

Original Research Article

In Vitro* Antibacterial and Antitubercular Activities of Leaf Extracts of *Senna occidentalis

ABSTRACT

Aim: This study aimed to evaluate the antibacterial and antitubercular activities of ethylacetate and ethanol leaf extracts of *Senna occidentalis*.

Study Design: Fresh leaves of *Senna occidentalis* collected from Suleja, Niger state were used for this study against some medically important micro-organisms viz; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumoniae*, *Mycobacterium bovis* and *Mycobacterium smegmatis*.

Place and Duration of Study: The study was conducted in Abuja, Nigeria at the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development [NIPRD], from February 2019 to April 2019.

Methodology: *Senna occidentalis* leaves were extracted successively with ethyl-acetate and ethanol. The obtained extracts were tested *in vitro* for antibacterial activity by agar well diffusion method, while anti-tubercular screening was carried out by broth micro-dilution method. A fixed-dose concentration of chloramphenicol was used as a control drug against the bacterial isolates while isoniazid was used as control drug against the mycobacterium isolates.

Result: The *in vitro* antibacterial screening showed that the crude extracts exhibited varying activity against the different microbes with highest zone of inhibition at 12 mm, and anti-tubercular activity with MICs ranging from 97.6-390.6 µg/mL. Among these extracts, ethyl-acetate extract showed significant antibacterial activity against most of the test micro-organisms. The most susceptible micro-organism was *P. aeruginosa* (12mm zone in ethyl-acetate at 80 mg/mL) followed by *B. subtilis* (10 mm zone in ethyl-acetate extract at 80 mg/mL) and *E. coli* (9 mm zone in ethyl-acetate extract at 80 mg/mL). The ethanol extract was the most effective in inhibiting the growth of *M. smegmatis* and *M. bovis* with MICs of 97.6 µg/mL and 195.3 µg/mL.

Conclusion: The activities observed could be attributed to the presence of some active metabolites contained in the extracts which could be useful in drug development for therapeutic purposes.

Keywords: *Senna occidentalis*; anti-bacterial activity; anti-tubercular activity; micro-organisms.

1. Introduction

Tuberculosis (TB) still remains one of the leading causes of death in the world. It is an infectious deadly disease caused by the organism, *Mycobacterium tuberculosis*, which emerged from East Africa for more than three million years ago [1]. About one-third of the world's population is at risk to develop active TB and contribute to the continued spread of *M. tuberculosis* [2, 3, 4]. Nigeria is ranked seventh in the world and second in Africa among the 30 countries with the highest burden of TB [5]. Globally, there are an estimated 9.3 million new cases and 13.7 million chronic active cases responsible for 1.7 million deaths worldwide yearly. Moreover, up to 50 million people are said to be infected with drug-resistant forms of TB from which about 500,000 cases of multidrug resistant (MDR) TB worldwide per a year [6]. Management of TB/MDR-TB patient requires intense multi-chemotherapy for at least six months to two years. It is very hurtful to a patient's health due to high levels of drug toxicity and its adverse effects [7, 8, 9]. The

emergence of MDR TB and extensively-drug resistant (XDR) TB to the medicines now in use makes urgent search for new anti-TB agents worldwide [10, 11].

Medicinal plants are important source of drugs especially in traditional medicine [12]. It is a common practice in Nigeria and other parts of the world to use medicinal plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. Medicinal plants contain a lot of bioactive constituents or phytochemical compounds which are secondary metabolite because they are not required for growth, respiration, transpiration or any primary function in plants [13]. The major secondary metabolites including alkaloids, carbohydrates, flavonoids, tannins, terpenoids, and steroids [13]. Plant initially produces these phytochemical compounds to protect themselves from pathogens and predator [14].

Senna occidentalis (Linn) (formerly *Cassia occidentalis* or *Ditremexa occidentalis*), also called Coffee Senna in English is a shrub that grows between 5 to 8cm in height, belongs to the plant family *Fabaceae* and the subfamily *Caesalpinaceae* which are commonly found in the tropical and sub-tropical regions of the world [15]. It is a straight, somewhat branched, smooth, semi-woody, fetid herb, hard, stout, with a few lateral roots on mid-section. The stem of the plant is reddish purple and the leaves are alternate, each leaflet 4-6 cm long and 1.5-2.5 cm wide [16]. It can be found in open pastures and in fields cultivated with cereals such as soybean, corn, sorghum and others; thus, during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crops [17, 18]. It is an ayurvedic plant with huge medicinal importance and also used for various therapeutic purposes in traditional medicine [19, 20, 21]. In Nigeria, this plant is locally called Sanga-sanga or Rai dore in Hausa language [22, 23]; Akidi agbara in Igbo language and Abo rere in Yoruba language [24].

