THE EFFECTS OF GINGER [*Zingiber officinale*] ON THE MICROBIAL LOAD OF A NIGERIAN TRADITIONALLY FERMENTED MAIZE PASTE (OGI)

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ABSTRACT

Various concentrations (1, 5 and 10 %) of milled oven-dried ginger and fresh ginger were incorporated into ogi at the beginning of fermentation. Physicochemical and microbiological changes were noted during fermentation. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of ethanolic ginger extract on selected bacterial isolates were also investigated. Physicochemical parameters such as pH and Titratable acid were analysed while microbiological changes were observed by inoculation of the various concentrations on Nutrient Agar, Salmonella-Shigella agar using the spread plate method. The pH of ogi samples decreased steadily and ranged between 4.11 at the beginning of fermentation and 5.74 at the end of fermentation and Titratable acidity (TA) ranged between 0.22 at the beginning of fermentation (0 hr) and 0.75 % at the end of fermentation (72 hrs). The total viable and coliform counts ranged between 2.8 and 7.3 ($x10^5$ cfu/ml) and 2.8 and 5.9 ($x10^5$ cfu/ml) while the Salmonella-Shigella agar counts ranged between 0 and 3.5 ($x10^2$ cfu/ml) from the beginning of fermentation to the end. Samples containing no ginger (D) had the highest coliform counts of 5.9 $x10^5$ cfu/ml and the highest total viable count of $7.3x10^5$ cfu/ml. Samples FC, DC and D were the only ones with recorded bacteria count on Salmonella-Shigella agar. Sample DB (5% dry ginger) had the lowest coliform counts, had the lowest total viable count and did not have any growth on Salmonella-Shigella agar. *Shigella dysenteriae* had the lowest MIC and MBC of 3 mg/ml while *Salmonella typhi* had the highest MIC and MBC of 5 mg/ml. This study revealed that incorporation of ginger into ogi significantly reduced its microbial load during fermentation which may lead to an improvement in its nutritional quality and the prevention of some food-borne diseases.

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INTRODUCTION

Ogi is a fermented cereal porridge from West Africa which can be produced from maize (*Zea mays*), guinea corn (*Sorghum bicolor*) and millet (*Pennisetum typhodenum*). It serves as supplement for infant's feeding, consumed as breakfast meal by many and it is also regarded as food of choice for the sick (Oyewole, 1997). In many West African countries, exclusive breastfeeding is usually adequate up to three to four months of age, but after this period it may become increasingly inadequate to support the nutritional demands of the growing infant. Thus, in a weaning process there is always the need to introduce soft, easily swallowed foods to supplement the infant's feeding early in life. However, in Nigeria the usual first weaning food is called pap, *akamu*, *ogi*, or *koko* and is made from maize (*Zea mays*), millet (*Pennisetum americanum*), or guinea corn (*Sorghum* spp.) (King and Ashworth, 1987).

Fermentation is one of the oldest and most economical methods of producing and preserving foods (Billings, 1998). The fermentation of ogi is performed by various lactic acid bacteria including *Saccharomyces* and *Candida* sp. as well as *Debaryomyces hansenii* (Odunfa and Adeyele, 1985). The fermentation processes involved in production of ogi improves the sensory and nutritional qualities, availability of proteins, amino acids (lysine, threonine, methionine), carbohydrates, certain b-group vitamins and minerals (Chavan and Kadam, 1989).

Ogi which is usually called pap, akamu and koko by people of West Africa can be processed into a slurry paste by heating in boiling water under constant stirring. It is a delicacy food product which does not receive any treatment designed to reduce its microbial load. Therefore, there is a necessity to improve the quality and shelf stability of ogi. Various foods have been preserved in order to decrease their microbial load and enhance their shelf stability by using chemicals such as benzoates, nitrites and sulphites. However, some of these chemicals could have adverse effects on human health and there is a resulting trend towards less processed food (Soomro *et al*., 2002).

