IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 2 Ver. III (Mar -Apr. 2015), PP 32-36
www.iosrjournals.org

Evaluation of the Antimicrobial, Antioxidant and Phytochemical activities of Methanolic and Aqueous Extract of *Cissus aralioides* Leaves on some selected pathogenic Microorganisms

[1]Eze, V.C., [2]Onoja, S.O. and [3]Chikezie, J.C.
[1,3]*Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State.*
[2]*Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.*

Abstract: *Plants used for traditional medicine contain a wide range of substances which can be used to treat various infectious diseases. Hence, methanol and aqueous extracts of Cissus aralioides leaves were analyzed for antioxidant, antimicrobial and phytochemical properties. The plant extract showed significant (P<0.05) antioxidant activities in both methanol and aqueous extracts. The antioxidant activity of both methanolic and aqueous extract of C. aralioides increased as the concentration increased with methanolic extracts showing higher antioxidant activity but both still lower than the standard (ascorbic acid).The methanolic and aqueous extracts of the test plant also showed presence of phytochemical constituents saponins, glycosides, flavonoids, alkaloids, tannins and sterol terpenes. The antimicrobial activity of the plant extract was evaluated against Escherichia coli, Salmonella enterica, Staphylococcus aureus and Candida albicans using the agar disc diffusion method. The highest antimicrobial potentials was observed for the methanol extract of C. aralioides against Salmonella enterica with a mean zone of inhibition diameter of 18.5mm mean while the aqueous extract had a mean zone of inhibition diameter of 15mm. The rest of the three test organisms were resistant to both the methanol and aqueous extracts. The Minimum Inhibitory Concentration (MIC) observed was 50 mg/ml and 25mg/ml for the methanol and aqueous extracts respectively. This indicates that C. aralioides are efficacious and can be used in the management of the disease cause by Salmonella enterica. This study demonstrate that C. aralioides has antioxidant and antimicrobial activities, which could be attributed to its phytochemical composition. The study suggests that methanol and water are both good extracting solvent for C. aralioides.*
Key words: *Antimicrobial, antioxidant, methanolic, aqueous, Cissus aralioides, pathogenic, microorganisms*

I. Introduction

In the last decade, there has been a global upsurge in the use of traditional medicine, complementary and alternative medicines in both developed and developing countries (e.g. Nigeria). Traditional Medicine is a group of diverse medicinal and health care systems, practices and products including acupuncture, herbalism, traditional Chinese medicine, naturopathy, Ayurveda, hypnosis, and homeopathy in addition to a range of other practices that are not generally considered to be part of conventional medicine and are not integrated into the dominant health care system in most countries (WHO, 2005). Today, traditional complementary and alternative medicines play an important role in health care and health sector reform globally. This is due to the fact that 80% of families in the developing world today still depend on traditional herbal remedies for the treatment of disease. This reason is that they are affordable and easily accessible to all (Bizimenyera et al., 2007; Adefuye and Ndip, 2013). Thus there have been increased research interests to verify the activity of the medicinal plants as claimed by the herbalist. The researches in medicinal plants have led to the isolation, characterization and formulation of some drugs in orthodox medicine. Examples of such drugs include vincristine, vinblastine, aspirin, morphine etc (Adefuye and Ndip, 2013). Cissus aralioides is one of the commonly used medicinal plants in South-Eastern Nigeria by native doctors.

