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Antibacterial Activity of Aqueous Extract of *Momordica charantia* and *Terminalia catappa* on Multidrug Resistant Invasive *Escherichia coli* isolated in Ready-to-Eat (RTE) Foods from Ekiti State

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Abstract. Antibacterial activity of aqueous extract of *Momordica charantia* and *Terminalia catappa* was investigated against multidrug resistant invasive *Escherichia coli* isolated from ready-to-eat foods such as sausage rolls, meat pie, egg roll, doughnut and smoked fish. From the samples tested, smoked fish was found to have the highest microbial load of $1.05x10^6$cfu/mL (55%) followed by meat pie $7.5x10^5$cfu/mL (37%); Sausage roll $7.0x10^5$cfu/mL (36%); and egg roll with $6.5x10^5$cfu/mL (31%). Doughnut was found to have the lowest microbial load of $58x10^5$cfu/mL (20%). The minimum inhibitory concentration (MIC) of the extract determined by agar dilution technique showed that *M. charantia* and *T. catappa* had an MIC of 0.5 g/mL and the degree of antibacterial potency of the extracts determined using broth dilution technique showed that at concentration of 0.5 mg/mL, *M. charantia* and *T. catappa* had diameter of zone of inhibition of 10.4mm and 11.2 mm, respectively. The observed antibacterial effect of the *T. catappa* was more pronounced. This investigation depicts positive results by the use of *T. catappa* extract in drug development for the treatment of infections caused by *E. coli* isolated from ready-to-eat foods.

Keywords: antibacterial activity, *Momordica charantia, Terminalia catappa*, *E. coli,* ready-to-eat food.

Introduction

Escherichia coli have been identified as an indicator microorganism for food safety (Adams and Moss, 2000). Pathogenic *E. coli* has been recognized as an increasingly important human diarrheagenic pathogen in all parts of the world, especially in young children of the developing countries (Porat *et al.*, 1998). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance to antimicrobials (Dubois *et al.,* 2010) and report of multiple antibiotic resistance is not infrequent (Kumarasamy *et al*., 2010). Because of the increasing resistance of most pathogenic bacteria to conventional antibiotics, it becomes imperative to search natural alternatives.

The universal role of plants in the treatment of diseases is exemplified by their employment in all the major systems of medicine. There is a great wealth of knowledge concerning the medicinal and other properties of plants that is transmitted from generation to generation by tribal societies. Now monographs on crude plant preparations have made their way to modern pharmacopoeia. The use of modern isolation techniques means that new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations (Evans, 2002).

Various studies have been conducted with the extracts of different plants' antimicrobial activity as well as for the discovery of new antimicrobial compounds (Parekh and Chanda, 2007; Chah *et al*., 2006). *Momordica charantia* (bitter melon) is an economically important medicinal plant belonging to the family Cucurbitaceae. Its fruit extract act as anti-diabetic agent in normal and alloxan-diabetic rats (Kolawole *et al*., 2011). It is indigenous to tropical areas including India, Asia, South America and Nigeria and cultivated throughout South America as food and medicine. Various preparations of *M. charantia* extracts from fruit juice to dried fruit bits have been employed traditionally worldwide, particularly for blood-sugar lowering effects (Kolawole *et al*., 2011).

Terminalia catappa is a large tree in the family, Combretaceae that is native to the tropical regions of Asia, Africa, and Australia. It is known by the common names Bengal almond, Singapore almond, ketapang (Indonesian) and in Benin (Nigeria) as ebelebo (Ajenifuja *et al*., 2015). The leaves contain several flavonoids such as kaempferol or quercetin, several tannins such as punicalin, punicalagin or tercatin, saponins and

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phytosterols. Due to this chemical richness, the leaves (and the bark) are used in different herbal medicines for various purposes. For instance in Taiwan, fallen leaves are used as herb to treat liver diseases. In Suriname, a tisane made from the leaves is prescribed against dysentery and diarrhoea. The leaves may contain agents for prevention of cancers (although they have not demonstrated anticarcinogenic properties) and antioxidants, as well as anticlastogenic characteristics. Extracts of *T. catappa* have shown activity against *Plasmodium falciparum* chloroquine (CQ)-resistant (FcB1) and CQ-sensitive (HB3) strains (Hnawia *et al*., 2011).

There is a need to search natural products that could deliver potent and quick therapy at low or no cost at all. The healthcare delivery for the larger proportion of the rural communities in Nigeria and most part of Africa today hinged to a large extent on medicinal plants based on traditional health care delivery system. According to the World Health Organization (WHO, 2009), as many as 80% of the world's population depend on traditional medicines for their primary health care delivery and needs. Plants have occupied a very important position in health care delivery in various regions of the world; this is even more prominent among rural parts of Nigeria. However, with plants gaining more recognition and relevance in health care delivery in humans, various agencies of government and non governmental organizations (NGOs) are supporting the development of traditional medicines (Briskin, 2000). Therefore, this study aims to determine the antibacterial activity of aqueous extract of *M. charantia* and *T. catappa* on multidrug resistance invasive *E. coli* isolated in ready-to-eat (RTE) foods from Ado-Ekiti, Southwestern Nigeria.

