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Statistical analysis on bio-efficacy of different solvent fraction of *Andrographis paniculata* against some bacteria

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Abstract

This research is concentrated on the Statistical Analysis of antimicrobial activity of different solvent extract of *Andrographis paniculata* leaves on bacterial that cause diseases. And it was aimed to compare the effect of *Andrographis paniculata* on some selected bacteria with the normal antibiotics. The bacterial isolates used in the study were *Staphylococcuc aurcus, Streptococcus pyogens, Proteus vulgaris* and *Escherichia coli.* The data was collected by experimental method from laboratory as source of primary data. Randomized complete block design with two-way analysis of variance were used to analyze the data in order to determine the significant difference in efficacy of fractions solvent extract of *Andrographis paniculata* leaves on bacteria. The normal antibiotics used are: Streptomycin, Gentamycin, Amoxillin, Chloramphenicol, Contrimoxazole, ofloxaacin, Erthrommycin, ERX, PT and Ceftriaxone. MINITAB 16 statistical software were used for computation analysis of the data collected. The result of the analysis reveal that there is significant difference in the efficacy of different antibiotics on bacteria that cause diseases. Although it was observed that the level of inhibition of each antibiotics were not the same but they all possess antibicteria properties that could be used to treat some bacteria diseases. Therefore, it was concluded that *Andrographis paniculata* is highly potent in the treatment of some selected bacterial diseases. I hereby recommended that, the effective drugs could be produced from *Andrographis paniculata* leaves used in traditional medicine, thereby enhance self-reliance, more active drugs can be produced to solve the present-day problems of drug resistance in the health management.

Keywords: *Andrographis paniculata*; Antibiotics; ANOVA; Bacteria; Treatment

1. Introduction

Andrographis paniculata leaf is an herbaceous plant, commonly known as king of bitters" in the family Acanthaceae. It is widely cultivated in southern Asia, mostly the leaves and roots have been traditionally used over the centuries for different medicinal purposes in Asia and Europe as a forklore remedy for a wide spectrum of ailments or as an herbal supplement for health promotion Anurag *et al.* [1], Ayak *et al.* [3], Dada *et al.* [8]. Anial *et al.* [2], Rajalakshmi *et al.* [10], Tapsel *et al.* [21] worked on the aerial parts of the plants (leaves and stems) are used to extract the active phytochemicals. It shows previous investigations on the chemical composition of the Andrographis develop antimicrobial compounds with the isolated compounds or their synthetic analogues and the present investigation study the antibacterial potential of extract of *Antigraphis paniculata* against selective human pathogens. Singha and Roys [20] Reported that crude powder suspended in water had no *invitro* antibacterial activity against *salmonella, shigella, Eschericia-coli* and *Staphylococcus aureus*, even at a concentration of 25 mg/ml crude powder. The same result was reported by Liu *et al.* [12], Parvataneni and Koduru [14] who found crude aqeous extract of leaves had no activity against *Eschericia coli* or klebsiella pneumonia but exhibited significant antimicrobial activity against gram positive aureus, methicilin-resistant *Staphylococcus aureus* (MRSA), and gram-negative Pseudomonas aeruginosa. Pholphana *et al.* [15],

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Praja *et al.* [16], Seeman and Wankhade [19], Singha and Roys [20] reported significant antibacterial activity of an aqeous extract and attributed it to the combined effect of Andropholides and arabinogalactan proteins.

In contrast, Kowti *et al.* [18], Seeman and Wankhade [19], Coon [7], Deng [9] investigated the antimicrobial activity using *Andrographolia paniculata* methanolic and aqeous extracts and authentic andrographolide did not show any activity. Recent research has thrown light on cultivation of this plant on large scale because of its high medicinal value. Hence, the presence investigation was taken up with an objectives to evaluate the antibacterial potential against the micro-organism Chooora *et al.* [6], Caceres [5] the bioactive compound andrographolide has been reported to be effective in the treatment of upper respiratory tract infection KanoKwan [11]. *Andrographis paniculata* is also used for other medical purpose. For example, digestive problem, blood cleanser, fever, sore throat Kowti *et al.* [18]. *Andrographis paniculata* is used to cure fever and cold and is one of the best antimalaria agents compared to the commercial product of quinine. The herb has shown an ability to reduce inflammation (heat) and fight viral infections and is used as a principal ingredients in traditional Chinese medicinal formular for lung support from colds Singha and Roys [20]. *Andrographis paniculata* acts to dispel heat and remove toxin. Andrographaloid was found to be more potent and a standard hepate protective agent Ayak *et al.* [3], Chang and Butpph [4]. Since ancient times, *Andrographis paniculata* has been known in traditional Asian medicine as an immune system booster to treat infections in the gastrointestinal tract and upper respiratory tracts, harps, sore throat and a variety of other chronic infectious diseases Praja pati *et al.* [16]. Parvataneni and Koduru [14] Reported the antibacterial activity of methanol extract of the leaves of *Andrographis paniculata* against *Staphylococcus auereus, Bacillus substitilis, Streptococcus epidemidis, Eschericia coli, Pneumonia* and *Pseudomonas aeruginosa*. Kowti *et al.* [18] Reported the antibacterial value of ethanol leaf extract against pathogenic bacteria. Bioactive compound andrapholides was isolated from the leaf, their results revealed that the ethanol leaf extract and andrographolide compound are potent in inhibiting these bacteria and his work highlights that the inhibitory effect is on part with standard antibiotics. Rajalakshmi *et al.* [10] reported antimicrobial activity of various organic and aqeous extracts of eight months old micro-propagated plantlets of *Andrographis paniculata* against gram negative bacterials such as *Klebsiella Pneumonia, Eschericia coli, Pseudomonas aeruginosa*, gram positive *Staphylococcus aureus* and *Bacillus subtilitis* bacteria. Flavonoids mainly exist in the root, but have also been isolated from the leaves. The aerial parts contain alkanes, ketones and aldehydes Tapsel *et al.* [21].

