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PHYTOCHEMICAL ANALYSIS OF *Gongronema latifolium* ("Utazi") LEAF AND ITS BIOACTIVITY ON MICROBIAL ISOLATES FROM AFRICAN SORGHUM BEER (*Burukutu*)

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ABSTRACT

Forty-five (45) samples of prepared burukutu from Kaduna were microbiologically examined for microbial isolates using standard methods. Leaf extract of *Gongronema latifolium* was obtained using ethanol, hot and cold water as solvents. Phytochemical analysis of the plant was carried out using standard chemical methods. Antimicrobial properties of the extracts were evaluated using agar well diffusion method. The mean bacterial count of burukutu samples ranged from $1.2 \pm 0.10 \times 10^6$ to $8.3 \pm 0.30 \times 10^6$ cfu/ml in Kachia, $1.6 \pm 0.32 \times 10^6$ to $5.1 \pm 0.44 \times 10^6$ cfu/ml (Kajuru) and $1.4 \pm 0.21 \times 10^6$ to $7.2 \pm 0.40 \times 10^6$ cfu/ml in Kudan. The mean fungal count ranged from $1.47 \pm 0.31 \times 10^6$ to $3.87 \pm 0.35 \times 10^6$ cfu/ml in Kachia, $1.27 \pm 0.12 \times 10^6$ to $3.37 \pm 0.35 \times 10^6$ cfu/ml (Kajuru) and $1.50 \pm 0.27 \times 10^6$ to $4.00 \pm 0.30 \times 10^6$ cfu/ml in Kudan. Seven bacterial and four fungal species were identified. The phytochemistry of ethanol extracts of *G. latifolium* leaves showed phenols had the highest concentration of 15.74 ± 0.03 mg/100g and the least was anthraquinones (0.87 ± 0.02 mg/100g). The minimum inhibitory concentration (MIC) of the ethanol extract showed that *Staphylococcus aureus* and *Lactobacillus fermentum* were most susceptible to the leaf extract of *G. latifolium* with MIC of 6.25 mg/ml. The leaf extract of *G. latifolium* have the potential to preserve and extend the shelf-life of burukutu haven inhibited the microorganisms associated with it.

KEYWORDS: Burukutu, *Gongronema latifolium*, Phytochemical, Proximate

INTRODUCTION

Gongronema latifolium is a non-woody herbaceous plant from the family of Asclepiadaceae. It is widespread in the tropical and subtropical regions, especially in Africa and South America, with a moderate representation in Northern and South-Eastern Asia (Morebise *et al.,* 2002). In South-Eastern and South-Western Nigeria, *G. latifolium* is commonly called "Utazi" and "Arokeke" respectively (Agbo and Obi, 2006). *G. latifolium* is utilizable in many different ways to prepare delicacies in homes but primarily used as a spice and vegetable in traditional folk medicine. It can be consumed fresh, cooked or dried (Ugochukwu *et al.,* 2003). Reports by some authors show that it contains essential oils,

saponins and pregnanes (Schneider *et al.*, 1993). Morebise *et al.* (2002) showed that it has anti-inflammatory properties. In many situations it is part of herbal prescriptions or preparations administered by herbalists for treatment and or management of certain health challenges: pains, infertility, hypertension and ulcers (Schneider *et al.*, 1993).

Beer is an alcoholic beverage made from cereal grains, usually barley as well as corn, sorghum, millet, rice, wheat, and oats (Okafor, 2007). Beer is the world's oldest and most widely consumed alcoholic beverage and the third most popular drink overall after water and tea. Beer is made using a process called fermentation, in which yeast convert sugars in the grain to alcohol and carbon dioxide (Gutcho, 1976; Grossman, 1995: Bamforth, 2017). The tropical beers (African local beers) are known by different names in different parts of the world: *burukutu* and pito in Nigeria, maujek among the Nandi's in Kenya, mawe in Malawi, kaffir beer in South Africa, merisa in Sudan, bouza in Ethiopia and pombe in some parts of East Africa (Okafor, 2007; Ibrahim and Aondover, 2013).

