

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 commonly used conventional antioxidants drugs have necessitated the search for natural antioxidants from plants extracts. **Objective:** The antioxidants and antimicrobial activities of methanol leaf extract of *S. occidentalis* obtained from North Central, Nigeria were evaluated against some pathogenic microorganism. **Methods:** The antioxidants activities were conducted using DPPH radical scavenging assay. The antibacterial activities were screened against *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* 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 organisms 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 IC_{50} value of the extract (263.53 ± 3.24 mg/mL) significantly ($p < 0.05$) higher than 83.63 ± 2.78 mg/mL obtained for standard drug. **Conclusion:** Methanol leaf extract of *S. occidentalis* contains some useful potential antimicrobial and antioxidant compounds 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

33 principles isolated from plant materials [2]. Antimicrobial agents from natural products
34 represent an immense untapped source of medicines and further investigation of plant
35 antimicrobials is highly welcome. Plant based antimicrobials are effective in the treatment of
36 infectious diseases while concurrently extenuating the side effects that are often linked with
37 synthetic anti-microbials [3].

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

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

54

55 On the array of traditional plants with antimicrobial reputation is *Senna occidentalis*. The
56 plant *Senna occidentalis* is a native plant of southern India, commonly known as Kasmard in
57 Sanskrit, Kasondi in Hindi and Coffee Senna in English. It belongs to family Fabaceae; it is a
58 medicinal plant which is widely used with other plants in Nupe ethno-medicine for treatment
59 of malaria and trypanosomiasis [12]. *S. occidentalis* is highly reputed for its numerous
60 medicinal uses and is known to be used ethno-medicinally as remedy for several human and
61 animal ailments. Biological activities including antimalarial, antitrypanosomal,
62 immunosuppressive, anti-inflammatory, larvicidal effect, anti-diabetic, anti-cancer, anti-ulcer
63 and hepatoprotectives [13-17], as well as the toxicological effects on blood components [18],
64 have been reported for *S. occidentalis*. The leaf extract have been found to contain important

65 phytochemicals such as tannins, alkaloids, glycoside, flavonoids, steroids, saponins,
66 anthraquinones and phlobatannins [19].

67 Odeja et al. [20] reported the antioxidant and antimicrobial activities of *S. occidentalis* leaves
68 from South West, Nigeria. However, bioactive components of plants varies with geographical
69 origin [21], environmental conditions such as weather parameters (temperature,
70 precipitation), and soil greatly contribute to the content of biological active compounds –
71 natural antioxidants - in plants. In order to contribute to knowledge on biological activities of
72 this plant, the present study aimed at evaluating the antioxidants and antimicrobial activities
73 of methanol leaf extract of this plant from North Central, Nigeria.

74 **MATERIAL AND METHOD**

75 **Plant Collection**

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

79 **Sources of Microorganisms**

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

84 **Sample preparation and Extraction of plant Materials**

85 Fresh leaves of *S. occidentalis* were grounded using a grinder mill. A 200 g of the powdered
86 plant was extracted with 600ml of methanol. The resulting extract was concentrated using
87 rotary evaporator and stored in a refrigerator at 4°C until required [22].

88 **Assay for antibacterial activity**

89 Stock cultures were maintained at 4°C on nutrient agar (HiMedia) slants. Active cultures for
90 experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth
91 (HiMedia) and incubated at 37 °C for 24 hours for bacterial proliferation [23]. Antibacterial
92 activity of methanol leaf of *S. occidentalis* was carried out using Agar-well diffusion method
93 as described by Jayaraman et al., [23], using Ciprofloxacin (40 µg/ml) as standard drug.
94 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
95 were determined by tube dilution method for each of the test organism in triplicates.

96 DPPH radical scavenging activity

97 DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity by methanol leaf of *S.*
98 *occidentalis* was measured by the spectrometric method as described previously [24]. The %
99 inhibition by the extract was calculated using the formula.

100 % inhibition=

$$101 \quad \frac{(A_1 - A_2)}{A_1} \times 100$$

102 Where

103 A_1 = absorbance of blank sample

104 A_2 = absorbance of *S. Occidentalis*

105 Statistical analysis

106 Data were analyzed using statistical package for social science (SPSS) version 16 and
107 presented as means $\pm$ SEM. Comparisons between .The results was done using Analysis of
108 Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P < 0.05$ were
109 considered as statistically significant as described by Mahajan, [25].