This work is focused on the analysis of the *Andrographis* extract against the selective micro-organisms. The study is based on primary data obtained by the experiment carried out at Science Laboratory Technology in Federal Polytechnic Offa, Kwara Sate Nigeria, in which the observations or the zones of inhibition (Tn mm) are recorded as data obtained. The broad aim of this work is to study statistically the antibacterial potential of *Andrographis peniculata* on selective bacteria and compare with normal antibiotics.

2. Data Presentation and Analysis

2.1. Data Presentation

This aspects preset the data obtained from the level of inhibition of solvent extract of *Andrographis paniculata* leaf against some selective bacterial.

Table 1 The level of inhibition of bacteria isolate on methanolic extract of *Andrographis paniculata* leaf

CONCENTRATION (Treatment)						
S/N	**BACTERIA (Block)**	**Gram's Reaction**	**A 100mg/ml**	**B 50mg/ml**	**C 25mg/ml**	**D 12.5mg/ml**
1	*Staphylococcus aureus*	Gram positive	20.0	16.0	15.0	14.0
2	*Staphylococcus aureus*	Gram positive	18.0	15.0	13.0	12.0
3	*Proteus vulgaris*	Gram negative	20.0	15.0	13.0	12.0
4	*Escherichia coli*	Gram negative	16.0	11.0	0.0	0.0

Table 2 The level of inhibition of Ethanolic extract of *Andrographis paniculata* leaves against gram positive and Gram negative bacteria

CONCENTRATION (Treatment)						
S/N	**BACTERIA (Block)**	**Gram's Reaction**	**A 100mg/ml**	**B 50mg/ml**	**C 25mg/ml**	**D 12.5mg/ml**
1	*Staphylococcus aureus*	Gram Positive	20.0	18.0	15.0	14.0
2	*Staphylococcus aureus*	Gram Positive	20.0	15.0	14.0	12.0
3	*Proteus vulgaris*	Gram Negative	19.0	18.0	16.0	14.0
4	*Escherichia coli*	Gram Negative	15.0	0.0	0.0	0.0

Table 3 The level of inhibition of Acetic acid extract of *Andrographis paniculata* leaves against gram positive and Gram negative bacteria

CONCENTRATION (Treatment)						
S/N	**BACTERIA (Block)**	**Gram's Reaction**	**A 100mg/ml**	**B 50mg/ml**	**C 25mg/ml**	**D 12.5mg/ml**
1	*Staphylococcus aureus*	Gram positive	16.0	14.0	12.0	10.0
2	*Staphylococcus aureus*	Gram positive	17.0	16.0	15.0	12.0
3	*Proteus vulgaris*	Gram negative	24.0	20.0	18.0	16.0
4	*Escherichia coli*	Gram negative	0.0	0.0	0.0	0.0

Table 4 The level of inhibition of Acetone extract of *Andrographis paniculata* leaves against gram positive and Gram negative bacteria

CONCENTRATION (Treatment)						
S/N	**BACTERIA (Block)**	**Gram's Reaction**	**A 100mg/ml**	**B 50mg/ml**	**C 25mg/ml**	**D 12.5mg/ml**
1	*Staphylococcus aureus*	Gram positive	18.0	16.0	15.0	14.0
2	*Staphylococcus aureus*	Gram positive	15.0	14.0	12.0	10.0
3	*Proteus vulgaris*	Gram negative	15.0	14.0	12.0	10.0
4	*Escherichia coli*	Gram negative	19.0	0.0	0.0	0.0