Africa sorghum beer '*burukutu*' is a brownish-pink traditional alcoholic beverage consumed in the Northern Guinea savanna region of Nigeria, Republic of Benin and in Ghana (Okafor, 2007). The age long drink, serves as a source of alcohol for those who lack the financial means to patronize refined brew like beer and other foreign or imported drinks. It is produced mainly from the grains of guinea corn (*Sorghum vulgare* and *Sorghum bicolor*). The process of production of *burukutu* involves malting, mashing, fermentation and maturation (Ekundayo, 1969; Egemba and Etuk, 2007) . The production process of these indigenous drinks involves fermentation at its initial production stage which results in alcoholic drink. Its traditional processing method makes it susceptible to microbial contamination which results in the short shelf life of the product (Bamforth, 2017). This study was conducted to ascertain the antimicrobial potential of *G. latifolium* on microbial isolates from *burukutu* with a view to using it to extend its shelf life.

MATERIALS AND METHODS

Sample Collection

A total of forty-five (45) samples of *burukutu* used for this study were obtained from Kaduna metropolis. Fifteen (15) samples each of freshly prepared *burukutu* were obtained from the following locations; Kachia, Kajuru and Kudan. The samples were collected in 500 ml sterile plastic bottles and were transported to the laboratory in a cooler ice box containing ice packs for microbiological and physico-chemical analyses. Freshly harvested *G. latifolium* leaves were obtained from Uselu market, Benin City, Nigeria.

Isolation and enumeration of Microorganisms

Bacteria in *burukutu* were enumerated and isolated using nutrient agar and fungi with potatoe dextrose agar. The microbial isolates were identified phenotypically following standard microbiological procedures as described by Cheesbrough (2002), Lyn *et al.* (2014), Barnett and Hunter (1998).

Preparation of Ethanol Extract of Utazi Leaf

The "Utazi" leaves were collected by hand plucking from the plant and cleaned of debris by rinsing in running sterile distilled water. The leaves were then air-dried at ambient temperature (28 ± 2

°C) for 14 days to eliminate moisture (Atata *et al.,* 2003). The dried leaves were milled using a blender. Five grams (5g) of the milled leaves was soaked in 25 ml of each of the solvents (hot water, cold water and 99 % ethanol) separately. Each mixture was stirred occasionally and allowed to stay overnight. The samples was periodically shaken for at least 3 h a day to ensure complete extraction (Nenaah and Ahmed, 2011). The extract was filtered using Whatman filter paper No. 1 placed in a glass funnel and was concentrated by evaporation using water bath.

Antimicrobial Activity Bioassay

Standardization of the Bacterial Cell Suspension

Five colonies of each test organisms were inoculated into sterile test tube containing sterile nutrient broth and incubated at 35 °C for 24 h. The turbidity produced by these organisms were adjusted and used to match the McFarland turbidity (opacity) standard.

Agar Well Diffusion Method

Sterile nutrient agar was poured into sterile petri dishes aseptically and allowed to solidify. The surface of the sterile nutrient agar plate was streaked with pure culture of the standardized bacterial cell suspension. A cork borer (8 mm in diameter) was sterilized by flaming and used to create a ditch at the centre of each plate. The holes so created were then filled with the plant extract. The plates were allowed to stand for one hour for pre-diffusion of the extract and incubated at 28±2°C for 24 h. At the end of the incubation period, the diameter of the zone of inhibition was measured in millimeter (Ahmed and Aqil, 2007). This procedure was also carried out on the fungal isolates using potato dextrose agar as the medium.

Minimum Inhibitory Concentration (MIC) of the Extract

Test tubes were arranged on a test tube rack. The initial concentration of the plant extract (100 mg/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract (stock solution) into 5 ml of sterile nutrient broth to obtain 50 mg/ml concentration. The above process was repeated several times to obtain the different concentrations of the extract. Each concentration was inoculated with 0.1 ml of each standardized bacterial cell suspension and incubated at 28±2°C for 24 h. Growth on the broth was indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organisms, was taken as the minimum inhibitory concentration (MIC). Negative controls were set up as follows: nutrient broth only, nutrient broth and sterile plant extract, and finally positive control containing nutrient broth and test organism. This procedure was done for fungal isolates using potato dextrose broth.

Phytochemical Analysis

The qualitative and quantitative determination of phytochemical constituents of *G. latifolium* were carried out following standard techniques (Trease and Evans, 1996; AOAC, 2016).

