

Antibacterial Effect of Edible Plant Extract on *Escherichia coli* 0157:H7

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Abstract: Sixteen preparation of the ethanol and aqueous extracts of four edible plants, *Entada africana* (bark), *Terminalia avicennoides* (bark), *Mitragyna stipulosa* (bark) *Lannae acida* (stem bark) were screened for their inhibitory effects on ten strains of *E coli* 0157:H7 (EHEC) using the agar diffusion method. It was shown that ethanol extracts of *Entada africana* inhibited all the ten strains used, some extracts showed variable antibacterial activities while some others could not cause any inhibition. The minimum inhibitory concentration (MIC) of the potent extracts ranged from 1.56mg/ml to 50.00mg/ml while the minimum bacteriocidal concentration (MBC) was between 6.25mg/ml to 25.00mg/ml. Pytochemical screening of the extracts revealed that all contained saponin. Some showed presence of tannins and glycosides while alkaloid was not detected in all samples.

Key words: Antimicrobial, plant extract, pytochemical, inhibitory and agar diffusion

Introduction

Intestinal disorders especially diarrhoea are a major cause of morbidity and mortality in developing countries. The rate of occurrence is usually high in infants and children (Bern *et al.*, 1992). Enterohaemorrhagic *E. coli* is one of the six groups of *E coli* recognized as aetiological agents of diarrhoea. It produces cytotoxins referred to as verocytotoxin or Shiga-like toxin which is responsible for haemorrhagic colitis. This organism was first identified as a cause of illness in 1982 and the infections have since been reported with increasing frequency. It is now of public health importance as it is readily isolated from human and animal waste that pollute the environment (Smith *et al.*, 2003). The disposal of human and animal waste is inadequate and potable water is not available in many parts of the city. The incidence of food and water contamination therefore poses serious health hazard to the people especially infants and the elderly. The consumption of contaminated food especially meat dishes have been found responsible for illness and cattle as major reservoirs of the organism (Wells *et al.*, 1991). In developed countries, good hygienic practices, adequate preservative technique for processed foods and use of antimicrobial substances such as antibiotics are the various ways of control and treatment. In Africa, traditional medicine is practiced and plants have been exploited for the treatment of many infections and diseases. Plants readily synthesize substances for defense against attack by insects, herbivores and microorganisms (Majorie, 1999). The antimicrobial nature of these substances have been well documented. The extract of some food spices, water soluble arrowroot tea extract were able to inhibit some

intestinal pathogens including *E. coli* 0157:H7 (Aboaba and Efuwape, 2001; Kim and Fung, 2004). Many plant extracts owe their potency to the presence of substances such as tannins, phenolic compounds and so on. These substances are usually found in various parts of the plants like roots, leaves, shoots and bark. Many plants have therefore become sources of important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicine. In recent times, emphasis is placed on use of natural materials in the control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable side effects. The aim of this research work was to find out possible antimicrobial action of the extracts of some edible plants traditionally used as herbal medicine on the growth of *E coli* 0157:H7 *in vitro*.

Materials and Methods

Preparation of plant extract: The four plants namely *Entada africana* (bark), *Terminalia avicennoides* (bark), *Lannae acida* (stem bark) and *Mitragyna stipulosa* (bark) were dried and pulverized into fine powder. Their ethanol and aqueous extracts were prepared using the cold maceration and soxhlet extraction techniques. The hot extracts were concentrated to dryness at 50°C in the vacuum oven while the cold extracts were dried under vacuum using the freeze drier. The 1g portion of each sample was reconstituted in 100mls of sterile distilled water and sterilized by membrane filtration.

Preparation of bacterial culture: The stock culture of each of the ten strains of *E.coli* 0157:H7 used was

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subcultured on MacConkey agar at 37°C for 24 hrs. The culture was emulsified in 3mls sterile saline and adjusted to obtain a concentration of 1.5×10^6 cells/ml.

Sensitivity test: The agar diffusion method was used. Wells (2) of 7mm were made into previously seeded Nutrient agar plates. Each well was filled with 0.1ml of each plant extract. The same quantity of sterile distilled water and 75% ethanol served as controls. Duplicate plates were prepared for each extract and controls, they were incubated at 37°C for 24hrs. The diameter of cleared zones was measured in mm. The transparently cleared zones showed bacteriocidal activity while the cleared zones containing micro colonies showed bacteriostatic activity.

