Effect of different Antibiotics on Bacteria that cause Typhoid Fever

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Abstract— **The leaf of Morinda lucida was screened for antibacterial activity. Aqueous and Ethanolic extract of the plant were useful in order to evaluate their antibacterial activities. Also, some conventional antibiotics were used to determine their effects on bacteria that cause Typhoid Fever. From the above given information, statistical application of analysis of variance was applied and the results revealed that among the bacteria screened, *Pseudomonas aeruginosa* was the most resistant bacteria strain, while *Flavobacterium* sp., the most susceptible one. *Morinda lucida* extract was active against all the tested bacteria, while some conventional antibiotics were active, some were not. The phytochemical screening of the leaf extract of *Morinda lucida* indicated the presence of alkanoid, tannis phlobatannin, saponnis, flavonoids, steroid, glycosides, anthraquinone, phenol, regins and reducing sugar. In conclusion, there exists significance difference among the effect of the antibiotics drugs on the bacteria that cause typhoid fever as well as the bacteria that cause typhoid fever due to the antibiotics.**

Keywords: Completely Randomized Block Design (CRBD), Flavonoid sp., Morinda lucida, Pseudomonas aeruginosa, Phytochemical.

INTRODUCTION

Typhoid fever also known simply as typhoid is common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the faeces of an infected person, which contains the *Bacterium salmonella enteric subsp. Enteric, Serovar typhi*.

The disease has received various names such as gastric fever, enteric fever, abdominal typhus, infatile remittent fever, slow fever, nervous fever, and pythogenic fever. The name typhoid means resembling typhus and come from neuro-psychiatric symptoms common to typhoid and typhus. Despite this similarity of their names, typhoid fever and typhus are distinct disease and are caused by different species of bacteria. The occurrence of this disease fell sharply in the developed world with the rise of 20^{th} century sanitation techniques and antibiotics. In 2013 it resulted in about 161,000 down from 181,000 in 1990.

Typhoid fever is contacted by drinking or eating the bacteria in contaminated food or water. People with acute illness can contaminate the surrounding water supply through stool, which contains a high concentration of the bacteria. Contamination of the water supply can in turn taint the food supply. The bacteria can survive for week in water or died sewage. About 3% - 5% of people become carriers of the bacteria after the acute illness. Others suffer a very mild illness that goes unrecognized. These people may become long term carriers of the bacteria even though they have no symptom and be the source of new outbreak of typhoid fever for many years.

The use of plant as source of medicine in treating disease is an ancient practice. People on all continent have long applied poultries and imbibed infusion of hundred, if not thousands of indigenous plants dating back to pre-history (Cowan, 1999). The widespread use of herbal remedies and health care preparation, such as those described in the ancient text like the bible and the Vedas has been traced to the occurrence of natural product with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them rich source of different types of medicine (Nair *et al*., 2005). In recent time, attention has been reverted back to plants as sources of therapeutic agent due to their higher properties. These include among others reduced cost, relative lower incidence of adverse reaction compared to modern conventional pharmaceutical and ready availability (Karachi, 2006).

Throughout the history of mankind, many infectious diseases have been treated with plant extracts (Buwa and Staden, 2006). In Africa, approximately 80% of the population still relies on traditional healing practice and medical plant for their daily healthcare needs, despite the immense technological advancement in modern medicine. The vegetation and floral biodiversity of Africa provide traditional health practitioner with an impressive pool of natural pharmacy from which plants are selected as remedies or ingredient to prepare herbal medicine (phytomedicine) for a plethora of human and veterinary disorders. It is estimated that today, plant materials are present in or have provided the model for 50% western drugs. Many commercial proven drugs used in modern medicine today were initially used in crude form in traditional or folk healing practice or for other purpose that suggested potentially useful biological activity (John and Ojewole, 2006). Until recently, effectively chemothera-peutical drugs for treatment of infectious disease have been largely antibiotics such as chloramphenicol, tetracycline, genta-mycin, cephalosporin, etc. these antibiotics have been available for so long in market hence the emergency of adulteration, drug resistance amongst the microorganism, which reduced their effectiveness, extends illness and risk of complications and death (WHO, 2005).

