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COMPARATIVE STUDY OF THE ANTIMICROBIAL PROPERTIES OF *ALOE VERA* JUICE AND GEL (LEAF) EXTRACTS AGAINST SELECTED CLINICAL ISOLATES.

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Abstract— **The comparative study of the antimicrobial properties of *Aloe vera* juice and gel (leaf) extracts against selected clinical isolates have been investigated using standard microbiological methods. The organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Candida albicans* and *Aspergillus niger*. The different extracts exhibited antimicrobial activities against the test organisms. Aqueous extract of *Aloe vera* juice had the highest antibacterial activity against *K. pneumoniae* at all the concentrations (50gm/ml, 25mg/ml and 12.5mg/ml) with the following mean zone diameters of 15.0 ± 0.02mm, 11.0 ± 01mm and 8.0 ± 0.04mm respectively. The ethanolic extract of the juice presented a zone diameter of 16.5 ± 0.01mm, 11.5 ± 0.02mm and 9.0 ± 0.01mmm at 50mg/Ml, 25mg/mL and 12.5mg/mL concentrations respectively. The least activity was recorded in *P. aeruginosa* with no zone of inhibitions at 25mg/ml and 12.5mg/ml concentrations in both juice extracts. The antifungal activity was not significant at P<0.05 in *A. niger* which was resistant in all the concentration except at 50mg/ml. The percentage weight yield of extracts was highest in the aqueous (70% and 60%) than in the ethanolic (30% and 40%) respectively. MIC was significantly different at P<0.05 for all the organisms except *P. aeruginosa* at 50mg/ml, for aqueous extracts of both juice and gel. At 25mg/ml and 12.5mg/ml concentration, there was no significant difference at P<0.05. The protective wall of gram-negative bacteria are known to hinder bioavailability of drug ingredients to organisms. The extracts of this plant possess compounds with antimicrobial properties, which will add credence to the ethano-medicinal use of this plant and since the plant is a reservoir for new bioactive compounds, it will contribute to the health care needs of developing and underdeveloped countries.**

Index Terms **: *Aloe vera*, juice, gel, antimicrobial activity.**

I. INTRODUCTION

Herbal medicine have been used in the treatment and cure of infectious diseases from ancient time in many countries of the world [1]. Also most of our medicines are distillation or combinations of substances which have been distributed abundantly in nature long before their value was demonstrated and understood by scientific methods [2]. The biological inhibitors by different natural plants such as essentials oils and plants extracts have been investigated widely [3], [4].

Aloe vera plant also known as ' burning or first –aid plant', is a succulent plant from the Aloe family with about 400 different species. It has its origin in the Sahara desert in North Africa[4],[1],[2]. *Aloe vera* is one of the oldest known therapeutic herb[5]. It has short stem with thick green leaves like structures that grow from central point[6].They store water in their stems, leaves and roots[7],[9]. *Aloe vera* plant could survive in wide temperature range of 104˚F to freezing temperature without damage to the roots [8]. It possesses serrated leaf margin as adaptive feature and forms arbuscular mycorrhiza which is a symbiotic interaction that permits better access to mineral nutrients in the soil.[9]. A cut leaf *Aloe vera* reveals an orange-yellow sap from the opening, known as the juice[10]. Similarly, the leaves contain a clear glooey mucilaginous substance known as gel, which is made up of fibres ,water and other ingredients[5].This *Aloe vera* gel consists of 99.3% water and 0.7% solid with glucose and mannose consisting a large part[11].

Phytochemical studies have shown that *Aloe vera* contains the following active substances example, salicylic acid, saponins, alkaloids, anthraquinones, vitamins, phenolic acid, ,lignin, coumarins terpenes, flavonoids, sesqueterpenes, xythones, sulphur, lupeol and cinnamic acids[12],[3],[9].. Numerous scientific research have revealed that these compounds have analgesic, anti-inflammatory, wound healing, ,immune modulating cytotoxic and antimicrobial properties against some bacterial, fungal and viral organisms.[13]. They also have cosmetic properties and aid in food preservation [14] [12].

II. AIMS

This research seeks to evaluate the antimicrobial effectiveness of *Aloe vera* juice(leaf) and gel extracts on

selected clinical isolates. Also to compare antimicrobial activity of extracts to standard antibiotics with similar activity.

III. MATERIAL AND METHODS

A. Sample Collection And Identification of plant material:

Mature fresh *Aloe vera* plants were harvested from the University Of Calabar Botanical garden and identified by Mr. Frank Adejoye, a plant taxonomist with the Department of Botany, University of Calabar and voucher specimen of the plant deposited in the herbarium of Botany Department of Botany, University of Calabar. Nigeria. The leaves were transported to the laboratory for processing.

