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MOLECULAR DETECTION OF *E. COLI* O157:H$_7$ ISOLATED FROM INFANTS DIARRHEAL STOOLS AND ITS SENSITIVITY TO *P. GUAJAVA* (GUAVA) AND D. *MICROCARPUM* (TAURA) EXTRACTS

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ABSTRACT
P. guajava and *D. microcaroum* are common medicinal plants grown naturally in most parts of tropical and subtropical regions of Northern Nigeria. They are used traditionally in treating Diarrhea in children, especially at the age of teething. This study was conducted to detect, isolate and identify *E. coli* O157:H$_7$ from diarrheal stools of children and evaluate the antibacterial activities of *P. guajava* and *D. microcarpum* extracts against the test bacteria. A total number of hundred (100) samples were collected from the Children's Clinic of General Hospital Gusau, Zamfara State, using standard microbiological procedures. *E. coli* O157:H$_7$ is an important human pathogen, implicated as one of the important causative agents of Diarrhea in children. The test organism was detected, isolated and identified using Cultural Growth Characteristics, Gram reactions, Serology, Biochemical tests and Molecular screenings. The plant's extracts were obtained using Soxhlex Apparatus with Methanol as solvent. Phytochemical screening of the plant extracts revealed Alkaloids, Flavonoids, Tannins and Saponins. Antibacterial activities of the aqueous methanolic extracts of the test plants against *E. coli* O157:H$_7$ were evaluated using Sensitivity Tests, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC). The most active phytochemical compounds were obtained by fractionation using Chromatography. The chemical structure of the most active phytochemical was elucidated using Nuclear Magnetic Resonance Spectroscopy. Confirmation of the antibacterial activities of the test plants lends credence to the traditional use of those plants in treating Diarrhea in children, especially those of teething age. It is therefore recommended that *P. guajava* and *D. microcarpum* be further purified to yield templates for the synthesis of Orthodox drugs for treating Diarrhea in children.

Keywords: Molecular Detection, Antibacterial Activity, *E. coli* O157:H$_7$, *P. guajava*

1. INTRODUCTION
Over the years, children at teething age (usually 2-6 months) have suffered severe diarrheal infections in many communities in northern Nigeria as well as parts of third-world countries. [15]. World Health Organization [41] indicates that Diarrhea is the second leading cause of death in children under five years and is responsible for killing around 525,000 (8%) children every year. About 80% of these deaths occur in Africa and Asia [42]. The myth that teething is the leading cause of Diarrhea is quite prevalent. However, with advances in modern medicine and infectious disease research, it has been discovered that teething is not the main cause of Diarrhea in children under teething. [32]. *P. guajava* is a common and well-grown tropical tree abundantly grown for fruits. Many parts of the world use

P. guajava for medicinal purposes [10]. Various pharmacological studies have shown the plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial antigenotoxic, antigenotoxic, antiplasmodial, cytotoxic, and antidiabetic [10]. It is used to treat Diarrhea, Dysentery, gastroenteritis, Hypotension, diabetes and the improvement of locomotor coordination [1]. It contains high organic and inorganic compounds such as polyphenols, antioxidant, and antiviral compounds [27]. *D. microcarpum* grows naturally in the drier West and Central Africa [2]. It has a wide range of uses due to its medicinal properties, edible fruits and hardwood [12]. It contains Alkaloids, Terpenoids, Flavonoids, Tannins, Phenolics reduction sugar and Glycosides [8], [6]. One of the common causative agents implicated in children's diarrheal infection or gastrointestinal disorder, especially at teething ages, is *E. coli* O157:H_7 [9],[5]. *E. coli* O157:H_7 is classified by the characteristics of their virulence properties. This virulent *E. coli* include Enterohemorrhagic (EHEC) –for example, E. coli O157:H_7, Enteroaggrasive (EggEc), Enteropathogenic (EPEC) and Enterotoxigenic [37], [11]. This particular research aimed to investigate the potential antibacterial properties of the sample plants against E coli O157:H7, which was identified to be one of the causative agents of Diarrhea in children m Significantly, the continued emergence of infectious diseases caused by *E. coli* O157:H_7. More so, there is scanty information on *E coli* O157:H_7 in Nigeria and the rising cost of conventional drugs. The success of this research will no doubt reduce the incidence of bacteria among the affected communities.

