Antimicrobial Activity of Some *Allium* Extracts on Microorganisms Associated With Soybean Daddawa Spoilage

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Abstract
Microorganisms associated with deterioration of Spontaneously Fermented Soybean Daddawa (SFSD) were isolated, classified and their enzyme profiles determined. The inhibitory activities of aqueous and ethanol extracts of three Alliums: garlic, onion and leek (3, 5 ad 7 % concentration) on the identified isolates were also assessed. Bacillus, Staphylococcus, Lactobacillus, Candida and Cryptococcus spp. were found to be associated with SFSD spoilage. All these isolates demonstrated activities of six to eleven different enzymes. High alkaline phosphatase and leucine arylamidase activities characterised all isolates. The esterase activities of the yeasts strains were higher than those of Bacillus and LAB strains. Extracts of all tested Allium spp. demonstrated significant ($p < 0.05$) abilities to arrest proliferation of the isolated organisms. These activities were Allium-type and concentration-dependent, with garlic at the highest extract concentration (7%) exhibiting the most significant ($p < 0.05$) inhibitory activity. The high levels of alkaline phosphatase, leucine arylamidase and esterase activity have implications on soybean daddawa spoilage. The results indicate that Allium spp., especially garlic, could be exploited in the preservation of soybean daddawa.

Keywords: Condiment, Garlic, Leek, Onion, Preservation, Soybean daddawa

Activité Antimicrobienne de Certains Extraits D'allium Sur des Microorganismes Associés à la Détérioration du Daddawa de Soya

Résumé
Les microorganismes associés à la détérioration du soja Daddawa fermenté spontanément (SFSD) ont été isolés, classés et leur profil enzymatique a été déterminé. Les activités inhibitrices des extraits aqueux et d'éthanol de trois alliums: ail, oignon et poireau (concentration de 3, 5 et 7 %) sur les isolats identifiés ont également été évaluées. Bacillus, Staphylococcus, Lactobacillus, Candida et Cryptococcus spp. ont été associés à la détérioration de la SFSD. Tous ces isolats ont démontré des activités de six à onze enzymes différentes. La phosphatase alcaline élevée et l'arylamidase leucinique caractérisaient tous les isolats. Les activités de l'estérase des souches de levures étaient plus élevées que celles des souches Bacillus et LAB. Extraits de tous les Allium spp testés. capacité démontrée (p 0,05) d'arrêter la prolifération des organismes isolés. Ces activités étaient de type Allium et dépendantes de la concentration, l'ail présentant la plus forte concentration d'extrait (7 %) affichant l'activité inhibitrice la plus importante (p 0,05). Les niveaux élevés de phosphatase alcaline, de leucine arylamidase et d'estérase ont des

répercussions sur la détérioration du daddawa du soya. Les résultats indiquent qu'Allium spp. et surtout l'ail pourraient être exploités dans la préservation du daddawa de soja.

Mots clés : Condiment, ail, poireau, oignon, conservation, daddawa de soya

Introduction

In rural population across Africa and Asia, traditional fermented foods of legumes and oilseeds origin are used to meet protein requirements of most poor households (Adedeji et al., 2017). In West Africa, *Bacillus* fermented legumes such as *Daddawa, Ogiri* (Adedeji *et al.,* 2017), *Soybean-daddawa* (Popoola and Akueshi, 1985, Omafuvbe *et al.,* 2000; Kolapo et al., 2007a, b) are very popular as condiments in soup and sources of dietary protein. However, industrialisation of the production process of these indigenous food products is far from being attained. This is attributable to the non-standardisation / optimisation of their production and poor / scanty information on their shelf stability.