The plant has been used in different parts of the world by the traditional healers in treating different forms of diseases. *Cassia occidentalis* plant extract (4–5 drops) is used in curing eye inflammations and also used in Jamaican folk medicines for curing diarrhoea, dysentery, constipation, fever, cancer, eczema and venereal diseases [25]. It has been documented in literatures that extract of *Senna occidentalis* has antimicrobial activity [26, 27], larvicidal and pupicidal activity [28], antioxidant and hepatoprotective activity [29], anti-inflammatory actions [20], antimalarial activity [30], antianxiety and antidepressant activity [31], analgesic activity [21] and antidiabetic activity [32, 33, 34]. Moreover, studies on this plant showed that the nature and amount of the phytochemicals varies according to the season and geographical location [35]. This present research was carried out to evaluate the antibacterial and antituberculosis activities of ethylacetate and ethanol leaf extracts of *Senna occidentalis*.

2. Materials and Methods

2.1 Plant Collection

Fresh leaves of *Senna occidentalis* were collected in the month of February, 2019 from Suleja, Niger State, identified and authenticated by the Taxonomist at the Herbarium Unit, National Institute for Pharmaceutical Research and Development (NIPRD). A voucher specimen was deposited at the herbarium of the institute.

2.2 Preparation of Plant Extract

The fresh leaves of *S. occidentalis* were dried at room temperature for 14 days, after which it was pulverized using a mechanical grinder. Ethylacetate and ethanol solvents of volume 500 mL each was used for the maceration for 24 hours. The solution obtained was filtered using a filter paper. The filtrate was allowed to dry on the water bath at 50°C to obtain the various extracts and kept in the refrigerator until needed for use.

2.3 Test Organisms

Pure clinical isolates of *Bacillus subtilis*, *Klebsiella pneumoniae*, collected and biochemically confirmed from Diagnostic Laboratory of NIPRD clinic and American Typed cultures of *Escherichia coli* [ATCC 25952], *Staphylococcus aureus* [ATCC 25923], *Pseudomonas aeruginosa* [ATCC 27853], *Salmonella paratyphi* [ATCC 9150], *Mycobacterium bovis* [27290], *Mycobacterium smegmatis* [607] were used in this study.

2.4 Inoculum Preparation

A loopful of the test organism (*S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *K. pneumoniae* and *S. paratyphi*) was taken from their respective agar slants, sub-cultured into 5 mL of nutrient broth and incubated at 37°C. Following incubation at 37°C for 24 hrs, organisms were diluted with normal saline to a turbidity that was equivalent to 0.5 Mc Farland standard (10^6 CFU/mL) [36].

Fifty micro-litre (50 μ L) of each freshly thawed stock test organism (*M. Bovis* and *M. smegmatis*) was inoculated into 50 mL of sterile Middle brook 7H9/ADC media and incubated at 37°C with shaking for 5-7 days. The activity grown *M. bovis* and *M. smegmatis* culture had its optical density adjusted to between 0.2 - 0.3 at a wavelength of 650 nm using Jenway 6405 UV-Visible spectrophotometer.

2.5 Antibacterial Activity

Prepared concentrations (80 mg/mL, 40 mg/mL, 20 mg/mL and 10 mg/mL) of each extract were tested against the test organisms using Agar well diffusion method as described by [37]. One hundred microliter (100 μ L) of the suspension of standardized microorganisms was inoculated into sterile molten Mueller Hinton agar, swirled and poured into sterile Petri dishes and allowed to solidify. Holes for each concentration of the extracts and positive control were bored aseptically using a sterile cork borer of 5 mm. The bottom of the bored holes was sealed using a drop of Mueller Hinton agar. One hundred microliters of different concentrations of the extracts and a fixed dose (30 μ g/mL) of the positive control, chloramphenicol being a drug of choice as a broad spectrum antibiotic was dispensed into appropriately labelled wells respectively. The plates were allowed to dry inside the biosafety cabinet as well as allowing the extracts to diffuse for about 2 hrs and then incubated at 37°C for 24 – 48 hours. Antibacterial activity was assessed by measuring the size of the zone of inhibition surrounding wells and taking the average of the readings of each duplicate plate post incubation.

