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# Molecular Detection of *E. coli* O157:$H_7$ Isolated from Infants Diarrheal Stools and Its Sensitivity to *Mangifera indica* (Mango) and *Bosweilia dalzeilii* (Hano) Extracts

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**Abstract:** *M. indica and B. dalzeilii* are common medicinal plants grown naturally in most parts of tropical and subtropical regions of Northern Nigeria. They are used traditionally in the treatment of diarrhea in children especially at teething age. This study was conducted to detect, isolate and identify E. coli O157:$H_7$ from diarrheal stools of children and evaluate antibacterial activities of M. *indica* and *B. dalzielii* extracts against the test bacteria. Total number of one hundred (100) samples were collected from Children Clinic of General Hospital Gusau, Zamfara State Nigeria using standard microbiological procedure. *E. coli* O157:$H_7$ is an important pathogens of human, implicated as one of the important causative agents of diarrhea in children especially at teething ages. The test organism was detected, isolated and identified using Cultural Growth Characteristics, Gram reactions, Serology, Biochemical tests and Molecular screenings. The sample plants extracts were obtained using Soxhlex Apparatus with Methanol as solvent. Phytochemical screening of the plants extracts revealed the presence of Alkaloids, Flavoids, Tannins and Saponnins. Antibacterial activities of the aqueous methanolic extracts of the test plants against *E. coli* O157:$H_7$ was evaluated using Sensitivity Tests, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC). The most active phytochemical compounds was obtained by fractionation using Chromatography. Chemical structure of the most active phytochemical was elucidated using Nuclear Magnetic Resonance Spectroscopy. Confirmation of antibacterial activities of the test plants, lend credence to the traditional used of those plants in the treatment of diarrhea in children especially at teething ages. It is therefore recommended that, *M. indica* and *B. dalzeilii* can be further purified to yield templates for synthesis of Orthodox drugs for the treatment of diarrhea in children.

**Keywords:** Molecular Detection, Antibacterial Activity, *E. coli* O157:$H_7$, *M. indica*, *B. dalzeilii,* Phytochemical Screening, Diarrhea

## 1. Introduction

*Mangifera indica* (Mango) juicy stone fruit belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. Over 1000 mango varieties are available worldwide [32]. Currently, mango is cultivated on an area of approximately 3.7 million hac worldwide. Mango fruits takes the second position as a tropical crop next to banana in terms of production and acreage used [18, 25]. It has been well documented that, mango fruits are important sources of micronutrients, vitamins, and other phytochemicals [42]. Various parts of mango are used for more than thousand years as wide variety of Ethanomedicinal uses to cure diseases such as throat disease, malaria, dysentery, diarrhea, and many others [20]. These include; the roots, bark, leaves, flowers, fruits and stony part of the seeds. Pharmacologically, mango is used as anti-cancer, antidiabetic, anti-inflammatory, hepotoprotective, anti-hemorrhagic, anti-tetanus, analgesic, antipyretic, kidney damage, anti-ulcer anti-diarrheal etc [39,

36, 40]. Antimicrobial activities of aqueous and ethanolic extracts mango leaves against different microorganisms have been reported. These organisms include; *S. aureus*, *Micrococcus*, viruses, *E. coli*, *Klebsella pneumoniae*, *P. aerogenosa*, *Candida albicans* and Fungi [7]. *Boswelia dalzielii- (Hano/Ararrabi)* Is a genus of tree in the order sapindales. There are four speices of Bosweilia; *B. bhau-dajia*, *B. frereana*, *B. papyrifera* and *B. seratu.* [31]. Boswellia species are moderate –sized flowering plants. Bark of the plant is used in traditional medicine. Gallic and protocatechnic acids were isolated as the main antibacterial and antioxidant principles of the stem bark of *B. dalzielii*, 4-methoxy-E-resver [30]. *Escherichia coli* O157:$H_7$ is a gram negative rod shaped, facultative anaerobic bacterium of family Enterebacteriaceae [10]. Based on its virulence, the bacterial organism is classified into five groups; Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohaemoorhagic *E coli* (EHEC), Attcahing and Effecting *E coli* AEEC and Shiga-Toxin Producing E coli (STEC) [14].

