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# Identification and Authentication of Microbes Causing urinary tract infection and Detection of Antibacterial Activity for Methanolic Extract of *Senna alexanderina* against these Pathogenic Bacteria in Khartoum State, Sudan

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**Abstract:** The aim of this study is to isolate and identify the microbes causing urinary tract infection and antibacterial of those microbes by used methanolic extract of plant and antibiotics. One hundred samples were collected for both genders in Khartoum State. From sixty-three out of one hundred samples obtained on different types of microbes are *Staphylococcus aureus* (33.3%), *Enterococcus fecales* (9.5%), *Escherichea coli* (19%), *Klebsiella pneumonae* (7.9%), *Protus mirabilis*, *Pseudomonas aeuroginosa* (9.5%), *Citrobacter ssp* (4.7%), *Candida .albicans* (7.9%). The antibacterial results against isolated microorganisms using methanolic extract of *Senna alexanderina* showed resistance to these microbes except S. aureus was sensitive; also, most microbes were sensitive to antibiotics.

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**Keywords:** urinary tract infection, methanolic extract, *Senna alexanderina*, Pathogenic Bacteria, Microbes

## Introduction:

Urinary Tract Infection represents one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today. It is estimated that about 35% of healthy women suffer symptoms of Urinary tract infection at some stages in their life. The incidence of UTI is greater in women as compared to men, which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (Sharma *et al*., 2009). An antimicrobial compound is a substance that kills or inhibits the growth of bacteria. Some plants have been investigated significantly for their antimicrobial activity and as large numbers of plant products have been shown to inhibit the growth of pathogenic microorganism. In recent years, works in medical field return back to nature particularly in the use of medical plants treat human ailments, this now trend was supported by the recent World Health Organization, orientation strategy that embarked on examination of historical position of modernization of biochemically based health care (WHO,1988 and Dole,2004) Consequently, medical practitioners are also prescribing herbal medicine teas and herbal extracts as a supplementary type of treatment in everyday problems caused by our modern civilization (Gomez *et al*., 2007) .Sudan has an immense diversity and variation in vegetation and is one of the richest countries with regard to phytopharmaca. Although herbal remedies are often perceived as being natural and, therefore, safe, they are not principally free from adverse effects. While many investigations of the quality values of medicinal plants are being reported in the current literature, less emphasis has been made on the metal content of herbal products (Gomez *et al*., 2007). Such as renal failure, symptoms of chronic toxicity and liver damage (Gomez *et al*., 2007). The aim of this study is to investigate the antimicrobial activity of *Alexandrian senna* (leaves) to ascertain the rationale for its use in traditional medicine.

## Material and Method:

### Study Area:

This study was conducted at Khartoum State, Sudan. Samples were taken from patients admitted at Khartoum Teaching Hospital and Omdurman Teaching Hospital.

### Samples Collection

Hundred individuals were recruited, for taking urine randomly irrespective of sex and age.

### Data collection

Data were collected from the patients using structured questionnaire involving ; age , sex.

**Identification of Isolated bacterial**
The colonial morphology and fermentation of lactose were examined macroscopically on Cystine lactose Electrolyte Deficient (CLED), this was observed by yellow color. Then uses staining test (gram stain) and biochemical test such as (Indole, Citrate Utilization, Urease, $H_2S$ production).

**Plant material:**
The leaves of *Alexandrian senna* were collected from the local market in Khartoum. The plant was identified in the microbiology department, Faculty of Pure and Applied Sciences, International University of Africa by. Ahmed Elshikh and by comparison with herbarium of the department. The dried plant samples were cleaned from dust and grass then they were separately crushed to a powder by using sterilized mortar and pestle.

**2. Preparation of plant extracts:**
Fifty grams from *Alexandria senna* were extracted into 500 ml methanol. Resulting extraction in the solvent was evaporated and concentrated using the rotary evaporator at 50 C°. (Abeysinghe, 2010).