Cissus aralioides is commonly called "eriri agwo" in Nigeria (Igbo) and "Kindamina" in Cameroun, and belong to the family Vitaceae. It is a lofty climber which is woody at the base with stout green succulent stems constricted at the nodes and sometimes sub-succulent leaves. Flowers are greenish or whitish, comparatively large and horizontal. The fruit is 2½ cm long and mostly red in colour. The whole plant is covered with irritating hairs and the leaves contain an acid and slightly acrid red sap. They are commonly found in deciduous forests and fringing jungle across the regions of Tropical Africa (Burkill, 2000; Ezeja et al., 2015). In African traditional medicine, different parts of Cissus aralioides are used in the treatment of different ailment either alone or in combination with other medicinal plants. The disease conditions that have been managed traditionally with C. aralioides include arthritis, rheumatism, dropsy, gout, swellings, edema, febrifuges, pain-killers, pulmonary troubles, venereal diseases etc. Cissus aralioides leaves and roots are also used as

antimicrobial agents against microorganisms of the gastrointestinal and urogenital tracts (Aluka 2010; Assob et al., 2011; Burkill 2000; Ezeja et al., 2015). This work was aimed at investigating the antimicrobial, antioxidant and phytochemical composition of the methanolic and aqueous extract of Cissus aralioides leaf.

II. Materials and Methods

Sample collection and identification

The medicinal plant used Cissus aralioides was collected from Michael Okpara University of Agriculture Umudike, Abia State Nigeria. It was identified by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology of the same institution.

The test bacterial isolates (Escherichia coli, Salmonella enterica and Staphylococcus aureus) and the fungal isolate (Candida albicans) were obtained from the Microbiology Laboratory of the Federal Medical Centre Umuahia, Abia State, Nigeria. They were purified, sub-cultured and re-identified to ensure purity of the isolates.

Confirmation of Bacterial and fungal Isolates

The bacterial isolates were confirmed using some biochemical tests such as catalase test, coagulase test, indole test, citrate test, methyl red-Voges Proskaurer reaction, urease test, H_2S production and carbohydrate fermentation tests while the Candida albicans was identified with the germ tube test [Fawole and Oso, 1998; Cheesbrough, 2006; Tamber and Khante, 2010].

Sample Preparation

The plant part used was the leaves and were washed in running tap water for dirt removal. The leaves were then spread in laboratory trays and allowed the water to drain out. They were dried in the oven at low temperature of 40 ^{0}C and ground in a laboratory mill. The ground sample was sieved through 1mm test-sieve to obtain powdered samples.

Preparation of Cissus aralioides Extracts

Twenty grams of the ground sample was weighed and mixed with 180 ml of the extracting solvents (methanol and water) in separate flasks. They were shaken very well and then allowed to stand for 48 hrs at room temperature. They were then shaken again and filtered through Whatman No.1 filter paper and the filtrates were collected in separate labeled beakers which were previously weighed. The filtrate from each sample was evaporated to dryness and the beakers were left in a dessicator to allow adhering condensing vapour to be removed (Borokini and Omotayo, 2012).

Phytochemical Analysis

The phytochemical screening of all the extracts was carried out to determine the presence of the alkaloid, flavonoids, cyanogenic glycoside, saponins, terpenes, and phytosterols using the method of Sofowora (1993).

Determination of the antimicrobial effect of the Herbal Preparation

The agar disc diffusion method was used to determine the antimicrobial properties of the crude extracts as described in the clinical and laboratory standards institute and the National center for infectious Disease, center for disease control and prevention. Within 15 minutes after adjusting the turbidity of the inocula suspension, a sterilized swab was aseptically dipped into the suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid to remove excess inocula from the swab. The dried surface of a Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface with bacteria. This procedure was repeated by streaking two more times, rotating the plate approximately 60^o each to ensure an even distribution of inocula. Whatman No. 1 filter paper was perforated and sterilized by putting it in a hot-air oven regulated at 100^{o}C for 2 hours. Standard drug 100 µg/ml of ciprofloxacin was used as positive control for the bacterial isolates and 1000 iu/ml of nystatin was used for the fungal isolates, while 10% v/v DMSO was used as negative control. Duplicates of each plate were made and the procedure was repeated for the other microorganisms. The plates were kept in the refrigerator for about 4 hours for the complete diffusion of the extract and incubated at 37 ^{o}C for 48 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a sterilized ruler clinical and laboratory standards institute (Cheesbrough, 2004).