Determination of Minimum inhibitory concentration (MIC) and Minimum bacteriocidal concentration (MBC):

The extracts that exhibited considerable activity were diluted double fold (2:2) with nutrient broth in a series of six test tubes. An aliquot of 1ml of the bacterial suspension (1.5×10^6) was inoculated into each tube. The control tubes were inoculated with same quantity of sterile distilled water and 75% ethanol. All tubes were incubated at 37°C for 24hrs. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration. The contents of all tubes that showed no visible growth were cultured on MacConkey agar, incubated at 37°C for 24hrs. The minimum bacteriocidal concentration was considered as the lowest concentration that could not produce a single bacterial colony.

Phytochemical properties of plant extracts: The reconstituted extracts were examined for the presence of alkaloids, tannins, glycosides, saponin and flavonoids as described by Okerulu and Chinwe, 2001.

Results

Sensitivity test: Six extracts were able to produce clear zones on all the ten strains used indicating bacteriocidal activity. There were however variations in diameter size, 28mm with *Entada africana* and 9mm with *Lanna acida*. Some extracts of *Terminalia avicennoides*, *Myragyna stipulosa* and *Lanna acida* however exhibited bacteriostatic properties.

Determination of MIC and MBC: The minimum inhibitory concentration of the extracts varied between 1.56 - 50.00 mg/ml while the minimum bacteriocidal concentration was between 6.25-25.00 mg/ml (Table 1).

Phytochemical properties of plants extracts: The most common substance found in all extracts was saponin. Some showed the presence of tannin, glycoside and

flavonoid. Alkaloid was not detected in any sample. Table 2.

Table 1: Minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) expressed in mg/ml

Sample	EHEC 3B		EHEC 8B	
	MIC	MBC	MIC	MBC
E	6.25	12.50	3.13	6.25
N	1.56	12.50	3.13	6.25
F	50.0	-	-	-
H	12.50	25.00	50.00	-
L	6.25	12.50	12.50	25.00
O	12.50	25.00	6.25	12.50

Discussion

The inhibitory effect of sixteen plant extracts on enterohemorrhagic *E.coli* was investigated in vitro. The results revealed the antibacterial potential of these extracts especially the ethanol extract of *Entada africana*. This is not surprising as the antimicrobial nature of many edible plant extracts such as cranberry, lime and lemon juices have been demonstrated (Mara *et al.*, 2003). The grape seed extract (Acti Vin) and pine bark extract (Pycnogenol) have also been found to be inhibitory to *E.coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* in vitro and in raw ground beef (Juhee *et al.*, 2004). The relatively high potency of the ethanol extract may be attributed to the dissolving power of alcohols over water.. (Majorie, 1999). This may be complemented with the fact that the bark was used as it has been reported that the stem bark of medicinal plants generally show high antimicrobial activity than the leaves (Binutu and Lajubutu, 1994).

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage. Araujo *et al.* (2003) confirmed the antimicrobial nature of essential oil of *M.officinalis* on five food spoilage yeasts and found that the main component of the oil which is citral showed high fungitoxic property. The active components usually interfere with growth and metabolism of microorganisms in a negative manner and is quantified by determining the minimum inhibitory concentration and minimum bacteriocidal activity. These values are used as guide for treatment of most infections. Results obtained showed that the MIC values for the most sensitive extracts were lower than their MBC values . This suggests that they were bacteriostatic at lower concentrations but bacteriocidal at higher concentrations.

Phytochemical screening showed the presence of saponins in all samples. Tannins and glycosides were found in some of the extracts but alkaloids were not detected at all. This is not surprising as alkaloids readily

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Table 2: Phytochemical properties of the plant extracts

Extract	Alkaloid	Tannin		Glycoside	Saponin		Flavonoid
		KOH	FeCl ₃		Frothing	Emulsion	
A	-	-	-	-	+	+	+
E	-	-	-	-	+	+	-
K	-	-	-	+	+	+	-
N	-	-	-	+	+	+	-
B	-	-	-	-	+	+	-
F	-	-	-	+	+	+	-
J	-	-	+	+	+	+	-
P	-	-	+	+	+	+	-
C	-	-	+	+	+	+	-
H	-	-	+	-	+	+	-
L	-	-	-	-	+	+	-
O	-	-	-	+	+	+	-
D	-	-	+	+	+	+	-
G	-	-	-	-	-	-	-
I	-	-	+	+	+	+	-
M	-	-	+	-	+	+	-

Key : + Positive. - Negative

decompose with time. Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties. Many plants contain non toxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens(Aboaba and Efuwape, 2001).

It should be noted that the combined form of all four extracts are traditionally used for treating diarrhoea as it is believed that each one contributes it's own active component for optimal efficacy. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components with the view of their use for *in vivo* studies.

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