Studies of Antimicrobial Properties of Indigenous Medicinal Plants on *Escherichia Coli* 0157:H7 Isolates from Cattle Faeces

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Abstract: The antimicrobial properties of indigenous medicinal plants *Azadirachta indica* (Neem seeds), *Vernonia amygdalina Linn* (Bitter leaves), *Ocimum gratissimumlinn* (Scent leaves), *Moringa oleifera* seeds, *Zingiber offtcinale(G\nger), Allium sativum* (Garlic), *Pterocapus santalinoides* (Nturukpa) against *Escherichia coli* 0157:H7 isolated from cattle faeces using Cefixime-Pottasium Tellurite Sorbitol McConkey (CT-SMAC) Agar supplemented with 4 methyl umbelliferyl D-glucuronide (MUG) was investigated. The colourless white colonies on the selective medium were subjected to several biochemical tests and a confirmatory test using immunological latex reagent 0157:H7 antiserum to detect agglutination. *Escherichia coli* 0157:H7 isolates were subjected to the medicinal plants extracts using well-in-agar method. The susceptibility of *Escherichia coli* 0157:H7 isolates to the plants extracts revealed that *Escherichia coli* 0157:H7 were susceptible to *Allium sativum* clove (methanolic extract), *Moringa oleifera seed* (ethanolic extract), *Vernonia amygdalina* leaf (ethanolic extract) and *Pterocarpus santalinoides* leaf (Ethanolic extract) in varying degrees and their mean zones of inhibition were 9.8 ± 0.05mm, 8.93 **O.O.3mn.7.35 ± OO(Hmmand 6.0 ± 0.04mm respectively. Susceptibility of the isolates to the extracts were in the* order *Allium sativum>Moringa oleifera> Vernonia amygdalina>Pterocarpus santalinoides.* Ethanol and methanol are the best solvents for the extraction of active constitutents of the plants used in the study. The demonstration of antimicrobial activity of crude extracts of *A. sativum, P. santalinoides, V. amygdalina and M. oleifera* extracts on *Escherichia coli* 0157:H7is an indication that these plants have antimicrobial properties and therefore are potential sources for drugs with better modes of action.

Keywords: Plant extracts, *Escherichia coli* 0157:H7, Antimicrobial -susceptibility test, cattle feaces, CT-SMAC

Introduction

Medicinal plants are known to contain substances which could be used for treatment purposes or used to produce drugs (Sofowora, 1999). Many of such plants known to be used primitively to alleviate symptoms of illnesses have been screened to have medicinal importance, some of which include: *Azadirachta indica* (Neem), *V. amygdalina* (Bitter leaf), *Allium sativum* (Garlic), *O. gratissimum* (Scent leaf), and *Zingiber officinale* (Ginger). These plants have been reportedly used in the treatment of ailments such as stomach disorder, fever symptoms and cough traditionally (Odugbemi, 2006). These medicinal plants also have bioactive constituents such as alkaloids, tannis, flavonoids and phenolic compounds (Nweze *et al.*, 2004). Enterohemorrhagic*E. coli* (EHEC) is a pathogenic *E. coli* strain that produces shiga toxins (Stxs) and cause Hemorrhagic Colitis (HC) and the life-threatening sequelae Hemolytic Uremic Syndrome (HUS) in humans. Ruminants represent the main natural host of EHEC and are generally healthy carriers of the organisms (Bauer and Welch, 1996; Deng *et al.*, 2004). This study focuses on isolation of *E. coli* O157:H7 from cattle faeces and subjecting the isolates to screened indigenous plant extracts.

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Materials and methods

Description of study area

The samples of cattle feaces used in this study were collected from Egbu metropolis which is in Owerri North Local Government Area of Imo State. The plant samples were collected from Chummy Chommy Garden which is also in Owerri North Local Government Area, in Imo State, Nigeria.

Collection of Samples

One hundred and fifty samples of cattle feaces were collected from the grazing fields, forty samples from the slaughter house, thirty samples from the intestinal colon and thirty rectal swabs of the cattle, making a total of two hundred and fifty (250) samples. Sterile universal collection bottles with spoon cork were used to aseptically collect a small portion of feaces from the cattle dumps as soon as they were excreted by the cattle in the grazing fields. Stool samples were collected from intestinal colon of the cattle as soon as they were cut open by the butchers in the slaughter rooms. Sterile swabs were used to collect rectal swabs from the rectum of the cattle. They were immediately placed in buffered glycerol transport medium and put into ice pack containers before being transported to the laboratory for analysis.

