

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Abstract

Background: *Mirabilis jalapa* is a perennial herbaceous bushy plant with several pharmacological activities, such as anti-diabetic, anti-inflammatory, anti-oxidative, anti-bacterial, anti-microbial, anti-fungal, anti-viral, diuretic and anthelmintic. Wound infections are a major public health concern in Rwanda, with 8% of the general population having difficulties controlling them and 25% of those above 60 years old. Traditional medicine is used to treat many conditions such as chest infections, coughs, snake-bites and burns. *Mirabilis jalapa* seeds have been reported to be effective against bacteria causing wound infections. **Aim**: This study evaluated the antibacterial activity of *Mirabilis jalapa* leaves extract against infected wound bacteria from patients attending Rango Health Center.

Methods: Cross sectional study design and maceration method was used in *Mirabilis jalapa* leaves extraction with Methanol, Ethanol, Diethyl ether and Distilled water as solvents.

Results: Different extracts were obtained according to the solvents with different volumes and concentrations. The highest extract volume obtained was Distilled water with 6 ml and the least was Diethyl ether with 2.7 ml. In 71 participants who had wound infection, 42% had a bacterial infection. Among them, 25% had *Staphylococcus aureus*, 7% had *Klebsiella pneumoniae*, 6% had *Escherichia coli*, and 4% had *Pseudomonas aeruginosa*. Through the agar well diffusion method, Methanol extracts showed the highest inhibition zones while distilled water extracts exhibited the lowest inhibition zones to the isolated bacteria.

Conclusion: The study confirms the antibacterial activity of the *Mirabilis jalapa* leaves extract against infected wound bacteria, with methanol and ethanol being the best solvents.

Keywords: *Mirabilis jalapa* leaves, wound infections, antibacterial activity

INTRODUCTION

Mirabilis jalapa is a perennial herbaceous bushy plant which belongs to the Nyctaginaceae family and can grow up to one meter.[1] It was named by Carl Von Linne in 1753, from the scientific Latin words, Mirabilis meaning "admirable" by allusion to the remarkable colors of its flowers.[2] It has worldwide distribution and the whole plant was reported to have several pharmacological activities including anti-diabetic, anti-inflammatory, anti-oxidative, anti-bacterial, anti-microbial, anti-fungal, anti-viral, diuretic, anthelmintic and is also effective against urinary tract disorders.[3] Parts of *Mirabilis jalapa* are used in the treatment of different diseases. Roots are used traditionally for allergic skin disorders and asthma. Stems are used in treating grand mal seizures. Flowers are used to treat herpes lesions, earaches, skin infections, infected wounds, bee and scorpion stings. The seed powder is used externally to treat infected wounds. Leaves are used in different treatments. A leaf infusion is applied topically to reduce swelling after bone fracture and is also used in inflammation, boils and emetic conditions.[4]

Injuries are one of the major causes of wounds. due to the high prevalence of injuries and antimicrobial resistance has been associated with many currently used antibiotics, The identification of alternative agents for wound healing is paramount. According to the world health organization (WHO), injuries cause 4.4 million deaths around the world each year and constitute 8% of all deaths.[5] The WHO indicates that high rates of microbial resistance against antibiotics are frequently observed worldwide among common bacterial infections that include; urinary tract infections, wound infections, sepsis and others. The rate of resistance to ciprofloxacin varies from 8.4% to 92.9% for *Escherichia coli* and from 4.1% to 79.4% for *Klebsiella pneumoniae*. Resistance to antibiotics has adverse effects on treatment where for example, people with methicillin-resistant *Staphylococcus aureus* (MRSA) infections are 64% more likely to die from infection complications than people with drug-sensitive infections.[6]

Mirabiris jalapa parts including leaves are potential candidates to overcome the challenges of antimicrobial resistance. *Mirabilis jalapa* flowers have the potential for wound healing. Flowers have flavonoids which limit the number of free radicals thus preventing excessive tissue damage in the inflammatory phase and also contain β-sitosterol-D-glucoside.