RESULTS AND DISCUSSION

The total microbial count of "*Burukutu*" samples sold in Kaduna metropolis is shown in Table 1. The mean bacterial count ranged from $1.2 \pm 0.10 \times 10^6$ cfu/ml to $8.3 \pm 0.30 \times 10^6$ cfu/ml in Kachia, $1.6 \pm 0.32 \times 10^6$ cfu/ml to 5.1 ±

0.44×10^6 cfu/ml in Kajuru and $1.4 \pm 0.21 \times 10^6$ cfu/ml to $7.2 \pm 0.40 \times 10^6$ cfu/ml in Kudan. The mean fungal count ranged from $1.47 \pm 0.31 \times 10^6$ cfu/ml to $3.87 \pm 0.35 \times 10^6$ cfu/ml in Kachia, $1.27 \pm 0.12 \times 10^6$ cfu/ml to $3.37 \pm 0.35 \times 10^6$ cfu/ml in Kajuru and $1.50 \pm 0.27 \times 10^6$ cfu/ml to $4.00 \pm 0.30 \times 10^6$ cfu/ml in Kudan. The presence, number and kind of microorganisms isolated in *Burukutu* could be as a result of processing and post-processing (fermentation) either from the handling or during mixing. The utensils used for storage may also be responsible for the microbial population and diversity in *burukutu*. Although *burukutu* is an acid fermented product, there is possibility of post processing contamination resulting from poor handling either at the processing site or from the market places during vending (Lyn *et al.*, 2014).

Microorganisms isolated and their percentage occurrence frequencies are shown in Table 2. Bacteria isolated from the samples were *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactobacillus fermentum, Lactobacillus brevis, Pseudomonas aeruginosa* and *Lactobacillus plantarum* (Table 2). Fungi isolated from the samples were *Aspergillus* spp., *Candida* spp., *Penicillium* spp. and *Saccharomyces* spp. (Table 2). The lowest percentage occurrence of bacterial isolates was 26.7 % (*Lactobacillus brevis*) while the highest percentage occurrence was 73.3 % (*Staphylococcus aureus*). The lowest percentage occurrence of fungal isolates was 13.3 % (*Penicillium* spp.) while the highest percentage occurrence was 66.7 % (*Aspergillus niger*). The high mean microbial counts recorded shows exposure of the samples to heavy contamination by different genera of bacteria and fungi. The main sources of contamination may include humans, utensils, processing equipment and environment, handling and storage conditions (Eze *et al*., 2008). This observation is in agreement with the report of Kolawole *et al.* (2007), who documented information on the proximate and microbial analysis of *burukutu* and pito produced in Ilorin, Nigeria.

Table 3 shows the results of the susceptibility pattern of test organisms to the leaf extracts of *G. latifolium*. Ethanol extracts had the highest inhibitory effect while cold water extracts had no effect on all the isolates. *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus plantarum, Aspergillus niger, Candida utilis* and *Saccharomyces cerevisceae,* were inhibited by both ethanol and hot water extracts. Results obtained showed that *G. latifolium* had no antimicrobial activity on *Pseudomonas aeruginosa* and *Penicillium* spp. The minimum inhibitory concentration (MIC) of the ethanol extract showed that *Staphylococcus aureus* and *Lactobacillus fermentum,* were most susceptible at 6.25 mg/ml followed by *Escherichia coli*, *Lactobacillus brevis and Lactobacillus plantarum* inhibited at 12.5 mg/ml. *Bacillus subtilis* and *Saccharomyces cerevisceae* were inhibited at 25 mg/ml, *Aspergillus niger and Candida utilis* were inhibited at 50 mg/ml, but *Pseudomonas aeruginosa* and *Penicillium* spp. were not susceptible at all concentrations used (Table 4).

