Global Journal of Science Frontier Research
Volume 11 Issue 8 Version 1.0 November 2011
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN : 2249-4626 & Print ISSN:0975-5896

Microbiological Risk Assessment and Anti-Microbial Activities of Azadirachta Indica Stem Extract against Sanitary Indicator Bacteria Associated with Kunnu Samples

By Ajao, A.T. , Yakubu,S.E
Ahmadu Bello University,Zaria

Abstract - Microbiological risk assessment of kunnu sold in Ilorin metropolis and therapeutic effect of methanolic extract of A. indica against sanitary indicator bacteria isolated from it was evaluated. Antibiotic susceptibility pattern and invitro therapeutic efficacy of Azadirachta indica stem extract was determined. This study indicated that the Kunnu samples were grossly contaminated with high plate count of 7.2 x 106 cfu/ml. Total Salmonella/Shigella plate count, Listeria monocytogenes counts, Total Staphylococcal count, total coliform count and E.coli were ranged between (12x101 – 7.6 x 103) cfu/ml, (4.8x102 – 25x104)cfu/ml, (9.4x101 – 1.0x104)cfu/ml (0.30x102 – 1.36x105)cfu/ml and (0.21x101 – 27x103)cfu/ml respectively. Erythromycin, Gentamycin, Cephalosporin, Ciprofloxacin and Chloramphenicol showed high level of antimicrobial activity against the tested isolates, while they displayed about 40% resistance to Penicillin, Streptomycin, Bacitracin, and Ampicillin. Methanolic extract of A. Indica stem showed inhibitory activity against all the bacterial isolates in which diameter of zone of inhibition, MIC and MBC ranged between 15-28mm, 3.125-50mg/ml and 3.125-100mg/ml respectively. E.coli showed highest zone of inhibition of 28mm with 3.125mg/ml and 6.25mg/ml of MIC and MBC respectively while pseudomonas sp showed lowest zone of inhibition of 15mm and 50-100mg/ml for MIC and MBC respectively. Control of both pathogens and spoilage bacteria in kunnu becomes important in order to produce food that when properly handled and stored, will have a long shelf life and reduce the incidence of food borne diseases.

Keywords : *Microbiological risk assessment, A. indica, susceptibility, kunnu, sanitary indicator bacteria.*

GJSFR Classification :

Strictly as per the compliance and regulations of :

Microbiological Risk Assessment and Anti-Microbial Activities of Azadirachta Indica Stem Extract against Sanitary Indicator Bacteria Associated with Kunnu Samples

Ajao, A.T.[α], Yakubu,S.E[Ω]

Abstract - Microbiological risk assessment of kunnu sold in Ilorin metropolis and therapeutic effect of methanolic extract of A. indica against sanitary indicator bacteria isolated from it was evaluated. Antibiotic susceptibility pattern and invitro therapeutic efficacy of Azadirachta indica stem extract was determined. This study indicated that the Kunnu samples were grossly contaminated with high plate count of 7.2 x 10^6 cfu/ml. Total Salmonella/Shigella plate count, Listeria monocytogenes counts, Total Staphylococcal count, total coliform count and E.coli were ranged between ($12x10^1$ – 7.6 x 10^3) cfu/ml, ($4.8x10^2$ – $25x10^4$)cfu/ml, ($9.4x10^1$ – $1.0x10^4$)cfu/ml ($0.30x10^2$ – $1.36x10^5$)cfu/ml and ($0.21x10^1$ – $27x10^3$)cfu/ml respectively. Erythromycin, Gentamycin, Cephalosporin, Ciprofloxacin and Chloramphenicol showed high level of antimicrobial activity against the tested isolates, while they displayed about 40% resistance to Penicillin, Streptomycin, Bacitracin, and Ampicillin. Methanolic extract of A. Indica stem showed inhibitory activity against all the bacterial isolates in which diameter of zone of inhibition, MIC and MBC ranged between 15-28mm, 3.125-50mg/ml and 3.125-100mg/ml respectively. E.coli showed highest zone of inhibition of 28mm with 3.125mg/ml and 6.25mg/ml of MIC and MBC respectively while pseudomonas sp showed lowest zone of inhibition of 15mm and 50-100mg/ml for MIC and MBC respectively. Control of both pathogens and spoilage bacteria in kunnu becomes important in order to produce food that when properly handled and stored, will have a long shelf life and reduce the incidence of food borne diseases. This indicates the need for more effective methods such as HACCP to control microbial access through efficient sanitation and good manufacture practices. However, it can be recommended that the stem extract of the A. indica can serve as preservatives to control microbial growth in kunnu.

