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INCIDENCE OF *STAPHYLOCOCCUS* SPP AND SOME MEMBERS OF ENTEROBACTERIACEAE FAMILY AND THEIR SUSCEPTIBILTY TO LEAF EXTRACTS OF *MOMORDICA BALSAMINA* (BALSAM APPLE)

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ABSTRACT
Study was carried out on the incidence of Staphylococcus spp. and some members of the family Enterobacteriaceae family, in lettuce and cabbage. And their susceptibility to Momordica balsamina leaf extracts. Forty samples each of lettuce and cabbage were analyzed. Salmonella species were isolated from 32 (80%) lettuce and 30 (75%) cabbage samples. Staphylococcus species were isolated from all the lettuce and cabbage samples. Only three (7.5%) of the lettuce samples yielded Shigella species, and none from cabbage. Escherichia coli was isolated from 5 (12.5%) lettuce and 6 (15%) cabbage samples. E. coli 0157:H7 was not detected in any of the samples. All the isolates were tested for susceptibility to the methanol, ethanol and aqueous leaf extracts of M. balsamina using disc diffusion method Disc concentrations of 1.0, 10, 100, 1000, 2000, and 3000µg/disc were prepared from the leaf ectracts of M. balsamina Highest activity was recorded with ethanolic extracts on Shigella spp and E. coli with zone diameter of inhibition of 14mm and 13mm respectively. Other extracts were active against the test organisms with varying degree of inhibition, while some were not active. The susceptibility of Staphylocuccus species to the extracts was insignificant and seemed to be the most resistant bacteria among the text organisms. The study therefore stresses the need to sanitize vegetables adequately before consumption.

Key words: Incidence, Staphylococcus, Enterobacteriaceae, susceptibility, leaf extracts, Momordica balsamina

INTRODUCTION

Naturally occurring foods such as fruits and vegetables normally contain some microorganisms and may be contaminated with additional organisms during handling (Pelczar *et al.,* 2005).

A vegetable is an edible plant or part of a plant. Vegetables are eaten in a variety of ways as part of main meals and as snacks. The nutritional content of vegetables varies considerably, though generally, they contain little protein or fat (Woodruff, 1995), and varying proportions of vitamins, dietary minerals, fibres and carbohydrates. Vegetables contain a great variety of other phytochemicals, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties (Gruda, 2005).

Raw fruits and vegetables have been known to serve as vehicles of human disease for at least a century (WHO, 2009) and the number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables and unpasteurized fruit juices has increased in recent years (Buck *et al.,* 2003). Clinical cases of Salmonellosis caused by contaminated fruits and vegetables have been recently documented or reported with increasing frequency (Hedberg *et al.,* 1994). Most of these cases involved or were suspected of involving ingestion of improperly stored or handled prepared foods that initially carried the bacteria as surface contamination (Geldreich and Bordner, 1970). The level of *Salmonella* occurring epiphytically on fruits and vegetables retailed in the market place is a concern to epidemiologists and to the food industry (Zhuang *et al.,* 1995). Lettuce (*Lectuca sativa*) and cabbage (*Brasica oleracea* var capitata) are among the most popular vegetables consumed raw or after being minimally processed.

Momordica balsamina, African pumpkin (Cucurbitaceae), is a tendril bearing, wild climber containing wide spectrum of medicinal and nutritional values and has been used as a traditional folk medicine in many countries. It is used in Hausa community of Northern Nigeria for the treatment of gastrointestinal diseases. The leaves, fruits, seeds and bark of the plant contain resins, alkaloids, flavonoids, glycosides steroids, terpens, cardiac glycoside, saponins having various medicinal importance viz: anti-HIV, anti-plasmodial, shigellocidal, anti-diarrheal, antiseptic, anti-bacterial, anti-viral, anti-inflammatory, anti -microbial, hypoglycemic, antioxidant, analgesic, hepatoprotective, anti-helmintic, management of high fever, management of excessive uterine bleeding, in the treatment of syphilis, rheumatism, hepatitis, skin diseases, and gastroenteritis (Otimenyin *et al.,* 2008). This research assessed the bacteriological quality of lettuce and cabbage and evaluated the in-vitro antibacterial activity of *Momordica balsamina* leaf extracts on the bacteria isolated from lettuce and cabbage.

MATERIALS AND METHODS

Samples Collection

Samples of lettuce (*Lectuca sativa*) and cabbage (*Brassica oleracea* var capitata) were collected from different retail markets in Kano metropolis in a sterile polyethene bags. The samples were taken immediately to the laboratory for analysis.