Materials and Methods

Sample collection. Samples of RTE foods (sausage roll, meat pie, egg roll, doughnut and smoked fish) were purchased from a standard eatery and local kiosk in Ado-Ekiti metropolis. The samples were aseptically collected in a clean polyethylene bag and transferred immediately to the laboratory for analysis. The methods of preservation of the food products in a dry state or refrigeration by the standard eatery and local kiosk were employed in this study.

Collection, identification and processing of plants. Young leaves of *M. charantia* and *T. catappa* were collected from farmlands at Ado - Ekiti. The plant samples were identified at the Department of Science Technology, Federal Polytechnic, Ado Ekiti and a voucher specimen was kept in the laboratory No: Med Plant 2011/098. The method described by Osho *et al.* (2007) was used. *M. charantia* and *T. catappa* leaves were air-dried at room temperature of (26°C±1°C) and milled using a Thomas Willey Milling Machine.

Isolation and identification of *E. coli.* Strains of *E. coli* were isolated from RTE food samples purchased from a standard eatery and local kiosk in Ado-Ekiti metropolis. The bacterial isolates were characterized and identified based on morphological features and biochemical tests as described by Cowan (1999); and were maintained on nutrient agar slants at 4 °C in the refrigerator until required.

Extraction of bioactive components from the plant materials. Extraction method described by Ajenifuja *et al*. (2015) was employed. Fifty grams (50g) of the powdered plant materials (*M. charantia* and *T. catappa*) were weighed into different beakers and 500mL of distilled water was poured into each beaker, respectively and were boiled at 100 °C. The contents were stirred using a sterile glass rod and allowed to stand for 72 h at room temperature (25 °C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrates concentrated and evaporated using water-bath at the temperature of =95 °C. Extracts were then kept in a cool dry place until further use.

Determination of the degree of antibacterial potency. The disk diffusion method described by Ajibade (2014) was employed. Various concentrations of the extracts were prepared in test tubes (0.50 – 3.00 mg/mL). Disks prepared from Whatman No. 1 filter paper were sterilized in an oven at 160 °C for 30 min. and soaked in the extracts for 24 h. A loopful of the final dilution (10^3) of the test bacterial suspension was spread on an oven-dried nutrient agar. The disks of different concentrations of the extracts were placed at equidistance on the agar and incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters (mm) with a meter rule.

Determination of minimum inhibitory concentration. This was carried out using the agar dilution method previously described by Odelola and Okorosobo (1988). A colony from stock was sub-cultured into 5mL of nutrient broth and incubated at 37 °C for 18 h. A 0.1mL culture of the overnight broth of each organism was added into 9.9 mL of the broth. The procedure was

continued to obtain a final dilution of 10^3 mL (Smith *et al*., 2000). A 2cm streak of bacterial strains were made on oven-dried nutrient agar plate containing increasing concentrations (0.50 – 3.00 mg/mL) of the extracts. The lowest concentration that gave no visible growth after overnight incubation at 37°C was taken as the Minimum Inhibitory Concentration (MIC) of each extract.

Statistical analysis. Statistical analysis of P-value was calculated by using Fisher exact test; a test of comparison of 0.50 – 3.00 mg/mL between plant extracts (*M. charantia* and *T. catappa*) was carried out. Variables with ≥ 1.0 diameter of zones of inhibition and a P-value of ≥ 0.10 in univariate analysis were subsequently analysed in a multivariate model.

Results and Discussion

Table 1 shows the bacterial colony count for different samples. Smoked fish had the highest colony forming unit of 1.05 x 10^6 cfu/mL, followed by meat pie, sausage roll, and egg roll which have microbial load of 7.5, 7.0 and 6.5 x 10^5 cfu/mL, respectively. Doughnut had the lowest microbial load of 5.8 x 105 cfu/mL. The incidence of *E. coli* isolated from RTE food samples on Eosin Methylene Blue (EMB) agar is shown in Table 2 that shows that smoked fish has the highest microbial load 58(55%), followed by meat pie with 28(37%), sausage roll with 25(36%) and egg roll with 20(31%). Doughnut has the lowest microbial load of 12(20%).