Keywords : Microbiological risk assessment, A. indica, susceptibility, kunnu, sanitary indicator bacteria.

I. Introduction

Kunnu is a cereal-based beverage in Nigeria. It is marketed in all parts of Nigeria; the cereals used in its production are Millet, sorghum, and maize in decreasing order of preference (Gaffa, et al., 2002, Nwachukwu, et al., 2009).

Non-alcoholic beverages play a very important role in the dietary pattern of people in developing countries like Nigeria. They are regarded as after meal drinks or refreshing drinks during the dry season in rural and urban centres. The sorghum grain Kunu-Zaki has about 76.3% starch, 11.6% proteins, 3.3% fat 1.9% fibre and 1.3% ash along with a wide array of amino-acids (Lichtenwalner *et al.*, 1979). The additive that is used is sweet potatoes; it contains essential amino acid and is a rich source of vitamins (Osuntogun and Aboaba, 2004).

It provides a source of income and a means of poverty alleviation and contributes to variety in the diet and the food security of millions. Small-scale food industry also provides linkages to local suppliers of agricultural raw materials and to income generating activities such as the manufacture of machinery, packaging and ingredients (FAO, 1997). The traditional production process involves: steeping the grain in a local household utensils such as calabashes, and earthen were vessels and grinding of the stepped grain with ginger in grinding machines to pulverize the grains for enzymatic actions. (Adeyemi and Umar, 1994; Onuorah, *et al.*, 1987).

The traditional production of Kunu is still at village technology level. The process of production involves wet milling of the cereal, wet sieving, partial gelatinization of the slurry, sugar addition and bottling (Adejuyitan *et al.,* 2008). The fermentation process may last for 12-72hours (Gaffa and Ayo, 2002) after which it is kept for acidification to develop.

The quality of the drinks therefore depends on the raw materials and the hygiene of the personnel, water and the production environment. Brief fermentation, involving mainly lactic acid bacteria and yeast, usually occurs during sleeping of grains in water over 8-48 hours (Odunfa and Adeleye, 1985). The consumption rate of the beverage has also been studied (Gaffa and Ayo, 2002). Owing to the high demand for this product and the high consumption rate, it is thought that the present traditional production process is outdated, inefficient, time consuming and with product quality varying between batches.

Author[α] : Department of Biology, Kwara State Polytechnic, Ilorin, Nigeria. E-mail : ajaoabdullahi@yahoo.com +2348060276738.
Author[Ω] : Department of Microbiology,Ahmadu Bello University,Zaria.

Most of these beverages are made up of about 90% of water, sugar, flavouring agents and sometimes preservatives (Osuntogun and Aboaba, 2004) but some of the waters used for kunu processing such as wells and boreholes are prone to contamination from various sources. It has been reported that the microbiological quality of most of the pipe borne and well water supplies to some communities in Nigeria is poor with *coliform* counts far exceeding the level recommended by WHO (Adesiyun 1983). Feacal contamination of water supplies and contaminated food handlers has most frequently been implicated in the outbreak of food poisoning caused by *Escherichia coli* (Adams and Moss, 1999). Unsafe water is a global public health threat, placing persons at risk for a host of *diarrheal* and other disease as well as chemical intoxication Hughes and Koplan, 2005,

Pathogens such as *Bacillus cereus, Salmonella sp* and *Escherichia coli* are naturally present in some soil, and their present on fresh produce is not rare. Salmonella, *Escherichia coli* 0157:H7, *Campylobacter jejuni, Vibrio cholerae,* parasites, and viruses are more likely to contaminate foods most especially ready to eat food.