2.6 Anti-tubercular Activity

The anti-tubercular test of the plant extracts was conducted using the broth micro-dilution method in 96 well micro-titre plates [38]. Each of the extract was first dissolved in tween 20 and then diluted in Middle brook 7H9 broth, to give a starting concentration of 100,000 μ g/mL which was diluted across the 96-well micro-litre plate in a two-fold serial dilution to give final testing concentrations of 50,000 μ g/mL to 97.7 μ g/mL. Twenty five (25) mg of Isoniazid (Sigma Aldrich Inc) was dissolved in 1 mL dimethylsulfoxide (DMSO) and 25 μ g/mL solution was made by diluting 25 μ L in 25 mL 7H9 broth, sterile filtered was used as positive control drug and extracts/drug free medium with culture suspensions were used as negative control. Each extract concentration was assayed in duplicate. The plates were then incubated for 5-7 days at 37°C. After the 7th day, 25 μ L of tetrazolium salt dye was added to all the wells, re-incubated over-night and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest drug/extract concentration that prevented the color change of the tetrazolium dye to pink. Colourless well was interpreted as there is no mycobacterial growth and pink color was interpreted as growth occurrence.

3. Results

For the antibacterial activity, ethylacetate extract was found to be active against all the test organisms (*S. aureus*, *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *S. paratyphi*) while only *E. coli*, *P. aeruginosa* and *K. pneumoniae* had activity at 40 mg/mL concentration (Table 1). The ethanol extract was active only against *S. paratyphi* at 80 mg/mL and 40 mg/mL respectively (table 1). All the organisms were resistant against both extracts at 20 mg/mL and 10 mg/mL concentrations.

For the anti-tubercular activity, the extracts were screened against *M. smegmatis* and *M. bovis*. Ethanol extract showed MIC of 97.6 µg/mL while ethyl-acetate showed MIC of 195.3 µg/mL against *M. smegmatis* whereas ethanol extracts showed MIC of 195.3 µg/mL while ethylacetate showed MIC of 390.6 µg/mL against *M. bovis* (Table 2).

4. Discussion

Tuberculosis remains a global infectious disease and with emergence of multi-drug resistance strains, there is need for research and development of new compounds that will serve as leads in drug development. *Senna occidentalis* is a well-known herb used as ayurvedic traditional medicine for their effectiveness against wide range of diseases due to the presence of diverse secondary metabolites responsible for their antibacterial activity [39]. Egharevba *et al.*, [24], reported the phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam. He also reported the isolation of anthraquinones; emodin and chrysophanol from the plant. Musa *et al.*, [40], reported the presence of anthraquinones, tannins, flavonoids while saponins was not detected in the ethanolic extract of *S. occidentalis*. Odeja *et al.*, [41], detected the presence of alkaloids, anthraquinones and resins in the ethyl acetate extract of *S. occidentalis*.

For instance, herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery [42], as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics [43]. Saponins lower the cholesterol level; have anti-diabetic and anti-carcinogenic properties [44], and are also expectorants, cough suppressants and for haemolytic activities [45, 46]. Alkaloids are known to possess anti-malaria property [47], and also have antispasmodic and analgesic properties [43]. Also, flavonoids and resins present in the plant might be responsible for its anti-inflammatory properties. Flavonoids are considered a key ingredient in Chinese folkloric medicine having anti-inflammatory effect on both acute and chronic inflammation [48, 49]. Emodin has been reported to have antibacterial effect against *Bacillus subtilis* and *Staphylococcus aureus* while chrysophanol has been reported to have some wound healing properties [50, 51].

The existence of these metabolites strongly suggests great potential of the plant as a source of phytochemicals. Various studies of phytochemicals of the plant shows that the amount and nature of phytochemicals varies according to climate condition for the growth of the plant and different geographical locations.