Some of these substances are also known to contribute to the self-defense of plants against infectious organisms (Kim *et al*., 2001). This study will therefore be undertaken to assess the effect of both fresh and dry ginger on the microbial load and quality of ogi. The microorganisms in ogi have been isolated and identified (Ohenhen, 2002). Moulds associated with the surface microflora of fermenting corn are *Cephalosporium* sp, *Rhizopus* sp, *Oospora* sp, *Cercospora* sp, *Fusarium* species and *Aspergillus* sp, including *Aspergillus niger* and *Penicillium* sp. *Cephalosporium* sp predominates. All are eliminated within 6h of steeping. The bacteria are *Corynebacterium* sp, *Clostridium* sp, *Enterobacter cloacae* and *Lactobacillus plantarum, Lactobacillus brevis* and *Acetobacter* sp. Yeast usually isolated are *Saccharomyces cerevisiae, Rhodotorula* sp and *Candida mycoderma*. The above microorganisms are not all found in all fermentations. The predominant microorganism in the ogi fermentation is *Lactobacillus plantarum* responsible for the production of lactic acid, the main acid (Banigo and Muller, 1972). The aim of this study is to assess the effect of dry and fresh extracts of ginger on bacterial populations during the fermentation of a locally prepared maize paste, ogi.

MATERIALS AND METHODS

Production of Ogi

A modified traditional preparation of ogi was carried out in this study as previously described by Odunfa and Adeyele (1985). The maize obtained was washed and steeped in clean water in a plastic container with cover. The water was decanted after two days and the maize wet milled into slurry. The slurry was sieved using a muslin cloth, which separated the pomace from the filtrate.

Preparation of ginger and incorporation into Ogi

The ginger was washed manually, peeled with a sharp knife and then dried in a hot air oven at 55 °C (Ziaur-Rehman *et al*., 2002). The dried ginger was ground to a fine powder in a mill. Then, different concentrations of the powdery ginger were added to the filtrate to prepare different batches of ogi. These batches of ogi were divided into seven groups giving rise to samples FA, FB, FC, DA, DB, DC and D.

Sample FA contained 90 % maize and 10 % fresh ginger, sample FB contained 95 % maize and 5 % fresh ginger, sample FC contained 99 % maize and 1 % fresh ginger, sample DA contained

90 % maize and 10 % dry ginger, sample DB contained 95 % maize and 5 % dry ginger, sample DC contained 99 % and 1 % while sample D contained 100 % maize and 0 % ginger. After filtration, the filtrate was allowed to settle and get fermented for three days to yield ogi (Odunfa and Adeyele, 1985).

Preparation of media

The media used in this investigation include Nutrient broth, Salmonella-Shigella agar, Eosine methylene blue and Nutrient agar which were all prepared according to the manufacturers' instructions. However, 0.015% Nystatin w/v was incorporated in Nutrient agar to inhibit the growth of fungi.

Preparation of extract

Ethanol extraction was used in this study. The fresh ginger rhizomes were washed, peeled, sliced and oven dried for seven days. After drying, ginger slices were ground to fine powder using sterile mortar and pestle. 25 g of powdered ginger was soaked in 250 ml of ethanol. The flask was incubated at room temperature for 72 hours with shaking at intervals. The extract was evaporated at 50 °C. The dried extract sample was dissolved in ethanol separately to different concentrations ranging from 1 mg/ml to 50 mg/ml. The extract solutions were stored at 4 °C in capped bottles until needed.

Microbiological Analyses

Serial dilution and Inoculation of media

One gram of each ogi sample was homogenized in 9 ml sterile distilled water and 10-fold serial dilutions were carried out. 0.1ml of 10^{-4} dilutions were spread on nutrient agar (NA) for bacterial growth and total viable count, Salmonella-Shigella agar (SSA) for enteric bacteria such as *Salmonella* spp and *Shigella* spp; Eosine Methylene Blue (EMB) agar for coliforms and were all incubated at 37 ^{0}C for 24 hours.

Characterization and Identification of microbial isolates

The bacterial isolates were characterized based on their cultural and biochemical properties which included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskaeur (MR-VP), triple sugar iron test, utilization of sodium azide and various carbohydrates (glucose, lactose, maltose, fructose, mannitol, sucrose, and arabinose). The isolates were identified to the species level by comparing their

characteristics with those of known taxa, as described by Buchanan and Gibbons (1974) in Bergey's Manual of Determinative Bacteriology (Krieg and Holt, 1974).