2. MATERIALS AND METHODS

2.1. Materials

All the microbiological reagents and microbial growth media were of analytical grades purchased from Hospital Affairs Gusau and Emmaco Stores Kaduna. Glassware, machines and Laboratory equipment 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 Plant Materials

The plant samples were collected from forests and botanical gardens on the outskirts of Kaduna, Kaduna State, and Gusau Zamfara State. The plant samples were collected during the dry season.[24]. The plants were carefully selected in clean polythene bags and taken to the Faculty of Biological Sciences Ahmadu Bello University Zaria for authentication and taxonomic identification. Leaves and stems were used in this study. The plant samples were dried at room temperature. After drying, the plants were crushed and ground using a pestle and mortar.[4]. The powdered plant materials were subjected to methanol extraction using Soxhlet Apparatus. Powdered plant samples were enclosed in filter paper and placed in the Thimble Chamber of the Soxhlet Apparatus. Extraction Solvent (Methanol) was used in the process. At the end of extraction, a clear solution of the crude extract appeared in the siphon tube. It was collected, filtrated, and concentrated to dry under reduced pressure using a rotary evaporator at 40°C. [22].The extracts were transferred into air-tight tubes for further analysis.

2.3. Phytochemical Tests

Using standard procedure, P. guajava and D. microcarpum extracts were evaluated for qualitative and quantitative screening. The screening was conducted to test the presence of the secondary metabolites: alkaloids, flavonoids, saponins, tannins, cardiac glycosides, and steroids, as described by [39].

3. MICROBIOLOGICAL ANALYSIS

3.1. Collection of Diarrheal Stool Samples

One hundred samples from infants with diarrheal infections who presented for the first time at the children's clinic at General Hospital Gusau Zamfara State were collected aseptically using sterile rectal swabs. The samples were immediately transferred to the Microbiology Laboratory of the same hospital.

3.2. Isolation and Identification

The stool samples were physically observed and prepared for injection. All samples were aseptically inoculated onto MacConkey Agar Media (MA) and incubated at 37°C for 24 h. [44], [40]. Colonies that appeared to have fermented lactose were further subjected to gram reactions and serological and biochemical tests. Based on those tests, isolates were confirmed to be *E coli*. The isolates were further Subcultured onto Sorbitol MacConkey Agar (SMA) and incubated at 37°C for 24 h.[31]. Based on cultural characteristics, such as Growth on Sorbitol MacConkey Agar and molecular screening, the isolates were confirmed to be *E. coli* O157:H_7. Identified isolates were grown on Muller Hinton Agar Media slants and preserved for further analysis.

3.3. Molecular Identification

Pure isolates of the test organism were transported in an ice bloc to the Molecular and Research Diagnostic Laboratory Kaduna for molecular characterization. The molecular screenings that were conducted included DNA extraction, DNA amplification, DNA visualization, and DNA sequencing. The results of all the screenings, based on bioinformatics analysis using the Basic Alignment Search Tool (BLAST), further confirmed that the test organism was *E. coli* O157:H_7 [33],[17].

3.4. 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 of normal saline and compared with 0.5 Macfarlands turbidity standard, which marched with 0.1 x 10^8 cfu/mL [4].

3.5. Antibacterial Sensitivity Tests

The antibacterial sensitivity of the test organisms was tested using the 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 the standard culture of the test organisms was flooded, and the excess was discarded. Wells were bored using a sterile corked borer of 4mm in diameter. A drop of molten Muller Hinton was poured into each well. About 100µL of prepared concentrations of the test

plant extracts were added into each well.[25]. The plates were allowed to stand at room temperature for one hour (Pre-diffusion time). Then, the plates were incubated at 37°C for 24 h. At the end of the incubation, the growth inhibition zone diameter was measured in millimeters (mm) using a calibrated ruler.

3.6. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the two test plant extracts were determined using the Agar Dilution Method. The plates were aerobically incubated at 37°C for 24 h. At the end of the incubation, the lowest concentration of the extract that inhibited the growth of the test organism was determined, starting from 25mg/mL, 12.5 mL, and 6.3mg/L. The apparent suspension was considered to have positive results, which inhibited the growth of the test organism (bacteriostatic) [13],[43].