Sample Preparation

Ten gram (10g) each of lettuce and cabbage samples was aseptically placed in a sterile blender and homogenized in 90ml of peptone water and was labelled as stock (10^{-1} dilution). Further dilution was obtained by pipetting one milliliter (1ml) from the homogenate into test tube containing nine milliliter (9 ml) of buffered peptone water and labeled 10^{-2} and so on, to 10^{-3} (Dahiru *et al.,* 2008), culture was made from the tubes as follows:-

Isolation and Identification of *Escherichia coli*

For *E. coli,* Eosin methylene blue (EMB) plates were streaked with a loopful of the sample from the dilution tubes and incubated at 37^0C for 24 hours. Bluish black colonies with green metallic sheen were streaked on to nutrient agar slant Gram stained and subjected to biochemical characterization (Indole, Methyl red, Kligler Iron agar (KIA) and citrate utilization test) (Cheesbrough, 2000).

Isolation and Identification of *E. coli* 0157:H7

Isolates that formed green metallic sheen on EMB were streaked on Sorbitol Macconkey Agar, and incubated at 37ºC for 24 hrs. After incubation the plates were observed for the presence of colorless colonies, and subsequent confirmation using serological kit for *E. coli* O157:H7 (Cheesbrough, 2000).

Isolation and Identification *of Staphylococcus species*

A loopful of the samples from the dilution tubes were streaked on to mannitol salt agar plates, the plates were incubated at 37^0C for 24 hours. Following incubation, mannitol fermenting organisms which showed a yellow zone surrounding their growth (yellow colonies) were streaked on to nutrient agar slant, gram stained, and subjected to biochemical characterization (Catalase and Coagulase test) (Cheesbrough, 2000).

Isolation and Identification of *Salmonella Spp*.

For *Salmonella* spp., deoxycholate citrate agar plates were streaked with a loopful of the samples from an enrichment broth (selenite F), the plates were incubated at 37^0C for 24 hours. Suspicious colonies (i.e. pale colonies with blackening were inoculated onto nutrient agar slant and subjected to gram staining, motility and other biochemical tests as applicable (Kligler iron agar and citrate utilization test using simmons citrate) (Shamsuddeen *et al*., 2009; Cheesbrough, 2000).

Isolation and Identification of *Shigella* Spp.

Deoxycholate citrate agar plates were streaked with a loopful of the samples from the dilution tubes. Suspicious colonies (i.e. pale colonies without blackening were inoculated onto nutrient agar slant and subjected gram staining, motility and other biochemical tests as applicable (Kligler iron agar and citrate utilization test using Simmons citrate) (Cheesbrough, 2000)

Collection and Identification of Plant Material

Momordica balsamina leaves were collected from Romi Village Dawakin Tofa Local government, Kano State, Nigeria. These were confirmed at the Herbarium Section of Biological Sciences Department of Bayero University, Kano, with the help of identification schemes (Aliyu, 2007). The leaves were air dried and reduced to powder using sterile motar and pestle as described by Mukhtar and Tukur (1999).

Extraction of Ethanol, Methanol and Water Fractions from the Leaves

Fifty grams (50g) of the powdered plant leaves was macerated in 500ml each of 99% ethanol, 99% methanol and distilled water in separate flasks. The suspensions were kept at room temperature and left for 14 days (ethanol and methanol) and 7 days (water) with regular shaking. The suspensions were then filtered and the solvents (ethanol, methanol and water) were removed by concentration using rotary evaporating machine (Fatope *et al*., 1993). The dried extracts were labeled and stored in the refrigerator at 4^0C.

BIOASSAY

Preparation of Extracts Impregnated Paper Discs

Whatman No. 1 filter paper was punched using a paper puncher to obtain discs of 6.0mm in diameter. The discs were then placed in a screw capped bijou bottles and sterilized in an autoclave at 121^0C for 15 minutes. The discs were allowed to cool until use. Two grams (2 g) of each extract were dissolved in 2 ml appropriate diluent (water for water extract and dimethysulphoxide (DMSO) for ethanol and methanol extract) to yield 1.0g/ml (1,000,000µg/ml) solution. This was labeled as the stock solution. From the stock solution, 0.1ml was transferred in to Bijou bottle containing 0.9 ml diluent, to effect 10 times dilution which gave a concentration of 100,000µg/ml. Subsequently, 0.1ml was transferred in to another Bijou bottle containing 0.9ml diluent, which gave a concentration of 10,000µg/ml and was further diluted to yield 1,000µg/ml and 100µg/ml respectively. Ninety (90) discs each of 6.0mm in diameter made from Whatman No. 1 filter paper were impregnated with the first three dilutions of the extracts (i.e. 90,000µg/0.9ml, 9000µg/0.9ml, 900µg/0.9ml, since 0.1ml was removed from each dilution to arrive at 1000,100,10µg/disc. For the last dilutions, (i.e. 100µg/ml), 100 discs each were impregnated with the dilutions of the extracts to arrive at 1µg/disc. Higher disc potencies of 2000 and 3000µg/disc were also prepared by diluting 0.2ml and 0.3ml of the stock solution of each extract with 0.8ml and 0.7ml of the appropriate diluents respectively. All the resultant impregnated filter paper discs were stored in refrigerator before use (Shamsuddeen *et al*., 2009).