Soybean daddawa is a condiment made by fermenting soybean (*Glycine max* (L.) Merr) seeds. Its preparation from dehulled soybean seeds is done mainly by traditional art using rudimentary utensils. However, few cases of commercial production on small scale basis exist (Popoola *et al.,* 2007). In the last four decades, the preponderance of studies on West African condiments such as soybean daddawa focused mainly on processing, nutritional evaluation and process optimisation (Popoola and Akueshi 1985, 1986; Omafuvbe 1994; Suberu and Akinyanju 1996; Obatolu *et al.,* 1998; Omafuvbe *et al.,* 2000, 2002, Okorie and Olasupo 2013, Afolabi and Abdulkadir, 2016; Kolapo *et al.,* 2019 a, b). This unwittingly generated lacuna of information on its storability. Few storage studies that exist on soybean daddawa suggest lipid peroxidation as a critical biochemical factor (Kolapo *et al.,* 2007a; Popoola *et al.,* 2007) in its spoilage. Consequently, dichloromethane extract of ginger was found capable to stem the peroxidation tide in stored soybean daddawa (Kolapo *et al.,* 2007b). However, the microbiological status of soybean daddawa undergoing spoilage has been scarcely reported.

Gottardi *et al*. (2016) stated that food spoilage can be driven by physical (oxygen, temperature, light) and/or biological (enzymatic activity and microbial growth) factors. Therefore, accumulation of information on the deteriorating soybean daddawa's microbiota and their enzyme profiles will probably provide more insights on changes during storage and hence translate to more success in its preservation attempts.

Reports have shown that extracts of some spices, herbs and *Allium* have demonstrated both antioxidant and antimicrobial activities in some foods (Ahn *et al*., 2007; Abdel-Salam *et al.,* 2014; Gottardi *et al*., 2016) and thus capable of extending their shelf-life. Recently, the abilities of ethanolic and aqueous extracts of some *Alliums* such as leek and onion to arrest lipid peroxidation in stored soybean daddawa has been reported (Kolapo and Popoola, 2019; Kolapo *et al.,* 2020). It is very possible that such extracts could be exploited for soybean daddawa preservation provided that they have proven antimicrobial activities against its spoilage microbiota. The present study therefore focused on evaluation of *in vitro* antimicrobial activities of aqueous and ethanolic extracts of selected *Allium* (garlic, onion and leek) on microorganisms associated with soybean daddawa deterioration. The enzyme profile of the spoilage microbiota was also reported.

Materials and Methods

Collection of seeds and soybean daddwa preparation

The Soybean seeds of variety TGX 1440-2E used for the present work were obtained from the Institute of Agricultural Research and Training, Ibadan. Nigeria. Soybean daddawa was prepared according to the method of Popoola and Akueshi (1985) as described by Omafuvbe *et al.* (2000).

Isolation and identification of micro-organisms from stored soybean daddawa

Popoola *et. al.* (2007) reported that deterioration of soybean daddawa usually begins after four days of storage. Therefore, isolation of microorganisms were made on 0, 5, 10 and 15th days of storage. The zeroth day sample was taken immediately after the completion of fermentation (72 h). Samples were taken in triplicates and were serially diluted using sterile peptone water. Nutrient agar and potato dextrose agar with 30 mg/ml of antibiotic (streptomycin sulphate) added were used for the isolation of bacteria and fungi, respectively. MRS agar was used for the isolation of lactic acid bacteria (LAB). Nutrient agar and MRS agar plates were incubated at 35^{o}C and 33 ^{o}C, respectively, for 48h. Potato dextrose agar plates were grown at 28^{o}C for 24 h. Colonies were grouped using their cultural and morphological features. Representative colonies from the incubated plates were purified by repeated streaking. In order to determine the genera of the different isolated bacteria, preliminary characterisation of the isolates was carried out by Gram staining, spore staining, catalase test, Voges-Proskauer test, Indole test and Oxygen relationship test as described by Skerman (1967). Also, growth in 7% NaCl, sugar utilisation, nitrate reduction, casein and starch hydrolytic potentials of the different strains were investigated.

