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# ANTIBIOGRAM COMPARISON OF *SALVIA OFFICINALIS* LEAF EXTRACT AGAINST *KLEBSIELLA PNEUMONIAE* AND *PROTEUS MIRABILIS*

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**Abstract**

*Salvia officinalis* is a culinary herb of 60-70 cm tall and petiole leaf of 6.0 cm long that belong to the Lamiaceae family and widely cultivated around the world, but a native in the Mediterranean region. This study aimed at the comparison and antibiogram of *Salvia officinalis* leaf extract against *Klebsiella pneumoniae* and *Proteus mirabilis.* The reference strains of *Klebsiella pneumonia* and *Proteus mirabilis* was obtained from NVRI Vom, Plateau state. Fresh leaves of *Salvia officinalis* were harvested pulverized and air dried. Both fresh and dried leaves were used and extracted using ethanol, cold and hot water as solvents. Standard microbiological techniques were employed in this study. The antibiogram of *Salvia officinalis* leaf extracts were determined by agar well diffusion method using 1000 mg/ml, 800 mg/ml, 600 mg/ml, 400 mg/ml and 200 mg/ml concentrations. At high concentration, *Klebsiella pneumoniae* was shown to be more susceptible to the ethanolic extract of the dry and fresh leaf extract than *Proteus mirabilis*. The zone of inhibition of the positive control, gentamicin against *Klebsiella* pneumoniae and *Proteus* mirabilis was higher than of both the cold and hot water (dry and fresh) extract but no significantly difference ($P<0.05$) with the ethanol (dry and fresh leaf) extract. Both test organisms were more susceptible to the dry leaf extracts of *Salvia officinalis* than the fresh leaf extracts. *Klebsiella pneumoniae* was more susceptible to the effect of the leaf extract than *Proteus mirabilis* with gradual increase in concentrations from 200mg/ml to 1000mg/ml. The ethanolic dry leaf extract was more effective against test organisms. In this study, *Salvia officinalis* leaf extract in high concentrations especially the ethanol extract were shown to be good alternative antibiotics against the test organisms. Therefore the leaf extract of *Salvia officinalis* can be effective against *Klebsiella pneumoniae* and *Proteus mirabilis* infections.

**Keywords:** Antibiogram, *Klebsiella pneumonia*, Leaf extract, *Proteus mirabilis, Salvia officinalis*

## Introduction

Traditional medicine has gained popularity throughout the world over the last decade. It has been used not just for inadequate primary health care in underdeveloped countries, but also in countries where conventional medicine dominates the national health care system (Hamidpour *et al.,* 2014; Imarenezor *et al.,* 2022). Because the specific flora available now did not exist when early men discovered the curative properties of plants and were only able to differentiate the plant afterwards (Kirubakari *et al.,* 2019). Higher plants and their extracts have long been utilized in traditional African medicine to treat infectious diseases (Wolde *et al.,* 2018).

Sage (*Salvia officinalis* L.) is a culinary herb that belongs to the Lamiaceae/Labiatae family and is also known as garden sage, common sage, or culinary sage. It is a fragrant perennial woody subshrub endemic to the northern Mediterranean area and widely dispersed over southern Europe's slopes and beaches. It is grown in Spain, Italy, Yugoslavia, Greece, Albania, Argentina, Germany, France, Malta, Turkey, England, and Canada, among other places (Glisic *et al.,* 2010). Its usage as a culinary herb has aided its growth into many nations, and it is currently grown all over the world for dried leaf, which are utilized as raw material in the medical, fragrance, and food industries (Ali *et al.,* 2017). Sage is mostly regarded as a culinary herb in western cookery and has been used in bird stuffing, pork, sausages, and fish flavors. Sage essential oil was utilized in perfumes, deodorants, insecticides, thrush, gingivitis, and as a sedative (Lopresti *et al.,* 2017). The herb is primarily used to improve cognition, but it is also used to treat cardiovascular diseases, excessive sweating, nervous disorders, depression, and cerebral ischemia, as well as to reduce nursing mother's milk when weaning (Imarenezor *et al.,* 2022). It is also recommended for gargling infectious throats and acts as an antiseptic for wounds (Lopresti *et al.,* 2017).