The presence of the amount of sanitary indicator organisms in foods are of importance in the assessment of the quality and safety of foods (Egwaikhide and Faremi, 2010); Edema *et al.,* (2008) reported that in developing countries, despite the appeared death of sustainable disease surveillance and reporting, it is widely known that cholera, Salmonellosis, Shigellosis, Typhoid, Brucellosis, Poliomyelitis and *Escherichia coli* infections are prevalent (FAO/WHO, 2003). A major obstacle in the consumption of Kunu is the outbreak of *listeriosis*, a food borne disease called *listeriosis*, is caused by *listeria monocytogenes*, a gram positive, facultative anaerobe which occurs singly or in pairs, also in short chains. (Murray et al, 2002). Even though, epidemiological evidence on outbreaks of food borne disease as a result of taking kunu is scarce, there are indications that it could still be contaminated to unsafe level at the point of consumption with air flora an other microorganisms from handlers, equipment serving containers, raw materials and lack of portable water for processing. This indicates the need for more effective methods to control microbial access to foods through efficient sanitation that helps to produce food that, when properly handled and stored, will have a long shelf life and reduce incidence of food born diseases (Marriot, 1989 Cords and Dychdala, 1993).

Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infections diseases (Natarajan et al, 2003). *Azadirachta indica* is one of such medicinal plants belonging to the family *Meliaceae* and is Indigenous to southern Asia (Akula et al., 2003).

It is an extensively popular tree in Nigeria and is commonly referred to as "Neem" (English), "Dogon Yaro" (Hausa), "*Gaadina*" (Fulfulde) and "Akun Shorop" (Igbo). *Azadirachta Indica* is a multi - purpose timber tree from which high value products are extracted for use as an insecticide, fertilizers and multipurpose medicines. *Azadirachta indica*, it is popularly known as the village dispensary (Akula et al., 2003).

The therapeutic efficacies of the *Azadirachta Indica* have been described by practioners of traditional medicine. Some of the *ethnomedicinal* uses included treatment of skin disorders, rashes and boils, stomach ulcer, rheumatism, respiratory tract infections, sore gums and throat, eye and ear infections, leprosy and diabetes (Isman *et al.*, 1990; Kaura *et al.*, 1998, Akula *et al.*, 2003). Also, the medicinal uses has been reported by several workers and these includes having antipyretic (Okpanyi and Ezenkwa, 1981), antimalaria (Tella, 1977), anti-tumour (Fujiwara *et al.,* 1982), anti-ulcer (Pillai and Santhakumari, 1984) antidiabetic (Shukla *et al.*, 1984) and cardiovascular properties (Thompson and Anderson, 1978). In a precious survey of plant used for the treatment of ear and eye infections, amongst the practitioners of traditional medicine and other knowledgeable rural dwellers in the northern parts of Nigeria, the neem seed was listed as one of the most popular source of medicaments.

The microbiological safety of food and water is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Edema and Omemu, 2004). Nearly, 90% of *diarrheal* related deaths have been attributed to unsafe or inadequate water supplies and sanitation (Younes and Bartram 2001; WHO, 2004).

This work is aimed to determine the microbiological risk assessment of kunnu sold in ilorin metropolis and also to evaluate the therapeutic values of *methanolic* extract of *A. indica* against isolated sanitary indicator bacteria associated with the purpose of improving its quality using botanical compound to serve as preservatives.

II. Materials and Methods

a) Sampling Procedures

Ten samples each of Kunnu beverages from ten different locations within Ilorin metropolis were purchased in plastic containers that were washed with 70% ethanol and rinsed twice with sterile distilled water. They were labeled at the point of purchased and transported to the lab within 4hours after sampling.

b) Microbiological Evaluation

Ten milliliter(10ml) of each sample were aseptically transferred into 90ml of 0.1% sterile peptone water, appropriate dilutions (up to 10^5) were prepared, 0.2ml of inoculums was plated on each plates (Harrigan

and McCance, 1976) has reported by Edema et al., (2008); (Fawole and Oso 2001); (Oranusi, 2003). *Aerobic mesophiles* were made on plate count agar (oxoid, UK) coliform count on *MacConkey* agar, *E.coli* on Eosin Methylene Blue agar, *Staphylococcus* sp on Manitol Salt Agar, PALCAM agar on *Listeria sp*. (oxoid, UK).