Collection of Plant Materials

The fresh samples of *Occissium gratissium* (scent leaves), *Venonia amygdalina* (bitter leaves), *Pterocarpus santoliniodies* (nturukpa leaves) and *Moringaoleifera* seeds were harvested from Chummy

Chommy Garden at Okwu Uratta in Owerri North Local Government of Imo State.

Zingiber officinale (Ginger) and *Allium sativum* (Garlic) were bought in Ekeukwu Owerri market. While *Azadirachta indica* (Neem) seeds were harvested from neem trees in St Mulumba Catholic Church and in FUTO environment in Owerri, Imo State, Nigeria.

Identification of Plant Materials

The plants were identified by botanist of the Plant Science and Biotechnology Department of Imo State University, Owerri.

Phytochemical Screening of Plant Materials

The plant materials were screened for tannins, saponins, flavonoids, steroids, alkaloids, Terpenoids (Thite *et al*., 2013).

Extraction of Plant Materials

The method of Ghamba *et al., (*2013) was adopted for the preparation of the crude extracts of the plant materials. Freshly harvested *Pterocarpus santalinoides* (nturukpa leaves), *Veronia amygdalina* (bitterleaves), *Occissium gratissium* (scent leaves) *Moringa oleifera* seed, *Zingiber officinale* (gingerrhizome), *Allium sativum* (garlic bulb), *Azadirachta indica* (neem seeds) were washed using sterile water and dried in the laboratory at ambient temperature (28-30°C). The various plant parts were ground into fine powder using the laboratory milling machine (Model Ed. 5 USA) and using ethanol, methanol, acetone and distilled water, the plant materials were extracted using standard microbiological methods (Nostro *et al*., 2000).

Culturing of Samples

The collected samples were cultured for 24 hours at 37°C in an enrichment medium, Cefixime Potassium Tellurite Sorbitol MacConkey Agar (CT-SMAC) supplemented with 4 methyl umbelliferyl D-glucouride (MUG). Biochemical analysis and confirmatory test for *Escherichia coli* O57:H7 was carried out using standard microbiological methods.

Antimicrobial Susceptibility Testing

Agar diffusion/well in agar method was used for plant extracts screening. The method of Ghamba *et al*., (2013), was employed. Solvents were evaporated using rotary evaporator and the crude extracts were kept in dessicators. The dried extracts were re-suspended in Phosphate Buffered Saline(PBS) and brought to the desired concentrations.

Statistical Analysis

The data generated from this analysis were analysed using SPSS-Statistics (Statistical Package for the Social Sciences) and Excel (Manning et al, 1999).

Results

The isolation frequency of *Escherichia coli* O157:H7 from different sample types (grazing field 65.27%, slaughter rooms 13.9%, intestinal colon 8.33% and rectum of cattle 12.5%) were ascertained and corresponding results were recorded (Table 1).

TABLE 1: ISOLATION FREQUENCY OF *E.COLI* O157:H7 FROM DIFFERENT SAMPLE TYPES

S/N	Source of Faecal Sample	Number of Samples Examined	Isolation Frequency/(%)
1	Grazing field	150	47(65.27)
2	Slaughter houses/rooms	40	10 (13.9)
3	Intestinal colon	30	6 (8.33)
4	Rectum	30	9(12.50)
	Total	250	72(100)

The phytochemical analysis of the plant extracts was determined using the appropriate reagent and the non-nutritional components of the plant extracts were recorded. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals (Table 2).