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More so, these drugs are not only expensive and inadequate but their administration are often accompanied with high toxicity and adverse effect (Babayi *et al*., 2004). Furthermore, for millions of people, particularly the rural African, poor and disadvantage, synthetic drugs are unavailable, unaffordable and unsafe or improperly used (Karaman *et al*., 2003; WHO, 2005). Again in industria-lized nation, despite the progress made in the understanding of micro-organism and their control, the emergency of unknown disease causing microbes pose serious public health concern (Iwu *et al*., 1999). These and other inherent limitations associated with conventional antimicrobial agent have redirected interest to the great potential of the herbal world for the search of all classes of drugs.

The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternative and offer profound therapeutic benefit and more affordable (Iwu *et al*., 1999). Further active component of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole safety and efficacy much superior to that of its isolated and pure active component (Babayi *et al*., 2004).

Aim and Objectives

The aim and objectives of this paper are as follow:

1. To determine the level of inhibition of bacteria that causes typhoid when treated with Morinda lucida.
2. To compare the level of the antibacterial effect of different conventional antibiotics.

Data Presentation

Data were collected through direct observation method from the laboratory

Table i: The phytochemical analysis of aqueous and ethanolic extract of morinda lucida leaf

Chemical constituent	Result	
	Water	Ethanol
Saponin	+	-
Tannin	-	+
Flavanoid	+	+
Resins	+	-
Anthraquinones	-	-
Alkaloid	-	-
Glycoside	-	-
Reducing sugar	+	+
Steroid	-	-
Phenol	+	+

Key	
+	Present
-	Absent

Table ii: The level of inhibition of selected angtibiotics against various bacteria that cause typhoid

Antibiotics	Sa	St	Pa	Kp	Ec	Bs	Fs
Chlorafenicol	26	2	27	0	0	19	10
Gentamycin	0	27	18	26	26	11	7
Erythromycin	0	0	0	28	28	0	21
Amoxillin	0	10	8	30	30	22	14
Streptomycin	29	0	15	30	30	13	0
Morinda lucida	14	18	0	16	18	3	25

Key	
Sa	*Staphylococus aureus*
St	*Salmonella typhy*
Pa	*Psuedomonas aeuroginosa*
Kp	*Klebsiella pneumia*
Ec	*Escherichia coli*
Bs	*Bacilus subtilis*
Fs	*Flavobacterium sp.*

Method of Analysis

Randomized Complete Block Design (RCBD)

Randomized complete block design is a design in which experimental units are first short into homogenous group called block. Treatments are then assigned at random within block. The RCBD is called a random effect model the experimental unit with similar feature are put together in group called blocks such that within each block there is homogeneity and between each block there is heterogeneity. A random process is then used to assign treatment to experimental units within a block. The objective is to keep variability among experimental unit within a block as small as possible and to maximize difference among blocks.

Characteristics of RCBD

1. It is a 2-way classification: namely treatment and block.
2. The block is a replication of the treatment and block constitutes replication.
3. Treatments are allocated at random sample within the block

4. Also if the treatments are random sampled out, the blocks are not the model is also mixed.
5. Randomized block can be complete or incomplete.

Complete blocks have equal replication of the treatment while incomplete block unequal replication of the treatment.

Advantages of RCBD
1. It can be effective grouping a more precise result than a completely randomized design of comparable size.
2. The statistical analysis is relatively simple.
3. Randomized complete block design can be accommodation any number of treatment and replication but all treatments are equally replication.

Disadvantages of RCBD
1. The degree of freedom of experimental error is not as large as that of a completely randomized design.
2. More assumptions are required for the model than the CRD model.
3. Using observation within block requires more complex calculations.