110 RESULTS

111 Zone of inhibition of methanol leaf extract of *S. occidentalis* against some pathogenic
112 microorganisms are presented in table 1. The zone of inhibition by the extract increases with
113 increased concentration. The inhibitory zone against *E. coli* ranged from 15.00 $\pm$ 0.50 mm to
114 30.00 $\pm$ 0.05 mm, for *K. pneumonia* from 11.00 $\pm$ 0.30 mm to 16.00 $\pm$ 0.05 mm, for *S. aureus* it
115 ranged from 11.00 $\pm$ 0.50 to 24.00 $\pm$ 0.20 mm, for *P. aeruginosa* it ranged from 15.00 $\pm$ 0.50 mm
116 to 28.00 $\pm$ 2.54 mm while the extract had the zone of inhibition in the range of 14.00 $\pm$ 0.50 mm
117 to 34.00 $\pm$ 0.40 mm against *S. typhic*. However, the organisms were not sensitive to the extract
118 at the dose of 80 and 120 mg/ml. The MIC of the extract against the organism ranges between
119 160 mg/ml- 280 mg/ml while the MBC range between 160 mg/ml – 320 mg/ml. The most
120 sensitive organisms were *S. aureus* and *P. aeruginosa* while *K. pneumonia* show some degree
121 of resistances compared to other organisms tested (figure 1). The extract and the standard
122 antioxidant (Vitamin E) promoted an inhibition of DPPH radical with increasing
123 concentrations. The IC₅₀ (concentration that inhibits 50% of the DPPH radical) value of
124 extract (263.53 $\pm$ 3.24 mg/mL) was significantly ($p < 0.05$) higher than 83.63 $\pm$ 2.78 mg/mL
125 obtained for standard drug (Table 2)

126

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

. Data represent means ± SEM of triplicate determination.

Table 2: DPPH radical scavenging activities of methanol leaf extract of *S. occidentalis* and standard drug (vitamin E)

S/NO	Concentration mg/ml	<i>S.occidentalis</i> (%)	Vitamin E (%)
1	10	23.56±1.11 ^a	31.10±1.13 ^b
2	50	34.34±0.89 ^a	49.20±0.90 ^b
3	100	28.45±1.21 ^a	55.20±3.44 ^b
4	150	39.23±2.47 ^a	56.66±2.45 ^b
5	200	44.34±3.56 ^a	77.70±2.45 ^b
6	250	49.45±3.32 ^a	82.22±3.45 ^b

. Data represent means ± SEM of triplicate determination.

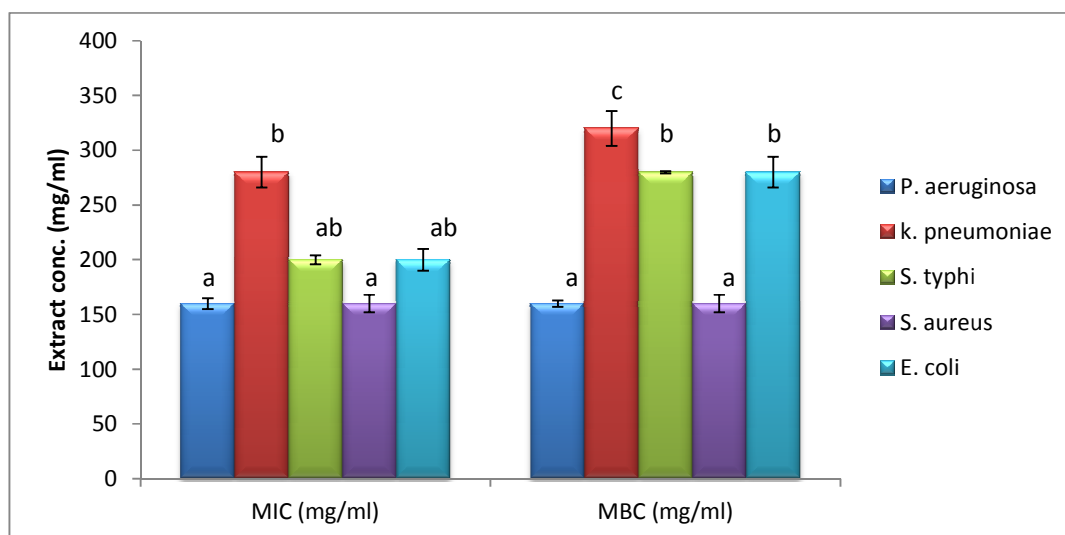


Figure 1; Minimal inhibitory concentration and Minimal bactericidal concentration of methanol leaf extract of *S. Occidentalis*. Each bar represents means $\pm$ SEM of triplicate determination.

DISCUSSION

The antimicrobial activities of methanol leaf extract of *S. occidentalis* obtained from North Central Nigeria were investigated against five bacteria, *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. An 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 [26]. 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 [27]. 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 IC_{50} 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 [28]. 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 [29]. These findings support earlier reports that plant metabolites like phenol, tannins and flavonoids possesses antioxidant and antimicrobial activity [21].

The antioxidants and antimicrobial activities demonstrated *S. occidentalis* leaves obtained from South West, Nigeria [20] were higher than that of the sample obtained from North Central Nigeria as reported in this study. These variations may be attributed to the amount and quality of secondary metabolites affected by genetic factors, climatic conditions, soil and cultivation techniques [30]. Furthermore, other factors that specifically determine the biological activities of plants include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized isolation procedures employed, the purity of active compounds, seasonal changes and the test system employed [31]. All these factors may influence the synthetic pathways of the active compounds in the plant extracts [32]

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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