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Antioxidants and Antimicrobial- Activities of Methanol Leaf Extract of *Senna occidentalis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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.

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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, Pseudimonas 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 bacterialcidal concentration (MBC) by plating various dilution of extract.
Results: The extract show significant antibacterial activities with MIC in the range of 160 mg/ml-280 mg/ml and MBC in range of 160 mg/ml – 320 mg/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 120 mg/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.

1. 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 and E 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* is 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].

Odeja et al. [20] reported the antioxidant and antimicrobial activities of *S. occidentalis* leaves from South West, Nigeria. However, bioactive components of plants varies with geographical origin [21], environmental conditions such as weather parameters (temperature, precipitation), and soil greatly contribute to the content of biological active compounds – natural antioxidants - in plants. 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 from North Central, Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Collection

Freshly harvested *S. ocidentalis* 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.

2.2 Sources of Microorganisms

Pure isolates 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.

2.3 Sample Preparation and Extraction of Plant Materials

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

2.4 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 [23]. Antibacterial activity of methanol leaf of *S. occidentalis* was carried out using Agar-well diffusion method as described by Jayaraman et al. [23], 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.

2.5 DPPH Radical Scavenging Activity

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

$$\% \text{ inhibition} = ((A_1-A_2)/A_1) * 100$$

Where

A_1= absorbance of blank sample
A_2= absorbance of *S. occidentalis*

2.6 Statistical Analysis

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

3. RESULTS

Zone of inhibition of methanol leaf extract of *S. occidentalis* against some pathogenic microorganisms are presented in Table 1. The zone of inhibition by the extract increases with increased concentration. The inhibitory zone against *E. coli* ranged from 15.00±0.50 mm to 30.00±0.05 mm, for *K. pneumonia* from 11.00±0.30 mm to 16.00±0.05 mm, for *S. aureus* it ranged from 11.00±0.50 to 24.00±0.20 mm, for *P. aeruginosa* it ranged from 15.00±0.50 mm to 28.00±2.54 mm while the extract had the zone of inhibition in the range of 14.00±0.50 mm to 34.00±0.40 mm against *S. typhic*. However, the organisms were not sensitive to the extract

at the dose of 80 and 120 mg/ml. The MIC of the extract against the organism ranges between 160 mg/ml- 280 mg/ml while the MBC range between 160 mg/ml – 320 mg/ml. The most sensitive organisms were *S. aureus* and *P. aeruginosa* while *K. pneumonia* show some degree of resistances compared to other organisms tested (Fig. 1). The extract and the standard antioxidant (Vitamin E) promoted an inhibition of DPPH radical with increasing concentrations. The IC_{50} (concentration that inhibits 50% of the DPPH radical) value of extract (263.53±3.24 mg/mL) was significantly ($p < 0.05$) higher than 83.63±2.78 mg/mL obtained for standard drug (Table 2).

Table 1. Zone of inhibition of methanol leaf extract of *Senna occidentalis*

Concen. (mg/ml)	*E. coli*	*K. pneumonea*	*S. aureus*	*P. aeruginosa*	*S. typhic*
	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	*S. occidentalis* (%)	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