Different strains of *Bacillus, Staphylococcus,* LAB, and yeast were identified by assaying selected cultures in API fermentation galleries. *Bacillus* strains were assayed on API 50 CHB galleries. Strains of *Staphylococcus* were assayed on API Staph galleries. LAB strains were assayed on API 50 CHL galleries, while yeasts were assayed on API 20^{o}C AUX. Respective strains were grown on appropriate agar medium (Nutrient Agar for *Bacillus* and *Staphylococcus;* MRS Agar for *Lactobacillus)* at 37^{o}C for 24 h and PDA for yeast at 28^{o}C for 24 h. Sterile swab was used to harvest microbial growth, and was suspended in sterile physiological saline and its turbidity adjusted to 2 McFarland. One hundred microlitres of the microbial suspension was transferred into one tube of respective medium.

The API strips were placed in the incubation trays into which 10 ml of sterile distilled water had been distributed into the honey comb to maintain moist conditions and the cupules of the strips containing the dehydrated substrate inoculated with the respective microbial suspension using a sterile pipette. The boxes were incubated at 37^{o}C and the strip was read at 24 and 48 hours of incubation. Different strains were identified by referring to a reference table provided with the kit for the purpose of identification.

Determination of the enzyme profile of isolated microorganisms

The enzyme profile of the microorganisms associated with stored soybean daddawa was assayed in API ZYM (Bio Merieux, France) galleries. The galleries test for 19 different enzymes. Respective bacterial strains were grown on appropriate agar medium at 37^{o}C for 18 h. Yeasts were grown on potato dextrose agar at 28^{o}C for 24 h. Sterile swab was used to harvest microbial growth, and was suspended in sterile physiological saline and its turbidity adjusted to 2 McFarland.

The API strips were placed in the incubation trays into which 5 ml of distilled water had been distributed into the honey comb to maintain moist conditions and the cupules of the strips containing the dehydrated substrate inoculated with 65 µl of microbial suspension using a sterile pipette. The boxes were incubated at 37°C for 5 h. After incubation 1 drop of each of ZYM A and ZYM B reagents was added to each cupule. The colour change and its intensity within 8-10 min were compared with the standard stated in the reading table to determine the enzyme profile of the test organisms.

***Allium* samples and preparation of extracts**.

Garlic (*A. sativum* L.), onion (*A. cepa* L.) and leek (*A. ampeloprasum* L.) were purchased from a local market in Ibadan, Nigeria. The samples were sorted to remove stones and dirt, washed and air dried. Both ethanol and aqueous extract of the three investigated *Alliums* were obtained using the method reported by Irkin and Korukluoglu (2007). Two hundred and seventy grammes of air dried, peeled garlic, leek or onion were blended in a Saphire mixer grinder (Mahavir Impex, India) with 300 mL distilled water and 99.5 % ethanol for 1 min at 18,000 RPM. The mixtures were macerated for 24 h at 4°C and was thereafter passed through a clean muslin cloth. The resulting extracts were filter sterilised using a 0.45 µm pore size cellulose acetate membrane filter (Cole-Parmer-47mm). The extracts were used immediately.

Evaluation of antimicrobial effect of *Allium* extracts on isolated microorganisms

The antimicrobial activity of the three tested *Allium* extracts on the bacteria, LAB and yeasts isolated from stored soybean daddawa was carried out using a modified method of Yano *et al.* (2006). An overnight culture of respective microorganism was diluted with peptone water (for *Bacillus* and *Staphylococcus*), MRS broth (for LAB) and saboraud broth (for yeast) to a level of 10^4 cfu/ml. Extracts of *Alliums* such as leek and onion have been reported to arrest lipid peroxidation in stored soybean daddawa (Kolapo and Popoola, 2019; Kolapo *et al.,* 2020) at 3, 5 and 7% preservative concentrations. Aliquot of each *Allium* extract suspension (3%, 5% and 7%) was prepared using sterile distilled water; 100 microlitre of the aliquot was then incubated at 30°C for 30 min. Thereafter, 100 microlitre aliquot of the microbial dilution was mixed with the incubated 100 microlitre extract suspension. The mixture was incubated at 30°C for 24h. For the control experiment, the microbial dilution was mixed with the same amount of sterile peptone water/MRS broth/saboraud broth and incubated as above. After incubation, 100 microlitre aliquot of the mixed solutions and of the 10-fold dilutions was pour plated on Nutrient agar/MRS agar/Saboraud agar and incubated. Nutrient agar plates were incubated at 37°C for 24 h while MRS agar plates were incubated at 33°C for 48 h. Saboraud agar plates were incubated at 28°C for 48 h. Viable count ($\log_{10}$cfu/ml) from each plate was then estimated.