**Preparation of bacterial suspensions:**
One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about $10^8$- $10^9$ C.F.U./ml. The suspension was stored in a refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension (C.F.U. /ml). Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

***In vitro* testing of extracts for microbial activity:**
The cup-plate agar diffusion method was adopted according to (Kavanagh, 1972) with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension $10^8$–$10^9$ C.F.U/ml were thoroughly mixed with 100ml of sterile molten nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar were left to dry and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic Microlitre-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extracts against each of the test organisms. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the resultants and growth inhibition zones were measured averaged and the mean values were tabulated.

**Analysis of Data**
Data were collected from the patients using structured questionnaire involving; age, sex. Data were analyzed using Microsoft Office Excel 2007.

**Result and Discussion**
Sixty six patients (66%) were collected from Khartoum Hospital and thirty four (34%) from Omdurman Hospital as shown in (figure 1). In addition, the sex ratio was determined for both sexes, where the proportion of females was 67% and the proportion of males 33% as shown in (figure 2). The frequency of bacterial growth was determined for the samples collected, where the percentage of bacterial growth (63%) and ratio of samples that did not grow (37%) as shown in (figure 3). The gram reaction of isolated bacteria revealed that (30) isolates were gram positive and (33) were gram negative shown in (figure 4).

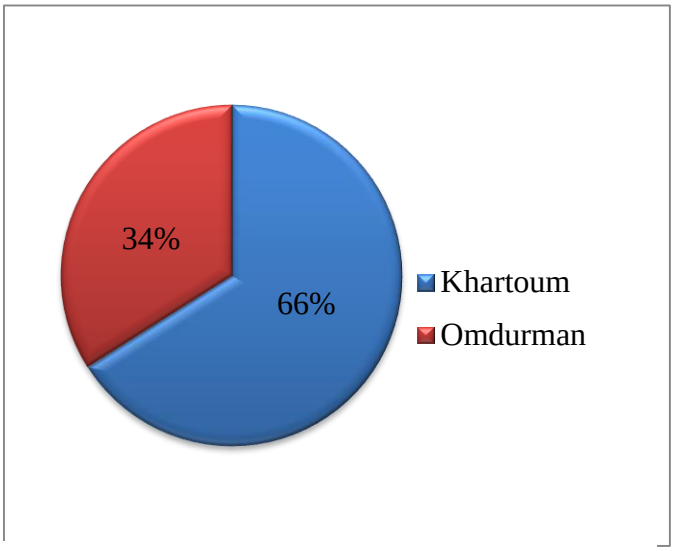


**Figure (1): Distribution of Specimens according to hospital**

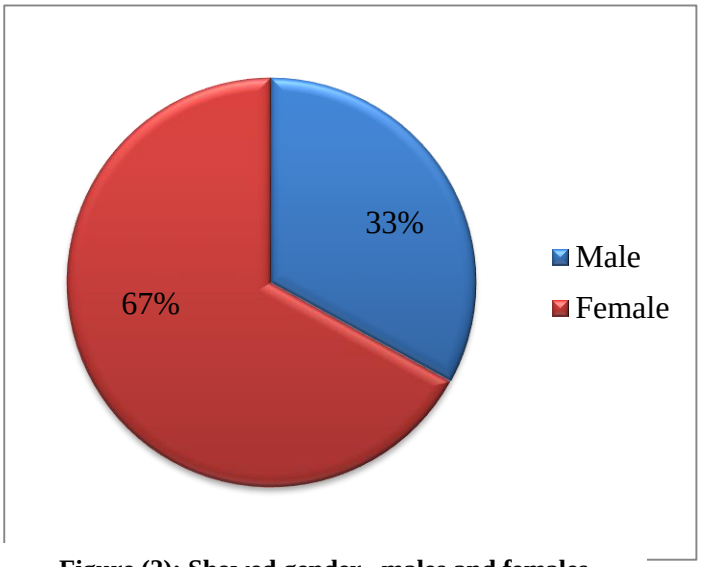


**Figure (2): Showed gender males and females distribution**

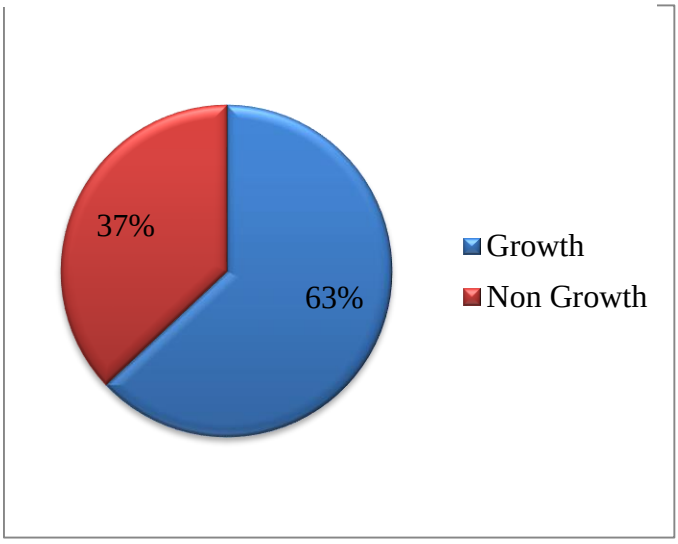


**Figure (3): the Frequency of bacterial growth**

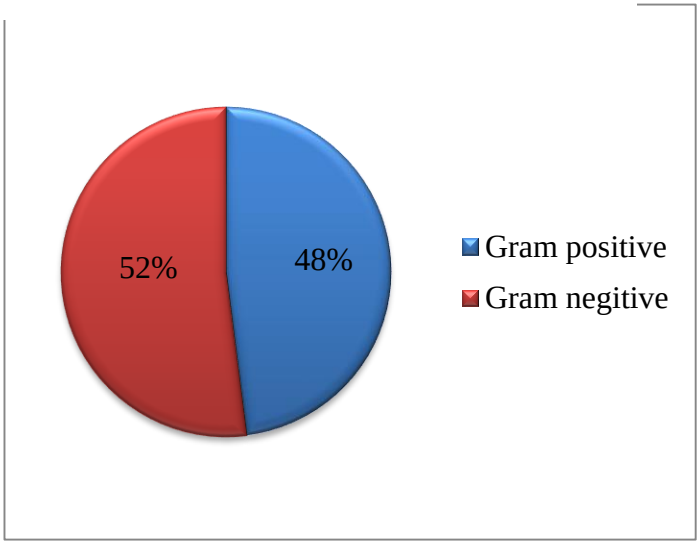


**Figure (4): Frequency the Gram reaction for isolated bacteria**

The isolated causative agents were identified to be *Staphylococcus aureus* (33.3%), *Klebsiella pneumonae* (7.9%), *Protus mirabilis* (11.1%), *Enterococcus fecales* (9.5%), *Escherichea coli* (19%), *Pseudomonas aeuroginosa* (9.5%), *Candida .albicans* (7.9%) and *Citrobacter* (4.7%) Table (1) and figure (5).

**Table (1) Type of isolated bacteria and the percentage**

| Bacteria | Frequency | Percentage |
|---|---|---|
| *Staphylococcus aureus* | 21 | 33.3% |
| *Enterococcus fecales* | 4 | 6.3% |
| *Escherichea coli* | 12 | 19% |
| *Klebsiella pneumonae* | 5 | 7.9% |
| *Protus mirabilis* | 7 | 11.1% |
| *Pseudomonas aeuroginosa* | 6 | 9.5% |
| *Citrobacter spp* | 3 | 4.7% |
| *Candida .albicans* | 5 | 7.9% |
| **Total** | **63** | **100%** |