One milliliter (1ml) of the aliquot from serially diluted samples was plated on each of the media using pour plate method for the enumeration of the bacteria associated with the kunnu samples. Ten milliliter (10ml) of the samples was enriched in *selenite* F and incubated for 24hours thereafter plated on *salmonella shigella* agar (SSA) .All the plates were incubated at 37^{0}C for 48hours. All the colonies were subcultured and stored in a stock culture before used.

c) Antimicrobial susceptibility testing

A standard disc diffusion method of (Jorgensen and Turnidge 2003) was used. The antibiotics used are, Penicillin G10 units, Cephalosporin 30µg, Bacitracin 10 units, Streptomycin 10µg, Ampicillin 30µg, Gentamicin 10µg, Erythromycin 10µg, Tetracycline 3µg, Ciprofloxacin 5µg, Chloramphenicol 30µg, Kanamycin 10µg.

d) Collection of plants

Stem of *Azadirachta indica* (Neem plant) were collected from Kwara State polytechnic main campus, Ilorin and authenticated at the department of Plant Biology, University of Ilorin.

e) Plant extraction preparation

The plant materials used (*A. indica*) were collected and dried in shade. The dried stem were grounded to power and suspended in petroleum ether and kept in refrigerator overnight for removing all the fatty substances, overnight incubation the supernatant was discarded and the residue was dried at room temperature. 50mgs of residues were soaked in 250ml of methanol and kept at 4^{0}C overnight; the supernatant was filtered and dried to evaporate the organic solvent at room temperature. The *sedimented* extract was weighed and dissolved in 0.1% *Dimethyl Sulfoxide* (DMSO) to get 100mg/ml concentration. (Natarajan *et al.,* 2003)

f) Standardization of inoculum

The selected sanitary indicator bacteria isolated from kunu samples were standardized to that of the 0.5 *Macfarland* standard (1.5 x 10^8 cfu /ml) by adding sterile distilled water.

g) Inhibition assays

Bacterial isolates were cultivated in nutrient broth at 35^{0}c for 2-6 hours to achieve standardized inoculum (1.5 x 10^{8}cfu/ml) of each of the isolated sanitary indicator bacterium (in duplicates) swabs were dipped into their suspension and then streaked over the surface of the Mueller Hinton agar and allowed to dry for 15minutes before the antibiotic discs were applied. The diameters of zone of inhibition were recorded after incubation at 37^{0}c for 24hours.

h) Evaluation of antimicrobial activity

The preliminary antimicrobial screenings of the *methanolic* extract of the plant was carried out using the agar diffusion techniques (Singleton, 1999, Ahmed and Beg, 2001; Pundir, *et al.*, 2010). Mueller Hinton agar plates were inoculated with 0.1ml of standardized *inoculum* (1.5 x 10^8 cfu/ml) of each selected bacterial isolate and spread with sterile swabs. A standard cork borer of 8mm diameter was used to cut uniform wells in agar plates containing the bacterial *inoculum* and the lower portion was sealed with molten agar medium. A 0.1ml volume of the crude plant extract was poured into a well of inoculated plates. The plates were incubated at 37^{0}c for 24hours after which diameters of zone of inhibition were measured (Obiukwu and Nwanekwu, 2009, Pundir *et al.*, 2010).

i) Antibacterial activity

Antibacterial activity was recorded if the zone of inhibition was greater than 8mm (Hammer et al, 1999) as reported by Pundir *et al.*, (2010). The antibacterial activity results were expressed in terms of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active, while 13-18mm as active and >18mm as very active (Junior and Zanil, 2000).

j) Determination of the minimum inhibitory concentration (MIC)

The MICs of the *methanolic* plant extract was determined using *macrodilution* broth method of (Pundir *et al.*, 2010) with little modification. A twofold serial dilution of the extract was prepared in sterile Mueller-Hinton broth to achieve a decreasing concentration ranging from (200 to 1.56mg/ml. Each dilution was (1.5 x 10^8 cfu/ml). The inoculated tubes were incubated at 37^{0}c for 24 hours. The MIC was taken as the lowest concentration that inhibited the growth of the organism from the tubes. A100µl of the content was plated out onto the surface of agar medium and then incubated for 24hours at 37^{0}c. MBC is then taken as the lowest concentration without growth of organism on the agar plate.