Table 1: Total microbial count of "*burukutu*" samples sold in Kaduna

Sample Source (LGA)	Sample point	Mean bacterial count ± S.D ($\times 10^6$)	Mean fungi count ± S.D ($\times 10^6$)
Kachia	Dk	2.37 ± 0.15	1.47 ± 0.31
	Aw	1.2 ± 0.10	2.10 ± 0.46
	Bh	4.7 ± 0.20	1.70 ± 0.50
	Ak	7.5 ± 0.31	3.87 ± 0.35
	Sb	8.3 ± 0.30	2.20 ± 0.27
Kajuru	Rm	1.6 ± 0.32	1.27 ± 0.12
	Dt	3.5 ± 0.31	2.27 ± 0.45
	Fg	2.2 ± 0.25	1.47 ± 0.42
	Kt	3.7 ± 0.25	3.37 ± 0.35
	Yh	5.1 ± 0.44	2.77 ± 0.40
Kudan	Kd	1.4 ± 0.21	2.57 ± 0.35
	Lb	2.6 ± 0.30	1.50 ± 0.27
	Ma	3.7 ± 0.31	3.53 ± 0.32
	Dm	4.6 ± 0.40	2.67 ± 0.25
	Kn	7.2 ± 0.40	4.00 ± 0.30

Key: S.D = Standard deviation, LGA: Local Government Area
Dk = Doka, Aw = Awon, Bs = Bishini, Ak = Ankwa, Sb = Sabon, Rm = Rimau, Dt = Dokta, Fg = Fuga, Kt = Kutura, Yh = Yahaya, Kd = Kadama, Lb = Labalo, Ma = Ma-aji, Dm = Damaski, Kn = Kanawa

Table 2: Percentage occurrence of microbial isolates

Microbial isolates	No. of samples examined	Kachia Market	Kajuru Market	Kudan Market	Frequency (%)
			Occurrence		
Bacteria					
Staphylococcus aureus	5	4	3	4	11 (73.3)
Bacillus subtilis	5	3	0	2	5 (33.3)
E. coli	5	2	0	3	5 (33.3)
Lactobacillus brevis	5	0	0	4	4 (26.7)
Pseudomonas aeruginosa	5	3	4	0	7 (46.7)
Lactobacillus fermentum	5	3	3	3	9 (60)
Lactobacillus plantarum	5	4	0	3	7 (46.7)
Fungi					
Aspergillus niger	5	3	3	4	10 (66.7)
Candida utilis	5	4	0	3	7 (46.7)
Penicillium spp	5	0	2	0	2 (13.3)
Saccharomyces cerevisceae	5	0	3	2	5 (33.3)

Table 3: Susceptibility pattern of leaf extracts of *Gongronema latifolium* on test isolates at 100 mg/ml

S/N	Isolates	Diameter of Zone of Inhibition (mm)		
		Ethanol Extract	Hot Water Extract	Cold Water Extract
1	*Bacillus subtilis*	14	5	-
2	*Escherichia coli*	18	6	-
3	*Staphylococcus aureus*	28	12	-
4	*Lactobacillus fermentum*	26	10	-
5	*Lactobacillus brevis*	22	9	-
6	*Pseudomonas aeruginosa*	-	-	-
7	*Lactobacillus plantarum*	20	7	-
8	*Aspergillus niger*	10	-	-
9	*Candida utilis*	12	-	-
10	*Penicillium* spp.	-	-	-
11	*Saccharomyces cerevisceae*	14	5	-

Key - = No zone of inhibition,

Table 4: Minimum inhibitory concentration (MIC) of ethanolic extract of *G. latifolium* on microbial isolates

Test Organisms	Concentration (mg/ml)					MIC (mg/ml)
	3.125	6.25	12.5	25	50	
Bacillus subtilis	+	+	+	−	−	25
Escherichia coli	+	+	−	−	−	12.5
Staphylococcus aureus	+	−	−	−	−	6.25
Lactobacillus fermentum	+	−	−	−	−	6.25
Lactobacillus brevis	+	+	−	−	−	12.5
Pseudomonas aeruginosa	+	+	+	+	+	> 50
Lactobacillus plantarum	+	+	−	−	−	12.5
Aspergillus niger	+	+	+	+	−	50
Candida utilis	+	+	+	+	−	50
Penicillium spp.	+	+	+	+	+	> 50
Saccharomyces cerevisceae	+	+	+	−	−	25

Key: + = Growth, - = No growth, MIC = Minimum Inhibitory Concentration

Table 5: Quantitative Phytochemical analysis of ethanolic leaf extracts of *Gongronema latifolium* (Utazi leaf)