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extract was determined according to the methods described by Shahidi (2004) and Kabir *et al*. (2005). The extract was diluted to concentrations ranging from 1.0 mg/ml to 50 mg/ml. 1ml of each dilution of ginger extract was added to nutrient broth tubes which were seeded with 0.1 ml of the standard bacterial inoculum. Negative control tubes with no bacterial inoculation, were simultaneously maintained. Tubes were incubated aerobically at 37 °C for 24 hours. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC. Dilutions showing no visible growth for the MIC were subcultured onto a fresh NA agar plate and incubated at 37 °C for 24 hours. The lowest concentration of the extracts yielding no growth on the NA plate was recorded as the minimal bactericidal concentration (MBC).

Physicochemical analyses

pH

The pH of the various ogi samples was determined at 24 h intervals as described by Adesokan *et al.* (2008) using a digital pH meter.

Titratable acid (%)

The titratable acid (TA) of ogi samples was analyzed at the same time interval by titrating 0.1 M NaOH solution and methyl red as an end point indicator. The titre volume of each homogenate was multiplied by 0.09 to give the percentage TA as lactic acid (Olubamiwa and Kolapo, 2008).

Statistical Analysis

Statistical Analysis of total viable, coliform and Salmonella-Shigella agar counts were evaluated using the t-test analysis. The mean was considered statistically significant at $P<0.05$ confidence limit.

RESULTS

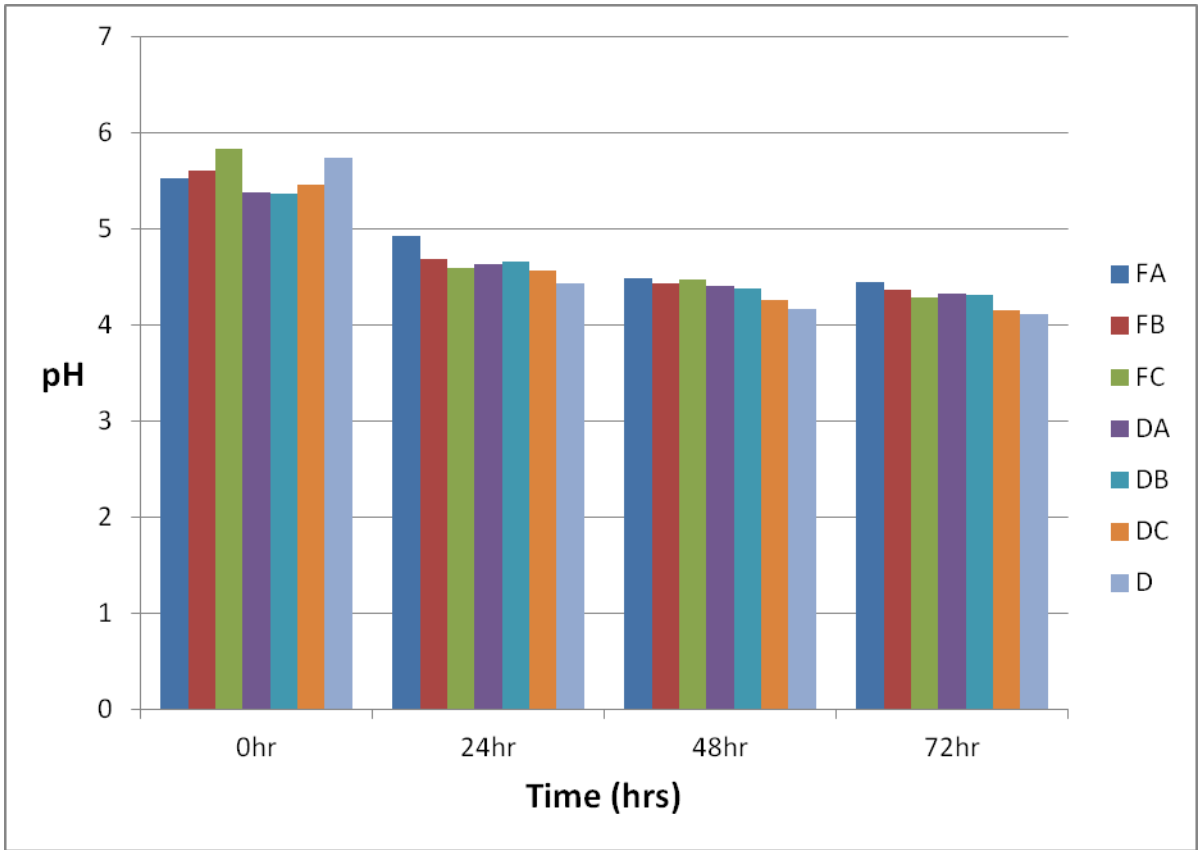

Figure 1: Effect of ginger on pH change during fermentation.

