

Antioxidants and Antimicrobial- activities of methanol leaf extract of *Senna occidentalis*

ABSTRACT

Background: Oxidative stress and infectious disease have always been considered as a leading cause of morbidity and mortality in humans. Free radicals damage other molecules by extracting electrons from them in order to attain stability. The side effects associated with chemotherapeutic agents has called for alternative from natural products. **Objective:** The antioxidants and antimicrobial activities of methanol leaf extract of *S. Occidentalis* against some pathogenic microorganism were evaluated. **Methods:** The antioxidants activities were conducted using DPPH radical scavenging assay. The antibacterial activities were screened against *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *S. aureus* and *E.coli* using agar well diffusion method at various concentrations (80-360 mg/ml). The minimum inhibitory concentration (MIC) was determined using serial dilution method while minimum bactericidal concentration (MBC) by plating various dilution of extract. **Results:** The extract show significant antibacterial activities with MIC in the range of 160mg/ml-280mg/ml and MBC in range of 160mg/ml - 320mg/ml. The most sensitive organisms were *S. aureus* and *P. aeruginosa* while *K. pneumonia* show some degree of resistances compared to other organism tested. However, the organisms were not sensitive to the extract at the dose of 80 and 120mg/ml. The extracts and the standard antioxidant (Vitamin E) promoted an inhibition of DPPH radical with IC₅₀ values of 263.53 mg/mL against 83.63 mg/mL for standard drug. **Conclusion:** Methanol leaf extract of *S. occidentalis* contains some useful potential antimicrobial and antioxidant that could serve as candidate for the development of drug for therapeutic purposes.

Keywords: Antioxidants, Antimicrobial, DPPH, *Senna occidentalis*

INTRODUCTION

Pathogenic bacteria have always been considered as a leading cause of morbidity and mortality in humans. Even though a number of new anti-biotics have been developed by pharmaceutical organizations bacterial genetic ability to transmit and acquire resistance to these drug and therapeutic agents has generated a worldwide predicament of antimicrobial resistance [1]. Antimicrobial resistance coupled with undesirable side effect associated with antibiotics has geared the scientist attention towards extracts and biologically active principles isolated from plant materials [2]. Antimicrobial agents from natural products represent an immense untapped source of medicines and further investigation of plant

antimicrobials is highly welcome. Plant based antimicrobials are effective in the treatment of infectious diseases while concurrently extenuating the side effects that are often linked with synthetic anti-microbials [3].

Free radicals especially reactive oxygen species (ROS) are highly reactive molecules produced during redox reaction which in turn initiate a chain of reactions with consequent implicative effect in the aetiology of certain degenerative disorder [4]. Natural antioxidants including vitamin A, C, E and ascorbic acid, as well as enzymes like peroxidises, catalase (CAT), superoxide dismutase (SOD) and glutathione exist in the living system to detoxify or counter the effect of these free radicals [5]. However, oxidative stress results from imbalance between the production of free radicals and the ability of the natural antioxidants to detoxify the free radicals or repair the damage tissue.

Africa is blessed with enormous diversity of natural product with healing practice as revealed by several citations [6,7]. The medicinal virtue of natural products lays on some bioactive principles that produce a definite physiological action on the human body and that could serve as a candidate for the developments of new drugs [8, 9]. The most important of these bioactive compounds are classified as alkaloids, tannins flavonoids and phenolic compounds. Many plant leaves have antimicrobial principles such as tannins, essential oils and other aromatic compounds [10]. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids [11].