3.7. Determination of Minimum Bactericidal Concentration (MBC)

From MIC results, plates with no growths were used. A loop full of positive MIC plates was streaked into 5mL sterile Mueller Hilton broth. The plates were labelled and incubated at 37°C FOR 24 h. After incubation, where no growth was observed (Bactericidal), it was recorded as Minimum Bactericidal Concentration (MBC). ([38[,[20].

3.8. 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 ≥0.05 was considered insignificantly different.

4. RESULTS AND DISCUSSION

Preliminary qualitative phytochemical evaluation of the two plant samples revealed various traces and quantities of bioactive chemicals, as shown in Table 1. Alkaloids, Flavonoids, Saponins, Cardiac glycosides, Steriods/Terpenoids, Phenols Tannins and Anthroquanones were detected from the methanol aqueous extracts of *P. guajava* and *D. microcarpum.* Crude extracts of the test plants revealed their respective antibacterial activities against the *E. coli* O157:H_7 isolated from children suffering from Diarrhea at teething ages. This result tallies with the findings of other researchers. [7],[35]. All relevant information related to the results is presented in Table 2-10 below:

Table 1. *Phytochemical Compounds were detected from the aqueous methanol extracts of P. guajava and D. micrcarpum.*

Extract	Alkn	Cgds	Sapn	Phcp	Tann	Str/Ter	Flv	Ant
P. guajava	++	+++	+	++	+++	++	++	+
D. microcarpum	+	++	++	+++	+	++	++	+

Key: (+) = Trace quantity, (++) = Moderate quantity, (+++) = Appreciable quantity.

The quantitative analysis of the test plant extracts revealed varied quantities of Alkaloids,

Flavonoids, Saponins, Phenols and Tannins, as shown in Table 2 and Table 3. [30],[16],[14].

Table 2. *Quantitative values of phytochemical compounds obtained from P. guajava crude extracts.*

Compounds	Absorbance	Average Abs	Conc. (mg/ml)	Conc. (mg/g)	Conc.%
Alkaloids	0.211, 0.213, 0.208	0.2106	0.0413	41.25	4.1
Flavonoids	0.355, 0.352, 0.359	0.3550	0.1042	104.15	10.4
Saponins	0.081, 0.083, 0.083	0.0823	0.00306	30.58	3.1
Phenols	0.058, 0.062, 0.060	0.0600	155.58	155.75	15.6
Tannins	0.030, 0.030, 0.030	0.0300	0.1090	109.00	10.9

Table 3. *Quantitative values of phytochemical compounds obtained from D. microcarpum crude extracts.*

Compounds	Absorbance	Average Abs	Conc. (mg/ml)	Conc. (mg/g)	Conc.%
Alkaloids	0.107, 0.108, 0.107	0.107	0.0225	22.5	2.3
Flavonoids	0.269, 0.272, 0.270	0.0270	0.00791	79.1	7.9
Saponins	0.085, 0.081, 0.084	0.083	0.0308	30.8	3.1
Phenols	0.040, 0.037, 0.037	0.083	0.1013	101.3	10.1
Tannins	0.031, 0.032, 0.035	0.00327	0.1179	117.89	11.8

The quantitative percentage of phytochemical compounds in each plant sample revealed that *P. guajava* and *D. microcarpum* contained appreciable % of Alkaloids, Flavonoids, Tannins and saponins, as shown in Table 4.[27].

Table 4. *Quantity (%) of phytochemical compounds in the two sample Plant Samples extracts.*

Phytochemical compound	*P. gaujava*	*D. microcarpum*
Alkaloids	15.20%	5.54%
Flavonoids	12.0%	4.52%
Tannins	7.3%	3.80%
Saponins	12.20%	9.21%

Results of the antibacterial sensitivity of the test organism against the different concentrations of the plant's sample extracts revealed varied inhibition zones, as shown in Tables 5. and 6. Amoxilin antibiotic was used as a positive control, which tallies with what was reported by [34],[26], [21].

