

The antibacterial activity of the traditionally used *Cymbopogon schoenanthus* and *Senna holosericea*, collected from Alabwa region, Saudi Arabia

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Abstract: The biodiversity of medicinal plants have many economic importances and improve human health. Many biologically active compounds were isolated from plants that were traditionally used in folkloric medicine. These compounds can be either a new drug or a base for new drug. The antibacterial activity of the traditionally used *Cymbopogon schoenanthus* and *Senna holosericea*, collected from Alabwa region, Saudi Arabia was determined using agar well diffusion method. The plants were collected, identified and extracted using both different organic solvents (methanol, ethanol and acetone) and hot water. The tested bacteria were multidrug resistant, *Escherichia coli*, *Micrococcus lutes*, *Klebsiella pneumoniae*, and *Shigella sonnei*. Maximum activity was recorded for the methanolic extract *Senna holosericea* against all tested bacteria with inhibition zone diameter ranged from 31-35 mm and MIC value of 37.5 µg/ml. Lower activities was recorded for the methanolic extract of *Cymbopogon schoenanthus* against all tested bacteria with MIC ranged from 37.5 -70 µg/ml. Weak antimicrobial activity or no activities were recorded for hot water extracts of the two tested plants. No toxicity was recorded for the methanolic extracts of the two tested plants using *Artemia salina* as test organism. In conclusion, the two tested plants, used traditionally in medicine by popular, recorded antimicrobial activities against the multidrug resistant bacteria with no toxicity.

Keywords: *Artemia salina*, *Cymbopogon schoenanthus*, *Senna holosericea*, antibiotic, extract

I. Introduction

Out of 7,000 recognized plants all over the world, only 900 plants recorded medical importance (Joshi *et al.*, 2011). Moreover, the flora of Saudi Arabia is rich with the medicinal plants (Rahman *et al.*, 2004) which provide many secondary products and drugs (Edith *et al.*, 2005). In the last years, using plant materials or their extracts in alternative and complementary medicine has increased and many antimicrobial agents have been discovered and identified (Kaur and Arora, 2009). About 70 -140 species of the genus *Cymbopogon*, grown in tropical and subtropical regions (Khanuja *et al.*, 2005) and can be used in different industries (Khanuja *et al.*, 2005). Two species of the genus *Cymbopogon* were recorded in Saudi Arabia, *C. commutatus* and *C. schoenanthus* (Chaudhary, 1999) which found in several regions of Saudi Arabia (Migahid, 1996) and used by popular to treat people with kidney or as antibacterial and antifungal agents (Hilo, 1996). Medicinal plants are rich in phytochemicals including flavonoids, tannins, terpenoids, and glycosides which have antibacterial activities against Gram positive and Gram negative bacterial isolates (Khalid *et al.*, 2011). Balasankar (2013) detected that anthraquinones of this herb can inhibit a variety of bacteria (staphylococci and bacillus) and dermatomyces (*Microsporum audouini*). *Senna holosericea* (Fresen.) Greuter is a small shrub, contained anthranoides derivatives, belong to Fabaceae and used to treat constipation (Ghazanfar, 1994, Nadal *et al.*, 2003). The antibacterial activity of methanol, n-hexane and aqueous extracts of *Senna* was revealed to the presence of alkaloids, steroids and flavonoids (Kumar *et al.*, 2009) which showed strong activity against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. The previous result suggested that this plant has therapeutic effect and can be used in curing human diseases. Purification and identification of the active compounds in the plant material or its extracts must be carried out. The aim of this study was studying the antibacterial activity of the traditionally used *Cymbopogon schoenanthus* (L.) Spreng and *Senna holosericea* (Fresen.) Greuter, collected from Alabwa region, Saudi Arabia.

II. Material and Methods

Plant materials

Healthy plants of *Cymbopogon schoenanthus* and *Senna holosericea* were collected from the Alabwa, Kingdom of Saudi Arabia during winter 2016. All the plant materials were identified at Biology Department, Faculty of Science, KAU, Jeddah, A voucher specimen was deposited in the herbarium, Faculty of Science, KAU, Jeddah.

