Antimicrobial Activity of Aqueous and Ethanolic Extracts of *Aloe vera*

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Abstract: This study evaluated the antimicrobial activities of crude ethanolic and aqueous extracts of *Aloe vera* using standard techniques. Results obtained revealed reasonable antimicrobial activities on the tested organisms to varying zones of inhibition. The activities however were found to be concentration dependent while no apparent statistical disparity was observed with the different extracting solvents (tvalue = 0.88, p>0.05). The minimum inhibitory dilution of both extracts range from 25-100mg/ml. It can thus be inferred that the tested *Aloe vera* has good antimicrobial properties.

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1. Introduction

Herbal medicine is an alternative form of therapy and has become the mainstream throughout the world due to the growing resistance of pathogens to conventional antibiotics (De Smet, 2002). The screening of plant extracts and plant products for their antimicrobial activity has in most cases involved higher plants, many of which have shown clinical relevance as sources of potential chemotherapeutic agents (Essawi and Srour, 2000; Srinivasan *et al.,* 2001; Hamil *et al.,* 2003; Arias *et al.,* 2004; Shilpi *et al.,* 2006; Van wyk, 2008; Kosalge and Fursule, 2009). However, the realization by scientists that many native shrubs contain several unique chemicals that could provide new benefits to man and its livestocks have triggers their cultivation.

Nigeria has a rich variety of medicinal plants distributed in the different geoecological regions of the country. The plant *Aloe vera* L. Burm. f. (Family *Liliaceae*) is an ancient semi tropical medicinal plant indigenous to Africa, Madagascar and Arabia (Adams *et al*.,2010). This plant has been designated as the legitimate synonym for *A. barbadensis* Mill. According to the International Code of Botanical Nomenclature (Newton,1979; Grindlay and Reynolds, 1986), *Aloe vera* is one of the approximately 420 species of the genus *Aloe* (Dagne *et al.,*2010), which is variously classified as belonging to the *Asphodelaceae, Liliaceae*, or *Aloaceae* families. The geographic origin of *Aloe vera* is believed to be in Sudan, with the plant subsequently being introduced in the Mediterranean region and most other warm areas of the world (Makanjuola *et al.,*2016). This plant has been reported to exhibit a wide range of biological characteristics such as antimicrobial, antifungal, anti-inflammatory, immune stimulant, antiseptic, wound and burn healing, antiulcer, antitumor and antidiabetic activities among other functions. However, the limitation of information on the inhibitory effect of *Aloe vera* on the strains of micro organisms present in our environment calls for confirmation of such observation using disease causing microorganisms in our clinical environment.

Source of plant

Aloe vera specimens were obtained from the Agronomy Unit of Dagwon farm (National Veterinary Research Institute, Vom). The plants were authenticated by the taxonomist at the Department of Botany and Biochemistry, University of Jos, Nigeria.

Preparation of Plant Materials and Extracts

The plucked leaves were washed with running tap water and then with distilled water several times. They were then disinfected, weighed using metler's balance and sliced longitudinally. One kilogram of the fresh leaves was air dried on the laboratory table at 27^{o}C after which they were shredded and preserved in airtight cellophane bags. The shredded leaves were milled into powder form using a warring commercial blender to give 600g. Hundred grams (100g) of each of the powdered plant materials was soaked in 500 ml of ethanol and water for 6h using soxhlet apparatus.

Phytochemical studies

Phytochemical tests were carried out to determine the presence of flavonoids, tannins, alkaloids, saponins and anthraquinones using the methods described by Odebiyi and Sofowora (1978).

Test organisms

Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella spp, Proteus vulgaris, Pseudomonas aeroginosa, Klebsiella aerogens, Trichophyton mentagraphytes, Candida albican and Cryptococcus neoformans were obtained from Bacteriology and Dermatophilosis Unit, Division of NVRI, Vom. The isolates identities were further confirmed in our laboratory using standard procedures (Cheesborough, 2005). The isolates were maintained on Tryptone Soy agar (TSA) (Oxoid) and Sabouraud dextrose agar (oxoid) at 4°C for bacteria and fungi respectively.

