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STATISTICAL ANALYSIS ON THE ANTI-MICROBIAL EFFECT OF SWEET POTATO AND GRAPE LEAF EXTRACTED AGAINST SOME PATHOGENIC MICRO-ORGANISMS

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Abstract—Sweet potato and Grape leaf have been extensively used in medicine. The present investigation was undertaken to statistically evaluate the antimicrobial activity of these leaves extracts (methanolic and ethanolic) to determine the most significant extract and concentration that will inhibit the growth of some micro-organisms causing some certain diseases in the human system. The experiment was conducted on two species of micro-organism (*Staphylococcus aureus* and *Escherichia coli*), each applied 5 different treatments; 18mg/ml, 16mg/ml, 14mg/ml, 12mg/ml and 10mg/ml of each extract. A randomized complete block design (two way ANOVA) was used to analyze the data (zone of inhibition) collected, while Duncan multiple range tests was used for the Post-hoc test (with the aid of Minitab 16 and SPSS 20). Result revealed that treatment A extract with 18mg/ml concentration is the best in inhibiting the growth of the micro-organisms in this study.

Keywords— Sweet potato, Grape, *Staphylococcus aureus*, *Escherichia coli*, ANOVA

I. INTRODUCTION

It was confirmed that plants are sources of nutrients and other bioactive moieties [3], responsible for vital functions in humans, therefore possess numerous health prospects [8], [13]. Energy and dietetic regimes linkages were reported in literature; also, the extraction of bioactive moieties and their useful on human metabolism need systematic and coherent which need further investigations, for establishment of meticulous and persuasive association for consumers [13]. Grape (*Vitis vinifera* L.) was a leading fruit crop producing 70 million metric tons grapes every year in the world with extraordinary taste and flavor [11]. Various food products were produced from its fruit, seeds and leaves were used for

production of herbal medicines, as well as dietary supplements [2, 9].

Sweet potato (*Ipomoea batatas* L.) is a member of morning glory family (Convolvulaceae) [14] Sweet potato is among the major food crop in the world and is cultivated in all tropical and subtropical regions particularly in Asia, Africa and Pacific [4]. Asia and Africa accounts for 95 percent of the production [5]. The crop is mainly grown for tubers, but a large volume of sweet potato vines (Stems and Leaves) are left after harvesting [9]. The vines can be used as an alternative feed resource for ruminants to reduce competition between animals and humans for grains that is, maize and soya bean [10]. The value of sweet potato vines as feed supplement is enriched by high palatability [7], moderate level of crude protein and water soluble carbohydrate [12], and high level of digestibility greater than 62% [6],[1]

II. MATERIALS AND METHODS

Freshly young leaves of *Sweet potato and Grape* were collected from a tree during hamathan period of 2018, in the Federal Polytechnic Offa, and authenticated by Mr. Abbah, E.A. Lecturer in the Biology/Micro-Biology unit, Department of Science Laboratory Technology, Federal Polytechnic Offa, Offa Kwara State, Nigeria, 30 grams of the powdered plant materials were extracted successively with 600ml of methanol and ethanol as solvents for 4 days by Rotary evaporator equipment.

A. Tests organisms—

The test organisms used were clinical strain of bacteria which are pure culture of gram negative and gram positive bacteria respectively. They are *Staphylococcus aureus* and *Escherichia coli* respectively, they were propagated and stored in slant McCartney bottle at 4°C before use.



B. Experimentation-

The agar diffusion method is used for the antimicrobial evaluations. The plates were sterilized in a hot air oven at 161°C for 2hrs. After the preparation of different volume of each extract, it was applied into each well of the agar containing various isolates of bacteria in the plate to give their corresponding zone of inhibition. The diameter of the inhibition zone was measured in mm. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm. All the run of the experiment were performed completely in randomized manner in Biology/Micro-biology laboratory, Science Laboratory Technology Department, the Federal Polytechnic Offa.

C. Method of analysis

The statistical tool used in the analysis is Randomized Complete Block Design (two way classification analysis of variance) to determine if there is any significant difference between the treatments (concentrations) and the plant extract, and Duncan multiple range test was also used to know which of these treatments and the plant extract is highly significant (if difference exist in the prior analysis).