Statistical Model for RCBD
$y_{ij} = \mu + T_i + B_j + \ell_{ij}$
where
i = 1,2,3 ……., r
j = 1,2,3, ……., b
μ = Grand mean
T_i = effect of the ith treatment
B_j = effect of the jth block
ℓ_{ij} = error term the error term are assumed to independent and normally distributed with mean zero constant variance that is ℓ_{ij} – NID (0, δ^2)

$$\sum_{i=1}^{t}\alpha_i = 0 \quad \sum_{j=1}^{b}\beta_i$$

Table iii: Layout of Two-Ways Classification

Block	1	2	3 ……………………….T	Total
1	Y_{11}	Y_{12}	Y_{13} ……………………….Y_{1t}	Y_1
2	Y_{21}	Y_{22}	Y_{23} ……………………….Y_{2t}	Y_2
3	Y_{31}	Y_{32}	Y_{33} ……………………….Y_{3t}	Y_3
.	.			
.	.			
.	.			
.	.			
B	Y_{b1}	Y_{b2}	Y_{b3} ……………………….Y_{bt}	Y_b
Total	Y_1	Y_2	Y_3 ……………………….Y_t	Y..

TREATMENT

Let $\sum_{i=1}^{t} y_{ij}$ be the total of all observation taken under treatment i

$Y_j = \sum_{i=1}^{t} y_{ij}$ be the total of all observation

N = tb = is the total number of observation
Y_i = is the average of the observation taken under treatment i
Y_j = is the average of the observation in block
$Y_{...}$ = is the grand average of all observation

Express Mathematically

$$Y_i = \sum_{i=1}^{t} y_{ij} \quad i = 1,2,3…….t$$

$$Y_j = \sum_{i=1}^{t} y_{ij} \quad j = 1,2,3……..b$$

$$Y.. = \sum_{i=1}^{t}\sum_{i=1}^{b} y_{ij} = \sum_{i=1}^{t} y_{ij} = \sum_{i=1}^{t} y_{ij}$$

$$\bar{y} = y_i/b, \ \bar{y}_j = y_j/t \quad \bar{y}.. = y../N$$

Procedure for Analysis RCBD
Test Hypothesis
H_0: $T_1 = T_2 … = T_t = 0$
H_1: $T_1 \neq T_2 … \neq T_t \neq 0$ for at least one i

For block
H_0: $B_1 = B_2 … = \alpha_t = 0$
H_1: $B_1 \neq B_2 … \neq \alpha_t \neq 0$ for at least one j

Statistical model
$Y_{ij} = \mu + \alpha_i + \beta_j + \ell_{ij}$

Table iv: Analysis of variance table for completely randomized block design (RCBD)

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F*
Treatment	a-1	SStrt	SStrt/a-1	MStrt/MSE
Block	b-1	SSblock	SSblock/b-1	MSblock/MSE
Error	(a-1)(b-1)	By Subtraction	SSE/(a-1)(b-1)	
Total	ab-1	SStotal		

Decision Rule and Conclusion
FOR TREATMENT
Reject H_o if Fcal > Ftab or P- value < α, otherwise do not reject H_o
FOR BLOCK

Reject H_o if Fcal > Ftab or P- value < α, otherwise do not reject H_o.
Table value $F_{v1, v2}$ for treatment
α = level of significance
V_1 = degree of freedom of treatment
V_2 = degree of freedom of error

Analysis

Hypothesis testing for Antibiotic

H_o: ($T_1 = T_2 \ldots = T_t = 0$) There is no significance difference among the effect of six (6) antibiotics drugs on bacteria that cause typhoid fever.

H_1: ($T_1 \neq 0$ for at least one i) There is significance difference among the effect of six (6) antibiotics drugs on bacteria that cause typhoid fever.

Hypothesis testing for Bacteria

H_o: ($B_1 = B_2 \ldots = B_t = 0$) There is no significance difference among the bacteria that cause typhoid fever due to the antibiotics.

H_1: ($B_1 \neq 0$ for at least one i) There is significance difference among the bacteria that cause typhoid fever due to the antibiotics.

Critical α = 0.05

Decision rule: Reject H_o if P-value is less than level of significance α = 0.05 otherwise do not reject.