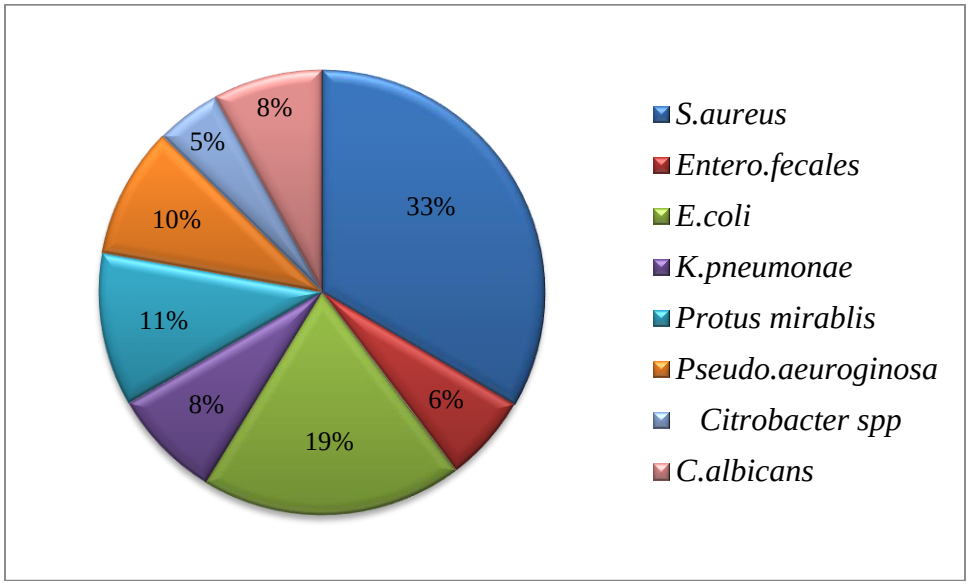


**Figure (5): Type of microbial isolated and percentage**

Antibacterial activity of *Senna alexandrina* of isolated bacteria organisms is shown in (Table 2) using different concentration of the methanol extracts (100, 50, 25, 12,5, 6,2 mg/ml), The highest activity of the methanolic extract was in the concentration (100%) against *S. aureus* (15 mm), while no good activity was shown against other organisms and other concentrations used. Compared with reference drugs (Table 3) and (figure 6), most of them were of active than methanolic extract of *Senna alexandrina*, except for amoxicillin, where *E. coli*, *K. pneumonae*, *Ps. aeroginosa* and *Citrobacter* were resistant to it, whereas *E. fecales* and *Prot. mirablis* were half-sensitive to it. In addition, *Ps. aeruginosa* and *Citrobacter* showed resistance against Co-trimoxa, while *K. pneumoniae* was half-resistant.

**Table (2) Number of isolated bacteria and their minimum inhibitory Conc. (MIC) of methanolic extract of *Senna alexandrina* leaves at different concentrations**

| Bacteria isolated | Concentrations of extract (mg/ml) and MDIZ | | | |
|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 |
| *Staphylococcus aureus* | 15 | 4 | 2 | - |
| *Enterococcus fecales* | 4 | - | - | - |
| *Escherichea coli* | 3 | 5 | 4 | - |
| *Klebsiella pneumonae* | 6 | - | - | - |
| *Protus mirabilis* | 4 | 3 | - | - |
| *Pseudomonas aeuroginosa* | 2 | 3 | - | - |
| *Citrobacter spp* | 2 | 1 | - | - |