Table 5 The level of inhibition of selective antibiotics against some bacteria

Bacteria (Observation Block)						Antibiotic Mean response
S/N	**ANTIBIOTICS (Treatment)**	**Proteus vulgaris**	***Escherichia Coli***	***Staphylococcus aureus***	***Streptococcus Pyogenes***	
1	AMX	0.0	14.0	0.0	5.0	4.75
2	ERY	0.0	10.0	6.0	6.0	5.50
3	CPX	2.0	0.0	8.0	6.0	4.00
4	COT	0.0	0.0	0.0	0.0	0.00
5	PT	8.0	0.0	8.0	7.0	5.75
6	GEN	2.0	0.0	11.0	8.0	5.25
7	CRO	8.0	0.0	0.0	0.0	2.00
8	CHL	3.0	0.0	0.0	0.0	0.75
9	STR	0.0	0.0	6.0	0.0	1.50
10	OFL	7.0	10.0	8.0	5.0	7.50
11	*Andrographis paniculata*	15.4	14.4	16	3.8	12.40
Bacteria Mean Response		4.1	4.4	5.7	3.7	4.5

AMX = Amoxylin, ERY = Erthromycin, COT = Cotrimoxazole, GEN = Genetamycin, CHL = Chloraphenicol, STR = Streptomycin, OFL = Ofloxacin, CRO = Ceftriaxone, Etc

2.2. Data analysis and results

Table 6 Two-Way Anova of Level of Inhibition of Methanolic Extract of *Andrographis paniculata* Leaf against the selective Bacteria

Two-way ANOVA: observation versus methanolic extract, bacteria					
Source	**DF**	**SS**	**MS**	**F**	**P**
Methanolic extract	3	206.25	68.7500	9.07	0.004
Bacteria	3	223.25	74.4167	9.81	0.003
Error	9	68.25	7.5833		
Total	15	497.75			

S = 2.754 R-Sq = 86.29% R-Sq(adj) = 77.15%

At 5% level of significant, p (0.004) <0.05 in table 6, a column reject Ho and conclude that the means of the methanolic extract against the bacteria are significant.

Interpretation: The table 6 indicate that the means of mathanolic extract fractions of (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml) against the selective Bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes, Proteus vulgaris, Escherichia coli*) are significantly different.

According to the two-way analysis in table 6, it reflects that the efficacy of methanolic extract of *Andrographis paniculata* leaves against the bacteria are significantly difference having p values <0.05

Table 7 Two-Way Anova of Level of Inhibition of Ethanolic Extract of *Andrographis paniculata* Leaves against the selective Bacteria

Two-way ANOVA: observation versus ethanolic extract, bacteria					
Source	DF	SS	MS	F	P
Ethanoli extract	3	169.25	56.417	7.08	0.010
Bacteria	3	474.75	158.250	19.85	0.000
Error	9	71.75	7.972		
Total	15	715.75			

S = 2.824 R-Sq = 89.98% R-Sq(adj) = 83.29%

At 5% level of significance, p (0.010) <0.05 in table7, reject Ho and conclude that the means of the ethanolic extract against the bacteria are significant.

Interpretation: The table 7 indicate that the means of ethanolic extract fractions of (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml) against the Bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris, Escherichia coli*) are significantly different.

According to the two-way analysis above it reflect that the efficacy of ethanolic extract of *Andrographis paniculata* leaves against the Bacteria are significantly difference having p-values <0.05.

Table 8 Two-Way Anova of Level of Inhibition of Acetic Acid Extract of *Andrographis paniculata* Leaves against some Bacteria

Two-way ANOVA: observation versus acetic acid extract, bacteria					
Source	DF	SS	MS	F	P
Acetic acid extract	3	48.25	16.083	6.98	0.010
Bacteria	3	840.75	280.250	121.55	0.000
Error	9	20.75	2.306		
Total	15	909.75			

S = 1.518 R-Sq = 97.72% R-Sq(adj) = 96.20%

At 5% level of significance, p (0.010) <0.05 in table 8, reject Ho and conclude that the means of the acetic acid extract against the Bacteria are significant.

Interpretation: The table 8 indicate that the means of acetic acid extract fractions of (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml) against the Bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris, Escherichia coli*) are significantly different.

According to the analysis above, it reflect that the efficacy of acetic acid extract of *Andrographis paniculata* leaves against the Bacteria are significantly difference having p-values <0.05.