Media And Ragents used: Different microbiological media were used. These include Nutrient Agar, Mueller Hinton Agar, Sabouraud dextrose Agar. All reagents used in this study were of analytical grade.

B. Collection Of Bacterial And Fungal Isolates:

Stock cultures of bacterial and fungal isolates which served as test organisms were obtained from the medical laboratory of the University Of Calabar Teaching Hospital, Calabar. These organisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Candida albicans* and *Aspergillus niger*. They were transported to microbiology laboratory. These isolates were further subcultured on Nutrient broth for viability and incubated at 37^0C for 18h to get fresh isolates.

IV. ANALYTICAL METHODS

A. Processing Of Aloe Vera Samples:

Extraction of *Aloe vera* gel and juice was done by the filleting method described by[15]. Freshly washed leaves of *Aloe vera* with initial weight of 1500g leaves were dissected longitudinally and the colourless parenchymatous gel scrapped out and liquefied using food blender at 10,000 rpm to remove fibres. The resultant solution was stored at 4˚C for future use.

Similarly, the processing of *Aloe vera* juice (leaf) involved sun-drying the leaves at 30^0C and pulverized leaves using a mechanical mill[16] and sieved. The difference (loss in weight) between the initial weight and final weight was recorded as sample moisture content[16]. This was stored up for future use at 4˚C.

B. Preparation of crude extracts Of Aloe vera Leaves;

A 50g weight of the dried pulverized leaves were dissolved in 250 ml flask of each solvent (aqueous and 70% ethanolic solvents) at the ratio of 1:5 dilutions[16]. The solutions were stirred at 30 min interval for 2hrs, before allowing it to settle for 24hrs for aqueous and 48h for70% ethanol. The process was repeated three times for 70% ethanol solution after 48h before extracts were later filtered through Whatman's No.1 filter paper. The filtrates were evaporated to dryness using a water bath at 60˚C, to eliminate the ethanol. The known amount of solvent free solid mass was used to determine 'concentrations 50, 25 and 12.5 in mg/mL[15]. And stored at 4˚C for future use[13].

C. Preparation of Sensitivity Discs:

Disc were prepared according to the method described by[16]. Regularly cut out discs from Whatman No 1 filter paper of 6mm in diameter were autoclaved at 120˚C for 30 min. The sterile blank discs in triplicates were soaked in 2mL of the different extracts (aqueous and 70% ethanolic)concentrations per millilitres (, 50mg/mL, 25mg/mL, 12.5mg/mL)per disc with control as distilled water, after which the discs were drained and dried.[14].

D. Standardization of known isolates:

Different media were used in the standardization of known isolates. Pure culture of each inoculum of test organism(*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Pneumoniae, Escherichia coli* and *Bacillus subtilis* were bacterial species, while *Candida albicans* and *Aspergillus niger* (fungal species) were streaked on a solid media and incubated at 37˚C for 24 hr and 25˚C at 72 hr respectively for bacterial and fungal isolate and later observed for growth. A loopful of each inoculum of bacterial isolates was cultured in a sterile nutrient broth for 18 hrs at 37˚C and fungal isolate in sabouraud dextrose broth for 48hr at 25˚C.

E. Antimicrobial Sensitivity Screening Of Extracts

This process was carried out using the Agar disc diffusion method by [15]. Two-fold dilutions was made. Mueller Hinton Agar plates and Sabouraud dextrose Agar plates were used. A .O. 1ml aliquot of each standardized culture was inoculated on the plates and spread evenly. Disc impregnated with extracts (aqueous and 70% ethanolic) of different concentration were aseptically placed on Agar surfaces at specified intervals .Antibiotic discs of known concentrations which served as control were also placed on the Agar surface for comparison and incubated at 37^OC for 24hr for bacterial species and 27^OC for 72hr for fungal isolates. These plates were observed for zones of inhibition and zone diameters measured in millimeter. This process was to determine the antimicrobial activity. The assay was performed in triplicates for the juice and gel and their mean as final result [16].

F. Minimum Inhibitory Concentration of Test Extracts

This process was carried out as described by Andrews (2001). Nutrient broth and sabouraud dextrose broth were used to determine in vitro activity of the new antimicrobial extracts against the bacterial and fungal isolates respectively, to get the Mcfarland standard, these tubes were incubated at 37^OC for 18hrs for bacterial isolates for turbidity and 27^OC for 48hrs for fungal species respectively. A loopful of each isolate was inoculated into a fresh broth tube. A 0.2ml of each extract with different concentration (100ug/ml, 50ug/ml, 25ug/ml and 12.5ug/ml) respectively were added to each tube in triplicates for each isolate and incubated and later observed for growth.