Determination of antimicrobial activity

The medium used was Mueller Hinton agar (Oxoid, U.K). The bacterial inoculum were adjusted to 0.5 McFarland turbidimetric standard and inoculated onto the medium using sterile swabs. For each extract, three replicate plates were prepared against the test organisms. Antimicrobial activity of the ethanolic and aqueous extracts of the plant samples were evaluated by the agar well diffusion method. Using sterile cork-borer of 6 mm diameter, equidistant wells were cut in each of the agar plates, while different concentrations of the extracts, 1000, 750, 250, and 100 mg/ml were introduced into the wells. The plates were left for 2 h at room temperature to allow the extract to diffuse. The solvents used for extraction served as control and was introduced into a separate well as appropriate. Ciprofloxacin /Fluconazole (250/150 mg/ml) was used as standard antimicrobial agent for comparison. The plates were then incubated at 37°C for 24 h. Antimicrobial activity was determined by measurement of zone of inhibition around each well using a pair of calipers (in mm) and read on a meter rule.

Serial dilution of *Aloe vera* juice

Twelve sterile tubes were arranged in the rack and 1ml of sabouraud dextrose broth/nutrient broth as appropriate was added to tubes 2 to 12 (except tubes 1 and 11) and 2.0ml of *Aloe vera* juice were put into tube 1. Then, 1.0ml of *Aloe vera* juice was transferred from tube1 to tube 2. Serial doubling dilution was made from tubes 2 to 10 by transferring 1.0ml of the homogeneous tube 2 content to tube 3, and from 3 to 4 and so on to 10 and the remaining 1ml was discarded. Then, 1ml of the *Aloe vera* juice was added to tube 11 (negative control) and 1ml of sabouraud dextrose broth/nutrient broth was added to tube 12 (positive control).

Determination of minimum inhibitory dilution (MID) of *Aloe vera* juice

1.0 ml of 0.5 McFarland turbidity of each of the organisms was added to the contents of all the tubes and incubated at 37°C for 7days. The highest dilution showing no turbidity was defined as the MID.

Results

The in vitro antibacterial activity of crude ethanolic and aqueous extracts of *Aloe vera* used locally for the treatment of diseases are presented in Tables 1. All the crude plant extracts possess reasonable antimicrobial activities on the tested organisms to varying zones of inhibition.

Table 1**:** In Vitro Antimicrobial activities of Crude Ethanolic and Aqueous Extracts of *Aloe vera*

	Concentration of extracts/ Zones of inhibition (mg/ml)											
Org	1000	750	500	250	100	1000	750	500	250	100	250	50
ETHANOLIC EXTRACT						AQUEOUS EXTRACT					CIP	50% ethanol
EC	26	23	20	15	12	25	21	17	14	11	9.2	0.00
SA	28	25	20	15	12	26	22	18	14	9	12.4	0.00
ST	24	23	21	17	14	20	18	15	11	9	16.0	0.00
PA	32	28	25	22	16	29	26	22	19	14	18.0	0.00
PV	24	21	18	12	10	22	20	15	13	9	17.6	0.00
KA	00	00	00	00	00	00	00	00	00	00	12.4	0.00
SS	30	27	25	22	14	28	25	22	17	9	19.4	0.00
TM	30	26	23	18	10	27	25	22	15	10	16.9	0.00
CA	00	00	00	00	00	00	00	00	00	00	19.0	0.00
CN	26	23	20	17	10	24	21	17	13	10	17.3	0.00

Key:

EC = *Escherichia coli, SA = Staphylococcus aureus, ST= Salmonella typhi, PA=Pseudomonas aeruginosa,*
PV= *Proteus vulgaris, KA= Klebsiella aerogenes, SS = Shigella species, TM = Trichophyton mentagrophyte, CA= Candida albicans, CN = Cryptococcus neoformans*

The activities of both ethanolic and aqueous extracts of *Aloe vera* shows no significant statistical variation in terms of efficacy. The activities however were found to be concentration dependent. The activities of the plants were much more enhance at higher concentration especially where there are activities. Both extracts also shows no activity on *Klebsiella aerogenes and Candida albicans* at all the concentrations tested. The highest antimicrobial activities for all the extracts were observed at concentration of 1000mg/ml. Generally, all the crude plant extracts showed broad spectrum activities against Gram positive and Gram negative bacteria. The effect of the different extracting solvents on the antimicrobial activities of the tested plants shows that all solvents were able to extract the active ingredients in the plants and no apparent statistical variation was observed (tvalue = 0.88, p>0.05) as judged by their zones of inhibition on the different organisms. The minimum inhibitory dilution of both extracts also corroborated the findings of the agar well dilution technique.