Growth Media
Mueller Hinton agar plates (oxoid) were used in this study, prepared according to the manufacturer's specifications and excess moisture was removed by drying in agar dryer for 15 minutes.

Turbidity Standard Preparation
One percent (1% v/v) solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid (H_2S0_4) in to 99ml of distilled water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water. 0.6ml of barium chloride solution was added to 99.4ml of sulphuric acid solution to yield 1.0% w/v barium sulphate suspension. 2ml of the resultant turbid solution was transferred in to a clean test tube and used as the standard for comparison (Cheesbrough, 2000).

Standardization of Inoculum
Few colonies from an overnight culture of the test organisms each (i.e. *E. coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus* spp) were transferred into a tube containing 2.0ml normal saline with a sterilized inoculation loop, until the turbidity of the suspension matched the turbidity of the standard (1% barium sulphate) (Cheesbrough, 2000).

Susceptibility Test Procedure
A sterilized swab stick dipped in a standardized suspension of the test organisms each was evenly streaked on to the plates of Mueller Hinton agar. The plates were allowed 3 – 5 minutes for the surface of the agar to dry. Discs of different concentrations as well as the control disc were arranged and pressed firmly to the inoculated agar surface by means of sterile syringe needle (which minimizes the chances of contamination and scraping of the media). Each disc was sufficiently spaced out and kept at least 15mm from the edge of the plate and atleast 25mm between discs to prevent distortion and overlapping of zones. The standard antibiotic disc used as a control was streptomycin (30µg/disc). The plates were incubated at 37^0C for 18 hours aerobically. Diameters of zones of inhibition were measured using meter rule (ruler) and the mean recorded to the nearest millimeter (mm) (Cheesbrough, 2000).

RESULTS AND DISCUSSION
A total of 40 samples each of lettuce (*Lectuca sativa*) and cabbage (*Brassica oleracea var capitata*) were collected, analyzed for *Salmonella* species, *Shigella* species, *Staphylococcus* species *Escherichia coli* and *E. coli* O157:H7.

Of the 40 lettuce samples analyzed, *Staphylococcus* spp, *Salmonella* spp, *Shigella* spp, *Escherichia coli and .E. coli* O157:H7 were isolated from 40, 32, 3, 5 and 0 samples respectively corresponding to 100%, 85% 7.5%, 12.5% and 0% respectively (Table 1). Also of the 40 cabbage samples analyzed, *Staphylococcus* spp, *Salmonella* spp, *Shigella* spp, *Escherichia coli and .E. coli* O157:H7 were isolated from 40, 30, 0, 6 and 0 samples respectively corresponding to 100%, 75% 0%, 15% and 0% respectively (Table 2). Table 3 shows some physical characteristics of the extracts with the ethanol, methanol extracts soluble in DMSO and water extracts soluble in water. The sensitivity test showed that, the highest activity of ethanolic and methanolic extracts is on *Shigella spp* with zone diameters of 14mm and 11mm respectively (Table 4).

Table 1: Incidence of *Staphylococcus* spp. and some enteric bacteria on lettuce.

NUMBER. OF SAMPLES (20-50g)	ISOLATES	FREQUENCY	PERCENTAGE (%)
n=40	*Staphylococcus* spp.	40	100
	Salmonella spp.	32	85
	Shigella spp	3	7.5
	Escherichia coli	5	12.5
	E. coli O157:H7	0	0

Table 2: Incidence of *Staphylococcus* spp. and some enteric bacteria on cabbage.

NUMBER. OF SAMPLES (20-50g)	ISOLATES	FREQUENCY	PERCENTAGE (%)
n=40	*Staphylococcus* spp.	40	100
	Salmonella spp.	30	75
	Shigella spp	0	0
	Escherichia coli	6	15
	.*E. coli* O157:H7	0	0

Table 3: Some Physical Characteristics of *M. balsamina* Leaves Extracts.