This natural estrogenic steroid maintains moisture in the wound area, promoting cell growth. In the inflammatory phase, β-sitosterol limits the amount of prostacyclin, thereby accelerating the inflammatory phase[7] and the most common bacterial species that are known to cause wound infection are *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli,* and *Corynebacterium spp*.[8]

In Rwanda, traditional medicine mainly uses plants in treatment. *Gynandropsis gynandra* (isogi), fresh leaves are used for chest infections. *Tetradenia riparia* (umuravumba), leaf juice is used to treat coughs. *Pennisetum purpureum Schum*(urubingo) is applied on snake-bite and burns.[9] Though *Mirabilis jalapa seeds* are used in wound treatment, little is known about the effect of *Mirabilis jalapa* leaves on wound infections. Therefore, this study aimed to evaluate the antibacterial activity of *Mirabilis jalapa* leaves extract against bacteria from infected wounds from patients attending Rango Health Center to generate knowledge and guidance on their pharmacological activity and leaves are abundantly available.

METHODOLOGY

Study Area

This study was conducted at Rango Health Center located at Rango B cell, Tumba sector, Huye district in the southern province of Rwanda. The centre is a public health institution that serves many patients including those with wound infections.

Study Design

A cross-sectional study design was used in this study. The wound swab samples were processed and analyzed in UR Huye biotechnology laboratory complex located in Huye District, Ngoma Sector, Butare Cell, Mamba Village.

Study Population

Participants who visited Rango Health Center's minor surgery ward for the treatment of wound infections in the period of data collection voluntarily provided consent from themselves or through guardians.

Study Sample

Wound swabs were obtained from 71 participants and the sample size was determined using Daniel`s formula:

Data collection methods and procedure

Collection and Treatment of *Mirabilis Jalapa* Leaves

Mirabilis jalapa leaves were collected from the Arboretum Forest of the University of Rwanda, Huye campus, Huye district, the southern province of Rwanda after being confirmed by Botanist from Agroforestry Department and put in a plastic container to be transported to the University of Rwanda Huye Biotechnology Complex Laboratory. In the laboratory, leaves were washed under running tap water to remove all traces of dust and insects followed by drying in a drying oven for four days at 40°c. The dried leaves were removed and ground using *ZM 200 Ultra Centrifugal Mill, Retsch* to obtain powder which was stored in the screw-cupped container at room temperature to be used for extraction.

Extract preparation

This study employed the maceration method of extraction according to Tambun et al. where Ethanol, Methanol, Diethyl ether and Distilled water were used as extraction solvents.[10] The dried ground leaves were weighed using an electrical balance, with a sensitivity of 0.0001g and a 10%W/V mixture (70g of powder was mixed with 700ml of each solvent) was prepared in a beaker and then covered tightly with Aluminium foil to make an initial concentration of 0.1 g/ml. The mixtures were left at room temperature for 24 hours with continuous agitation using an orbital shaker / OS-340C at 60rpm and was subsequently filtered using a Whatman filter paper of 11 µ pore size to obtain filtrates which were by evaporation using *RE-100 Pro Rotary Evaporator* at 40^{0}C for 4 hours. After 3.5 to 4 hours, the presence of green color in the evaporates was checked to determine the completeness of evaporation procedures. The obtained extracts were stored in a refrigerator for further analysis.

Preparation of culture media

Routinely used microbiological culture media like MacConkey Agar (MAC), Blood Agar, Mueller Hinton Agar, Kilgrer Iron Agar, Simmon's citrate agar, Sulfur Indole Motility agar and Mannitol Salt Agar were prepared according to their manufacturers' instructions and were stored in the refrigerator (2^0 C to 8^{0}C) for future use.