FA = sample containing 90 % maize and 10 % fresh ginger
FB =sample containing 95 % maize and 5 % fresh ginger
FC =sample containing 99 % maize and 1 % fresh ginger
DA =sample containing 90 % maize and 10 % dry ginger
DB =sample containing 95 % maize and 5 % dry ginger
DC =sample containing 99 % maize and 1 % dry ginger
D = sample containing 100 % maize and 0 % ginger

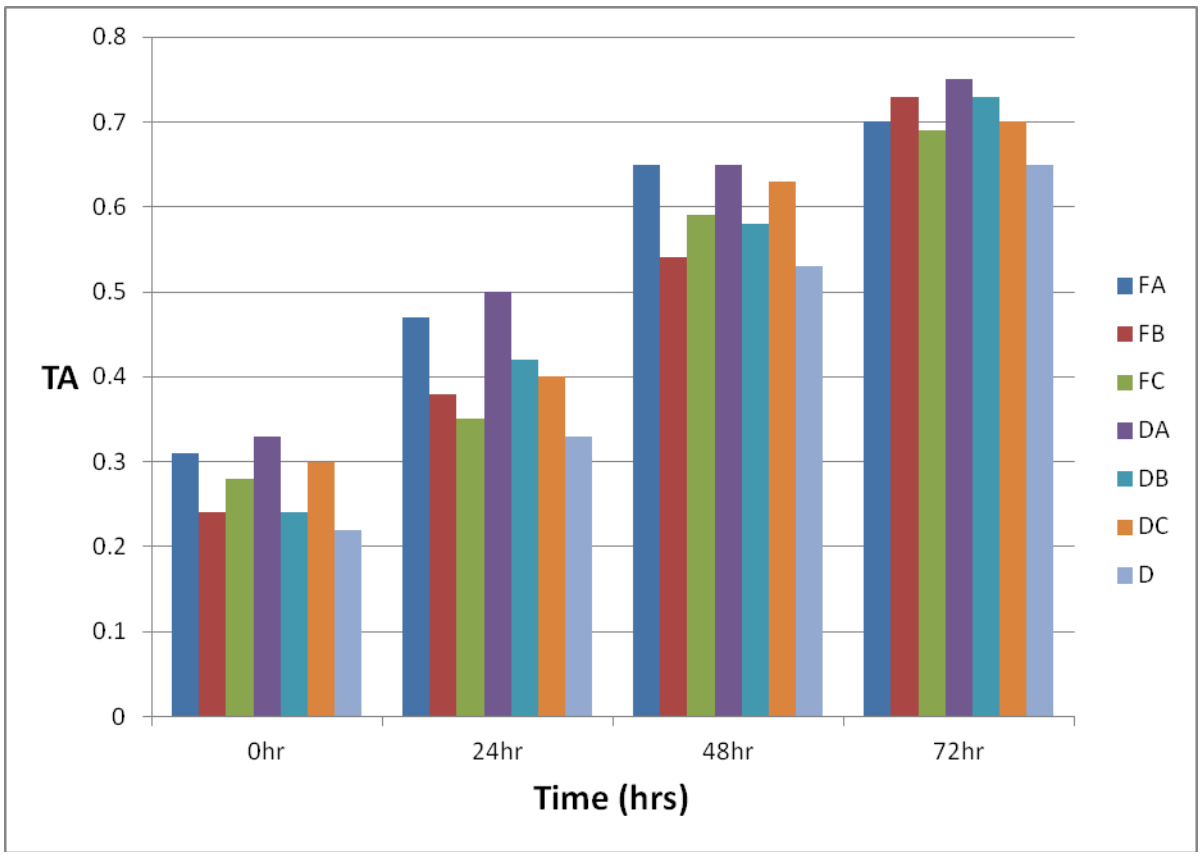

Figure 2: Effect of ginger on Titratable acidity change during fermentation.