V. RESULTS AND DISCUSSION

The percentage yield of the different extracts of *Aloe vera* juice and gel varied between the different solvents. Aqueous extracts of *Aloe vera* juice (leaf) recorded a higher field of 70% as against 30% of the ethanolic extract. Similarly, this was also the case in the aqueous extract of *Aloe vera* gel, with a weight

yield of 30.05g and 60% compared to ethanolic extract with 20.01g weight at 40%. The percentage loss in weight on drying was indicative of moisture content of the plant material. This is important in determining the rate of deterioration due to fungal attack or enzymatic activities. These enzymatic activities may lead to degradation of secondary metabolites responsible for the pharmacological activity or conversion of the constituents to less potent ones [16] [15] [9]. The weight loss did not affect the antimicrobial properties of the extract.

The aqueous and ethanolic extracts of *Aloe vera* juice (leaf) exhibited high antibacterial activity against certain grain positive and gram negative (*S. aureus, P. aeruginosea, K. pneumoniae, E. coli* and *B. subtilis*) bacterial species (table 2a). Also in Table 2b, antifungal activity was recoded against *C. albicans* and *A. niger*, with a mean zone diameter of 12.5mm and 10.0mm respectively at 50mg/ml concentration of the extract.

Similar results have been reported by [3], who observed very high antifungal and antibacterial activity of *Aloe vera* juice among pathogenic isolates in *in vitro* experiment. The least activity was recorded in P. *aeruginosa*, with mean zone diameter of 7.0mm at 50mg/ml concentration in aqueous extract, but was resistant at 25mg/ml and 12.5mg/ml concentrations respectively for the aqueous juice extract. The pattern of inhibition was similar for the ethanolic juice extract for *P. aeruginosa*, with zone diameter of 8.0mg/ml and was resistant at concentrations 25mg/ml and 12.5mg/ml respectively. This result is in agreement with that obtained by [5], where he observed that most gram negative organisms were resistant to most antimicrobials. This result is in agreement with [1] [6], who observed that depending on the methodology adopted in the studies on antimicrobial activity, diverse results may be obtained. The potency of the extracts was assessed by the presence or absence of inhibitory zones and their corresponding minimum inhibitory concentrations. All the organisms were sensitive to the standard antimicrobials (Gentamicin 30mg/ml and Fluconazole 30mg/ml) activities with a mean zone diameter of 17.0mm for *S. aureus*, 15.0mm for *P. aeruginosa*, and highest zone diameter of 21.0mm for *K. pneumoniae*. This result is in agreement with that obtain by [7] in a related experiment.

Pseudomonas aeruginosa was completely resistant to the antibacterial activity of *Aloe vera* gel extract, with no zone of inhibition, in Table 3a, but sensitive to ciprofloxacin (30ug) with a mean zone diameter of 23.00mm. *K. pneumoniae, E. coli, S. aureus* and *B. subtilis* were sensitive to the antibacterial activity of *Aloe vera* gel extract, with a mean zone diameters of 17.0mm, 14.0mm, 12.0mm and 8.0mm respectively at concentration 50mg/ml of extract. This result is similar to that obtain by [5]. The lack of maximum inhibitory effect of the get extract against gram negative bacteria may be due to the multilayered and complex cell wall which prevents entry of growth inhibitors [8]. Similarly, Table 3b high antifungal activity against *C. albicans* and *A. niger*, with a mean inhibitory zone diameter of 14.0mm and 8.0mm respectively. The organisms were also sensitive to Fluconazole antibioticswith C. *albicans* 14.0mm and 13.0mm for *A. niger* respectively. Similar results have been obtained by [5].

The minimum inhibitory concentration (MIC) values of the aqueous extract of *Aloe vera* juice (Table 4a) revealed that at 50mg/ml, *S. aureus, P. aeruginosa, K. pneumoniae, E. coli*, and *B. subtilis* were highly significant at $P<0.05$. Aqueous juice extract exhibited higher antibacterial activity than the ethanolic extract. At 25.0mg/ml and 12.5mg/ml the MIC were not significantly different at $P<0.05$. There was minimal effect in the fungal isolates of *C. albicans* and *A. niger* at $P<0.05$ MIC. The MIC for *Aloe vera* gel extract has a lower inhibitory effect at 25mg/ml and 12.5mg/ml concentrations respectively. The presence of some phytochemicals, have been shown by [5] [7] to provide synergistic effect which modifies the bioavailability and efficacy of the active ingredients.