Collection of samples

After receiving written consent from each participant wound swabs were collected using a Sterile Cotton Tip Applicator, Fusion Biotech and put in a sterilized centrifugation tube containing normal saline as a transport media. The tubes were transported in a cooler box containing ice packs with temperature monitoring of between 2-8°C to UR Biotechnology Complex Laboratory in the Microbiology department for immediate analysis.

Culturing and Identification

The wound swabs were initially cultured on Mannitol salt agar, MacConkey and blood agar and incubated for 24 hours at 37°c. The growth of bacteria was observed after 24 hours followed by a sub-culturing and biochemical test for bacterial identification on positive cultures. A coagulase test was done on bacterial colonies grown on Mannitol Salt Agar to identify *Staphylococcus aureus* while bacteria grown on Mac Conkey were sub-cultured on KIA, SIM and Simmon citrate agar to differentiate *Enterobacteriaceae* species. In addition, bacteria grown on Blood Agar were tested for their catalase and coagulase activities to single out *Staphylococcus aureus* from coagulase-negative bacteria. The following day, sub-cultured media were interpreted and positive *Klebsiella pneumoniae* was isolated from a positive Simmon citrate whereas a positive indole test helped to identify *Escherichia coli* from *Enterobacteriaceae* and a positive oxidase test was used to identify *Pseudomonas aeruginosa.*

Preparation of Bacterial Suspension

The bacterial suspension was prepared according to SOP 10 version 009 in the bacteriology department at UR biotechnology complex laboratory. In this procedure, a single colony was taken from a cultured media with aid of a sterile wire loop and transferred into 4 ml of sterile normal saline in a durham tube and the mixture was compared to 0.5 McFarland Turbidity Standard solution. Adjustments were done where needed to obtain an ideal suspension to be used On Muller Hinton agar media.

Antibacterial activity testing of Leaf Extracts

The agar well and Kirby Bauer diffusion techniques were used in this study. To begin with, a pure bacterial suspension was inoculated onto Mueller-Hinton agar by spreading. Then, a sterile pipette tip was used to create 4 wells of an estimated 6mm in diameter and 3mm in depth on the media following microbiological aseptic conditions. Thereafter 50 µL of each extract were placed into each of the created wells.[11]

In addition, antibiotic discs were used to control the interpretation of the antibacterial activity of *Mirabilis jalapa* leaves extract using the Kirby Bauer diffusion technique where Tetracycline and Oxacillin were used for gram-positive bacteria while Gentamicin, Ciprofloxacin and Amikacin were used for gram-negative bacteria. The diameter of the clear zone around the disc was measured and the results were interpreted as Sensitive, Intermediate, or Resistant according to CLSI 2023 recommendations.[12]

Data Analysis

The data were entered into a Microsoft Excel database, version 2016 (16.0.4266.1001 version) and then imported into SPSS analyzing tool version 22.0.0.0 for statistical analysis. The results of isolated bacteria, the concentration of extracts and the antimicrobial activity of *Mirabilis jalapa* were presented using tables and graphs.

RESULTS

Common Bacteria Isolated from Wound Infections

The study included 71 participants, 24 of whom were female (34%), and 47 of whom were male (66%). Of these participants 41 (58%) showed no bacterial growth and 30 (42%) swabs showed bacterial growth. Among those who tested positive, 18 (25%) had *Staphylococcus aureus*, 5 (7%) had *Klebsiella pneumoniae*, 4 (6%) had *Escherichia coli*, and 3 (4%) had *Pseudomonas aeruginosa*. **Figure 1** depicts the most common bacteria isolated on participants' pus swabs and their prevalence.