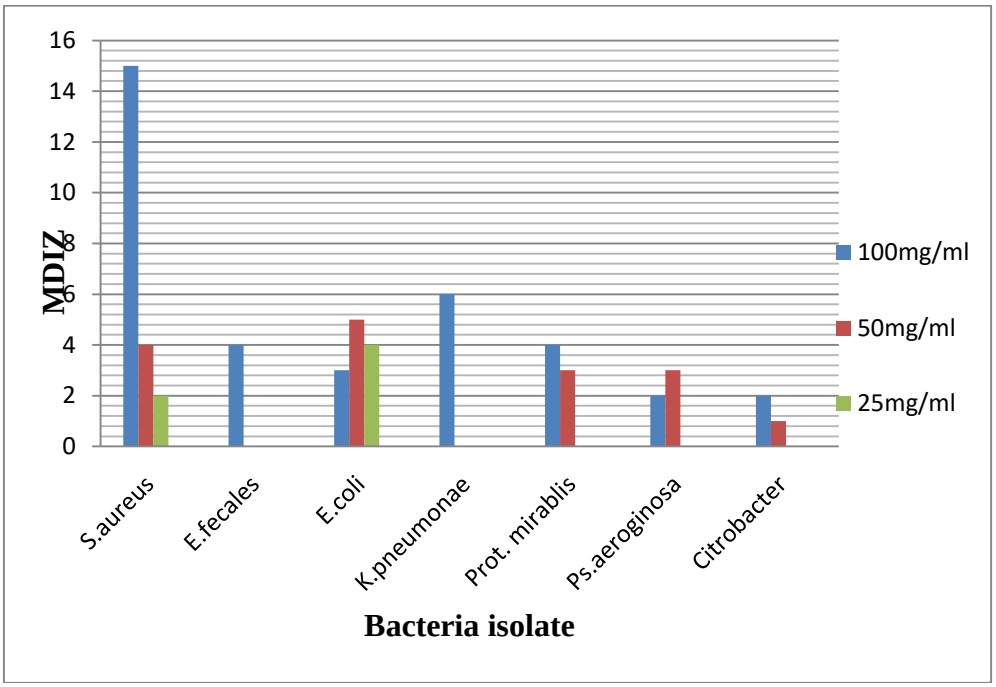


**Figure (6): Antibacterial activity of methanolic extract of *Senna alexandrina* (leaves) at different concentrations**

**Table (3) Antibacterial activity of reference drugs against isolated bacteria**

| Bacteria | Ciprofloxacin | Gentamicin | Amoxacillin | Co-trimoxa. |
|---|---|---|---|---|
| *Staphylococcus aureus* | S | S | S | S |
| *Enterococcus fecales* | S | S | S/R | S |
| *Escherichea coli* | S | S | R | S |
| *Klebsiella pneumonae* | S | S | R | S/R |
| *Protus mirabilis* | S | S | S/R | S |
| *Pseudomonas aeuroginosa* | S | S | R | R |
| *Citrobacter spp* | S | S | R | R |

* S= Sensitive, R= Resistant .

**Dissection:**

Bacterial infection is one of the most serious global health issues in 21st century. The emergence of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems (Sharma *et al*., 2009). In the modern world, multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Saranraj and Sivasakthivelan, 2012). According to result the percentage of *S. aureus*, *E. coli* and Proteus mirabilis was higher in the studied samples. These results differed from those obtained by (Sharma et al., 2009). The antibacterial results of methanolic extract of *Senna alexandrina* showed a poor activity against isolated bacteria. These results are similar to those of (Sami), who used different solvents of Senna alexandrina against some of the standard bacteria used in this study. While (Viswanathan and Nallamuthu, 2012) reports have shown the effectiveness of the methanolic extract of *Senna alexandrina* against *E. coli* and *P. aeruginosa*, these results are different from those of the present study. Results of antibiotic susceptibility showed that nearly all the isolates were sensitive and other resistant against most of the antibiotics tested during the present investigation. The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements/plasmids (Sharma *et al*., 2009).

**Conclusion:**

This study revealed some microbes causing urinary tract infection that were isolated and identified and activity of methanolic extract of *Senna alexanderina*

was evaluated for these microbes, as well as their sensitivity to antibiotics.

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