Preparation of plant extracts

The collected *Cymbopogon schoenanthus* and *Senna holosericea* were washed individually with distilled water, oven dried for 24 hrs at 60°C, cut into small pieces, and grinded into fine powder using electrical blinder. About 30 g of each dried plant was extracted using 250 ml of hot water or organic solvents (methanol, ethanol, and acetone) for 24 hrs. The slurry was filtered using a sterile filter paper, and the obtained extract was concentrated on a rotary evaporator at 42°C until dry and the residue was dissolved in 1 ml DMSO and was kept in sterile bottle under refrigerated conditions until use.

Pathogenic bacterial strains

Standard local pure culture of *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumonia*, and *Shigella sonnei* were provided by King Abdulaziz Hospital, Jeddah, Saudi Arabia. All cultures were checked up again for purity.

Antimicrobial activities

Antimicrobial activities of the obtained extracts were studied using Muller Hinton agar plates and Agar well diffusion method (Holder and Boyce, 1994). Further, susceptibility of the tested bacteria to different antibiotics was determined on Muller Hinton agar using the method described by Bauer *et al* (1966). The plates were incubated at 37°C for 24 hours and the diameter of the inhibition zones was measured in mm. Minimal inhibitory concentration was determined using Broth microdilution method and ELISA reader as described by Bonnavero *et al* (1998). Muller Hinton broth medium was used to grow the bacteria overnight and the growth was diluted to approximately 10^4 cell/ml and phenol red was used as a colorimetric indicator. MIC was determined by changing in broth color from yellow to pink.

Toxicity assay of plant extracts

Bioassay test using Brine shrimp lethality assay was carried out to investigate the toxicity of the methanolic plant extracts and *Artemia salina* was used as test organism. The percentage of mortality was determined and LC₅₀ was calculated (Adoum, 2009).

III. Results

The Gram negative pathogenic isolates *Escherichia coli*, *Klebsiella pneumonia*, *Shigella sonnei* in addition to the Gram positive *Micrococcus luteus* were obtained and checked up again in terms of purity and species characteristics using growth on specific media, API 20E and detection of some enzyme. Two common plants, *Cymbopogon schoenanthus* and *Senna holosericea* were collected from Alabwa region during winter 2016 and identified (Table 1 and Fig. 1). The evergreen *C. schoenanthus*, also called Camel or lemmon grass, is aromatic perennial grass which reached to 30 – 60 cm, enclosed by tight bundles of old sheaths at the base. This plant is wild and rarely being cultivated while *Senna holosericea* is small shrub or herb, legume and flowering plant of the family Fabaceae. The *Senna* plant has pinnate leaves, opposite paired leaflets, large brilliant yellow flowers and pods of medium size. The leaves of the two plants were extracted using different solvents. The results in Table 2 showed the antimicrobial activity of *Cymbopogon schoenanthus*, extracted using different solvents. Maximum inhibitory effect was recorded for the methanolic and ethanoilic extract of *Cymbopogon schoenanthus* while lower activity was recorded for acetonic extract (Fig 2) and no activity was recorded for the water extract. On the other hand, excellent antibacterial activity (inhibition zone diameters) against all tested bacteria was recorded for methanolic extract of *Senna holosericea* while moderate activity was recorded for both ethanoilic and acetonic extracts (Fig. 3) and lower activities was recorded for hot water extract (Table 3). The methanolic extract of *S. holosericea* gave inhibition zone diameter ranged from 31-35 mm with MIC 37.5 µm/ml, lower activities was recorded for the methanolic extract of *Cymbopogon schoenanthus* against all tested bacteria with MIC ranged from 37.5 -70 µm/ml (Table 4). Weak antimicrobial activity or no activities were recorded for hot water extracts of the two tested plants. *Klebsiella pneumonia* was resistant to all tested antibiotic except Cotrimoxazole while *E. coli* and *Shigella sonnei* were resistant to Cephalothin and Ampicillin. Furthermore, *Micrococcus luteus* was resistant to Nalidixic Acid and Nitrofurantion (Table 5). Toxicity of the two tested methanolic extracts was determined (Table 6) and no toxicity was recorded for the two tested extracts up to 100 µg/ml.