Table 1. Bacterial colony counts of the food samples

Samples	Microbial load (cfu/mL)
Sausage roll	7.0 x 10^5
Meat pie	7.5 x 10^5
Egg roll	6.5 x 10^5
Doughnut	5.8 x 10^5
Smoked fish	1.05 x 10^6

Table 2. The incidence of *E. coli* isolated from ready-to-eat food samples

Samples	Pigmentation on EMB Agar No. of *E. coil* n(%)
Sausage roll	25(36)
Meat pie	28(37)
Egg roll	20(31)
Doughnut	12(20)
Smoked fish	58(55)

n = microbial number

Table 3 shows antibacterial activity of aqueous extracts of *M. charantia* and *T. catappa* on *E. coli* isolated from RTE foods. *T. catappa* being the plant extract with the highest diameter of zone of inhibition (11.20 ± 0.03 23.00 ± 0.00 mm) at the concentration of 0.50 – 3.00 g/mL; *M. charantia* have the diameter of zone of inhibition of 10.40 ± 0.01–22.30 ± 0.15 mm at the concentration of 0.50 – 3.00 g/mL.

In this study, the detection of *E. coli* in RTE foods was exceptional. Biological contaminants of bacterial origin presents a major cause of food–borne diseases which results to acute or chronic illnesses such as *E. coli* gastroenteritis, brucellosis and camplylobacteriosis (Edema *et al*., 2005). The presence of this organism in RTE foods depicts a deplorable state of unhygienic and sanitary practices employed in the processing and packaging of these food products. Among the RTE food samples, smoked fish and meat pie showed high level of microbial isolates of *E. coli* and other pathogenic microorganisms such as *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* These results agree with previous report by El-Gohany (2004) that foods of animal origin either cooked or uncooked were predominantly contaminated with *E. coli* and *Salmonella.* From recent findings, food mixtures such as pastries, salads, sauces, soups have been frequently incriminated in food poison outbreaks (FDA, 2007). On comparing the bacterial contamination between the eating outlets (standard eatery and local kiosk), *E. coli* remain the predominant food contaminant. This is an indication of recontamination in food handling hygiene techniques starting from the processing of raw material to the finished product, a correlated finding of Ojeibun (2004). According to Okonko *et al*. (2008) the presence of *E. coli* is an indication of fecal contamination of the water

Table 3. Antibacterial activity of aqueous extracts of *M. charantia* and *T. catappa* on *E. coli* isolated from ready-to-eat foods

Conc. (mg/mL)	Plant extracts Zones of inhibition (mm)		*P-value*
	M. charantia	*T. catappa*	
0.50	10.40 ± 0.01	11.20 ± 0.03	0.80
1.00	12.30 ± 0.03	13.00 ± 0.00	0.70
1.50	16.50 ± 0.21	15.10 ± 0.09	1.40
2.00	19.30 ± 0.12	17.80 ± 0.20	1.50
2.50	21.70 ± 0.24	19.50 ± 0.53	2.20
3.00	22.30 ± 0.15	23.00 ± 0.00	0.70

sources that were utilized in the processing of these food products.

From this investigation, the issue of food poisoning is of paramount importance particularly in developing world where there are limited social amenities such as power and access to potable water. This study clearly confirmed the deplorable state of food consumed in such settings. Food poisoning/illnesses are entirely preventable by practicing good sanitation and food handling techniques (Betty and Richard, 1994). Thus to safeguard against the risks of this disease of moderate severity as described by Mossel and Van Netten (1990) on incidence of staphylococcal food poisoning and *E. coli* contamination in foods, there is need to educate and advocate for good manufacturing practices among food processors and food vendors.

The isolated organisms were susceptible to crude extracts of *M. charantia* and *T. catappa. T. catappa* had the highest potency ranging from the diameter of inhibition of 11.20 $\pm$0.03mm – 23.00 $\pm$0.00mm at concentration ranging from 0.5 – 3.00 mg/mL, respectively. Susceptibility pattern of the extracts were not highly pronounced with *M. charantia* as observed in *T. catappa* except at a higher concentration of 3.00 mg/mL. The result showed that *T. catappa* is efficacious in the treatment of diarrhoea from the diameter of zone of inhibition observed against *E. coli* isolated from RTE foods. This is an indication that the plant extract could be useful in the therapy of diarrhoea and other infections that may occur from RTE food poisoning.

RTE foods vended in Ado-Ekiti, Ekiti State Nigeria had unsatisfactory levels of contamination with *E. coli*. Unhygienic practice may reveal the risk factors associated with contamination of post processing of food. This research study helps to understand the menace caused by contamination of RTE foods and the fatal consequences accompanying it. It further shows the predominant microorganisms and their incidence on various food items. With the present findings, the public is made aware of the need to increase their hygienic practices. However, further detailed scientific study is necessary to develop rapid and easy detection method of pathogenic organisms in food and treatment of diseases that may arise due to intake of contaminated RTE foods.

Conclusion

These findings demonstrate that RTE food sold in Ado-Ekiti metropolis constitutes a likely potential hazard to human health. The isolation of *E. coli* in RTE foods that are fully cooked is a good indicator of post-processing contamination or inadequate cooking. Therefore, access to pure water and health education to the vendors on personal hygiene, food safety and proper disposal of waste would improve food quality thereby reducing incidence of food-borne diseases.

Conflict of Interest. The authors declare no conflict of interest

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