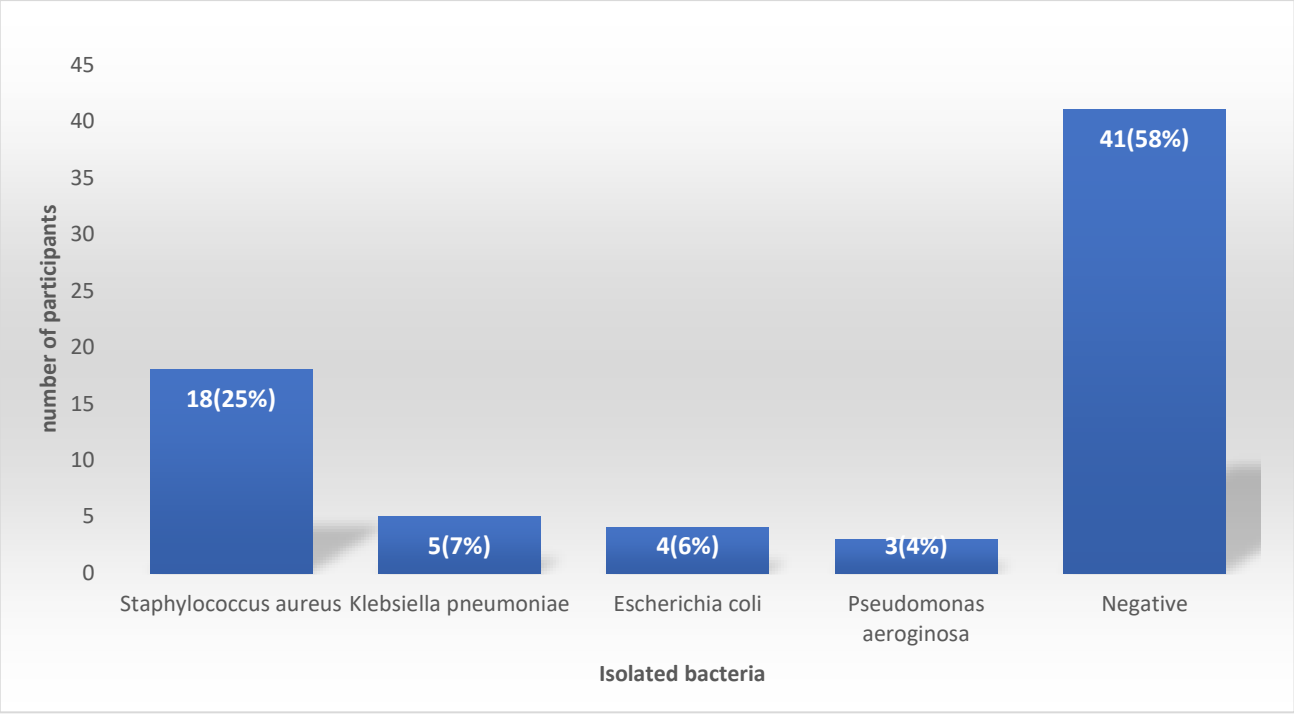

Figure 1. Common bacteria isolated from wound infections (n=71) and their Prevalence.

Antibacterial activity of *Mirabilis Jalapa* Leaves Extracts

According to **Figure 2** which illustrates the Mean antibacterial activity of *Mirabilis jalapa* leaves extracts, Methanol extracts had inhibition zones on *Staphylococcus aureus* of between 26-33mm ($\bar{x}$ = 30 mm) followed by ethanol extracts with inhibition zones of between 20-28mm ($\bar{x}$ = 25 mm). Diethyl ether extracts had inhibition zones of between 10-15mm ($\bar{x}$ = 12 mm) while the lowest inhibition was seen on distilled water extracts with between 8-12mm ($\bar{x}$ = 10 mm). In addition, the range of inhibition zones for *Klebsiella pneumoniae* varied from between 18-22mm ($\bar{x}$ = 20 mm) for methanol and ethanol extracts to between 8-11mm ($\bar{x}$ = 10 mm) and between 6-8mm ($\bar{x}$ = 70 mm) of diethyl ether and distilled water extracts respectively. Furthermore, the range inhibition zones for *Escherichia coli* for methanol and ethanol extracts were between 15-21 mm with a mean of 20 mm and 17mm respectively while diethyl ether and distilled water extract inhibited the growth of bacteria by 9-13 mm ($\bar{x}$ = 11 mm) and 6-7 mm ($\bar{x}$ = 6 mm) respectively. Lastly, the range inhibition zones on *Pseudomonas aeruginosa* were significant for methanol and ethanol extract with 21-23 mm ($\bar{x}$ = 22mm) and 18-20 mm ($\bar{x}$ = 19 mm) respectively. Diethyl ether extract inhibited the growth of bacteria by 7-10 mm ($\bar{x}$ = 9 mm) while inhibition for distilled water extracts was 6-8mm ($\bar{x}$ = 7 mm).