Table 9 Two-Way Anova of Level of Inhibition of Acetone Extract of *Andrographis paniculata* Leaves against some Bacteria

Two-way ANOVA: observation versus acetone extract, bacteria					
Source	**DF**	**SS**	**MS**	**F**	**P**
Acetone extract	3	159.5	53.1667	3.20	0.077
Bacteria	3	267.0	89.0000	5.36	0.022
Error	9	149.5	16.6111		
Total	15	576.0			

S = 4.076 R-Sq = 74.05% R-Sq(adj) = 56.74%

At 5% level of significance, p (0.077) >0.05 in table 9, do not reject Ho and conclude that the means of acetone extract against the bacteria are not significant.

Interpretation: The table 9 indicate that the means of acetone extract fractions of (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml) against the bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris, Escherichia coli*) are not significantly different.

According to the analysis, it rejects that the potency of acetone extract of *Andrographis paniculata* leaves against the bacteria are not significantly difference having p-values >0.05

Table 10 Two-Way Anova of Level of Inhibition of selective Antibiotic against some Bacteria

Two-way ANOVA: Response versus Antibiotics, bacteria					
Source	**DF**	**SS**	**MS**	**F**	**P**
Antibiotics	10	497.64	49.7636	2.90	0.012
Bacteria	3	25.08	8.3612	0.49	0.694
Error	30	514.84	17.1612		
Total	43	1037.56			

S = 4.143 R-Sq = 50.38% R-Sq(adj) = 28.88%

At 5% level of significance, p value (0.012) <0.05, we reject the null hypothesis. Therefore there is significant difference among the effect of different antibiotics used in the treatment of bacteria diseases.

The p value of bacteria 0.694 >0.05, we do not reject the null hypothesis. Therefore there is no significant difference among the bacteria treated with antibiotics. As such any differences in the effect of antibiotics can be traced to difference in bacteria.

Also, using the mean effect of antibiotics, it can be seen that the mean effect are: 0.00, 0.75, 1.50, 2.00, 4.00, 4.75, 5.25, 5.50, 5.75, 7.50 and 12.40 for Cotrimoxazole, Chloraphenicol, Streptomycin, Ceftriaxone, CPX, Amoxylin, Gentamycin, ERY, PT, Streptomycin and *Andrographis paniculata* respectively. Simultaneously, the mean responses of bacteria to antibiotics are: 3.7, 4.1, 4.4 and 5.7 for *Streptococcus pyogenes, Proteus vulgaris, Escherichia coli* and *Staphylococcus aureus*, respectively.

3. Results and discussion

There has been considerable interest in the use of plant material as an alternative method to control microorganisms and many plants have been showed to be specially targeted against resistance pathogenic bacteria.

Tables 1-4 was on the level of inhibition exhibited by the Bacteria on the different solvent extract of *Andrographis paniculata*. Table 1 was on the methanolic extract level of inhibition, table 2 was on the ethanolic extract, table 3 was on the Acetic acid extract while table 4 was on the Acetone extract level of inhibition. The Bacteria are *Staphylococcus aureus*, *Streptococcus pyogenes*, *proteus vulgaris* and *Escherichia coli*. Table 5 was on the level of inhibition of selected antibiotics against some bacteria with the Antibiotics Means of response and also Bacteria Means response.

Table 6 was on the two-way analysis of variance on methanolic extract of *Andrographis paniculata* against the some Bacteria where the p-values is 0.004. Table 7 was on the two-way analysis on ethanolic extract of *Andrographis paniculata* leaves against the Bacteria where the p-values is 0.010. Table 8 was on the two-way ANOVA of acetic acid extract of Andrographis leaves against micro-organism where the p-values is 0.010. Table 9 was on the two-way ANOVA of Acetone extract of Andrographis against the Bacteria where p-values is 0.077. Table 10 was also on the two-way ANOVA of Antibiotics against the some Bacteria in which p-values is 0.012. Also, the ranking scores of the mean effect of antibiotics are: 0.00, 0.75, 1.50, 2.00, 4.00, 4.75, 5.25, 5.50, 5.75, 7.50 and 12.40 for Cotrimoxazole, Chloraphenicol, Streptomycin, Ceftriaxone, CPX, Amoxylin, Gentamycin, ERY, PT, Streptomycin and *Andrographis paniculata* respectively. *Andrographis paniculata* exceed expectation by actively inhibiting the growth and survival of the various Bacteria.

4. Conclusion

Results obtained from this Analysis showed that *Andrographis paniculata* leaf extract, exhibited the strongest antimicrobial activity than the control. *Andrographis paniculata* is concluded to be highly potent in the treatment of some selected bacteria diseases. Also, the study revealed that the effective drugs could be produced from *Andrographis paniculata* leaves used in traditional medicine. This could lead to development of modern pharmaceutical industries, thereby enhancing self-reliance. More active drugs can be produced to solve the present day problem of drug resistance in the health management.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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