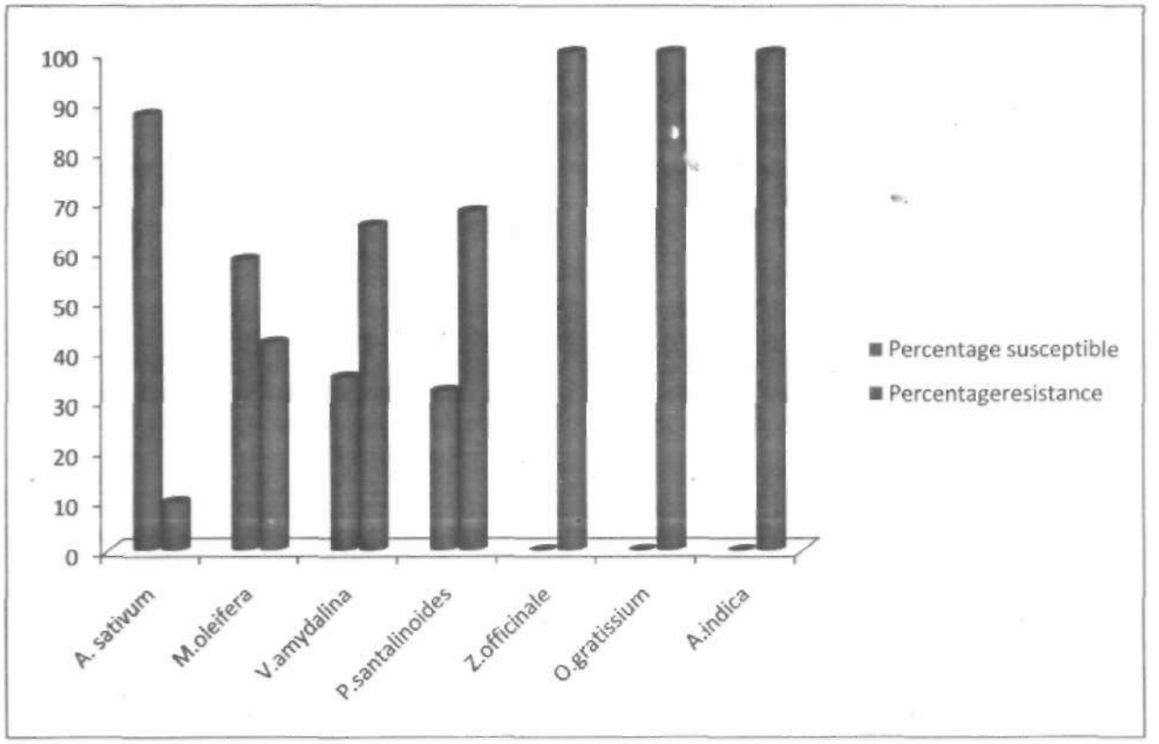
Table 2: Qualitative Phytochemical Composition of The Plant Extracts

Phytochemicals	Garlic bulbs	Ginger rhizomes	Bitter leaves	Nturukpa leaves	*Moringa oleifera* seeds	Neem seeds	Scent leaves
Alkaloids	+	+	+	+	-	+	+
Saponins	+	+	+	+	+	+	-

Flavonoids	+	+	+	+	+	+	-
Tannins	+	+	+	+	+	+	+
Anthraquinones	-	-	+	-	-	-	-
Steroids	-	-	+	-	+	+	-
Phenol	+	-	-	+	+	-	+
Volatile oil	+	-	-	-	+	+	+
Cardiae glycosides	+	+	+	+	+	+	+

+= Presence; - = Absence

The effectiveness of the plant extracts against *Escherichia coli* O157:H7 isolates used in this study reveals that *Allium sativum* clove (methanolic extract), *Pterocarpus santalinoides* leaves (Ethanolic extract), *Vernonia amygdalina* leaves (ethanolic extract) and *Moringa oleifera seeds* (ethanolic extract) had antibacterial effects against *Escherichia coli* O157:H7 as shown in(Figure 1).



Discussion

Seventy two samples out of two hundred and fifty samples tested were positive for *Escherichia coli* O157:H7. This shows that *Escherichia coli* O157:H7has a prevalence rate of 28.8%. This is in agreement with the studies of Nsofor and Ukachukwu, (2014) whose findings stated that *Escherichia coli* O157:H7has prevalence rate of 10%, 20%, 30% and 33.3% in the different samples analyzed.

In the present study, the efficacy of the plant extracts against *Escherichia coli* O157:H7 isolates used in this study reveals that *Allium sativum* clove (methanolic extract), *Pterocarpus santalinoides* leaf (Ethanolic extract), *Vernonia amygdalina* leaf (ethanolic extract) and *Moringa oleifera seed* (ethanolic extract) were observed. This is in agreement with the studies of Gaherwal *et al.* (2014) and Vidya and Pwar, (2016) whose findings showed that methanolic extracts of garlic was effective on *Escherichia coli.* This study also reveals that *Allium sativum* was the most effective extract among the plant extracts used. Antimicrobial properties were in the order *Allium sativum>Moring oleifera>Vernonia amygdalina>Pterocarpus santalinoides*. Whereas crude extracts of *Azadrichta indica, Zingiber officinale* and *Occimum gratissum* showed no visible inhibitory effects on *Escherichia coli*

O157:H7 isolates. This study disagrees with the findings of Vidya and Pwar, (2016) whose findings states that *Zingiber officinale* acetone and methanolic extract had inhibitory effects on *E.coli.* This however, could be attributed to the fact that the antibacterial activities of plant extracts depend on the species of plant, the type of solvent and the type of tested organism(Maher *et al.*,2012).

Conclusion

The demonstration of antimicrobial activity of crude extracts of *Allium sativum methanolic extract Pterocarpus santalinoides, Vernonia amygdalina and Moringa oleifera ethanolic* extracts is an indication that these plants have antimicrobial properties and therefore are potential source for drugs with better mode of action and can be used as an alternative drug for therapy.

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