Sage is a potential fragrant plant utilized for food, home treatments, and commercial medicines in both the tropics and temperate regions (Pyrzynska and Sentkowska, 2019). It is a versatile plant, and various research papers have been published to support its traditional use, biological benefits, and method of action. As a result, an attempt has been made to investigate the study on sage's traditional use, growing techniques, chemistry, and medical benefits that have been documented (Pyrzynska and Sentkowska, 2019). The use of medicinal plants to remove pathogenic organisms is becoming more common since these plants include a variety of bioactive compounds that may be beneficial to these pathogens. Several additional plants have antibacterial properties that have been documented (Ighodaro *et al.,* 2018).

*Klebsiella pneumoniae* is a Gram-negative bacterium that is encapsulated, non-motile, and facultatively anaerobic (Caldararo, 2019). Edwin Klebs isolated it from the airways of a dying pneumonia patient in 1875, and Carl Friedländer characterized it in 1882, earning it the moniker Friedlander's bacillus for a period (Verwey *et al.,* 2017).

*Klebsiella* can cause infections in the urinary tract, lower biliary system, and surgical incision sites, in addition to pneumonia. Pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia, and sepsis are among the clinical illnesses (Mulatu *et al.,* 2021). Contamination of the device creates a problem for individuals who have an intrusive device in their body; neonatal ward devices, respiratory support equipment, and urine catheters put patients at risk (Scheen *et al.,* 2020). Additionally, the use of antibiotics may raise the risk of nosocomial infection with *Klebsiella* bacteria. After germs enter the bloodstream, sepsis and septic shock can occur (Mulatu *et al.,* 2021).

Gustav Hauser discovered *Proteus mirabilis* in 1885 (Fallah *et al.,* 2021). *Proteus* got its name from Homer's classic 'The Odyssey' (Daas, 2021). *Proteus mirabilis* is well-known in clinical laboratories and microbiology survey courses as the species that swarms over agar surfaces, quickly displacing any other organisms there. This organism's two distinguishing characteristics are urease synthesis and vigorous swarming movement (Mobley, 2019). This species is a Gram-negative, motile, urease-positive, lactose-negative, indole-negative rod that generates hydrogen sulfide (Algburi *et al.,* 2020). It belongs to the same bacterial family as *E. coli (Enterobacteriaceae)* (Mobley, 2019). *Proteus mirabilis* bacteria are abundantly spread in soil and water in the natural environment (Gufe *et al.,* 2019). *Proteus spp* is present in the natural flora of the human intestine (Shimizu *et al.,* 2019). Its major pathogenic role is in urinary tract infections, although it may also cause wound infections and septicemia (Shimizu *et al.,* 2019). *Proteus mirabilis* will be found in the feces of around one-quarter of the human population (Gufe *et al.,* 2019). It is not a major source of urinary tract infections in anatomically normal urinary tracts, but it frequently invades when normal tract function is disrupted by apparatus such as catheterization (Shimizu *et al.,* 2019). Infection is frequently caused by strains found in the patient's feces (Gufe *et al.,* 2019). It can also be spread between catheter users by care professionals who are in charge of catheter administration.

Antibiotics are medications that are used to treat infectious diseases caused by bacteria (Ramrez-Rendon *et al.,* 2022). Bacteria are becoming less vulnerable to some formulated types of these medications as a result of long-term usage and increasing patient overuse (Nadeem *et al.,* 2020). Bacterial resistance involves numerous processes, including antibiotic efflux, drug degradation by bacterial enzymes, and target protein modification (Miranda *et al.,* 2018). The use of medicinal plants to remove pathogenic organisms is becoming more common since these plants include a variety of bioactive compounds that may be beneficial to these pathogens. Several additional plants have antibacterial properties that have been documented (Ighodaro *et al.,* 2018). *Salvia officinalis* has been used medicinally for centuries. This study is initiated to assess the Comparison and antibiogram of *Salvia Officinalis* Leaf extract against *Klebsiella Pneumoniae* and *Proteus Mirabilis.*