On the array of traditional plants with antimicrobial reputation is *Senna occidentalis*. The plant *Senna occidentalis* a native plant of southern India, commonly known as Kasmard in Sanskrit, Kasondi in Hindi and Coffee Senna in English. It belongs to family Fabaceae; it is a medicinal plant which is widely used with other plants in Nupe ethno-medicine for treatment of malaria and trypanosomiasis [12]. *S. occidentalis* is highly reputed for its numerous medicinal uses and is known to be used ethno-medicinally as remedy for several human and animal ailments. Biological activities including antimalarial, antitrypanosomal, immunosuppressive, anti-inflammatory, larvicidal effect, anti-diabetic, anti-cancer, anti-ulcer and hepatoprotectives [13-17], as well as the toxicological effects on blood components [18], have been reported for *S. occidentalis*. The leaf extract have been found to contain important phytochemicals such as tannins, alkaloids, glycoside, flavonoids, steroids, saponins, anthraquinones and phlobatannins [19]. In order to contribute to knowledge on biological

activities of this plant, the present study aimed at evaluating the antioxidants and antimicrobial activities of methanol leaf extract of this plant.

MATERIAL AND METHOD

Plant Collection

Freshly harvested *S. occidentalis* leaves were procured from Bosso, area of Minna, Niger State, Nigeria. The plant was authenticated by a botanist at National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

Sources of Microorganisms

Pure isolate of *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *E.coli* and *S. typhi* were procured from Microbiology Unit, Faculty of Life Sciences Federal University of Technology, Minna, Nigeria. Biochemical test and gram staining test were used to confirm the identity of the organism.

Sample preparation and Extraction of plant Materials

Fresh leaf of *S. occidentalis* were grounded using a grinder mill. A 200 g of the powdered plant was extracted with 600ml of methanol. The resulting extract was concentrated using rotary evaporator and stored in a refrigerator at 4°C until required [20].

Assay for antibacterial activity

Stock cultures were maintained at 4°C on nutrient agar (HiMedia) slants. Active cultures for experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth (HiMedia) and incubated at 37 °C for 24 hours for bacterial proliferation [21]. Antibacterial activity of methanol leaf of *S. occidentalis* was carried out using Agar-well diffusion method as described by Jayaraman et al., [21], using Ciprofloxacin (40 µg/ml) as standard drug. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by tube dilution method for each of the test organism in triplicates.

DPPH radical scavenging activity

DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity by methanol leaf of *S. occidentalis* was measure by the spectrometric method as described previously [22]. The % inhibition by the extract was calculated using the formular

$$\% \text{ inhibition} = \frac{A_1 - A_2}{A_2} \times 100$$

95 Where

96 A_1 = absorbance of blank sample

97 A_2 = absorbance of *S. occidentalis*

98 RESULTS

99 Zone of inhibition of methanol leaf extract of *S. occidentalis* against some pathogenic
100 microorganisms are presented in table 1. The zone of inhibition by the extract increase with
101 increase concentration. The inhibitory zone against *E. coli* ranged from 15.00±0.50 mm to
102 30.00±0.05 mm, for *K. Pneumonia* it ranged from 11.00±0.30 mm to 16.00±0.05 mm, for *S.*
103 *aureus* it ranged from 11.00±0.50 to 24.00±0.20 mm, for *P. aeruginosa* it ranged from
104 15.00±0.50 mm to 28.00±2.54 mm while the extract had the zone of inhibition in the range of
105 14.00±0.50 mm to 34.00±0.40 mm against *S. typhic*. However, the organisms were not
106 sensitive to the extract at the dose of 80 and 120 mg/ml. The MIC of the extract against the
107 organism range between 160 mg/ml- 280 mg/ml while the MBC range between 160 mg/ml –
108 320 mg/ml. The most sensitive organisms were *S. aureus* and *P. aeruginosa* while *K.*
109 *pneumonia* show some degree of resistances compared to other organism tested (figure 1).
110 The extract and the standard antioxidant (Vitamin E) promoted an inhibition of DPPH radical
111 with increasing concentrations. The IC₅₀ (concentration that inhibits 50% of the DPPH
112 radical) values of extract was 263.53 mg/mL against 83.63 mg/mL for standard drug (Table 2
113 and Figure 2)

114 **Table 1:** Zone of inhibition of methanol leaf extract of *Senna occidentalis*