EXTRACTS	AMOUNT RECOVERED(g)	COLOR	TEXTURE/SOLUBILITY
ETHANOL	3.5	Dark green	Gummy/DMSO
METHANOL	3.4	Dark green	Gummy/DMSO
AQUEOUS	3.5	Dark brown	Loose/water

Table 4: Susceptibility of food bacterial isolates to ethanol and methanol extracts of *Momordica balsamina* leaves.

ISOLATES	DIAMETER OF ZONE OF INHIBITION(mm)												
	EEM						MEM						
	A	B	C	D	E	F	A	B	C	D	E	F	S
Staphylococcus spp	00	00	07	07	09	10	00	00	00	00	00	00	17
Salmonella spp.	00	00	00	08	08	10	00	00	00	07	09	10	18
Shigella spp.	00	07	08	10	12	14	00	00	00	08	10	11	30
Escherichia coli	00	00	08	10	11	13	00	00	00	00	08	10	20

Keys: EEM=Ethanol extract of *M. balsamina* leaves, MEM=Methanol extract of *M. balsamina* leaves, A = 1µg/disc, B = 10µg/disc, C = 100µg/disc, D = 1000µg/disc, E = 2000µg/disc, F = 3000µg/disc, S = Streptomycin 30µg/disc.

DISCUSSION

From Table 1 it is clear that, there is evidence of contamination of the vegetables because presence of organisms like *Staphylococcus* spp, *Salmonella* spp, *Shigella* spp, and *Escherichia coli* could all be hazardous. *Staphylococcus* spp can produce enterotoxins in food in addition to being a potential pathogen. This work agrees with that of Mukherjee *et al.,* (2004) and Dahiru *et al.,* (2008), who also isolated *Salmonella* species, *Shigella* species, *Escherichia coli*, and *E. coli* 0157:H7 from fresh fruits and vegetables. Presence of such organisms on lettuce and cabbage may be attributed to the kind of manure used, the nature of the vegetables and also the water used in the irrigation. According to Kudva *et al.,* (1996), *E. coli* 0157:H7 has been isolated from feces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs and a variety of wild animals. Fresh vegetables have been identified as the vehicle for *E. coli* 0157:H7 infection in approximately 19 outbreaks in the United States (Olsen *et al*., 2000). Among fresh fruits and vegetables, lettuce appears to be more susceptible to bacterial contamination. Also some studies have shown that food borne pathogens can be internalized into lettuce leaves (Solomon *et al.,* 2002)

Mukherjee *et al.,* (2004) found the prevalence of *E. coli* on organically grown lettuce to be 22.4% and 10.2% on cabbage. In this study how ever, the percentage of *E. coli* positive samples of lettuce and cabbage were found to be 12.5% and 15% respectively.

Many works have shown that a number of potent herbs don't show activity or show reduced activity after separation of the active components. This largely has been attributed to the fact that, some of the components in the plants acts synergistically or inhibit the actions of other components in the plant (Otimenyin *et al.,* 2008).

According to Bukar *et al.,* (2009), the antimicrobial activity of any plant material is attributable to the phytochemicals present such as alkaloids, steroids, saponins etc., which act in protecting the plant against pathogens in the wild and the protection is equally conferred on humans when plant parts are drunk as concoctions or decoctions in ethnomedicine.

The results of the bioactivity of *Momordica balsamina* leaves extracts against the bacterial isolates found in this study, is consistent with the results obtained by Otimenyin *et al.,* (2008), even though modified cup plate method was adopted. In this study, ethanolic and methanolic extracts were active against the test organisms whereas aqueous extract had no activity against the test organisms. The result of the susceptibility test further confirm the Shigellocidal properties of *M. Balsamina*, due to the higher level of susceptibility of *Shigella* spp. to the ethanol and methanol extracts.

CONCLUSION

Presence of organisms like *Salmonella* spp. *Staphylococcus* spp, *E. coli* and *Shigella* from some of the vegetable samples is indicative of contamination.

Momordica balsamina plant has potentials for use in the treatment of gastroenteritis.

Recommendations

Measures should be taken to reduce the level of contamination of vegetables both in the field and in storage, through the observation of strict personal hygiene by farmers marketers and consumers.

Manure used as fertilizer should be adequately composted to reduce the level of microbial contaminants.

More works should be conducted on *M. balsamina* plant to ascertain its safety/toxicity for the possible development of drugs for the management of gastrointestinal disturbance.

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