Table 2: Influence of concentration on the antimicrobial activity of *Aloe vera*

Concentrations	n	Antimicrobial activity (Mean±SEM) mm
1000mg/ml	10	22.0±3.76
750mg/ml	10	19.6±3.38
500mg/ml	10	17.2±2.95
250mg/ml	10	9.8±1.74
100mg/ml	10	6.9±0.76

Fvalue = 8.8, P<0.05

Table 3: Influence of solvent of extraction on the antimicrobial activity of *Aloe vera*

Solvent of extraction	n	Antimicrobial activity (Mean±SEM) mm
Ethanolic extract	60	13.7±1.41
Aqueous extract	60	12.1±1.28

tvalue = 0.88, P>0.05

Table 4: Minimum Inhibitory Dilutions of *Aloe vera* on the tested pathogens

Organisms	MID(Aqueous)	MID(Ethanolic)
Escherichia coli	100	50
Staphyloccous aureus	50	50
Salmonella typhi	100	100
Pseudomonas aeruginosa	25	25
Proteus vulgaris	100	100
Klebsiella aerogenes	>100	>100
Shigella spp	25	25
Trychophyton mentagrophyte	25	25
Candida albicans	>100	>100
Cryptococcus neoformans	50	50

Discussion, Conclusion And Recommendation

The use of plants for treating infectious diseases can be dated back to antiquity. In this study, tested *Aloe vera* displayed very reasonable antimicrobial activities but to varying zones of inhibition. This observation is in consonance with that of Makanjuola *et al.,*(2016) who also documented similar findings. The fact that both Gram positive and Gram negative organisms as well as fungal pathogens were inhibited by these plants is an indication that they possess a broad spectrum based activities. The dependence of the antimicrobial activity on concentration suggest that the molecular weight of the extract that has contact with the micro organisms is more imperative than the diffusion rate through the media. The influence of solvent of extraction on the antimicrobial activities of the tested plants shows no statistical significant disparity on the activities of all the tested plant extracts for the different solvents. This is unexpected as Obi and Onuohia (2000) have earlier reported ethanol as

the solvent of choice when extracting plant active ingredients. Their findings however negate that which documented normal hexane as the best for extracting active ingredients of plant (Ijeh *et al.,* 2005; Junaid *et al.,* 2006). These two studies buttressed that, solubilization of required active ingredients in solvent may probably be the major factors influencing the selection of the most appropriate solvent of choice (Agu and Thomas, 2012). The minimum inhibitory dilution shows that the extracts exhibited definite bacteriostatic and fungistatic activities except on for *Klebsiella aerogenes and Candida albicans*. This result signify the probable optimum concentration of such extracts that could inhibit the tested pathogens (Brooks *et al.,* 2001), thereby guiding against abuse such as overuse or under dosage of the tested extracts. The results of the phytochemical screening of the extracts reveal the presence of saponins, tannins alkaloids, flavonoids, glycosides and anthraquinones. These phytochemicals have been shown to possess several biological activities including antimicrobial activity (sofowora, 1993). The flavonoids are mostly recognized for their antioxidant activity while their role in modifying the body reaction to allergens, viruses and carcinogens has also been reported (Balch and Balachi, 2000; Ekam and Ebong, 2007). According to Jiksika *et al*. (1992), alkaloids are organic compounds that contain nitrogen having sedative and analgesic properties. In another studies, the toxigenic effect of this phytochemical was reported (Obochi, 2006; Ekam and Ebong, 2007). According to these studies, such toxic effect is as a result of a stimulatory effect leading to neurological dysfunction. Edeoga (2006) reported that the phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of some herbal plants studied showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins which is in collaboration with the result of this study. In conclusion, the results of this study have shown that plant based therapy may just be the lasting panacea to the problem of multi drug resistance in microorganisms especially if selected properly. It can therefore be recommended that proper characterization of the plants active ingredients after fractionation be done in future using bioassay guided principle while elucidation of the most active ingredients can be done first using gas chromatography and then nuclear magnetic resonance.