Parameter	Concentration (mg/100g)
Alkaloids	1.43 ± 0.03
Phenols	15.74 ± 0.03
Flavonoids	5.97 ± 0.02
Tannins	11.32 ± 0.02
Saponins	3.06 ± 0.03
Anthraquinones	0.87 ± 0.02
Cyanogenic glycosides	2.65 ± 0.01

In this study antimicrobial effect of *Gongronema latifolium* leaf extract was evaluated on strains of bacteria and fungi isolated from *burukutu*. The results of the antimicrobial properties of *G. latifolium* (Utazi leaf) revealed inhibitory activities of the ethanol extract against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus plantarum, Aspergillus niger, Candida utilis* and *Saccharomyces cerevisceae*. At 50 mg/ml, ethanol and hot water extract of *G. latifolium* were observed to possess varying degree of antimicrobial activities against isolated microorganisms. The zone of inhibition with ethanol extract ranged from 14 mm against *L. fermentum* to 28 mm against *Staphylococcus aureus*. The values ranged from 10-14 mm against fungal isolates. No antimicrobial activity was observed against *Pseudomonas aeruginosa* and *Penicillium* spp with the ethanol extract. In the aqueous extract, inhibition zone ranged from 5 - 12 mm against bacterial isolates and 5 - 7 mm against fungal isolates. No antimicrobial activity was observed against *P. aeruginosa* and *Penicillium* spp. It was observed that ethanol extract of *G. latifolium* was more active against microbial isolates than the hot water extract. Cold water extract had no antimicrobial activity against microbial isolates. This may be due to the fact that the extracting solvent (cold water) is less effective in extracting the phytochemicals present in the leaves of *G. latifolium* thus no activity was exhibited (Atata *et al*., 2003; Ahmed and Aqil, 2007).

A higher zone of inhibition was recorded for *Staphylococcus aureus* indicating the inhibitory effect on Gram-positive bacteria. This result is in agreement with the observation of Eleyinmi (2007) who reported inhibitory effect of methanol extract on *Bacillus subtilis* and *Staphylococcus aureus. Pseudomonas aeruginosa* and *Penicillium* spp were not inhibited by all the extracts of *G. latifolium* (Utazi leaf). The resistance exhibited by *P. aeruginosa* is in agreement with the report of Jarwertz *et al*. (1998) who showed that *P. aeruginosa* is highly resistant to many antimicrobial agents. Akortha *et al*. (2011) also showed the resistance of *P. aeruginosa* to antibiotics. Barry (1976) observed that the susceptibility of some test organisms varies with the concentration of the antimicrobial substance. Of all the extracts ethanol showed greater inhibitory activity. Cold water did not show any inhibitory property. A minimum inhibitory concentration of 6.25 mg/ml observed for *S. aureus* and *L. fermentum*; 12.5 mg/ml for *E. coli*, *L. brevis* and *L. plantarum* is a plus for the potential application of this plant extract for preserving and extending the shelf-life of sorghum beers.

The result of the phytochemistry of ethanol extracts of *G. latifolium* leaves showed it contains alkaloids, phenols, flavonoids, anthraquinones, tannins and saponins. Phenols had the highest concentration of 15.74 ± 0.03 mg/100g and the least which was anthraquinones (0.87 ± 0.02 mg/100g) as shown in Table 5. The phytochemical constituents of extracts of *G. latifolium* (Utazi leaf) revealed the presence of alkaloids, phenols, flavonoids, cyanides, tannins and saponins. These constituents are common in medicinal plants however, their concentration vary (Sofowora, 1993). In this study, results of the quantitative phytochemical analysis shows that phenols had the highest composition of

15.74 ± 0.03 mg/100g. Thus the antimicrobial activity of this plant leaf may be attributed to its phytochemical content (Egbung *et al*., 2011) Several plants possess phytochemicals like tannins, phenols and flavonoids and saponins which have been shown to exhibit antimicrobial activities against microorganisms (Funatogawa *et al*., 2004). The effective antimicrobial activity observed in this study is in concord with earlier reports (Banso and Adeyemo, 2007). This study has shown that *G. latifolium* extract has antimicrobial activity against the isolated organisms and hence may be used as a hop substitute in brewing industries to extend shelf life of the product by inhibiting several varieties of bacteria that can cause spoilage of the locally brewed alcoholic beverage.

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