IV. Discussion

Pathogenic bacteria and fungi recorded resistance to antibiotics and new resources of antibiotics must be developed (Amer *et al.*, 2004, Al Masoudi *et al.*, 2013). The data obtained by many authors reported that plants from Saudi Arabia demonstrated antibacterial activity and many plants are found and needed to be studied (Aly and Bafeel, 2008, 2010, Aly *et al.*, 2013). The plants, *Cymbopogon*

schoenanthus and *S. holosericea* were commonly found in Alabwa region and used by the popular to treat many diseases, thus the two plants were collected, identified and extracted. Methanol, ethanol and chloroform were used for active material extractions while agar well-diffusion method was used to detect any antibacterial activities (Aly and Gumgumjee, 2011, El Sayed and Aly, 2014). The MICs of both extracts were determined and were different to that recorded before (Hashim *et al.*, 2016) which could be due to the method of extraction, the part of the plant used and method of detection. Our results showed that *Senna holosericea* methanolic extract was more active compared to *Cymbopogon schoenanthus* extract. Significant differences were recorded between *Cymbopogon schoenanthus* and control antibiotic while the difference was not significant between *Senna holosericea* extract and control. These variations might be due to many factors, including the method of extraction, solvent used, used pathogen, type of the plant, site of collection and harvest time. Thus, the methods of extraction and specify all the conditions that may affect the extraction. Moussa *et al.*, 2012 reported that Gram-negative are sensitive to antimicrobial agent than Gram-positive bacteria but Deans and Ritchie (1987), found no clear difference in bacterial sensitivity. On contrast, *Escherichia coli* was affected by the tested antimicrobial agents more than *S. aureus* (Gustafson *et al.*, 1998). The highest antibacterial activities of *Senna* leaves was recorded for the acetone extract, followed by dichloromethane, methane and hexane while water extracts gave the least activity against the test pathogens and Saponins, Tannins, Alkaloids and Flavonoids were recorded in the extracts (Doughari *et al.*, 2008). They added that *Senna* extract may have a role in many fungal and bacterial dangerous urinary tract and mycotic infections in addition to gonorrhoea, and pneumonia. The methanolic extract of *Cymbopogon schoenanthus* oil inhibited 50% bacterial pathogens (Hashim *et al.*, 2016) while more activates was found by El-Kamali *et al.* (2005). Our study showed that Gram-negative and Gram positive bacteria were highly susceptible to both tested plant extracts. Takaisi-Kikuni *et al.* (2000) found that the *C. schoenanthus* oil has bacteriostatic effect on bacteria. McLaughlin *et al.* (1998) studied the composition of the plant extracts and detected alkaloids, steroids, saponins, glycosides and flavonoids which may possess medicinal activities and antibacterial action (Gronhaug *et al.*, 2008; Kumar *et al.*, 2009). The toxicity of any plant extracts and/or essential oils must be carried out because active agents may be toxic in high concentrations. The two tested methanolic plant extracts showed no toxicity up to 100 µg/ml.

The effect of plant extract on bacteria may be on bacterial cell wall and cell membrane leading to cell lysis and death or on DNA, RNA, proteins and polysaccharides inhibition on microbial cells (Kalemba and Kunicka, 2003). In conclusion, scientific validation for the use of the two tested plant extracts and future studies should be carried out to enhance the antimicrobial activities of the extracts. Organic solvents have the ability to dissolve photochemical agents (Kawo (2007) and Kawo *et al.* (2009) while water extracts extract only the water soluble substances. The locations of a plant, time of collection, climate, soil, propagation method are all factors that affect active constituents and effectiveness of the plant used (Adoum *et al.*, 1997 and Odugbemi, 2008).