Table 1: ANOVA table for Randomized Complete Block Design

Sources of Variance	Degree of freedom	Sum of Squares	Mean Squares	F_0
Treatments	$a - 1$	$SS_{Treatments}$	$\frac{SS_{Treatments}}{a - 1}$	$\frac{MS_{Treatments}}{MS_{Error}}$
Blocks	$b - 1$	SS_{Blocks}	$\frac{SS_{Blocks}}{b - 1}$	
Errors	$(a - 1)(b - 1)$	SS_{Error}	$\frac{SS_{Error}}{(a - 1)(b - 1)}$	
Total	$N - 1$	SS_{Total}		

Statistical model: $y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$ $\begin{cases} i = 1, 2, \dots, a \\ j = 1, 2, \dots, b \end{cases}$

The hypotheses of interest are:

- i. H_0 : There is no significant difference between the treatments
 H_1 : There is significant difference between the treatments
- ii. H_0 : There is no significant difference between the plant extracts
 H_1 : There is significant difference between the plant extracts

Level of significance: 0.05

III. EXPERIMENTAL AND RESULTS

The antimicrobial activities of methanol and ethanol extracted from the leaves of *Sweet potato and Grape* against two pathogenic micro-organisms; such as *Staphylococcus aureus* and *Escherichia coli* were presented below.

Table 2: Methanolic extracted from sweet Potato leaf zone of inhibition (mm)

Organisms	Treatments					Total
	A (18mg/ml)	B (16mg/ml)	C (14mg/ml)	D (12mg/ml)	E (10mg/ml)	
<i>Staphy. aureus</i>	24	22	18	16	12	92
<i>E. coli</i>	26	20	17	15	10	88
Total	50	42	35	31	22	180
Mean	25.0	21.0	17.5	15.5	11.0	

Table 3: Ethanolic extracted from sweet Potato leaf zone of inhibition (mm)

Organisms	Treatments					Total
	A (18mg/ml)	B (16mg/ml)	C (14mg/ml)	D (12mg/ml)	E (10mg/ml)	
<i>Staphy. aureus</i>	18	16	14	10	8	66
<i>E. coli</i>	20	18	15	12	9	74
Total	38	34	29	22	17	140
Mean	19	17	14.5	11	8.5	

Table 4: Methanolic extracted from Grape leaf zone of inhibition (mm)

Organisms	Treatments					Total
	A (18mg/ml)	B (16mg/ml)	C (14mg/ml)	D (12mg/ml)	E (10mg/ml)	
<i>Staphy. aureus</i>	26	21	19	15	12	93
<i>E. coli</i>	29	26	20	18	14	107
Total	55	47	39	33	26	200
Mean	27.5	23.5	19.5	16.5	13.5	

Table 5: Ethanolic extracted from Grape leaf zone of inhibition (mm)

Organisms	Treatments					Total
	A (18mg/ml)	B (16mg/ml)	C (14mg/ml)	D (12mg/ml)	E (10mg/ml)	



<i>Staphy. aureus</i>	23	20	18	15	10	86
<i>E. coli</i>	13	9	0	0	0	22
Total	36	29	18	15	10	108
Mean	18	14.5	9	7.5	5	

Table 6: The most significant treatment in each extracted were collated
Zone of inhibition (mm)

Organisms	Extracts				Total
	S/Potato Leaf (Methanol)	S/Potato Leaf (Ethanol)	Grape Leaf (Methanol)	Grape Leaf (Ethanol)	
<i>Staphy. aureus</i>	24	18	26	23	86
<i>E. coli</i>	26	20	29	13	22
Total	50	38	55	36	108
Mean	25	19	27.5	18	

A. Statistical analysis

The data collected was analyzed with the aid of Minitab and SPSS software and the following results were obtained

Table 7: Methanolic extracted from sweet Potato leaf

Two-way ANOVA: observation versus Treatments, Organisms

Source	DF	SS	MS	F	P
Treatments	4	227.0	56.75	42.04	0.002
Organisms	1	1.6	1.60	1.19	0.338
Error	4	5.4	1.35		
Total	9	234.0			

S = 1.162 R-Sq = 97.69% R-Sq(adj) = 94.81%
 FINDING: P-value under treatments (0.002) < level of significant (0.05)