III. Results and Discussion

Microbiological risk assessment of Kunu and *antimicrobial* activity of *Azadirachta indica* stem extract against sanitary indicator bacterial was evaluated. Antibiotic susceptibility pattern and *invitro* therapeutic efficacy of *Azadirachta indica* stem extract was determined. This study indicated that the Kunnu samples were grossly contaminated with high plate count of 7.2 x 10^6 cfu/ml. Total *Salmonella/Shigella* plate count, *Listeria monocytogenes counts,* Total Staphylococcal count, total *coliform count* and *E.coli*

were ranged between ($12x10^1$ – 7.6×10^3) cfu/ml, ($4.8x10^2$ – $25x10^4$)cfu/ml, ($9.4x10^1$ – $1.0x10^4$)cfu/ml ($0.30x10^2$ – $1.36x10^5$)cfu/ml and ($0.21x10^1$ – $27x10^3$)cfu/ml respectively as shown in table 1.

All the tested *Enterobacteriacea* were not susceptible to all the selected β-lactam antibiotics, the investigation showed considerable variation in susceptibility pattern depending on the species as shown in table 2.

Erythromycin, Gentamycin, Cephalosporin, Ciprofloxacin and Chloramphenicol showed high level of antimicrobial activity against the tested isolates, while they displayed about 40% resistance to Penicillin, Streptomycin, Bacitracin, and Ampicillin. Methanolic extract of *A. Indica* stem showed inhibitory activity against all the bacterial isolates in which diameter of zone of inhibition, MIC and MBC ranged between 15-28mm, 3.125-50mg/ml and 3.125-100mg/ml respectively. *E.coli* showed highest zone of inhibition of 28mm with 3.125mg/ml and 6.25mg/ml of MIC and MBC respectively while *Pseudomonas sp* showed lowest zone of inhibition of 15mm and 50-100mg/ml for MIC and MBC respectively.

Table1 : prevalence and occurrence of sanitary indicator bacteria associated with kunnu (cfu/ml)

Location	Total Plate count	*Salmonella / Shigella*	*Listeria mono-cytogenes*	*Total Staphyl-ococcal count*	*Total Coliform Count*	*E.coli*
A	$7.2x10^6$	$12x10^2$	45×10^3	5.7×10^3	1.36×10^5	$27x10^3$
B	1.36×10^5	-	1.02×10^2	4.3×10^2	7.5×10^3	$13x10^2$
C	3.3×10^5	76×10^3	-	6.1×10^2	1.20×10^5	$81x10^2$
D	1.60×10^5	22×10^2	67×10^1	9.4×10^1	0.80×10^4	$61x10^2$
E	8.0×10^4	-	-	-	0.30×10^2	$0.21x10^1$
F	118×10^6	12×10^1	25×10^4	4.6×10^3	4.4×10^3	$15x10^1$
G	1.0×10^6	49×10^2	7.8×10^3	1.8×10^2	1.5×10^4	$30x10^2$
H	1.30×10^5	-	-	1.0×10^4	2.30×10^3	-
I	1.10×10^5	-	-	-	1.48×10^3	$23x10^1$
J	5.0×10^4	10×10^2	4.8×10^2	5.5×10^2	4.0×10^3	$1.0x10^2$

Table 2 : Drug susceptibility pattern of sanitary indicator bacteria isolated from kunnu samples (mm)