## MATERIALS AND METHODS

### *Study Area and Population*

The study was carried out within Wukari. Wukari is a Local Government Area is in the Southern Senatorial District of Taraba State with headquarter in the town of Wukari on the Federal A4 highway. The Donga River flows through the area and the Benue River forms a boundary with Nasarawa State to the northwest. The town is the base of the Wukari Federation, a traditional state. It has an area of 4,308 km² and a population of 241,546 at the 2006 census. The major languages spoken are Jukun, Kutep, Tiv, Hausa and Fulani (Imarenezor, 2017). The occupation of the inhabitants of the area is basically farming. Although some are civil servants while others are involved in one form of trade or the other.

### *Sample Collection*

*Salvia officinalis* was harvested in Zaria, Kaduna State, Nigeria. After cleaning of the plant material, it was washed with water after the harvest to guarantee the good conservation of the plant and then brought to the Federal University Wukari Taraba State's Microbiology Laboratory in sterile packs.

### *Plant Extraction Preparation*

In the experiment, leaves were employed for extraction. The obtained plant materials were dried in a shady and well aired place for two weeks. Thoroughly sieved and cleansed to eliminate debris and then mashed into small portions with a mortar and pestle before being stored in sterile container. The pulverized plant materials were employed for solvent extraction (ethanol, hot and cold aqueous).

To make an aqueous (water) extract, 10 g of each dried powdered plant material was immersed in 100 ml of distilled water in a sterile conical flask for 48 hours with steady shaking. It was then centrifuged for 10 minutes at 5000 rpm after being filtered through eight layers of muslin cloth. The supernatant was collected and concentrated in a vacuum at temperatures below 40°C using a Heidolph VE-11 rotary evaporator (Misal *et al.,* 2013). The samples were then kept in labeled sterile vials in a freezer at 4 degrees Celsius until further usage (Aneja *et al.,* 2012). Same procedure was done for the hot water extract with the water boiled to 100ºC then mixed with extract and allowed to cold a little before filtering (Aneja *et al.,* 2012).

Preparation of ethanolic extract: The plant powder was prepared in the same manner as described in the preceding section. For hydro-alcoholic extraction, 10 grammes of each dried powdered plant material were steeped in 80 percent ethanol in a sterile conical flask for 48 hours with continual shaking. The supernatant was collected and centrifuged at 5000 rpm for 10 minutes after filtration through 8 layers of muslin cloth, and was then concentrated in a vacuum below 40ºC using a Heidolph VE-11 rotaevaporator to make the final volume half of the original volume (stock solutions) (Misal *et al.,* 2013), and was then stored in labeled sterile bottles in a freezer at 4ºC until further use (Aneja *et al.,* 2012).

### *Preparation of Extract Concentration*

For the synthesis of various concentrations of *Salvia officinalis* leaf extracts. Plant extract stock solutions were made by dissolving 0.2g, 0.4g, 0.6g, 0.8g, and 1g of each crude plant extract in 100ml of dimethylsulphuroxide (DMSO) to achieve 200%, 400%, 600%, 800%, and 1000%

concentrations in mg/ml of the extract, respectively. These concentrations were stored in a container and labeled for future use.

***Preparation of Test Organisms***

The reference bacterial species, *Klebsiella pneumoniae* (ATCC 13884) and *Proteus mirabilis* (ATCC 29906), were obtained from the National Veterinary Research Institute in Vom, Plateau State, Nigeria. Following that, each organism was sub-cultured on nutrient agar. CLSI guidelines were followed for all laboratory work (Kebede *et al.,* 2021).

***Preparation of Media***

Mueller Hinton agar was used, and it was prepared per the manufacturer's instructions. The agar was made by dissolving 7 g of each agar in 250 m of distilled water in a separate conical flask. The media were then autoclaved for 15 minutes at 121°C. After allowing the sterilized medium to cool to 45°C, roughly 20 ml was placed into a sterile petri plate and allowed to gel (Kebede *et al.,* 2021).