Table 5. *Antibacterial Sensitivity of E. coli to Methanolic Extract of Psidium guajava (Guava) Zones of inhibitions of different extract concentrations.*

Isolate	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	100mg/ml Ctrl
E1	19±0.30	15±0.48	7 ±0.33	6±0.30	18.±0.48
E2	16±0.30	7±030	6±0.48	6±0.00	
E3	15±0.48	6±0.30	6±0.00	6±0.00	
E4	19±0.34	13±0.48	7±0.33	6±0.30	
E5	20±0.33	15±0.30	7±0.30	6±0.34	
E6	21±0.48	16±0.48	13±0.33	7±0.48	
E7	19±0.54	15±0.33	7±0.48	6±0.30	
E8	18±0.48	14±0.23	7±0.54	6±0.02	
E9	20±0.33	16±0.48	14±0.48	7±0.62	
E10	17±062	14±0.54	7±0.34	6±0.33	

Key: E = Isolate Ctrl = Control.

Table 6. *Antibacterial Sensitivity of E. coli O157:H_7 to Methanolic Extract of Deterium microscarpum mm.*

Isolate	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	100mg/ml Ctrl
E1	16 ±0.48	14±0.11	6 ±0.00	6 ±0.06	13.6±
E2	20±0.30	17±0.34	13±0.07	6±0.00	
E3	19±0.54	19±0.11	6±0.06	6±0.03	
E4	18±0.72	14±0.22	6±0.00	6±0.12	
E5	18±0.20	13±0.11	7±0.72	7±0.54	
E6	20±0.33	16±0.37	14±024	7±0.08	
E7	17±0.15	12±0.12	6±0.09	6±0.00	
E8	20±0.48	16±0.54	6±0.13	6±0.00	
E9	21±0.72	13±0.12	6±0.00	6±0.08	
E10	16±0.30	7±0.23	6±0.00	6±0.06	

Key: E= Isolate Ctrl = Control.

The test plant samples' Minimum Inhibitory Concentration (MIC) revealed varied and inconsistent values at different plant extract concentrations in milligrams per liter (mg/L). Tables 7 and 8. [23], [28].

Table 7. *Minimum Inhibitory Concentration (MIC) of Methanolic extract of Psidium guajava to E. coli O157:H7.*

Isolate	Concentration (mg/ml)			
	50mg/ml	25mg/ml	12.5mg/ml	β6.3mg/ml
E1	-	-	β	+
E2	-	β	+	+
E3	-	β	+	+
E4	-	β		+
E5	-	β	+	+
E6	-	-	+	+
E7	-	-	-	+
E8	-	-	β	+
E9	-	-	-	
E10	-	-	β	+

Key: β = MIC value - Turbidity observed + No turbidity observed.

Table 8. *Minimum Inhibitory Concentration (MIC) of Methanolic extract of Deterium micrscarpum to E. coli O157:H7.*

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	-	B	+	+
E8	-	-	-	β
E9	-	-		β
E10	-	B	+	+

KEY: β- MIC Value + = No turbidity observed - = Turbidity observed.

The results of Minimum Bactericidal Concentration (MBC), tables 9 and 10, showed the bactericidal effects of the various concentrations of the test plant extracts against E. coli O157:H7. This result agrees with what was reported by [29],[8].

Table 9. *Minimum Bactericidal Concentration (MBC) of Metanolic Extract of Psidium guajava on E. coli O157:H7.*

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

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

αα

Table 10. *Minimum Bactericidal Concentration (MBC) of Metanolic Extract of Deterium microscarpum on E. coli O157:H7.*

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

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

5. CONCLUSION

The crude extracts of *P. guajava* and *D. microcarpum* in this study revealed their respective antibacterial activities against *E. coli* O157:H_7 isolated from diarrheal stools of children under teething. The study confirmed that *E. coli* O157:H_7 is one of the primary causative agents of diarrheal infections in children. Methanol was the solvent for extracting bioactive phytochemical compounds from *P. guajava* and *D. microcarpum* crude extracts. Limitations of this particular research include inadequate modern equipment and facilities, scanty previous research studies on similar research areas, and poor financial resources. Confirmation of bacterial activities of the test plant extracts lends scientific credence to the traditional uses of those plants in treating 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 conflict of interest.

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