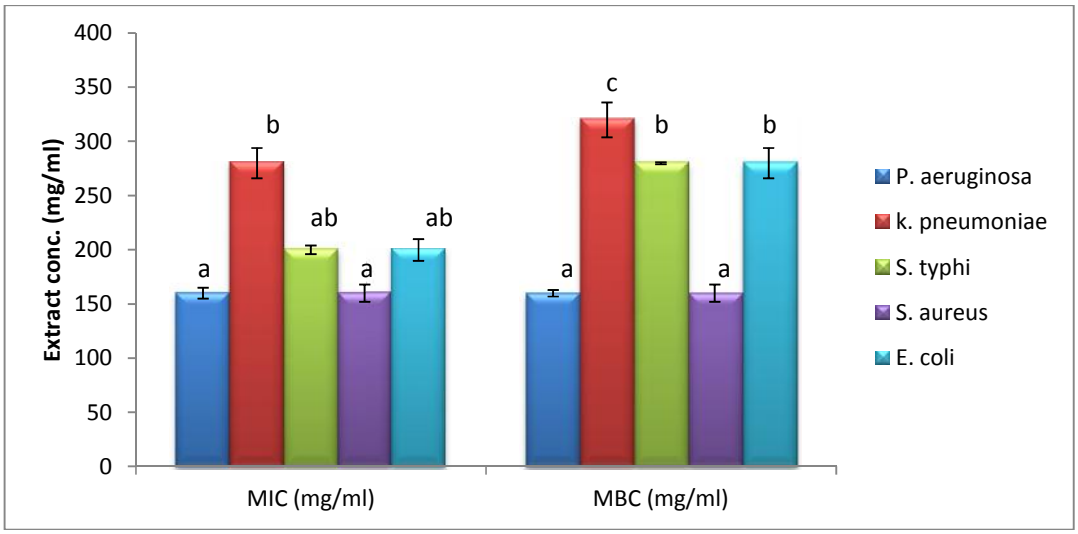

Fig. 1. Minimal inhibitory concentration and Minimal bacterialcidal concentration of methanol leaf extract of *S. occidentalis*

Each bar represents means ± SEM of triplicate determination

4. DISCUSSION

The antimicrobial activities of methanol leaf extract of *S. occidentalis* obtained from North Central Nigeria were investigated against five bacteria, *S. aureus, S. typhic E. coli, P. aeruginosa* and *K.* pneumonia. 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].

5. 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 authentification and subsequent commercialization.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000;31:247-256.

2. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turk J Biol. 2005;29:203-210.
3. Tsado AN, Lawal B, Ossai PC, Jagaba A, Gwadabe NK, Jiya AG, Umar AM, Oladunjoye JO. Antioxidants and anti-microbial activities of methanol extract of *Newbouldia laevis* and *Crateva adansonii*. J. Pharm. Allied Health Sci; 2016. DOI: 10.3923/jpahs.2016
4. Lawal B, Shittu OK, Ossai PC. Abubakar AN, Ibrahim AM. Antioxidant activities of giant African snail (*Achachatina maginata)* Haemolymph against CCl_4- induced hepatotoxicity in albino rats. Brit. J. Pharm. Res. 2015;6(3):141-154.
5. Halliwell B. How to characterize an antioxidant? An update. Biochem. Soc. Symp. 1995;61:73-101.
6. Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, Alozieuwa BU. Potential antimalarials from African natural products: A review. J. Intercult Ethno-pharmacol. 2015;4(4):318-343. DOI: 10.5455/jice.20150928102856
7. Bashir L, Shittu OK, Sani S, Busari MB, Adeniyi KA. African natural products with potential antitrypanosoma properties: A review. Inter. J. Bioch. Res. Rev. 2015; 7(2):45-79.
8. Lawal B, Shittu OK, AbdulRasheed-Adeleke T, Ossai PC, Ibrahim AM. GC-MS determination of bioactive constituents of giant African snail (*Archachatina maginata)* Haemolymph. IOSR Journal of Pharmacy and Biological Sciences. 2015;10(2):59-64.
9. Lawal B, Shittu OK, Abubakar AN, Olalekan IZ, Jimoh AM, Abdulazeez AK. Drug leads agents from methanol extract of Nigerian bee (*Apis mellifera)* propolis. J. Intercult Ethnopharmacol. 2015;4(4):318-343.
10. Yusuf OK, Bewaji CO. Evaluation of essential oils composition of methanolic *Allium sativum* extract on *Trypanosoma brucei* infected rats. Res. Pharm Biotechnol. 2011;3(2):17-21.
11. De Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, Levenfors JJ. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J Ethno-pharmacol. 2005;96:461-469.
12. Mann JM, Mark C, John T. The chemotherapy of trypanosomes; Marcel Dekker Inc. New York. 2008;2:233.
13. Onakpa MM, Ajagbonna OP. Antidiabetic potentials of *Cassia occidentalis* leaf extract on alloxan induced diabetic albino mice. Int J PharmTech Res. 2012;4:1766–1769.
14. Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa Congo. J Ethnopharmacol. 1999;68:193–203.
15. Jafri MA, Subhani MJ, Javed K, Singh S. Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxification in rats. J Ethnopharmacol. 1999;66:355–61.
16. Sadique J, Chandra T, Thenmozhi V, Elango V. Biochemical modes of action of *Cassia occidentalis* and *Cardiospermum halicacabum* in inflammation. J Ethno-pharmacol. 1987;19(2):201-12.
17. Bilal Bin-Hafeez, Iqbal Ahmad, Rizwanul Haque, Raisuddin S. Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. J Ethnopharmacol. 2001;75(1):13-18.
18. Berinyuy EB, Lawal B, Olalekan AA, Olalekan AA, Yusuf AA, Sakpe S, Ossai PC. Hematological status and organs/body-weight parameters in wister rats during chronic administration of *Cassia occidentalis.* International Blood Research and Reviews. 2015;4(3):1-7.
19. Ibrahim AM, Lawal B, Tsado NA, Yusuf AA, Jimoh AM. Phytochemical screening and GC-MS determination of bioactive constituents from methanol leaf extract of *Senna occidentalis.* Journal of Coastal Life Medicine. 2015;3(12):992-995.
20. Odeja O, Obi G, Ogwuche CE, Elemike EE, Oderinlo Y. Phytochemical screening, antioxidant and antimicrobial activities of *Senna occidentalis* (L.) leaves extract. Clin Phytosci. 2015;1:6. DOI: 10.1186/s40816-015-0007-y
21. Lawal B, Ossai PC, Shittu OK, Abubakar AN. Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam peel, maize chaff and bean coat. Inter J Appl Biol Res. 2014;6(2):01-17.
22. Tsado NA, Lawal B, Santali ES, Mohammed AS, Balarabe MM, Ibrahim HA, George JJ. Phytochemicals and acute toxicity profile of aqueous and methanolic extracts of *Crateva adansonii* leaves in

swiss albino rats. Asian J. Biochem. 2015;10(4):173-179.
23. Jayaraman S, Manoharan MS, Illanchezian S. *In-vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Tropical J. Pharma. Res. 2008;7(4):1143-1149.
24. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African J. Microbiol Res. 2009;3(13):981-996.
25. Mahajan BK. Significance of differences in means. In: Methods in Biostatistics for Medical and Research Workers, 6th edition. New Delhi: JAYPEE Brothers Medical Publishers. 1997;130-155.
26. Kumar N, Mueen AK, Raman D, Ahmed H. Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. J. Medic Plants Res. 2008;2(12):361-364.
27. Asquith TN, Butter LG. Interaction of condensed tannins with selected proteins. Phytochem. 1986;25(7):1591-1593.
28. Nahak G, Sahu RK. *In vitro* antioxidant activity of *Azaderachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. Nat. Sci. 2010;6(6):123-128.
29. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridth JB. The relative antioxidant activities of plants derived polyphenolics flavonoids. Fre. Rad. Res. 1995;22:375-383.
30. Bhatti HN, Iqbal Z, Shaid SA, and Bukharii H. Variations in oil potential and chemical composition of *Eucalyptus crebra* among different districts of Punjab–Pakistan. Internationa J Agric. Biol. 2007;1:136–138.
31. Hussain AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 2008;108:986-995.
32. Rached W, Benamar H, Bennaceur M, Marouf A. Screening of the antioxidant potential of some Algerian indigenous plants. J. Biol.Sci. 2010;10:316–324.

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