*E. coli* O157:$H_7$ is an emerging serotype of *E. coli* that accounts for most human diseases caused by enterohaemorrhagic *E. coli* (EHEC) [12, 19]. It is associated with threatening diseases conditions in human such as Haemolytic Uremic Syndrome (HUS), Thrombolic Thromcytic Purpura (TTP) and Haemorrhagic colitis (HC). The organism was first recognized as human pathogens following outbreak of haemorrhagic colitis associated with consumption of contaminated beef hamburger [35]. *E. coli* O157:$H_7$ has been transmitted via several routes including beef, roast beef, dairy products etc [8, 42]. The number of serotypes of vero-toxins producing *E. coli* causing human diseases is increasing but, *E. coli* O157:$H_7$ continues to be the dominant caused of Hemorrhagic Colitis and HUS [44].

Children at teething ages suffered serious diarrheal infections in many communities of northern Nigeria and many other parts of third world countries [3, 37, 43]. However with the advances medical and infectious diseases researches, it has been discovered children diarrheal infections have nothing in connection with teething as it as mythically believe. This particular research was conducted to address real causes of children diarrheal infection and provide solution using plants especially particularly *M. indica* and *B. dalzeilii* as the best option for treatment of such infections [12, 13]. Medicinal plants have been used in virtually all cultures as sources of medicine.

# 2. Materials and Methods

### *2.1. Materials*

All the microbiological reagents and microbial growth media were of analytical grades and purchased from Hospital Affairs Gusau and Emmaco Stores Kaduna Nigeria. Glassware, machines and Laboratory equipment used were from Microbiological Laboratories Federal Medical Centre Gusau, General Hospital Gusau and Genomics Laboratory Barau Dikko Teaching Hospital Kaduna.

### *2.2. Collection and Extraction of Plants Materials*

The plant samples were collected from forests and Botanical Gardens within the outskirts of Kaduna, Kaduna State and Gusau Zamfara State. The plant samples were during dry season. The plants were carefully selected in clean polythene bags and taken to Faculty of Biological Sciences Ahmadu Bello University Zaria, Ngeria for authentication and taxonomic identification. Leaves and stems were used in this study. The plant samples were dried under room temperature. After drying, the plants materials were crushed and ground using pestle and mortar [2]. The powdered plants materials were subjected to methanol extraction using Soxhlet Apparatus [22]. Powdered plant samples were enclosed in a filter paper and placed in the Thimble Chamber of the Soxhlet Apparatus. Extraction Solvent (Methanol) was used in the process. At the end extraction, clear solution of the crude extract appeared in the siphon tube and collected, filtrated and concentrated to dryness under reduced pressure using rotary evaporator at 40°C [23]. The extracts were transferred into air tight tubes for further analysis.

### *2.3. Phytochemical Tests*

Qualitative phytochemical screenings of the two test plants extracts revealed the presence of the following bioactive compounds; Alkaloids, Cardiac glycoside, Sapanin, Phenol compounds, Tannins, Flavonoids, and Anthroquanones (Table 1). Quantitative analysis of the plants extracts indicated that they contain appreciable quantity of some important secondary metabolites; Alkaloids, Tannins, Flavonoids and Saponins (Tables 2 and 3) [9, 16].

### *2.4. Microbiological Analysis*

#### *2.4.1. Collection of Diarrheal Stool Samples*

Total number 100 samples from infants with diarrheal infections and presented for the first time at children clinic at General Hospital Gusau, Zamfara State was collected aseptically using sterile rectal swabs and plastic bottles. The samples were immediately transferred to Microbiology Laboratory of the same hospital.

#### *2.4.2. Isolation and Identification*

The stool samples were physically observed and prepared for inoculation. All samples were aseptically inoculated onto MacConkey Agar Media (MA) and incubated at 37°C for 24 h. Colonies appeared to have fermented lactose were further subjected to gram reactions, serological and biochemical tests. Based on those tests, isolates were confirmed to an *E coli*. The isolates was further Sub-cultured onto Sorbitol MacConkey Agar (SMA), incubated at 37°C for 24 h [33]. Based on cultural characteristics non-sorbitol fermentation growth on Sorbitol MacConkey Agar, molecular screening, the isolates was confirmed to be an *E. coli* O157:$H_7$. Identified isolates were

grown on Muller Hinton Agar Media slants and preserved for further analysis [34].

#### *2.4.3. Molecular Identification*

Pure isolates of the test organism was transported in an ice blocs to Molecular and Research Diagnostic Laboratory Kaduna for molecular characterization. Molecular Screening conducted were; DNA Extraction, DNA Amplification, DNA Visualization and DNA Sequencing. Results of all the screenings, based on bioinformatics analysis using Basic Alignment Search Tool (BLAST), it was further confirmed that, the test organism was *E. coli* O157:$H_7$ [38].