***Antibacterial Activity***

The antibacterial activity of *Salvia officinalis* extracts (Aqueous and Ethanolic) against the test organisms was determined using the susceptibility test agar well diffusion technique (Balouiri *et al.,* 2016). The standard organisms were sub-cultured into peptone liquid broth for 18-24 hours. The turbidity of pure fresh cultures was calibrated using McFarland turbidity standards of 0.5. Later, the isolates were sub-cultured on Mueller-Hinton agar. A sterile 6 mm cork borer was then used to bore wells into the agar medium. On each agar plate, 0.2 mls of the extracts were put to each well, and the wells were then filled with the extract solution, taking care not to allow the solution to spill to the medium's surface. The plates were incubated at 37°C for 24 hours before being tested for antibacterial effectiveness against the test species using a sensitivity disc containing common antibiotics. Before incubating the plates in an incubator at 37°C for 24 hours, the plates were allowed to stand on the laboratory bench for 1-2 hours to allow appropriate inflow of the solution into the medium. The zones of inhibition were afterwards noticed on the plates by measuring the diameters of the zones of inhibition to the closest millimeter (Barnard, 2019).

***Statistical Analysis***

Three duplicates of each experiment were carried out twice for every single experiment. In the tests involving cell culture, duplicates of the cell replicate were utilized on two different occasions. Data were analyzed using analysis of variance (ANOVA) and treatment means were separated using Duncan's multiple range test. All data are presented as the mean ± SEM and a value of $P<0.05$ was considered to indicate a statistically significant difference.

**Results**

The results of the study are as represented in tables below. **Table 1** represents the cold and hot water extracts of the dry leaf of *Salvia officinalis* against *Klebsiella pneumoniae*. The hot water extract (24.17 ± 0.88 mm) was not significantly higher than the cold extract (21.50±0.58 mm) at the highest concentration of 1000 mg/ml. As same concentration the positive control (gentamicin) (25.17±0.88 mm) was shown to significantly higher than the cold dry extract but no significantly difference with the hot water dry extract. **Table 2** represents the cold and hot water extracts of the fresh leaf of *Salvia officinalis* against *Klebsiella pneumoniae*. The hot water extract (19.33±0.60 mm) was significantly not higher than the cold extract (15.33±0.60 mm) at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (25.17±0.88 mm) was shown to significantly higher than both the cold fresh extract and the hot water fresh extract. **Table 3** represents the result that indicates the cold and hot water extracts of the dry leaf of *Salvia officinalis* against *Proteus mirabilis.* The hot dry leaf water extract gave a zone of inhibition of (23.00±1.15 mm) which was significantly higher than the cold water dry leaf extract (19.33±0.60 mm) at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (22.33±1.17 mm) was shown to have no significant difference with both the cold dry extract and the hot water dry extract.. **Table 4** represents the effect of the cold and hot water extracts of the fresh leaf of *Salvia officinalis* against *Proteus mirabilis.* The hot water fresh leaf extract zone of inhibition of (21.33±0.60 mm) was significantly higher than the cold water fresh leaf extract (15.43±0.58 mm) against *Proteus* at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (22.33±1.17 mm) was shown to have no significant difference with the hot water fresh leaf extract but have significant difference with the cold water fresh leaf extract.