Table 8: Post Hoc Tests

Duncan^{a,b}

Treatments	N	Subset			
		1	2	3	4
E	2	11.00			
D	2		15.50		
C	2		17.50		
B	2			21.00	
A	2				25.00
Sig.		1.000	.160	1.000	1.000

FINDING: Treatment A has the highest value in the subsets

Table 9: Ethanolic extracted from sweet Potato leaf

Two-way ANOVA: observation versus Treatments, Organisms

Source	DF	SS	MS	F	P
Treatments	4	147.0	36.75	245.00	0.000
Organisms	1	6.4	6.40	42.67	0.003
Error	4	0.6	0.15		
Total	9	154.0			

S = 0.3873 R-Sq = 99.61% R-Sq(adj) = 99.12%
 FINDING: P-value under treatments is < level of significant (0.05)

Table 10: Post Hoc Tests

Duncan^{a,b}

Treatments	N	Subset				
		1	2	3	4	5
E	2	8.50				
D	2		11.00			
C	2			14.50		
B	2				17.00	
A	2					19.00
Sig.		1.000	1.000	1.000	1.000	1.000

FINDING: Treatment A has the highest value in the subsets

Table 11: Methanolic extracted from Grape leaf

Two-way ANOVA: observation versus Treatments, Organisms

Source	DF	SS	MS	F	P
Treatments	4	260.0	65.0	59.09	0.001
Organisms	1	19.6	19.6	17.82	0.013
Error	4	4.4	1.1		
Total	9	284.0			

S = 1.049 R-Sq = 98.45% R-Sq(adj) = 96.51%
 FINDING: P-value under treatments is < level of significant (0.05)

Table 12: Post Hoc Tests

Duncan^{a,b}

Treatments	N	Subset				
		1	2	3	4	5
E	2	13.00				
D	2		16.50			
C	2			19.50		
B	2				23.50	
A	2					27.50
Sig.		1.000	1.000	1.000	1.000	1.000

FINDING: Treatment A has the highest value in the subsets



Table 13: Ethanolic extracted from Grape leaf

Two-way ANOVA: observation versus Treatments, Organisms

Source	DF	SS	MS	F	P
Treatments	4	226.6	56.65	8.92	0.028
Organisms	1	409.6	409.60	64.50	0.001
Error	4	25.4	6.35		
Total	9	661.6			

S = 2.520 R-Sq = 96.16% R-Sq(adj) = 91.36%

FINDING: P-value under treatments is < level of significant (0.05)

Table 14: Post Hoc Tests

Duncan^{a,b}

Treatments	N	Subset		
		1	2	3
E	2	5.00		
D	2	7.50	7.50	
C	2	9.00	9.00	
B	2		14.50	14.50
A	2			18.00
Sig.		.193	.053	.237

FINDING: Treatment A has the highest value in the subsets

Table 15: Most significant treatment in each extracted

Two-way ANOVA: observations versus Treatments, Organisms

Source	DF	SS	MS
Treatments	3	127.375	42.4583
Organisms	1	1.125	1.1250
Error	3	57.375	19.1250
Total	7	185.875	

S = 4.373 R-SQ = 69.13% R-SQ(ADJ) = 27.98%

IV. DISCUSSION OF RESULTS

From the table of result the followings were found:

The p-value of treatments in the table 7, 9, 11, and 13 were less than there corresponding level of significant (0.05), this indicates that significant difference exist between the treatments applied. Also, in table 8, treatment C and D were in the same subset column, meaning that difference exist between all the treatments except C and D. Treatments in table 10 and 12 were in separate subset column, meaning that difference exist between all the treatments. From table 14 significant difference only exist between treatment AC, AD, AE and BE. Then treatment A in table 8, 10, 12 and 14 has the highest value of comparison. But in determining which of these plants extract is highly significant, the most significant treatment (treatment A) in each plant extracted were compared and the p-value of treatments in the table 15 is greater that the level of significant

(0.05), this indicates that there is no significant difference among these plant extracted.

V. CONCLUSION

18mg/ml of the methanol and ethanol extracted from sweet potato and grape leaf were the best inhibiting the growth of these micro-organisms in this study. Therefore, any of these plants extracted at 18mg/ml concentration were useful in preventing any of these micro-organisms.

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