#### *2.4.4. Standardization of Bacterial Inoculums*

The isolates were sub-cultured onto sterile Muller Hinton Agar plates incubated at 37°C for 18-24°C for 24 h. The sub-cultured isolates were inoculated onto a Test tube containing 5 mL normal saline and compared with 0.5 Macfarlands turbidity standard which marched with 0.1 x $10^8$ cfu/mL [1].

#### *2.4.5. Antibacterial Sensitivity Tests*

Antibacterial sensitivity of the test organisms was carried out using Agar Well Diffusion method on Muller Hilton Agar (MHA). About 20 mL of sterile Muller Hinton Agar was poured onto petri dishes and allowed to solidify. 2mL of standard culture of the test organisms was flooded and excess discarded. Using sterile corked borer of 4mm in diameter, wells were bored. A drop of molten Muller Hinton was poured into each well. About 100µL of prepared concentrations of the test plants extracts were added into each well [26]. The plates were allowed to stand at room temperature for 1 h (Pre-diffusion time). Then the plates were incubated at 37°C for 24 h. At the end of the incubation, diameter of the growth inhibition zones were measured in millimeter (mm) using calibrated ruler (Tables 4 and 5).

#### *2.4.6. Determination of Minimum Inhibitory Concentration (MIC)*

Minimum inhibitory concentration of the two test plants extracts against the bacterial isolates under investigation was determined using Agar dilution method at 100mg/L, 50mg/L, 25mg/L and 12.5m/L. Bottles each containing 10mL double strength sterile Mueller Hilton Agar were prepared. 10ml of the prepared extract is added to the first 10mL of double strength Mueller Hinton Agar and properly mixed. About 10mL of the mixture in the first bottle is withdrawn to the second bottle of double strength Mueller Hilton Agar. This process was repeated up to the $10^{th}$ bottle to obtain doubling dilutions of the extract and after which the last 10ml was taken from the $10^{th}$ bottle is discarded into a container containing disinfectant solution. The mixture was allowed to stand on the bench to solidify. Sterile paper disc was placed on the solidified Agar. About10µL of the standardized culture was added to each of the filter paper, and allowed to stand for 1 hour for pre-diffusion. The plates were all incubated at 37°C for 24 hours. At the end of the incubation period, growth was observed. The MIC was recorded as the Minimum Concentrations where no growth (Clear suspension) was shown (Tables 4 and 5) [24, 11].

#### *2.4.7. Determination of Minimum Bactericidal Concentration (MBC)*

Minimum Bactericidal concentration of the test plants extracts were also determined. From MIC results, plates with no any growth were used by picking the filter paper into 5mL sterile Mueller Hilton broth. The plates were labelled and incubated at 37°C for 24 h. At the end of the incubation period, the tubes were checked for growth. The minimum concentration where no growth was observed was MBC (Tables 6 and 7) [15, 6].

### *2.5. Statistical Analysis*

The experiments were conducted in triplicates. Data obtained was subjected to statistical analysis using ANOVA on SPSS version 16.0. Turkey-kramer Multiple Comparisons Test was used to separate the means. $P \geq 0.05$ was considered insignificantly different.

## 3. Results and Discussion

Quantitative phytochemical evaluation of the two test plants samples were shown in table 1 below: Phytochemicals detected were; Alkaloids, Flavoids, Saponins, Cardiac glycosides, Steriods/Terpenoids, Phenols, Tannins and Anthroquanones.

***Table 1.*** *Presence of the bioactive compounds in each sample plant extract.*

| Extract | Alkn | Cgds | Sapn | Phcp | Tann | Str/Ter | Flv | Ant |
|---|---|---|---|---|---|---|---|---|
| *M. indica* | ++ | +++ | ++ | + | ++ | +++ | ++ | + |
| *B. dalzielii* | +++ | ++ | + | ++ | +++ | ++ | ++ | + |

Key:
Akln = Alkaloids, Cgds= Cardiac glycosides Sapn= Saponins
Phcp= Phenol Compounds Tann= Tannins Str/Ter= Steriods/Terpenoids
Flv= Flavonoids Ant= Anthroquanones
(+) = Trace quantity, (++) = Moderate quantity,(+++) = Appreciable quantity

Quantitative determination of the all the test plants extracts indicated that, they all contained an appreciable quantity of some important bioactive compounds; such as Alkaloids, Flavonoids, Tannins, and Saponins [4, 16, 17]. Quantitative values and percentage of each bioactive compounds in all the sample plants extracts were shown in tables 2 and 3 below:

***Table 2.*** *Quantitative values of bioactive compounds in Mangifera indica (Mango).*

| Compounds | Absorbance | Average Abs | Conc. (mg/ml) | Conc. (mg/g) | Conc. % |
|---|---|---|---|---|---|
| Alkaloids | 0.061,.0.060,0.060 | 0.0603 | 0.0138 | 13.8 | 1.4 |
| Flavonoids | 0.340, 0.350,0.340 | 0.3433 | 0.1006 | 100.6 | 10.1 |
| Saponins | 0.048,0.0503,0.045 | 0.0480 | 0.0201 | 20.1 | 2.0 |
| Phenols | 0.048, 0.047, 0.047 | 0.0473 | 0.1238 | 123.8 | 12.4 |
| Tannins | 0.027,0.027,0.027 | 0.0270 | 0.0990 | 99.0 | 9.9 |

***Table 3.*** *Quantitative values of bioactive compounds in Boswelia dalzeili (Hano/Ararrabi).*

| Compounds | Absorbance | Average Abs | Conc. (mg/ml) | Conc. (mg/g) | Conc. % |
|---|---|---|---|---|---|
| Alkaloids | 0.191,0.195,.189 | 0.1917 | 0.0378 | 37.81 | 3.8 |
| Flavonoids | 0.401,0.399,0.399 | 0.3997 | 0.1178 | 117.81 | 11.8 |
| Saponins | 0.089,0.089,0.092 | 0.0900 | 0.0329 | 32.91 | 3.1 |
| Phenols | 0.806,.082,0.082 | 0.0833 | 0.2141 | 214.08 | 21.4 |
| Tannins | 0.028,0.029,0.028 | 0.0283 | 0.0283 | 102.33 | 10.2 |

Quantitative percentage of phytochemical compounds in each test plant sample screened revealed that, *M. indica* and *B. dalzeili* contain appreciable percentage of Alkaloids, Flavoids, Tannins and Saponins [28], as shown in table 4 below:

***Table 4.*** *Quantity (%) of phytochemical compounds in each sample Plant Extract.*

| Phytochemical compound | *M. indica* | *B. dalzielii* |
|---|---|---|
| Alkaloids | 6.4% | 5.00% |
| Flavonoids | 9.20% | 7.44% |
| Tannins | 6.33% | 5.65% |
| Saponins | 10.00% | 7.90% |

Tables 5 and 6 show the revealed varied inhibition zones produced against different concentrations of the test plants samples against E. coli O157:H7. Amoxilin antibiotic was used as a positive control. This findings tally with the works of other researchers [27, 21].

***Table 5.*** *Antibacterial Sensitivity of E. coli to Methanolic Extract of Mangifera indica (Mango) (Concentrations).*

| Isolate | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 100mg/ml Ctrl |
|---|---|---|---|---|---|
| E1 | 18 ±0.34 | 13±0.02 | 6 ± 0.13 | 6 ±0.00 | 7±0.33 |
| E2 | 17±0.08 | 14±0.09 | 6±0.14 | 6±0.06 | 27±0.48 |
| E3 | 14±0.11 | 7±0.14 | 6±0.00. | 6±0.07 | 28±0.30 |
| E4 | 16±0.12 | 6±0.01 | 6±0.06 | 6±0.09 | 7±0.24 |
| E5 | 17±0.48 | 13±0.30 | 6±0.08 | 6±0.72 | 7±0.28 |
| E6 | 15±0.37 | 6 ±0.12 | 6±0.13 | 6±0.24 | 20±0.18 |
| E7 | 17±0.08 | 14±0.15 | 6±0.11 | 6±0.33 | 6±0.31 |
| E8 | 18±0.24 | 13±0.08 | 6±0.08 | 6±0.11 | 8±0.34 |
| E9 | 16±0.20 | 6±0.11 | 6 ±0.00 | 6±0.07 | 29±0.06 |
| E10 | 18±0.24 | 14±0.15 | 7±0.06 | 6±0.48 | 28±0.11 |

Key: E = Isolate Ctrl = Control

***Table 6.*** *Antibacterial Sensitivity E. coli O157:$H_7$ to Methanolic Extract of Bosweilia dalzielii - (Hano/Ararrabi).*

| Isolate | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | 100mg/ml Ctrl |
|---|---|---|---|---|---|
| E1 | 20±0.33 | 16±0.15 | 13±0.11 | 6±0.24 | 6±0.00 |
| E2 | 17±0.54 | 13±0.22 | 7±0.12 | 6±0.72 | 26±0.20 |
| E3 | 18±0.48 | 14±0.33 | 6±0.00 | 6±0.06 | 28±0.24 |
| E4 | 18±0.48 | 14±0.72 | 7±0.33 | 6±0.00 | 31±0.54 |
| E5 | 20±0.62 | 16±0.08 | 13±0.11 | 7±0.30 | 28±0.18 |
| E6 | 22±0.34 | 17±0.12 | 15±0.72 | 6±0.62 | 28±0.11 |
| E7 | 20±0.30 | 16±0.08 | 13±0.12 | 6±0.00 | 7±0.24 |
| E8 | 20±0.34 | 16±0.48 | 13±0.11 | 6±0.12 | 8±0.72 |
| E9 | 19±0.06 | 15±0.08 | 8±0.07 | 6±0.48 | 30±0.05 |
| E10 | 18±0.72 | 14±0.20 | 7±0.33 | 6±0.30 | 29±0.2 |