There is no significant difference between the effect of the fresh leaf water extracts and the dry leave water extracts against *Proteus mirabilis***. Table 5** below represents the result that indicates the effects of ethanolic extracts of the dry leaf of *Salvia officinalis* against *Klebsiella pneumonia.* The ethanolic dry leaf extracts (28.00 ±0.58 mm) was significantly higher than the cold dry leaf (21.50±0.58 mm) but have no significant difference with hot water dry leaf extract (24.17±0.88 mm) and the positive control gentamicin (25.17±0.88 mm) at the highest concentration of 1000 mg/ml. The ethanolic fresh leaf extracts (24.33±0.60 mm) was significantly higher than both the cold fresh leaf (15.50±0.58 mm) and hot water fresh leaf extract (19.33±0.60 mm) but have no significant difference with the positive control gentamicin (25.17±0.88 mm) at the highest concentration of 1000 mg/ml. **Table 6** below represent the result that indicates the ethanolic extracts of the dry leaf of *Salvia officinalis* against *Proteus mirabilis*. The ethanolic extracts (26.00 ±0.58 mm) was significantly higher than the cold water dry leaf (19.33±1.17 mm) and hot water dry leaf extract (23.00±1.15 mm) with no significant difference with the control, gentamicin (22.33±1.17) at the highest concentration of 1000 mg/ml. The ethanolic fresh leaf extracts (24.33 ±0.58 mm) was significantly higher than the cold fresh leaf (15.43±0.58 mm) but have no significant difference with hot water fresh leaf extract (21.33±0.60 mm) and the positive control gentamicin (22.33±1.17 mm) at the highest concentration of 1000 mg/ml.

The ethanolic extract of the dry leave of *Salvia officinalis* was shown to be more efficient against *Klebsiella pneumonia* and *Proteus mirabilis* than the ethanolic extract of the fresh leaf**.**

**Table 1: Effects of hot and cold water extract of dry leaf of Salvia officinalis on Klebsiella pneumoniae**

| Concentration (mg/mL) | *Klebsiella pneumonia* | | | |
|---|---|---|---|---|
| | Cold water dry leaf extract (mm) | Hot water dry leaf extract (mm) | Negative control (No extract) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 9.50±0.29$^{d}$ | 12.00±0.29$^{d}$ | NZI | 13.33±0.60$^{e}$ |
| **400** | 10.67±0.60$^{d}$ | 15.00±0.76$^{c}$ | NZI | 16.43±0.58$^{d}$ |
| **600** | 13.33±0.60$^{c}$ | 17.00±0.87$^{c}$ | NZI | 20.33±0.73$^{c}$ |
| **800** | 17.17±0.60$^{c}$ | 21.00±0.76$^{b}$ | NZI | 22.83±0.88$^{b}$ |
| **1000** | 21.50±0.58$^{a}$ | 24.17±0.88$^{a}$ | NZI | 25.17±0.88$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Table 2: Effects of hot and cold water extract of fresh leaf of *Salvia officinalis* on *Klebsiella pneumoniae***

| Concentration (mg/mL) | *Klebsiella pneumonia* | | | |
|---|---|---|---|---|
| | Cold water extraction Fresh leaf (mm) | Hot water extraction Fresh leaf (mm) | Negative control (No extracts) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 7.50±0.58$^{d}$ | 8.33±0.60$^{e}$ | NZI | 13.33±0.60$^{e}$ |
| **400** | 9.00±0.87$^{cd}$ | 11.10±0.87$^{d}$ | NZI | 16.43±0.58$^{d}$ |
| **600** | 10.33±0.60$^{c}$ | 14.00±0.87$^{c}$ | NZI | 20.33±0.73$^{c}$ |
| **800** | 13.00±0.87$^{b}$ | 16.33±0.60$^{b}$ | NZI | 22.83±0.88$^{b}$ |
| **1000** | 15.33±0.60$^{a}$ | 19.33±0.60$^{a}$ | NZI | 25.17±0.88$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Table 3: Effects of hot and cold water extract of dry leaf of *Salvia officinalis on Proteus mirabilis***

| Concentration (mg/mL) | *Proteus Mirabillis* | | | |
|---|---|---|---|---|
| | Cold water extraction dry leaf (mm) | Hot water extraction dry leaf (mm) | Negative control (No extracts) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 7.23±0.96$^{d}$ | 9.27±0.72$^{e}$ | NZI | 12.00±0.87$^{c}$ |
| **400** | 9.10±0.87$^{d}$ | 13.33±0.60$^{d}$ | NZI | 15.00±0.87$^{b}$ |
| **600** | 12.33±0.60$^{c}$ | 17.33±0.60$^{c}$ | NZI | 19.00±1.15$^{b}$ |
| **800** | 15.33±0.60$^{b}$ | 20.33±0.88$^{b}$ | NZI | 21.00±0.87$^{a}$ |
| **1000** | 19.33±1.17$^{a}$ | 23.00±1.15$^{a}$ | NZI | 22.33±1.17$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Table 4: Effects of hot and cold water extract of fresh leaf of Salvia officinalis on Proteus mirabillis**