Antibiotics	*Salmonella.sp*	*Staph. aureus*	*Pseudomonas,sp*	*K. pneumonia*	*L.monocytogene*	*E.coli*
Penicillin G.(10 units)	2.0	27.0	0.0	4.0	11	0.0
Cephalosporin(30µg)	23	11.0	14.0	30	21	28
Bacitracin (10units)	0.0	19.0	0.0	0.0	13	0.0
Streptomycin (10µg)	17	8.0	10	24	17.0	21
Ampicillin (30 g)	3.0	26.0	04	06	14.0	13
Gentamicin (10µg)	23.0	15.0	11	20	15.0	23
Erythromycin (10µg)	10.0	26.0	11	14	13.0	18
Tetracycline (30µg)	6.0	19.0	4.0	14	19.0	17
Ciprofloxacin (5µg)	23.0	13.0	15	25	14.0	18
Chloramphenicol (30µg)	15.0	18	17	21	11.0	20

Table 3 : Susceptibility of the Sanitary Indicator Bacteria to Methanolic extract of *Azadirachta* indica (Neem plant)

Test Organisms	Zone Of Inhibition (Mm)	MIC (Mg/Ml)	MBC (Mg/Ml)
Salmonella sp	22	6.25	12.5
Staphylococcus aureus	26	3.125	3.125
Pseudomonas sp	15	50	100
Listeria monocytogene	17	25	50
Klebsiella pneumoniae	25	6.125	12.5
E.coli	28	3.125	6.25

Sanitary indicator bacteria such as total *coliform*, *E.coli*, *listeria monocytogenes*, *Staphylococcus* and *Salmonella* were used to measure hygienic level of kunnu and handling process in this study. The occurrence of food borne pathogens and sanitary indicator bacteria in this study is an indication that the Kunnu samples sold in Ilorin are neither microbiologically safe nor hygienic.

Poor hygiene practices of the food handlers during preparation might have been contributed to their presence as suggested by Mosupye (1999) that the presence of indicator organisms in food may be attributed to poor personal hygiene, poor practices among food handlers and cross contamination from either the environment, water used for processing or serving bottles and this can lead to foodborne illnesses.

High bacterial load of Kunnu in this investigation agreed with the result of (Gaffa *et al.,* 2002, Chukwu *et al.,* 2006). Waikhide and Faremi, (2010); suggested that the possible sources of these organisms in the food samples could be from nose, hand, skin and clothing of handlers, coughing, talking and sneezing droplets which could settle on the food during storage and retailing (Omonigho and Osubor, 2002 and Ojokoh and Tabowei 2002). Besides, high number of bacterial load can also be attributed to raw materials and water used for production process (Nwachukwu *et al*., 2009).

The presence of the most frequently isolated index of water quality and indicators of *faecal* contamination such as *E.coli*, total *coliform* and *salmonella sp* in this study is an indication of *faecal* contamination of the water used for processing coupled with poor environmental sanitation (Trevett et al., 2005).

Water fetched from wells and taps were transferred into containers, facilities that are not washed for several days, leaving sediments to settle at the bottom which might served as source of contamination and unhygienic handling of food. The isolation of *staphylococcus sp*, *Salmonella sp* and *Pseudomonas sp* in this study is of practical importance and it is an evidence of poor sanitary condition and lack of adequate portable water.*Salmonella* contamination is usually associated with food and animal *feaces* and its presence in this study is a signal of *faecal* contamination of both human and animal origin (Dondero, 1977).

In this study a multidrug resistance pattern was observed for *E.coli, Salmonella sp* and *Pseudomonas sp* with ampicillin, bacitracin and tetracycline. Bacteria species were susceptible to the ciprofloxacin, gentamicin. erythromycin, ciproploxacin and chloraphenicol. Resistance to tetracycline and ampicillin might be related to their overuse as opposed to gentamicin and ciprofloxacin which are not used for treating enteric infection in agreement with (Onyuka *et al*., 2011).

The high prevalence of resistance to tetracycline, ampicillin in *E.coli* has also been reported by Sifuna, in which E.coli was resistance mostly to ampicillin and tetracycline. Sack, 2001 and Shapiro, 1999 attributed resistance to use of tetracycline of mass prophylaxis during cholera or diarrhea.

None of the pathogens were resistant to *ciprofloxacin*, several studies have shown that ciprofloxacin offers advantages in the treatment of *salmonellosis* reaching high concentrations in serum and *faeces* (Threlfall, 2001;Eduardo, *et al*., 2001).