Test statistics:

$$F_{cal} = \frac{MS_{treatment}}{MS_{error}}$$

Computation

Table v: Two-way ANOVA: Response versus Bacteria, Antibiotic

Source	DF	SS	MS	F	P
Bacteria	6	923.24	153.873	1.14	0.364
Antibiotic	5	231.07	46.214	0.34	0.883
Error	30	4050.76	135.025		

S = 11.62 R-Sq = 22.18%
R-Sq(adj) = 0.00%

Individual 95% CIs for Mean
Based on Pooled StDev

Bacteria	Mean
1	11.5000
2	9.5000
3	21.3333
4	21.6667
5	22.0000
6	14.6667
7	12.8333

Individual 95% CIs for Mean Based on Pooled StDev

Antibiotic	Mean
1	12.0000
2	16.4286
3	11.0000
4	16.2857
5	16.7143
6	16.2857

Summary of result Antibiotics

There is significance difference among the effect of six (6) antibiotics drugs on bacteria that cause typhoid fever.

Summary of result Bacteria

There is significance difference among the bacteria that cause typhoid fever due to the antibiotics.

General Summary

This study has shown that the aqueous and ethanolic extracts of the *Morinda lucida* of the lucida leaf have antibacterial property which may offer a scientific basis for the traditional curative use of the plant. The bacteria tested are implicated in a wide variety of infection, basically Typhoid Fever, therefore, constituents of the leaf could be useful in chemotherapy. Among the bacterial screened, *Pseudomonas aeuroginosa* was the most resistant bacteria strain, while *Flavobacterium sp*., the most susceptible one. *Morinda lucida* extract was active against all the tested bacteria, while some conventional antibiotics were active some were not.

Recommendations

I hereby recommend that more researches should be carried out on the plant against typhoid fever to ascertain the needed herbs to use with it to have a definite curative power.

Also, the government and non-government organizations should explore the potentials in order to meet the need of human healthful lifestyle.

Since the average mean effect of *Morinda lucida* and Amoxillin are the same, I recommend that the government and professional scientists should explore this plant and use it in the treatment of several bacterial infections.

Conclusively, since we used ethanolic and aqueous extracts of the leaves of *Morinda lucida*, more research can be carried on the roots and barks to test for the effectiveness of this plant against certain bacteria.

REFERENCE

[1] Adomi, P. O. (2006): Antibacterial activity of aqueous and ethanol extract of the stem bark of Alstonia and Morinda lucida. Sci Res Essay 1(2): 50-53.

[2] Anani, K., Hudson, J. B. Desouuza, et al., (2003) Investigation of medicinal activities. Pharm. Biol 38: 40-45.

[3] Babayi, H., Kolo, I., Okogu, J. J. and Ijah, U. J. (2004): The antimicrobial activities of methanolic extract of Eucalyptus camadulensis and Terminalia catappa against some pathogenic micro-organisms. Biokemistri 16(2):106-111.

[4] Buwa, L. V. and Staden, V. J. (2006): Antibacterial and antifungal activity of traditional medicinal plant used against veneral diseases in South African Journal of Ethnopharmacology 103:139-142.

[5] Cowan, M. M. (1990): Plant product as antimicrobial agent, Chn. Microbiol. Rev 12:564-584.

[6] Douglas, C. Montgonery (2001): Design and analysis of experiment, Arzona State University. ISBN: 0-471-316490-0

[7] John, A. and Ojewole, O. (2006): Antinocieptive, Anti-inflammatory and Antidibetics properties of Hypoxis Hemerocallidea Fisch and CA Mey (Hepoxidaceae) Corn (African Potato) aqueous extract in Mice and Rate Journal of Enthnopharmacology 103:126-134.

[8] Karama, I., Sahin, F. and Sengui, M. (2003): Antimicrobial activity of aqueous and methanolic extract of Juniperous Exycedrus Journal of Enthnopharamacology 18(37):1-5.

[9] McNaught, A. D. and Wilkinson, A. (1997): IUPAC compendium of Chemical Terminology, 2nd edition (The Gold Book).

[10] Sofowora, E. A. (2006): Medicinal plant and traditional medicine in African Spectrum Book Ltd. Ibadan pg. 289.

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