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References

1. De Smet PAG (2002). Herbal remedies. New Engl. J. Med. 347: 2046- 2056.
2. Essawi T, Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 70: 343-349.
3. Hamil FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soerjato DD (2003). Traditional herbal drugs of southern Uganda: Literature analysis and antimicrobial assays. J. Ethnopharmacol. 84: 57-78.
4. Odebiyi A, Sofowora EA (1978). Phytochemical screening of Nigerian medicinal plants. Lloydia 41: 234-246. Olafimihan CA (2004). Effects of seasonal variation on the antibacterial activity of aqueous extract of Azadirachta indica fresh stem bark. Biosci. Res. Commun. 16(1): 13-16.
5. Shilpi JA, Taufiq-ur-Rahman MD, Amal US, Shahanum AM, Samir KS, Seidel V (2006). Preliminary pharmacological screening of Bixa orrelana L. leaves. J. Ethnopharmacol. 108: 264-271.
6. Srinivasan D, Nathan S, Suresh T, Lakshmana A, Perumalsamy P (2001) Antimicrobial activity of certain Indian medicinal plants used in folk loric medicine. J. Ethnopharmacol. 74: 217-220.
7. Van Wyk BE (2008). A broad review of commercially important southern African medicinal plants. J. Ethnopharmacol. 119: 342-355.
8. Kosalge SB, Fursule RA (2009). Investigation of ethnomedicinal claims of some plants used by tribals of Satpuda Hills in India. J. Ethnopharmacol. 121: 456-461.
9. Arias ME, Gomez JD, Cudmani N, Vattuone MA, Andisla MI (2004). Antibacterial activity of ethanolic and aqueous extract of Acacia aroma Gill ex Hook et. Life Sci. 75: 191-202.
10. Adams SP, Leitch IJ, Bennett MD, Chase MW and Leitch LH (2000). Ribosomal DNA evolution and phylogeny in Aloe (Asphodelaceae). Am. J. Bot., 87(11): 1578-1583.
11. Newton LE (1979). In defense of the name *Aloe vera*, t he cactus and succulent. J. Great Britain, 41: 29-30.
12. Grindlay D and Reynolds T (1986). The Aloe vera phenomenon: a review of the properties and leaf parenchyma gel. J. Ethnopharmacol., 102(6): 0074-0276.
13. Dagne E, Bisrat D, Viljoen A and Van Wyk BE (2000). Chemistry of *Aloe* species. Current Organic Chemistry, 4:1055-1078.
14. Makanjuola SO, Oluwadun A, Thomas, BT and Folorunso JB (2016). Influence of genetic

diversity on the antibacterial activities of *Aloe vera* accessions. International Journal of Genetics, 5(2): 25-29, 2015.
15. Obi VI and Onuoha C (2000). Extraction and characterization methods of plants and plant products. In: Biological and Agricultural technique. Ogbuile, J.N. and Ojiako, O.J. Ed. Websmedia Publications, Owerri. 271 –286.
16. Ijeh II, Omodamiro OD and Nwanna IJ (2005). Antimicrobial Effect of Aqueous and Ethanolic Fraction of Two Species: *Ocimum gratissimum* and *Xylopia gethiopica*. Afr. J. Biotechnol, 4(9), 953 – 956.
17. Junaid SA, Olabode OA, Onwuuri FC, Okwori AEJ and Agina SE (2006). The antimicrobial properties of *Ocimum gratissum* extracts on some selected Bacterial gastrointenstinal isolates. Afr. J. of Biotechnology, 5(22):2315-2321.
18. Agu GC and Thomas BT (2012). Antibacterial activities of ethanol and aqueous extracts of five Nigerian medicinal plants on some wound pathogens. Nature and Science, 10(2): 78-84.
19. Sofowora A (1993). Medical plant Traditional Medicine in Africa 2nd Edition, Spectrum Books Ibadan Nigeria 134- 136.
20. Brooks GF, Butel JS and Morse SA (2001). Cell structure. In: Jawetz, Melnick and Adelbergs Medical Microbiology. 22nd ed Lange Medical Books/McGraw-Hill USA, pp 7-37.
21. Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4:685-688.

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