The antibacterial activities exhibited by this plant extracts reported here corroborates the finding of other researchers who worked on the antimicrobial activities of this plants on the isolated indicator bacteria (Rao et al, 1986, Tuhin et al; 2007, Koona and Budida, 2011). That methanol extract in this study might have had higher solubility for more *phyto* constituents, consequently the highest antibacterial activity. The *methanolic* extract of *Azadirachta indica* exhibited antibacterial effect (Jafri and Jalis – sub-Hani 1999, Samy and Ignacinauth, 2000). (Koona and Budida 2011) and also demonstrated how MIC and MBC values is an indication that the *pyto* constituents of the plant have therapeutic properties (Doughari *et al*, 2008).

The antibacterial activity of *Azadirachta Indica* might be due to the presence of *triterperiods*, *phenolic* compounds, *carotenoids*, steroids, *valavinoids*, *ketones* and tetra-*triterperiods Azadirachtin* (Koona and Budida 2011).

The findings from the agar diffusion methods showed that the extract exhibit a favourable antimicrobial activity against indicator bacteria. Some of the MIC values obtained in this study were lower than MBC values indicating that the plant extract is *bacteriostatic* at lower concentration and *bacteriocidal* at higher concentration (Zakaria *et al*, 2007).

The standard organization of Nigeria (1985) stated that *coliform* bacteria and pathogenic microorganisms should not be present in beverages. This applies also to other food products. It was reported that counts of 10^7 Cells/g for *Bacillus* cereus (1CMSF, 1974), and 10^6 cells/g for *enterotoxigenic staphylococcus aureus* (Bergdoll, 1979) are required to present a risk of intoxication.The presence of *coliform* and *staphylococcus aureus* and processing and packaging in a contaminated environment could present a risk (Okonko *et al.,* 2008).The need for microbial assessment of water for production of sea food and food drinks should also be emphasized to reduce possible contamination as reported by Fagade *et al.,* (2005).

IV. Conclusion/Recommendation

Control of both pathogens and spoilage bacteria in kunnu becomes important in order to produce food that when properly handled and stored, will have a long shelf life and reduce the incidence of food borne diseases. This indicates the need for more effective methods such as HACCP to control microbial access through efficient sanitation and good manufacture practices. However, it can be recommended that the stem extract of the A. indica can serve as preservatives to control microbial growth in kunnu.

References Références Referencias

1. Afolabi O.R, Adams R.F, Popoola Tos (2004). Comparative studies and microbial risk assessment of fruit juices and fruit drinks available in the market. In the book of abstract no.59 of the 1st international conference on Science and National Development Organized by COLNAS, UNAAB, from 25-28th October, 2004. p.56.
2. Afolabi O.R, Adams RF, Popoola Tos. (2004). Comparative studies and microbial risk assessment