FA= sample containing 90 % maize and 10 % fresh ginger
FB= sample containing 95 % maize and 5 % fresh ginger
FC= sample containing 99 % maize and 1 % fresh ginger
DA= sample containing 90 % maize and 10 % dry ginger
DB= sample containing 95 % maize and 5 % dry ginger
DC= sample containing 99 % maize and 1 % dry ginger
D= sample containing 100 % maize and 0 % ginger

Table 1: Effect of Ginger on the total viable counts of ogi during fermentation

Ogi/ ginger samples	Time Interval (hrs)			
	0	**24**	**48**	**72**
	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)
FA	6.0 ± 0.02	4.6 ± 0.01	3.3 ± 0.01	3.6 ± 0.03
FB	5.5 ± 0.01	4.0 ± 0.03	3.5 ± 0.02	3.0 ± 0.01
FC	5.7 ± 0.01	4.1 ± 0.02	4.0 ± 0.02	3.2 ± 0.01
DA	6.8 ± 0.02	5.8 ±0.01	4.6± 0.03	4.1 ± 0.02
DB	5.0 ± 0.01	3.7 ± 0.02	3.2 ± 0.02	2.8 ± 0.01
DC	6.6 ± 0.02	5.4 ± 0.03	4.1± 0.02	3.7 ± 0.02
D	7.3 ± 0.03	5.9 ± 0.02	4.8± 0.01	4.5 ± 0.03

Values are means of triplicate treatments ± standard error

FA= sample containing 90 % maize and 10 % fresh ginger
FB =sample containing 95 % maize and 5 % fresh ginger
FC =sample containing 99 % maize and 1 % fresh ginger
DA =sample containing 90 % maize and 10 % dry ginger
DB =sample containing 95 % maize and 5 % dry ginger
DC =sample containing 99 % maize and 1 % dry ginger
D =sample containing 100 % maize and 0 % ginger

Table 2: Effect of ginger on the coliform counts in ogi during fermentation

Ogi/ ginger samples	Time Interval (hrs)			
	0	**24**	**48**	**72**
	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)	($x10^5$ cfu/ml)
FA	5.3± 0.02*	3.9± 0.01	3.4± 0.02	3.0± 0.03
FB	5.0± 0.02	4.2± 0.02	3.5± 0.01	3.6± 0.02
FC	5.0± 0.02	4.3± 0.03	3.9± 0.02	3.4± 0.01
DA	4.5± 0.01	3.4± 0.02	2.8± 0.03	2.4± 0.02
DB	4.8± 0.03	3.7± 0.02	3.1± 0.02	3.0± 0.02
DC	4.9± 0.03	3.9± 0.01	3.4± 0.01	3.3± 0.01
D	5.9± 0.01	4.8± 0.03	4.5± 0.01	4.4± 0.02

*Values are means of triplicate treatments ± standard error

FA =sample containing 90 % maize and 10 % fresh ginger
FB =sample containing 95 % maize and 5 % fresh ginger
FC =sample containing 99 % maize and 1 % fresh ginger
DA =sample containing 90 % maize and 10 % dry ginger
DB =sample containing 95 % maize and 5 % dry ginger
DC =sample containing 99 % maize and 1 % dry ginger
D =sample containing 100 % maize and 0 % ginger

Table 3: Effect of Ginger on the SSA count of Ogi during Fermentation

Ogi/ ginger samples	Time Interval (hrs)			
	0	**24**	**48**	**72**
	($x10^2$ cfu/ml)*	($x10^2$ cfu/ml)	($x10^2$ cfu/ml)	($x10^2$ cfu/ml)
FA	0	0	0	0
FB	0	0	0	0
FC	1.8± 0.01	1.8± 0.01	1.5± 0.02	1.6± 0.02
DA	0	0	0	0
DB	0	0	0	0
DC	0.9± 0.02	0.8± 0.01	0.8± 0.02	0.6± 0.02
D	3.5± 0.01	3.1±0.02	2.9± 0.01	2.9± 0.01

*Values are means of triplicate treatments ± standard error

FA =sample containing 90 % maize and 10 % fresh ginger
FB =sample containing 95 % maize and 5 % fresh ginger
FC =sample containing 99 % maize and 1 % fresh ginger
DA =sample containing 90 % maize and 10 % dry ginger
DB =sample containing 95 % maize and 5 % dry ginger
DC =sample containing 99 % maize and 1 % dry ginger
D =sample containing 100 % maize and 0 % ginger

Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of different concentrations of ginger extract on bacteria isolated from Ogi

Concentration (mg/ml)	*Escherichia coli*	*Shigella dysenteriae*	*Salmonella typhi*
50	no growth	no growth	no growth
40	no growth	no growth	no growth
30	no growth	no growth	no growth
20	no growth	no growth	no growth
10	no growth	no growth	no growth
5	no growth	no growth	no growth
4	no growth	no growth	+
3	+	no growth	+
2	+	+	++
1	++	+	+++

+ = 1 to 10 colonies
++ = 11 to 20 colonies
+++ = 21 to 30 colonies

DISCUSSION

There was a steady decrease in the pH during the fermentation while there was a significant increase in the tritatable acids (TA) in all the ogi samples (Figs. 1 and 2). This might be as a result of production of lactic acid by fermentative organisms responsible for the fermentation of ogi. This observation agrees with the report of Odunfa and Adeyele (1985). There was a reduction in the population of coliform organisms and total viable counts during the fermentation of ogi samples containing different quantities of both fresh and dry ginger. This might be due to the presence of antibacterial compounds such as gingerols, shogaols, vitamin A and B, paradol

and zingerine in ginger (Kolapo *et al.*, 2007). Ogi samples (FA, FB, FC, DA, DB, DC) produced with different concentrations of both fresh and dry ginger had lower microbial counts than the sample D produced without ginger (Tables 1 and 2). This might be as a result of antimicrobial activities of ginger incorporated into the samples. However, 5 % w/w concentration of ginger (both fresh and dry) had a significant difference ($P<0.05$) in counts when compared to other concentrations. The antimicrobial properties of ginger have been reported by Kolapo *et al*. (2007), Sasidharan and Menon (2010) and Auta *et al*. (2011).

From this study, it can be seen that samples DA, DB and DC had lower total viable counts compared to FA, FB and FC. It was also observed that DA, DB and DC had a significant difference ($P<0.05$) in coliform counts when compared to FA, FB and FC indicating that dry ginger is more effective against coliforms than fresh ginger. Hydrocarbon compounds have been reported to be more in dry ginger than fresh (Sasidharan and Menon, 2010). It was also observed that no detectable growth was seen in 10 % and 5% concentrations of both fresh and dry ginger in the Salmonella-Shigella agar (Table 3). The minimum inhibitory concentration (MIC) values of ginger extract on *Escherichia coli, Salmonella typhi, Shigella* spp indicated that *Shigella* had the lowest MIC at 1mg/ml, followed by *E. coli* at 2mg/ml and *Salmonella* at 3mg/ml. The minimum bactericidal concentration (MBC) followed the same pattern (Table 4).

There was also a pleasant aroma in the samples that had ginger incorporated in them which may increase the palatability of ogi especially for children. In addition, the low concentration incorporated did not cause the ogi to be pepperish which could cause some toddlers or children to dislike the meal. Use of ginger as a natural supplement is considered a healthy choice for the treatment of cardiovascular diseases (Mahmoodi *et al.*, 2006), hypertension (Benavides *et al.*, 2007), diabetes (Banerjee and Maulik, 2002), Alzheimer's disease (Chauhan, 2006) inflammation, thrombosis (Fukao *et al*., 2007) and even for cancer (Hsing *et al*., 2002). Recently, ginger was also reported for the treatment of nonalcoholic fatty liver diseases (Sahebkar, 2011). The antibacterial activities of the ginger extract are expected perhaps due to the presence of compounds like flavonoids and volatile oils which were dissolved in organic solvents. It is reported that sesquiterpenoids are the main component of ginger which attributes its antibacterial activity (Sahebkar, 2011).

CONCLUSION

It is concluded that 5 % w/w concentration of both fresh and dry ginger considerably reduced and inhibited the growth of food pathogens. However, dry ginger had a greater inhibitory effect on pathogens than fresh. Therefore, the use of ginger in ogi and probably other food items would decrease the chances of food poisoning, reduce the risk of food contamination, protect the consumer from different food-borne diseases and improve health status by using a small quantity of it. It is therefore concluded that ginger reduces the quantity of pathogenic microorganisms to the minimum therefore increasing its nutritional quality and consumption safety.

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