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Table 1. The used plant parts, common names and their families

| Scientific name | Common name | Plant family | Extracted part |
|--|-------------|--------------|----------------|
| <i>Cymbopogon schoenanthus</i> (L.) Spreng | Ethkhor | Poaceae | Leaf |
| <i>Senna holosericea</i> (Fresen.) Greuter | Sana | Fabaceae | Leaf |

Table 2. The antimicrobial activities of leaf extracts of *Cymbopogon schoenanthus* obtained with different solvents against Gram positive and Gram negative bacteria

| Pathogenic isolates | Solvent used | | | | Tetra-cycline (control) |
|------------------------------|------------------|-----------------|-----------------|-------------------|-------------------------|
| | Methanol extract | Ethanol extract | Acetone extract | Hot water extract | |
| <i>Escherichia coli</i> | 23±2.88 | 23±5.77 | 20±0.0 | ND | 30±0.0 |
| <i>Klebsiella pneumoniae</i> | 23±2.88 | 20±0.0 | 13±0.0 | ND | 20±0.0 |
| <i>Shigella sonnei</i> | 25±0.0 | 20±0.0 | 13±0.0 | ND | 30±0.0 |
| <i>Micrococcus luteus</i> | 20±0.0 | 20±0.0 | 20±0.0 | ND | 40±0.0 |
| Bacterial Index | 22.75* | 20.75* | 16.5* | ND | 30 |

*: Significant result compared to control

Table 3. The antimicrobial activities of leaf extracts of *Senna holosericea* obtained with different solvents against Gram positive and Gram negative bacteria

| Pathogenic isolates | Solvent used | | | | Tetra-cycline (control) |
|-----------------------------|------------------|-----------------|-----------------|-------------------|-------------------------|
| | Methanol extract | Ethanol extract | Acetone extract | Hot water extract | |
| <i>Escherichia coli</i> | 35±0.0 | 31±0.0 | 28±2.9 | 13±5.8 | 30±0.0 |
| <i>Klebsiella neumoniae</i> | 31±2.9 | 30±0.0 | 23±2.9 | 20±0.0 | 20±0.0 |
| <i>Shigella sonnei</i> | 33±2.9 | 33±2.9 | 28±2.9 | 20±0.0 | 30±0.0 |
| <i>Micrococcus luteus</i> | 35±0.0 | 28±7.69 | 20±0.0 | 13±5.8 | 40±0.0 |
| Bacterial Index | 33.5 | 26.0 | 24.8 | 16.5* | 30 |

*: Significant result compared to control

Table 4. The MIC of the tested plants for some pathogenic Gram negative bacteria using Broth Microdilution method.

| Pathogenic isolates | MIC of the methanolic extracts (µg/ml) | | |
|-----------------------------|--|--------------------------|---------------|
| | <i>Cymbopogon schoenanthus</i> | <i>Senna holosericea</i> | Tetra-cycline |
| <i>Escherichia coli</i> | 75 | 37.5 | 8.0 |
| <i>Micrococcus luteus</i> | 75 | 37.5 | 6.0 |
| <i>Klebsiella pneumonia</i> | 37.5 | 37.5 | 4.0 |
| <i>Shigella sonnei</i> | 37.5 | 37.5 | 4.0 |

Table 5. The antimicrobial activity of some antibiotics against some pathogenic Gram positive and negative bacteria using Disk diffusion method.

| Pathogenic isolates | Antibiotics | | | | | |
|------------------------------|------------------------|------------------------|--------------------|--------------------|-----------------------|----------------------|
| | Nalidixic Acid (30 µg) | Nitrofurantion (30 µg) | Cephalothin (30µg) | Ampicillin (25 µg) | Cotrimoxazole (25 µg) | Norfloxacine (10 µg) |
| <i>Escherichia coli</i> | 25±0.0 | 13±0.0 | ND | ND | 20±0.0 | 25±0.0 |
| <i>Micrococcus luteus</i> | ND | ND | 13±0.0 | 25±0.0 | 25±0.0 | 10±0.0 |
| <i>Klebsiella pneumoniae</i> | ND | ND | ND | ND | 15±0.0 | ND |
| <i>Shigella sonnei</i> | 25±0.0 | 15±0.0 | ND | ND | 25±0.0 | 30±0.0 |