| Concentration (mg/mL) | *Proteus Mirabillis* | | | |
|---|---|---|---|---|
| | Cold water extraction fresh leaf (mm) | Hot water extraction fresh leaf (mm) | Negative control (No extracts) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 7.33±0.60$^{d}$ | 8.03±0.58$^{e}$ | NZI | 12.00±0.87$^{c}$ |
| **400** | 9.43±0.58$^{c}$ | 10.33±0.60$^{d}$ | NZI | 15.00±0.87$^{c}$ |
| **600** | 10.33±0.60$^{c}$ | 14.33±0.60$^{c}$ | NZI | 19.00±1.15$^{b}$ |
| **800** | 13.43±0.58$^{b}$ | 17.03±0.58$^{b}$ | NZI | 21.00±0.87$^{ab}$ |
| **1000** | 15.43±0.58$^{a}$ | 21.33±0.60$^{a}$ | NZI | 22.33±1.17$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Table 5: Effects of ethanolic extract of dry and fresh leaf of *Salvia officinalis* on *Klebsiella pneumoniae***

| Concentration (mg/mL) | *Klebsiella pneumonia* | | | |
|---|---|---|---|---|
| | Ethanolic Extracts dry leaf (mm) | Ethanolic Extracts Fresh leaf(mm) | Negative control (No extracts) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 15.33±0.60$^{e}$ | 13.00±0.58$^{d}$ | NZI | 13.33±0.60$^{d}$ |
| **400** | 18.33±0.60$^{d}$ | 16.00±0.58$^{c}$ | NZI | 16.43±0.58$^{c}$ |
| **600** | 22.00±0.58$^{c}$ | 17.33±0.60$^{c}$ | NZI | 20.33±0.73$^{b}$ |
| **800** | 25.00±0.58$^{b}$ | 21.17±0.44$^{b}$ | NZI | 22.83±0.88$^{a}$ |
| **1000** | 28.00±0.58$^{a}$ | 24.33±0.60$^{a}$ | NZI | 25.17±0.88$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Table 6: Effects of ethanolic extract of dry and fresh leaf *of Salvia officinalis* on *Proteus mirabilis***

| Concentration (mg/mL) | *Proteus mirabilis* | | | |
|---|---|---|---|---|
| | Ethanolic Extracts dry leaf (mm) | Ethanolic Extracts Fresh leaf (mm) | Negative control (No extracts) (mm) | Positive control (Gentamicin) (mm) |
| **200** | 12.33±0.60$^{d}$ | 10.00±0.58$^{e}$ | NZI | 12.00±0.87$^{c}$ |
| **400** | 14.00±0.58$^{d}$ | 12.33±0.60$^{d}$ | NZI | 15.00±0.87$^{c}$ |
| **600** | 19.33±0.60$^{c}$ | 16.33±0.60$^{c}$ | NZI | 19.00±1.15$^{b}$ |
| **800** | 23.00±0.87$^{b}$ | 19.00±0.58$^{b}$ | NZI | 21.00±0.87$^{ab}$ |
| **1000** | 26.00±0.58$^{a}$ | 24.33±0.60$^{a}$ | NZI | 22.33±1.17$^{a}$ |

Key: NZI= No zone of inhibition, x= highest zone diameter of inhibition

Mean with the same alphabets ($^{a\ b\ c\ d}$) in a column are not significantly different.

