Effect of different Antibiotics on Bacteria that cause Typhoid Fever

Salau Ganiyat M., Ojo Olufemi D., Adeoye Akeem. O

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 alkanoids, tannins phlobatannin, saponins, 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, infafile 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 20th 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 a plethora of herbal remedies or ingredient to prepare herbal medicine (phytomedicine) for a plethora of human and veterinary diseases. 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).

Salau Ganiyat M., Department of Statistics, Federal Polytechnic Offa, Kwara State
Ojo Olufemi D., Department of Statistics, Federal Polytechnic Offa, Kwara State
Adeoye Akeem. O, Department of Statistics, Federal Polytechnic Offa, Kwara State
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 industrialized 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.

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

<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Result</th>
<th>Key</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sa</th>
<th>St</th>
<th>Pa</th>
<th>Kp</th>
<th>Ec</th>
<th>Bs</th>
<th>Fs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorafenicol</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>19</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>26</td>
<td>26</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Amoxillin</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>30</td>
<td>30</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>30</td>
<td>30</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Morinda lucida</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>18</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

**Key**
Sa: *Staphylococcus aureus*
St: *Salmonella typhi*
Pa: *Psuedomonas aeuruginosa*
Kp: *Klebsiella pneumia*
Ec: *Escherichia coli*
Bs: *Bacillus subtilis*
Fs: *Flavobacterium sp.*

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### 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
\[ y_{ij} = \mu + T_i + B_j + \epsilon_{ij} \]
where
\[ i = 1,2,3, \ldots, r \]
\[ j = 1,2,3, \ldots, b \]
\[ \mu = \text{Grand mean} \]
\[ T_i = \text{effect of the } i\text{th treatment} \]
\[ B_j = \text{effect of the } j\text{th block} \]
\[ \epsilon_{ij} = \text{error term} \]
\[ \text{the error term are assumed to be independent and normally distributed with mean zero constant variance that is } \epsilon_{ij} \sim NID(0, \sigma^2) \]

\[ \sum_{i=1}^{t} \alpha_i = 0 \quad \sum_{j=1}^{b} \beta_j = 0 \]

Table iii: Layout of Two-Ways Classification

<table>
<thead>
<tr>
<th>Block</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y_{11}</td>
<td>Y_{12}</td>
<td>Y_{13}</td>
<td>Y_{1}</td>
</tr>
<tr>
<td>2</td>
<td>Y_{21}</td>
<td>Y_{22}</td>
<td>Y_{23}</td>
<td>Y_{2}</td>
</tr>
<tr>
<td>3</td>
<td>Y_{31}</td>
<td>Y_{32}</td>
<td>Y_{33}</td>
<td>Y_{3}</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B</td>
<td>Y_{b1}</td>
<td>Y_{b2}</td>
<td>Y_{b3}</td>
<td>Y_{b}</td>
</tr>
<tr>
<td>Total</td>
<td>Y_{1}</td>
<td>Y_{2}</td>
<td>Y_{3}</td>
<td>Y_{..}</td>
</tr>
</tbody>
</table>

TREATMENT

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

\[ Y_j = \sum_{i=1}^{t} y_{ij} \]

\( N = tb = \) is the total number of observation
\( Y_i = \) is the average of the observation taken under treatment \( i \)
\( Y_{..} = \) is the grand average of all observation

Express Mathematically

\[ Y_i = \sum_{j=1}^{b} y_{ij} \quad i = 1,2,3 \ldots t \]

\[ Y_j = \sum_{i=1}^{t} y_{ij} \quad j = 1,2,3 \ldots b \]

\[ Y_{..} = \sum_{i=1}^{t} \sum_{j=1}^{b} y_{ij} = \sum_{i=1}^{t} y_{ij} = \sum_{j=1}^{b} y_{ij} \]

\[ y = \frac{Y_i}{b} \quad \bar{y}_j = \frac{Y_j}{t} \quad \bar{y}_{..} = \frac{Y_{..}}{N} \]

Procedure for Analysis RCBD

Test Hypothesis
\[ H_0: T_1 = T_2 = \ldots = T_t = 0 \]
\[ H_i: T_1 \neq T_2 = \ldots = T_t \neq 0 \text{ for at least one } i \]