ND: not detected

Table 6. Toxicity (% of cell mortality) of different concentration of the plant extracts using brine shrimp lethally test.

| Tested plants | Concentration of methanolic extract (µg/ml) | | | | |
|--------------------------------|---|-----|----|-----|------|
| | 25 | 50 | 75 | 100 | LD50 |
| <i>Cymbopogon schoenanthus</i> | 0.0 | 10 | 10 | 20 | >100 |
| <i>Senna holosericea</i> | 0.0 | 0.0 | 10 | 20 | >100 |

0.0: No cell mortality



Fig1. Whole plant of *Cymbopogon schoenanthus* (A) and extracted parts (B): and whole plant of *Senna holosericea* (C) and extracted parts (D).

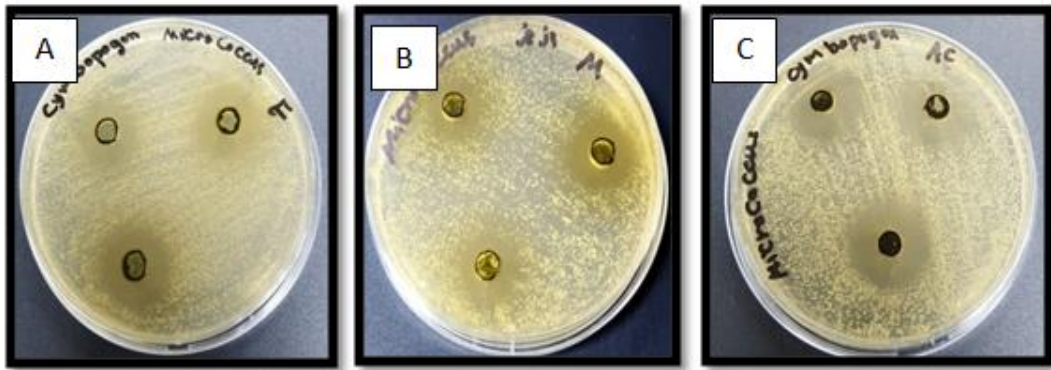


Fig 2. The antimicrobial activity of methanol (A), ethanol (b) and acetone (C) leaf extracts of *Cymbopogon schoenanthus* on *Micrococcus luteus* .

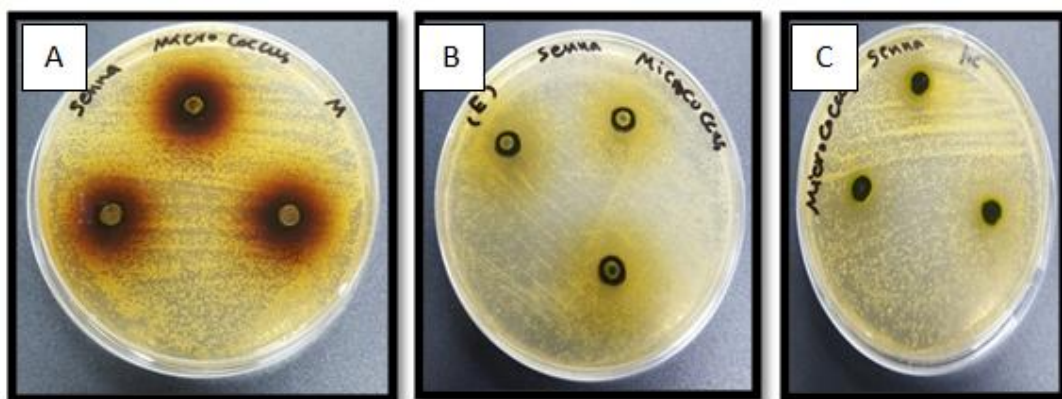


Fig 3. The antimicrobial activity of methanol extract (A), ethanol extract (B) and acetone extract (C) of leaves of *Senna holosericea* on *Micrococcus luteus* .