The antibacterial activities of ethanol and ethylacetate leaf extracts of *S. occidentalis* were investigated against two Gram-positive bacteria viz; *Staphylococcus aureus*, *Bacillus subtilis*, four Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella pneumoniae* and two mycobacterium species namely; *Mycobacterium smegmatis* and *Mycobacterium bovis*.

From Table 1 above, the highest activity (zone of inhibition of 12 mm) was shown by the ethylacetate extract against *Pseudomonas aeruginosa* and ethanol extract (zone of inhibition of 12 mm) against *Salmonella paratyphi* at 80 mg/mL while the lowest activity was at 6 mm. The result obtained for *P. aeruginosa* in this study is contrary to that of Egharevba *et al.*, [24], which showed no activity against *P. aeruginosa* across all concentrations, which may be due to the level of concentration or strain of *P. aeruginosa* used in this study. This result suggests that the antibacterial activity of ethylacetate and ethanol extracts of *S. occidentalis* increases when used in higher concentrations, which is in agreement with a work done by Sadiq *et al.*, [23], on the antibacterial activity of ethanol and water extracts of *Cassia occidentalis*. Result of this study shows the ethylacetate extract inhibited the growth of various species of gram negative bacteria while ethanol extract only inhibited the growth of *Salmonella paratyphi*. However, *S. aureus* was resistant to both extracts at all concentrations except for ethylacetate extract which was

susceptible at 80 mg/mL concentration. This is in accordance with a report by Sadiq *et al.*, [23], in which *S. aureus* was resistant to the ethanol and water extract used. This probably could be attributed to the cell membrane permeability or genetic factors. The result obtained from this study shows that ethyl acetate extract of *S. occidentalis* has higher antibacterial activity against the test organisms compared to the ethanol extract and this may be due to the different polarity of the solvents as well as the solubility of the active compounds that were able to dissolve.

Also in this study, the crude extracts of *S. occidentalis* showed varying degree of anti-tuberculosis activity. The ethanol extract was the most effective in inhibiting the growth of *M. smegmatis* and *M. bovis* with MICs of 97.6 µg/mL and 195.3 µg/mL respectively while the ethylacetate extracts had MICs of 195.3 µg/mL and 390.6 µg/mL respectively, but not as active as the control drug, isoniazid with an MIC of 0.02 µg/mL. In a previous study, Yeragamreddy *et al.*, [52], reported an MIC of 25 µg/mL for the ethylacetate fraction of *Cassia occidentalis* against *Mycobacterium tuberculosis*. Non-pathogenic tuberculosis strains are acceptable as surrogate in drug development research [53].

Table 1: Effect of ethylacetate and ethanol extracts of *S. occidentalis* at various concentrations (mg/mL) against bacterial isolates

Bacterial isolates	Zone of Inhibition (mm)							
	Ethylacetate				Ethanol			
	80	40	20	10	80	40	20	10
<i>Salmonella paratyphi</i>	7	-	-	-	12	7	-	-
<i>Staphylococcus aureus</i>	7	-	-	-	-	-	-	-
<i>Escherichia coli</i>	9	7	-	-	-	-	-	-
<i>Bacillus subtilis</i>	10	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	8	6	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	12	8	-	-	-	-	-	-

- = No zone of inhibition

Table 2: Effect of ethylacetate and ethanol extract of *S. occidentalis* against *M. smegmatis* and *M. bovis*

Organisms	Minimum Inhibitory Concentration Values (µg/mL)		
	Ethanol	Ethylacetate	Isoniazid
<i>M. smegmatis</i>	97.6	195.3	0.02
<i>M. bovis</i>	195.3	390.6	0.02

CONCLUSION

From the present study conducted, it can be concluded that the antibacterial and antituberculosis activity of *Senna occidentalis* may be due to the phytochemicals present in the plant. The antibacterial activity of these plants shows the importance of the extracts in traditional preparations which may be helpful in treating diseases such as urinary tract infections and diarrhea. Thus, it may be considered as a natural source of antimicrobials and anti-tuberculosis for therapeutic purposes. Further research should be carried out to identify the active compounds responsible for the plant biological activity and also screen the plant against multi drug resistance tuberculosis (MDR-TB).

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