VI. CONCLUSION

The different plant extracts exhibited inhibitory effects on growth of microorganisms studied; though of distinct effect, these differences may be as a result of concentration of extracts used to determine the antimicrobial activity. Plants are unlimited source for treatment of diseases. There is growing interest in investigation of different plant species for their potentials for therapeutic applications, since they have medicinal properties, with little or no side effects, low cost and toxicity. The results obtained in this study adds credence to the ethnomedicinal uses of *Aloe vera* plant for treatment of pathogenic infections, microbial infections, a reservoir of new bioactive compounds and antimicrobial agenst in new drug therapy. Further and detailed study of *Aloe vera* leaves is recommended.

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RESULTS

Weight and percentage field of the different solvent extracts of *Aloe vera* Juice and gel (leaf) in presented in Table 1.

TABLE 1: Weight and percentage field of crude extracts of *Aloe vera* juice and gel (leaf)

Extracts	Weight yield (g)	Percentage (%)
A. vera Juice: Aqueous	35.40	70%
Ethanolic	15.02	30%
A. vera gel: Aqueous	30.05	60%
Ethanolic	20.01	40%

TABLE 2a: Antibacterial activity of *Aloe vera* Juice (leaf) extracts against known bacterial isolates.

Microorganism:	Aqueous extract			Ethanolic extract			Gentamicin
	Conc. 150,	25,	125 (mg/ml)	50,	25,	12.5(mg/ml)	10ug/ml
Staphylococcus aureus	9.0[a]	7.5	0	11.5	8.5	0	17.0
Pseudomonas aeruginosa	7.0[a]	0	0	8.0	0	0	15.0
Klebsiella pneumoniae	15.0	11.0	8.0	16.5	11.5	9.0	21.5
Escherichia coli	9.5	7.5	0	11.0	8.0	0	12.0
Bacillus subtilis	12.0	8.0	0	13.5	10.0	8.0	13.5

Legend: a zones of inhibition ≤ 8 mean ± SD – intermediate

O = Resistant or no inhibition zone

Extract concentration – mg/ml

Zone diameter - mm

Zone diameter ≥ 8 – sensitive

TABLE 2b: Antifungal activity of *Aloe vera* juice (leaf) extract against known fungal isolates

Fungal isolates	*Aloe vera* juice extracts			Ethanolic			Fluconazole
	Conc. 50ug/ml	25	12.5	50	25	12.5	(30ug/ml)
Candida albicans	12.5	10.0±	8.0±	15.0 ±	14.5 ±	10.0	18.0
Aspergillus niger	10.0±	0	0	0	0	0	12.5

TABLE 3a: Antibacterial activity of *Aloe vera* gel (leaf) extract against known bacterial

Isolates	Aqueous			Ethanolic			Ciprofloxacin (30ug)
Concentration(mm/mL)	50	25	12.5a	50	25	12.5	
Staphylococcus aureus	12.0	9.5	0	15.0	11.0	0	17.0
Pseudomonas aeruginosa	0	0	0	0	0	0	22.0
Klebsiella pneumoniae	17.0	13.5	9.5	20.0	15.5	10.5	23.05
Escherichia coli	14.0	10.0	0	12.0	8.0	7.5	20.0
Bacillus subtilis	8.0	7.5	5.0				14.0

TABLE 3b: Antifungal activity of *Aloe vera* gel (leaf) extract against known fungal isolates

	Aloe vera gel extracts						
	Aqueous			Ethanolic			Fluconazole (30ug)
Fungal isolates	Conc. 5025		12.5[a]	50	225	12.5	
Candida albicans	14.0	11.5[b]	0	17.5	13.0	9.0	15.0
Aspergillus niger	8.0	7.5	0	9.0	8.5	0	13.0

Legend: (a) Extract concentration = mg/ml

Zones of inhibition = mean ± SD

[b] Zone diameter measured in (mm)

Zone diameter ≤ 8 – intermediate

≥ 8 – sensitive

0 – resistant

TABLE 4a: Minimum inhibitory concentration (MIC) values of *Aloe vera* juice (leaf) extract for bacterial isolates

Extracts	Conc (mg/ml)	*Staphylococcus aureus*	*P. aeruginosa*	*K. pneumonia*	*E. coli*	*B subtilis*
Ethanol	50	-	+	-	-	-
	25	+	++	-	+	-
	12.5	++	++	+++	++	+
Aqueous	50	-	-	-	-	-
	25	+	++	-	+	+
	12.5	++	+	+	++	++