With regards to the control antibiotic discs used, all gram-negative bacteria were sensitive to Ciprofloxacin, Amikacin, and Gentamicin, except *Pseudomonas aeruginosa*, which was intermediate for Gentamicin. Tetracycline and Oxacillin was effective against *Staphylococcus aureus.*

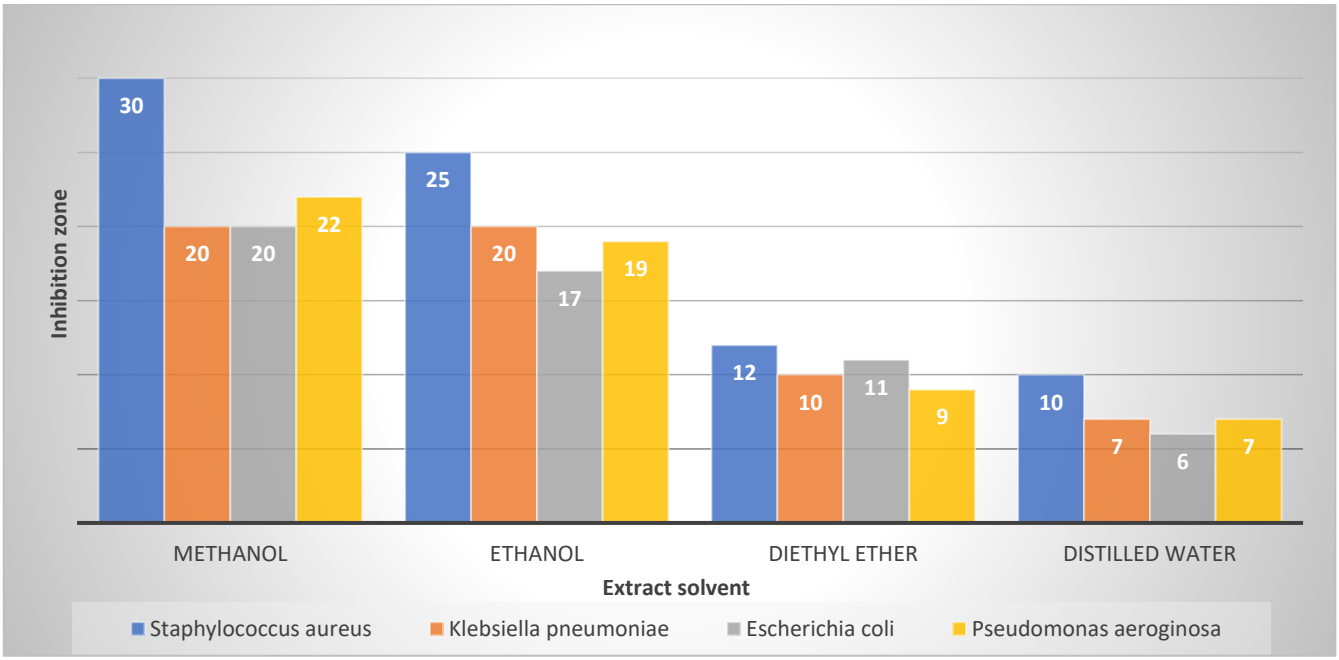

Figure 2. Mean antibacterial activity of *Mirabilis jalapa* leaves extracts

***Mirabilis Jalapa* Leaves Extraction Output**

Mirabilis jalapa leaves extraction and its antimicrobial activity analysis involved varieties of different methods and were used in the present study. Different extracts were obtained according to the **Table 3** with different volumes and concentrations. The highest extract volume obtained was Distilled water with 6 ml (11.6g/ml) followed by Methanol and Ethanol extracts with 5.2 ml (13.4 g/ml) and 4 ml (17.5g/ml) respectively while the least extract volume was Diethyl ether with 2.7 ml (25.9 g/ml). The table below represents the obtained results from each extract.

Table 3: Types of solvents used for plant extracts, volume and their final concentration.

Solvent	Obtained volume in ml	Concentration in g/ml
Methanol	5.2	13.4
Ethanol	4	17.5
Diethyl ether	2.7	25.9
Distilled water	6	11.6

DISCUSSION

Antimicrobial activity and plant extraction require studies in various solvents and numerous laboratory techniques which implied that different solvents needed to be used in this study. Diethyl ether was chosen as a solvent because of its low solubility in water. Distilled water was used and preferred due to its solubility in a wide range of substances, nontoxicity, nonflammability, and availability, as well as its high polarity and lack of impurities such as dissolved ions and the possibility of side reactions interfering with the results.[13] Because methanol is miscible with water, it was chosen as the most effective solvent for the extraction. It could extract polar secondary metabolites and required only a small amount of heat to concentrate the extract.[14] Ethanol retrieves a large amount of oil from the plant and evaporates completely.[15]