of fruits juices and fruit drinks available in the market in: the book of abstract No. 59 of the 1st international conference on science and National Development, Organized by COLNAS, UMAAB, from 25-88th October, 2004 p 56.
3. Alabaster JS, Llyod R (1980). Water quality for fresh fish 1st edition Butterworth, London, p. 283.
4. Beny A.J, Garcia F, Thrupp LD (1970). Interpretation of sensitivity test results *American Journal of clinical pathology* 53:140.
5. Bergdoll M.S (1979). Staphylococcal intoxications. In: Riemann H, Bryan F (eds). Food borne infection and intoxication. Adacemic press, New York, pp. 444-490.
6. D. A. Sack. (2001) Antimicrobial resistance in shigellosis, cholera and compylobacteriosis. In Background document for the World Health Organization global strategy for containment of antimicrobial resistance.
7. Daniyan S.Y, Muhammed H.B, (2008). Evaluation of the antimicrobial activities and phytochemical properties of extracts of Tamarindus Indica against some disease causing bacteria *Afr. J Biotech* (14): 2451-2453.
8. E. J. Threlfall, J. A. Skinner, and L. R. Ward. (2001). Detection of decreased in vitro susceptibility to ciprofloxacin in *Salmonella enterica* serotypes Typhi and Paratyphi *A. J Antimicrob Chemother* Vol. 48(5), pp 740-1.
9. Edema MO, Omemu AM, Fapetu OM (2001). Microbiology and physiochemical analysis of different sources of drinking water in Abeokuta.*Nigeria journal pf microbiology* 15 (1): 57-61.
10. Food and Agriculture organization (FAD) (1997). Annual Report on food quality control; 1:11-13.
11. Food Quality Control, Ministry Of Health Malaysia Manual for Microbiological Examination of food.
12. G. Eduardo, S. Carlos, E. Juan, C. Carlose, M. Rose, and R. Rose. (1995).Ciprofloxacin in the treatment of cholera: A randomized double blind control clinic trial of a single dose in Peruvian adults.
13. Gaffa T., I.A Jideani and I. Nkama 2001. Traditional production, consumption and storage of kunu a non-alcoholic cereal beverage plant food human Nutr., 57:73-81.
14. ICMSF (1978). Micro-organisms in foods. International commission on Microbiological specifications for foods. London: Blackie Academic and professional.
15. Inatsu Y, Bari M.L; Kawasaki S, Isshiki K (2004). Survival of *Staphylococcus aureus*, *Escherichia coli* 0157:H7, *Salmonella enteritis* 30 and *Listeria monocytogens* in *kimchi: J. food protect* 67 (7): 1497-1500.
16. J.C Rutzin, E.H Marth. 1980. Behaviours of *Enterobacter* species during fermentation by lactic acid bacteria journal of food processing pp 698-709, *Journal of Biotechnology* 7 (3): 617-621.
17. Kosek M, Bern C, Guerrant RL. (2003). The global burden of diarrhea disease, as estimated from studies published between 1992 and 2000. Bulletin of world health organization 81:197-204.
18. M.B Adams, M.O Moss. 1999. Food Microbiology. The Royal Society of chemistry Cambridge pp: 398.
19. Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz J. Microbiology* (eds), 31, pp 247-256.
20. Obiukwu, C.E and Nwanckwu, K.E (2010) In vitro antibacterial studies of five medicinal plants as alternative therapy for methicillin-resistant *Staphylococcus aureus* (MRSA) infection, *International Science Research Journal* 2:66-69.
21. Odunfa S.A (1985). African fermented food in microbiology of fermented foods Vol. 2ed. BjB wood Elsevier Appl. Sci. pp 155-191.
22. Okonko I.d, Ogunjobi A.A, Adejoye O.D, Ogunnusi T.A, Olasogba M.C (2008) comparative studies and microbial risk assessment of different water samples used for processing frozen sea-foods in Ijora-Olopa, Lagos State Nigeria. *African journal of biotechnology* 7 (16): 2902-2907.
23. Okonko I.O, Adejoye O.D, Ogunnusi T.A, Fajobi, E.A, Shittu O.B. (2008). Microbiological and physiochemical analysis of different water samples used for domestic purpose in Abeokuta and Ojota, Lagos State, Nigeria African.
24. Pundir, R.K, Jain, P and Sharma, C. (2010) Antimicrobial activity of ethanolic extracts of syzygium aromaticum and Allium sativum against food associated bacteria and *Fungi Ethnobotanical leaflets* 14:344-60.
25. R. Kakai (2009) Antibiotic resistance in Western Kenya. Global Antibiotic Resistance Partnership (GARP) inaugural meeting. Fairview Hotel Nairobi, Kenya. 6th – 7th Aug 2009. Available at: http://www.docstoc.com.
26. R. L, Shapiro, P. Waiyaki, B. L. Nahlen, L. Slutsker, and R. O. Muga.(1999)Transmission of *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera. *Am. J. of Tropical Medicine and Hygiene.* Vol. 60, 271-276,
27. Rao DVK. Singh K, Chopra P, Chabra P.C, Ramanujalu G.(1986) in vitro antibacterial activity of neem plant *Indian Med Research* 314-316.
28. Satyavati G.V, Raina M.K, Sharma M (1976) Medicinal plants of India Vol. 1.
29. Sexena K (1997) Antimicrobial screening of selected medicinal plants from *India Journal of Ethanopharmacology* 58 (2): 75-85.