Determination of Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) of the crude Extracts

The MIC value of the crude extracts was determined by adding dilutions of methanolic and aqueous extracts of Cissus aralioides to the nutrient broth in test tubes. A standard inoculum of the test organism was then added. After 24 hours, the MIC was reported as the lowest concentration of the extract showing visible turbidity or growth (Cheesbrough, 2004).

The MBC values were deduced from those test tubes with lowest concentrations at which no growth took place after incubation for 24 hours (Nester et al., 2004). Small colonies from each of those test tubes were transferred to fresh nutrient agar plates and incubated at 37^{o}C for 24 hours and plates examined for the presence or absence of growth. Plates with no microbial growth were regarded as the minimum bactericidal concentrations (Nester et al., 2004).

III. Results

The results of the antimicrobial, antioxidant and phytochemical activities of the methanolic and aqueous extracts of the C. aralioides are shown in Tables 1 – 3 and Fig. 1 respectively.

The phytochemical activities of the C. aralioides are shown in Table 1. The phytochemical parameters present were saponins, glycosides, flavonoids, tannin and sterols/terpenes. The flavonoids were moderately present in both the methanol and aqueous extracts. The glycosides were also moderately present in methanol extract and slightly present in aqueous extract. The saponins, alkaloids, tannin sterols/terpenes were all slightly present in aqueous and methanol extracts.

The mean zone of inhibition diameter is shown in Table 2. At 100 mg/ml, the methanol extract had a mean zone of inhibition diameter of 18.5mm on Salmonella enterica, the aqueous extract had 15 mm zone of inhibition on Salmonella enterica. However, the methanol and aqueous extracts of C. aralioides had no activity over Escherichia coli, Staphylococcus aureus and Candida albicans.

The MIC and MBC are shown in Table 3. The MIC of aqueous and methanol and aqueous extract on Salmonella enterica was 25 mm and 50 mm respectively. At the MIC values, Salmonella enterica showed no growth on sub culturing. Thus the MIC and MBC was the same value.

The antioxidant activity of the methanolic and aqueous extracts of the C. aralioides is shown in Fig. 1. There is significant antioxidant activity in both the methanol and aqueous extracts of C. The methanol extracts had a higher antioxidant activity compared with the aqueous extract but the antioxidant activity of the methanol and aqueous extracts were lower than the standard.

IV. Discussion

The aqueous and methanolic extracts of Cissus aralioides leaves were subjected to phytochemical screening and the results showed that the extracts contained saponins, glycosides, flavonoids, alkaloids, tannins and sterol/terpenes.

The plant extract used in this study also showed significant antioxidant activity; with the methanol extract having higher antioxidant effect compared to the aqueous extract. This could be due to the difference in solubility of the components in the extracting solvents. The antioxidant activities may be due to the presence of some of the phytoconstituents. However, the antioxidant effects of the test extracts were lower when compared with the standard.

The antimicrobial activities of the aqueous and methanol extracts of Cissus aralioides were also investigated. The aqueous and methanol extracts of Cissus aralioides inhibited Salmonella enterica. This confirms that the plant has antimicrobial activity against Salmonella enterica. It inhibited the organism with the average zone of 18.5 mm methanol extract and 15 mm aqueous extract. The results of antimicrobial activity of the aqueous and methanol extract of Cissus aralioides showed no inhibitory effect on the Staphylococcus aureus, Escherichia coli and Candida albicans. The result of the antimicrobial activities of C. aralioides is not in agreement with the report of Assob et al. (2011). However, Nwinyi et al. (2009) reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials. Also, the lack of activity against E. coli, Candida albicans and Staphylococcus aureus may be due antibiotic resistance and variation in the strains of the microorganisms used.