**Discussion**

Many scientists have reported on the medicinal use of plants, particularly as antibacterial (Modi *et al.,* 2022). *Salvia officinalis* antibacterial activity reports demonstrate varying amounts of microbial growth suppression against various pathogens (Barbălată-Mândru *et al.,* 2022). The plant leaf extract has been shown in studies to be effective against pathogenic bacteria such as *Staphylococcus aureus, Bacillus subtilis, Streptomyces alboniger, Micrococcus luteus, Staphylococcus epidermis, Pseudomonas aeruginosa and Bordetella bronchiseptica* (Kebede *et al.,* 2021). *Klebsiella pneumoniae* and *Proteus mirabilis* are significant pathogens that cause a variety of clinical illnesses, including urinary tract infection, pneumonia, skin and soft tissue infections, and bacteremia septicemia (Hashiguchi *et al.,* 2020).

The antibacterial activity of ethanolic, cold and hot water extract was determined in this study. The result of the ethanol extract of *Salvia officinalis* exhibited inhibitory effects against *klebsiella pneumonia* with zone of inhibition ranging from 15 mm to 28 mm for the dry leaf extract and 13 mm to 24 mm for the fresh leaf extract which was similar to the study carried by Benabdesslem *et al.* (2020) which reported the zone of inhibition of ethanol extract of *Salvia officinalis* against *Klebsiella pneumonia* varying from 8 to 20 mm. The hot water dry leaf extract (24.17 ± 0.88 mm) was not significantly higher than the cold water dry leaf extract (21.50±0.58 mm) at the highest concentration of 1000 mg/ml. As same concentration the positive control (gentamicin) (25.17±0.88 mm) was shown to significantly higher than the cold dry extract but no significantly difference with the hot water dry extract against *Klebsiella pneumoniae* (Table 1). The hot water fresh leaf extract (19.33±0.60 mm) was significantly not higher than the cold water fresh leaf extract (15.33±0.60 mm) at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (25.17±0.88 mm) was shown to significantly higher than both the cold fresh leaf extract and the hot water fresh leaf extract against *Klebsiella pneumoniae* (Table 2). *Klebsiella pneumoniae* was more sensitive to both water and ethanolic extracts of the dry and fresh leaf of *Salvia officinalis* than *Proteus mirabilis*, with the ethanolic extract of dry leaf having the highest zone of inhibition (28 mm) against *Klebsiella pneumoniae*, as similarly reported by Benabdesslem *et al.* (2020), with range of inhibition varying from 19 to 24 mm.

Against *Proteus mirabilis*, the hot water dry leaf extract gave a zone of inhibition of (23.00±1.15 mm) which was significantly higher than the cold water dry leaf extract (19.33±0.60 mm) at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (22.33±1.17 mm) was shown to have no significant difference with both the cold dry extract and the hot water dry extract (Table 3). The hot water fresh leaf extract zone of inhibition of (21.33±0.60 mm) was significantly higher than the cold water fresh leaf extract (15.43±0.58 mm) against *Proteus mirabilis* at the highest concentration of 1000 mg/ml while the positive control (gentamicin) (22.33±1.17 mm) was shown to have no significant difference with the hot water fresh leaf extract but have significant difference with the cold water fresh leaf extract (Table 4). There is no significant difference between the effect of the fresh leaf water extracts and the dry leave water extracts against *Proteus mirabilis* as similar reported was made by

Pogorzelska-Nowicka *et al.* (2021), who worked on the effect of cold plasma pretreatment on water-suspended herbs measured in the content of bioactive compounds, antioxidant activity, volatile compounds and microbial count of final extracts.

The ethanolic dry leaf extracts (28.00 ±0.58 mm) was significantly higher than the cold dry leaf (21.50±0.58 mm) but have no significant difference with hot water dry leaf extract (24.17±0.88 mm) and the positive control gentamicin (25.17±0.88 mm) at the highest concentration of 1000 mg/ml (Table 5). The ethanolic fresh leaf extracts (24.33±0.60 mm) was significantly higher than both the cold fresh leaf (15.50±0.58 mm) and hot water fresh leaf extract (19.33±0.60 mm) but have no significant difference with the positive control gentamicin (25.17±0.88 mm) at the highest concentration of 1000 mg/ml (Table 6). Similar study was reported by Benabdesslem *et al.* (2020), which showed the ethanolic extract of *Salvia officinalis* leaf with zone of inhibition of greater than 20 mm.