Concen. (mg/ml)	<i>E. coli</i>	<i>K. pneumonea</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhic</i>
Zone of inhibition(MM)					
80	-	-	-	-	-
120	-	-	-	-	-
160	15.00±0.50	-	-	16.00±1.00	14.00±0.50
200	18.00±0.11	-	11.00±0.50	15.00±0.50	16.00±0.10
240	20.00±0.56	-	11.00±0.05	23.00±0.60	20.00±0.05
280	21.00±0.05	11.00±0.30	18.00±0.60	23.00±0.50	26.00±0.78
320	26.00±0.00	15.00±0.00	19.00±0.56	26.50±0.40	29.00±0.55
360	30.00±0.05	16.00±0.05	24.00±0.20	28.00±2.54	34.00±0.40

115

Table 2: DPPH radical scavenging activity of methanol leaf extract of *S. occidentalis*

S/NO	Concentration mg/ml	<i>S.occidentalis</i>	Vitamin E.
1	10	23.56	31.10
2	50	34.34	49.20
3	100	28.45	55.20
4	150	39.23	56.66
5	200	44.34	77.70
6	250	49.45	82.22

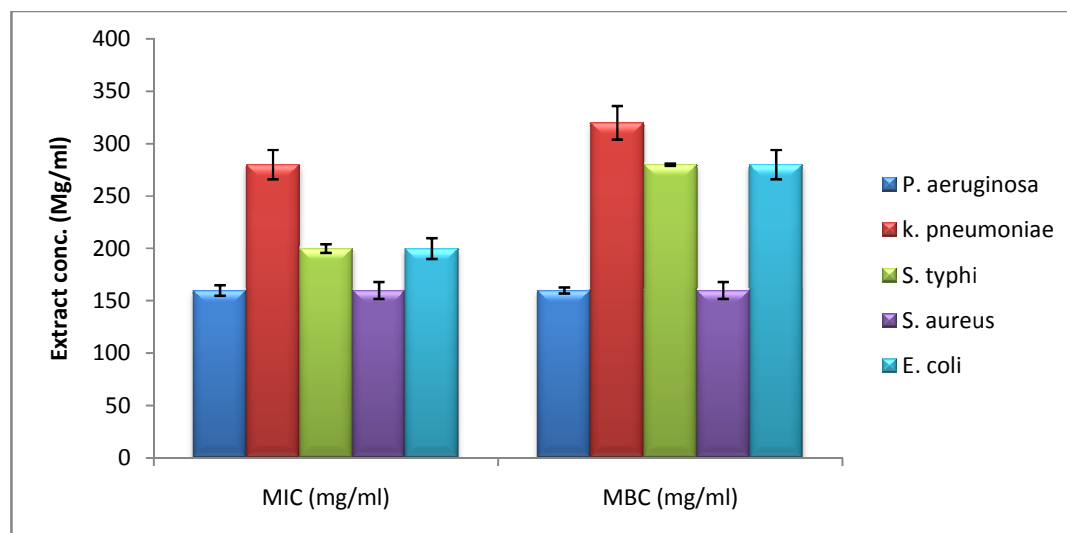


Figure 1; Minimal inhibitory concentration and Minimal bactericidal concentration of methanol leaf extract of *Senna occidentalis*