For block
\[ H_0: B_1 = B_2 = \ldots = B_b = 0 \]
\[ H_i: B_1 \neq B_2 = \ldots = B_b \neq 0 \text{ for at least one } j \]

Statistical model

\[ y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \]

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

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of Freedom</th>
<th>Sum of Square</th>
<th>Mean Square</th>
<th>F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment t</td>
<td>a-1</td>
<td>SStrt</td>
<td>SStrt/a-1</td>
<td>MStrt/MSE</td>
</tr>
<tr>
<td>Block</td>
<td>b-1</td>
<td>SSBblock</td>
<td>SSBblock/b-1</td>
<td>MSBblock /MSE</td>
</tr>
<tr>
<td>Error</td>
<td>(a-1)(b-1)</td>
<td>By Subtraction</td>
<td>SSE/(a-1)(b-1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>ab-1</td>
<td>SStotal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Decision Rule and Conclusion

FOR TREATMENT
Reject \( H_0 \) if \( F_{\text{cal}} > F_{\text{tab}} \) or \( P<\alpha \), otherwise do not reject \( H_0 \)

FOR BLOCK
Reject $H_0$ if $F_{cal} > F_{tab}$ or $P$-value $< \alpha$, otherwise do not reject $H_0$.

Table value $F_{\alpha, \nu_1, \nu_2}$ for treatment

$\alpha$ = level of significance
$\nu_1$ = degree of freedom of treatment
$\nu_2$ = degree of freedom of error

### Analysis

#### Hypothesis testing for Antibiotic

$H_0$: $T_1 = T_2 = \ldots = T_i = 0$ 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

$H_0$: $B_1 = B_2 = \ldots = B_i = 0$ 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 $\alpha = 0.05$

### Decision rule:

Reject $H_0$ if P-value is less than level of significance $\alpha = 0.05$ otherwise do not reject.

### Test statistics:

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

### Computation

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>6</td>
<td>923.24</td>
<td>153.87</td>
<td>1.1</td>
<td>0.36</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>5</td>
<td>231.07</td>
<td>46.214</td>
<td>0.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>4050.76</td>
<td>135.02</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

$S = 11.62$ R-Sq = 22.18%

R-Sq(adj) = 0.00%

### Individual 95% CIs for Mean Based on Pooled StDev

- Bacteria Mean
  - 1: 11.500
  - 2: 9.5000
  - 3: 21.3333
  - 4: 21.6667
  - 5: 22.0000
  - 6: 14.6667
  - 7: 12.8333

- 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 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.

### 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


Salau Monishola Ganiyat, BSc. MSc.
She obtained her MSc. from the prestigious University of Ilorin in 2019 and BSc from the University of Danfodiyo (both Statistics) in 2015 and 2010 respectively. She is presently a lecturer in the department of statistics, Federal Polytechnic, Offa, Kwara State-Nigeria. She has attended several workshops and conferences within Nigeria and has many journals publication at both national and international to her credit. She specializes in Social Statistic. She is an active member of Professional Statisticians Society of Nigeria (PSSN)

Ojo Olufemi David, BSc. MSc.
Born in 1983 in Niger State of Nigeria. He obtained his first degree (Bsc in Statistics) and Master Degree from Ladoke Akintola University of Technology in 2010 and 2015 respectively. He is presently a lecturer in the department of statistics, Federal Polytechnic, Offa, Kwara State-Nigeria. He has attended several workshops and conferences within Nigeria. He has many journals publication at both national and international to his credit. He specializes in Demography, and Applied general statistics. He is an active member of Professional Statisticians Society of Nigeria (PSSN)

Akeem Olanrewaju ADEOYE, Ph.D MSc.
He obtained his PhD and MSc. in Statistics in 2015 and 2010 respectively from the prestigious University of Ilorin. He is presently a lecturer in the department of statistics, Federal Polytechnic, Offa, Kwara State-Nigeria. He attended several workshops and conferences within and outside Nigeria. He has many journals publication at both national and international to his credit. He specializes in Operations Research, and Applied general statistics. He is an active member of Professional Statisticians Society of Nigeria (PSSN)