The antimicrobial activity of C. aralioides against S. enterica may be attributed to some of the phytochemical composition of C. aralioides. Some of the phytoconstituents; tannins, flavonoids, terpenes and alkaloids has been shown to possess antimicrobial activity. The results suggest that C. aralioides has bactericidal activity against S. enterica. This could be due to the inhibition of cell wall or cell membrane synthesis or inhibition of DNA replication. According to Rang et al. (2003), penicillin and cephalosporin prevent bacterial cell wall synthesis and in doing so, are bactericidal in action.

The MBC of the methanol and water extract are 25 and 50 mg/ml respectively. This indicates that the methanolic extract is more potent than the aqueous extract, but does not necessarily mean that the methanolic

extract would be more efficacious (Rang et al., 2003). The difference in the potency of the extract may be due to variation in the solubility of the phytoconstituents in the solvent used in the extraction (Nwinyi et al., 2009).

The demonstrated antioxidant activity of the extract may help to correct the effects of oxidative stress which may manifest due to bacterial toxin and disease condition in patient, following treatment with C. aralioides. To protect the body against oxidative stress, antioxidant supplementation or improvement in antioxidant nutrition is essential and antioxidants from natural sources have been proved to have higher bioavailability and therefore higher protective efficacy than synthetic antioxidants (Benedetti et al., 2004; Ezeja et al., 2015).

V. Conclusion

The study has demonstrates that methanolic and aqueous extract of C. aralioides leaf have a potent bactericidal activity against Salmonella enterica and also has a potent antioxidant activity. The methanolic extract of C. aralioides was more potent than the aqueous extract of C. aralioides in both antioxidant and bactericidal activity. Further work is required to isolate and characterize the active principle responsible for the antibacterial and antioxidant activity.

References

[1]. Adefuye, A.O. and Ndip, R.N. (2013). Phytochemical analysis and antibacterial evaluation of the ethyl acetate extract of the stem bark of Bridelia micrantha, Pharmacognosy Magazine; 9(33): 45–50.
[2]. Aluka, P. (2010). Plant Database. Retrieved from www.aluka.org/action/showcom on August 7, 2014.
[3]. Assob, J.C., Kamga, H.L., Nsagha, D.S., Njunda, A.L., Nde, P.F., Asongalem, E.A., Njouendou, A.J., Sandjon, B. and Penlap, V.B. (2011). Antimicrobial and toxicological activities of five medicine plant species from Cameroon Traditional Medicine", BMC Complement. Altern. Med.; 11: 70-75.
[4]. Benedetti, S., Benvenah, F., Pagharami, S., Francegh, S., Stephano, S. and Canestrari, F. (2004). Antioxidant properties of a novel phycocyania extract from the blue–green alga, Aphanizomenonflos-aquae. Life Sci.; 75. 2353-2362.
[5]. Bizimenyera, E.S., Swan, G.E., Samdumu, F.B., McGraw, L.J. and Eloff, J.N. (2007). Safety profiles of Peltophorum africanum Sond (Fabaceae) Extracts. South Afr. J. Sci.; 7: 66 –78.
[6]. Borokini, T.I. and Omotayo, F.O. (2012). "Comparative phytochemical analysis of selected medicinal plants in Nigeria. Int'l. J. Adv. Chem. Res.; 1(1): 011- 018.
[7]. Burkill, H.M. (2000). The useful plants of West Tropical Africa, Families vol. 5, Royal Botanical Gardens Kew, pp. 301- 302.
[8]. Cheesbrough, M. (2006). District laboratory practice in tropical countries, Part 2. Cambridge University Press,
[9]. United Kingdom, pp. 143-180.
[10]. Ezeja, M.I., Omeh, Y.N., Onoja, S.O. and Ukaonu, I.H. (2015). Anti-inflammatory and Antioxidant Activities of the Methanolic Leaf Extract of Cissus aralioides. Amer. J. Pharmacol. Sci.; 3(1): 1-6.
[11]. Fawole, M.O. and Oso, B.A. (1998). Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan; p.126.
[12]. Nester, E.W., Anderson, D.G., Rogers, C.E. and Nester, M.T. (2008). Microbiology, A Human Perspective, International edition, McGraw-Hill, New York, USA, pp. 508-641.
[13]. Rang, H. P., Dale, M. M., Ritter, J. M. and Moore, P. K. (2003). Pharmacology, Elsevier Science Ltd, New Delhi, pp. 368-378
[14]. [13] Sofowora, A. (1993). Medicinal plants and traditional medicine. Spectrum Books Limited, Ibadan, Nigeria; pp. 224-227.
[15]. Tamber, D.H. and Khante, B.S. (2010). Antimicrobial properties of traditionally used medicinal plants for enteric infections. Int'l J. Pharm. Sci. Res.; 1(9): 120-128.
[16]. WHO, (2005). National Policy on Traditional Medicine and Regulation of Herbal Medicine Report of a WHO global Survey. WHO, Geneva; pp 1-156.