The ethanolic extracts (26.00 ±0.58 mm) was significantly higher than the cold water dry leaf (19.33±1.17 mm) and hot water dry leaf extract (23.00±1.15 mm) with no significant difference with the control, gentamicin (22.33±1.17) at the highest concentration of 1000 mg/ml. The ethanolic fresh leaf extracts (24.33 ±0.58 mm) was significantly higher than the cold fresh leaf (15.43±0.58 mm) but have no significant difference with hot water fresh leaf extract (21.33±0.60 mm) and the positive control gentamicin (22.33±1.17 mm) at the highest concentration of 1000 mg/ml (Table 6). According to Pogorzelska-Nowicka *et al.* (2021) and Imarenezor *et al.*, (2022), both test organisms were responsive to the ethanol, cold and hot water extract of both dried and fresh leaf of *Salvia officinalis*. The antibacterial activity of ethanolic extracts is higher than that of cold and hot water extracts of dry and fresh *Salvia officinalis* leaf (Kebede *et al.,* 2021). The inhibitory zone expands as concentration increases gradually.

Using the leaf extract at a concentration of 1000 mg/ml, *Klebsiella pneumoniae* was more susceptible to the ethanolic extract of the dry leaf (28.0 ± 0.60 mm) than *Proteus mirabilis* with zones of inhibition of 26.00 ± 0.58 mm (dry leaf extract) while the control, gentamicin was 25 17 ± 0.88mm. The ethanol fresh leaf extract (24.33 ± 0.60 mm) against *Klebsiella pneumoniae* and 24.33 ± 0.60 mm (ethanol fresh leaf extract) against respectively. The zone of inhibition of the positive control, gentamicin against *Klebsiella* pneumoniae and 22.33 ± 1.177 mm against *Proteus* mirabilis was higher than of the cold and hot water (dry and fresh) extract but no significantly different ($P<0.05$) than the ethanol (dry and fresh) extract. The test organisms were more susceptible to the dry leaf extracts of *Salvia officinalis* than the fresh leaf extracts. *Klebsiella pneumoniae* was more susceptible to antibiogram activity of the leaf extract than *Proteus mirabilis* with gradual increase in concentrations from 200mg/ml to 1000mg/ml. With increase in concentrations, zone of inhibition also increased as was also reported by Barbălată-Mândru *et al.* (2022) and Imarenezor *et al*., (2022) who obtained similar findings when biomaterials were discovered to have antibacterial properties when poly (vinyl alcohol) was combined with the extracts obtained from various selected plants from Romania.

**Conclusions**

Plant extracts and plant-based chemicals are excellent antibacterial agents that have little impact on the host cell. Furthermore, they are ecologically benign, renewable, and sustainable. This study backs up the traditional usage of *Salvia officinalis* for the treatment of several drug uropathogenic disorders including diseases caused by *Klebsiella pneumoniae* and *Proteus mirabilis* and suggests that it might be a promising source of new bioactive chemicals. Both *Klebsiella pneumoniae* and *Proteus mirabilis* are sensitive to *Salvia officinalis* dried and fresh leaf extracts in this study. Therefore, *Salvia officinalis* has the potential to be employed as an alternative antibacterial agent against *Klebsiella pneumoniae* and *Proteus mirabilis* infections. In the agar well diffusion technique, the dry leaf of *Salvia officinalis* in the solvents used (ethanol, cold and hot water) showed more effectiveness than the fresh leaf extract as active phytochemicals can be easily extracted due to high polarity of the dry leaf in contrast to the fresh leaf . Ethanolic extract of sage also showed a higher inhibitory impact on the pathogens than water extracts while there was no significant difference between the ethanol extract and the control, gentamicin which indicated that at proper dosage the ethanol dry leaf extract has the potential to be used as a more effective mixture in tackling diseases or infections caused by the test organisms. The study scientifically confirms the usefulness of plant product of *Salvia officinalis* in the production of an effective antibacterial agent, but more research is needed to improve the action of plant extracts.

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