Key: E= Isolate Ctrl= Control

Minimum Inhibitory Concentration (MIC) of the test plants samples revealed varied and inconsistent values at different concentrations against different concentrations of the plants extracts as shown in tables 7 and 8 [29].

***Table 7.*** *Minimum inhibitory Concentration (MIC) of Methanolic extract of Mangifera indica to E. coli 0157:$H_7$.*

| Isolate | Concentration (mg/ml) | | | |
|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.3mg/ml |
| E1 | - | β | + | + |
| E2 | - | β | + | + |
| E3 | - | β | + | + |
| E4 | - | β | + | + |
| E5 | - | β | + | + |
| E6 | - | β | + | + |
| E7 | - | | β | + |
| E8 | - | - | β | + |
| E9 | - | - | + | + |
| E10 | - | - | | + |

Key: β = MIC Value + = No Turbidity observed -=Turbidity observed

***Table 8.*** *Minimum Inhibitory Concentration (MIC) of Methanolic extract of Boswelia dalzielii to E. coli 0157:$H_7$.*

| Isolate | Concentration (mg/ml) | | | |
|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.3mg/ml |
| E1 | - | - | - | B |
| E2 | - | β | + | + |
| E3 | - | - | β | + |
| E4 | - | - | β | + |
| E5 | - | - | - | β |
| E6 | - | - | - | - |
| E7 | - | - | - | β |
| E8 | - | - | - | B |
| E9 | - | - | β | + |
| E10 | - | - | β | + |

Key: β-- = MIC Value + = No turbidy observed - = Turbidity observed

Tables 9 and 10 showed Minimum Bactericidal Concentration MBC of effects of concentrations of the test plants extracts against E. coli O157:$H_7$. This results tallies with the works of other researchers [5].

***Table 9.*** *Minimum Bactericidal Concentration (MBC) of Methanolic Extract of Mangifera indica on E.coli 0157:$H_7$.*

| Isolate | Concentration (mg/ml) | | | |
|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.3mg/ml |
| E1 | - | α | + | + |
| E2 | - | α | + | + |
| E3 | - | - | α | + |
| E4 | - | - | A | + |
| E5 | - | α | + | + |
| E6 | - | α | + | + |
| E7 | - | α | + | + |
| E8 | - | - | α | + |
| E9 | - | - | A | + |
| E10 | - | α | + | + |

Key: α = MBC Value + = Absence of growth - = Growth observed

***Table 10.*** *Minimum Bactericidal Concentration (MBC) of Metanolic Extract of Boswelia dalzielii on E. coli 0157:$H_7$.*

| Isolate | Concentration (mg/ml) | | | |
|---|---|---|---|---|
| | 50mg/ml | 25mg/ml | 12.5mg/ml | 6.3mg/ml |
| E1 | - | | + | |
| E2 | + | α | + | |
| E3 | + | α | + | |
| E4 | + | α | + | |
| E5 | - | α | + | |
| E6 | - | - | α | |
| E7 | - | α | + | |
| E8 | - | α | + | |
| E9 | α | + | + | |
| E10 | α | + | + | |

Key: α=MBC Value + = Absence of growth - = Growth observed

# 4. Conclusion

The crude extracts of *M. indica* and *B. dalzeilii* in this study revealed their respective antibacterial activities against *E. coli* O157:$H_7$ isolated from diarrheal stools from children under teething age. The study confirmed that *E. coli* O157:$H_7$ is one the major causative agents of diarrheal infections in children. Methanol was found to be the solvent of choice for extraction of bioactive phytochemical compounds from *M. indica* and *B. dalzeilii* crude extracts. Confirmation of bacterial activities of the test plants extracts lend scientific credence to the traditional uses of those plants in the treatment of diarrhea in children. The test investigation in this research can be further purified and processed appropriately to develop modern orthodox drugs for the treatment of diarrhea in children under teething ages.

# Conflict of Interest

The authors declared that they have no any conflict of interest.

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