Table 1: Qualitative phytochemical characteristics of methanolic and aqueous extracts of Cissus aralioides leaves

	Saponins	Glycosides	Flavonoid	alkaloid	Tannin	Sterol/Terpens
Aqueous extracts	+	+	+	+	+	+
Methanol extracts	+	+	+	+	+	+

Key: + present

Table 2: The mean zone of inhibition diameter (mm) of Cissus aralioides leaves extract against the test organisms.

	Staphylococcus aureus	Escherichia coli	Salmonella enterica	Candida albicans
Methanol extract (100mg/ml)	0.00	0.00	18.5	0.00
Aqueous extract (100mg/ml)	0.00	0.00	15	0.00
ciprofloxacin (50 µg/ml)	13	30.5	27.5	NR
Nystatin (1000iµ/ml	NR	NR	NR	23.5

Key: NR = Not Required

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Cissus aralioides against Salmonella enterica

Extract	MIC (mg/ml)	MBC (mg/ml)
Water extract	50	50
Methanol extract	25	25

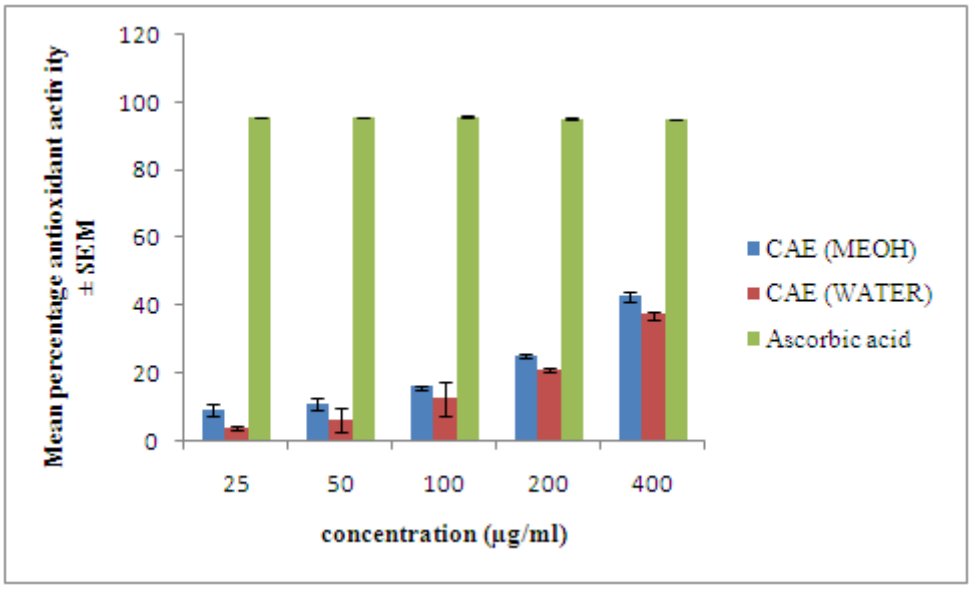

Fig. 1: Antioxidant Effect of Cissus aralioides