The results revealed that different solvents resulted in different extraction yields. This is because differences in the polarity of the extraction solvents can result in a wide range of bioactive compound levels in the extract.[16] When compared to Diethyl ether, distilled water extract, methanolic extract, and ethanolic extract had higher extraction yields, indicating that the extraction efficiency favors polar solvents.[17] In comparison to the other extracts used, distilled water extract had the highest yield (33.5%), contrary to reports by Truong et al., who found that Methanolic extract had the highest yield.[18] Diethyl ether extract had the lowest yield (15.08%), the same as reported by Abubakar and Haque, because it is highly volatile and evaporated during the experiments.[16]

In contrast to the current study, *Klebsiella pneumoniae* was not isolated in the study conducted by Bessa et al[19] whereas the same strains were isolated from wounds in the study conducted by Esebelahie et al. on aerobic bacterial isolates from infected wounds where *Staphylococcus aureus* had the highest prevalence, and *Pseudomonas aeruginosa* had the lowest.[20] The results showed that some extracts of *Mirabilis jalapa* leaves were significantly effective against the bacteria tested. In contrast to P. Poovendran's study, which found that ethanolic extract had high antibacterial activities,[21] the current study found that methanol extracts had higher antibacterial activities for all isolated bacteria. The same study found similar results with distilled water extract by demonstrating low antibacterial activity.[22] Staphylococcus aureus was sensitive to methanol and ethanol extracts when compared to control antibiotic discs, but resistant to diethyl ether and distilled water extracts. In addition, these extracts were sensitive to all gram-negative bacteria, whereas Diethyl ether and Distilled water extracts were resistant compared to Amikacin and Gentamicin control discs. Only methanol extracts were sensitive to Pseudomonas aeruginosa when compared to ciprofloxacin.

CONCLUSION

The current study confirms that *Mirabilis jalapa* leaf extract has antibacterial activity against bacteria from infected wounds. In comparison to other solvents, methanol and ethanol are effective solvents to use in *Mirabilis jalapa* leaves extraction because their extracts showed the highest antibacterial activity. *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* were the bacteria most associated with wound infections at Rango Health Center with *Staphylococcus aureus* the most prevalent while *Pseudomonas aeruginosa* was the least prevalent.

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