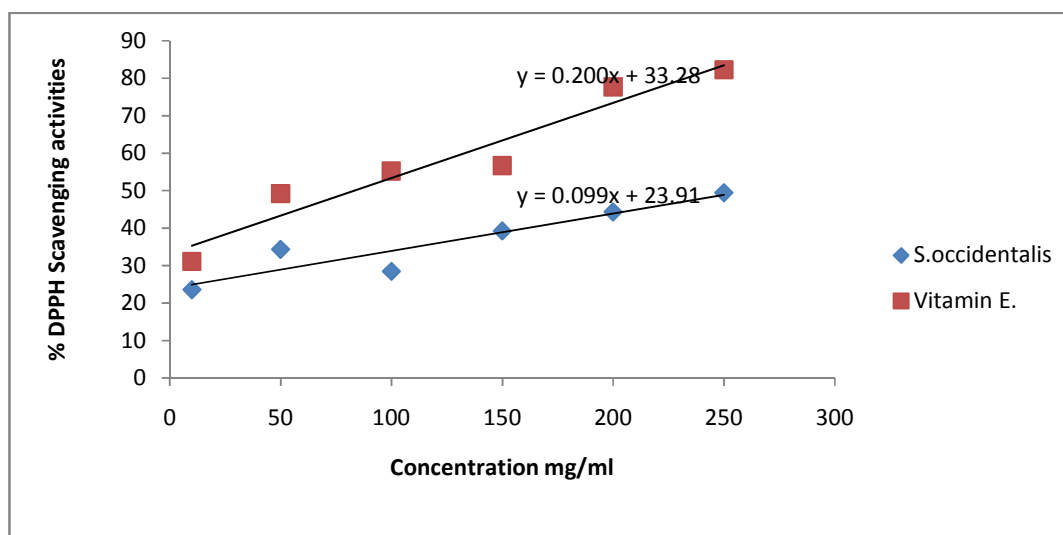


Figure 2: DPPH radical scavenging activity of methanol leaf extract of *Senna occidentalis*

Determination of IC₅₀

For vitamin E

From the equation $Y = 0.200x + 33.27$

At $Y=50$

$$IC_{50} (X) = 83.65 \text{ mg/mL}$$

For *S. occidentalis*

From the equation $Y = 0.099x + 23.91$

At $Y=50$

$$IC_{50} (X) = 263.53 \text{ mg/mL}$$

DISCUSSION

The antimicrobial activities of methanol leaf extract of *S. occidentalis* was investigated against five bacteria, *S. aureus*, *S. typhic*, *E. coli*, *P. aeruginosa* and *K. pneumonea*, Increase in the concentration of the extract yielded increase in the zones of inhibition. This linear relationship between extract concentrations and zones of inhibition could be that the extract was able to diffuse into the inoculated nutrient agar. The ability of methanol leaf extract of *S. occidentalis* to inhibit the growth of *E. coli*, a diarrhoea causing bacteria, explains why it is used in folk medicine to treat diarrhoea in Nigeria and other tropical countries [23]. The extract was found highly active at concentration of 360 mg/ml. The antimicrobial activities demonstrated by this plant extract could be linked to its phytochemical constituents especially tannins which has been reported to exert antimicrobial activities [24]. However, the lack of

activities at the dose of 80 and 160 mg/ml of the extract could indicate low concentration of the antimicrobial phytochemicals at those doses.

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Methanol leaf extract of *S. occidentalis* was found to exert significant antioxidants effect in DPPH radical scavenging assay with IC₅₀ value of 263.53 mg/ml. The decrease in absorbance of DPPH caused by methanol leaf extract of *S. Occidentalis* is due to the reaction between antioxidant molecules and radicals, which results in the scavenging of the radical by hydrogen donation. Many antioxidants compounds are present in natural product. Flavonoids are phenolic compounds with important roles in scavenging free radicals and thus play vital roles in preventing oxidative stress associated disorder [25]. The antioxidant effect demonstrated by *S. Occidentalis* could be attributed mainly to the presence of phenolic compounds in it [19]. This high scavenging property was attributed to hydroxyl groups existing in the chemical structure of phenolic compounds that can provide the necessary components as a radical scavenger [26] This findings support earlier reports that plant metabolites like phenolic contents, tannins and flavonoids possesses antioxidant and antimicrobial activity [27].

CONCLUSION

This study has shown that the methanol leaf extract of *S. occidentalis* contains some useful potential antimicrobial and antioxidants principles. Thus, it may be considered as a natural source of antimicrobials and antioxidants for therapeutic purposes. It is however, hoped that pertinent scientist and stakeholders should look further into this plant for detailed authentication and subsequent commercialization.

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