Statistical Analysis

Data obtained were expressed as means. The statistical significance of differences was assessed using analysis of variance followed by Student – Newman – Keuls post hoc test. A two-tailed p-value<0.05 was considered to be statistically significant. Values that were significantly different were separated using Duncan Multiple Range test using SPSS for windows Version 11.0 statistical package.

Results and Discussions

Biochemical characteristics of bacterial and yeast isolates

A total of sixty-two bacterial and twelve yeast strains were isolated from samples collected

from 0, 5, 10 and 15th day of storage. Thirty eight, eleven and thirteen strains belonged to genera *Bacillus*, *Staphylococcus* and *Lactobacillus*, respectively. Representative isolates from different sampling day were assayed on API fermentation galleries. Table 1 shows the sugar fermentation on API fermentation galleries by *Bacillus, Lactobacillus Staphylococcus* and yeast strains isolated from stored soybean daddawa. The groups of microorganisms isolated from stored soybean daddawa produced naturally were similar to those that mediated its fermentation. This is true with respect to *Bacillus*, *Staphylococcus* and lactic acid bacteria. Similar findings have been reported by Kolapo and Sanni (2006) and Kolapo (2008), thus, orchestrating the concept that microorganisms that fermented soybean daddawa can proceed to spoil it. Earlier, Oyeyiola (1988) stated that further fermentation after the normal 2-3 days of daddawa production may not cause any significant beneficial effect. In addition to the above mentioned groups of organism, various yeasts were found to be associated with stored soybean daddawa. Yeasts have not been reported in soybean daddawa fermentation (Omafuvbe *et al.,* 2000). However, their presence in the soybean daddawa samples undergoing spoilage as evident in the present investigation might be consequent upon post-fermentation contamination.

Enzyme activity of isolated micro-organisms

The enzymatic profiles of different strains of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeasts isolates are shown in Table 2. The number of demonstrable enzymes and its degree differed both between and within genus. Within the genus *Bacillus*, between six and seven different enzymes were produced while *Staphylococcus* spp. also produced seven enzymes. *Lactobacillus* spp. equally produced seven different enzymes. Within the genus *Candida*, between eight and eleven enzymes were produced by the two examined species. The tested species of *Cryptococcus* also produced between five and eleven enzymes.

All the examined organisms demonstrated a very high alkaline phosphatase and leucine arylamidase activities. The esterase activities of the yeasts strains were higher than those of *Bacillus* and LAB strains. Valine arylamidase activity was exhibited by only two yeasts-*Candida tropicalis* and *Cryptococcus humicola*. Trypsin activity was only exhibited by *B. pumilus, S. xylosus* and *C. humicola* while *Candida famata* and *C. humicola* were the organisms which demonstrated α-chymotrypsin activity. Most yeast strains and *S. xylosus* had a high level of acid phosphatase activity while Naphthol-AS-BI-phosphohydrolase activity was highest in the two LAB strains. It was only *Candida tropicalis* which had both α and β-galactosidase. Alpha-glucosidase activity was only characteristic of the LAB and yeast strains while β-glucosidase activity was only demonstrated by *Candida tropicalis* and *Cryptococcus humicola*.

While suggesting the role of organisms associated with stored soybean daddawa, Kolapo (2008), opined that the proteolytic activity of these organisms may have led to the production of by-products such as amines and other products which resulted to the development of off flavour.

However, the results from the present study suggest that there is likely more than proteolysis that is responsible for spoilage of stored soybean daddawa as all the organisms isolated demonstrated between six to eleven different enzyme activities. It is noteworthy that there is similarity between these enzymes and those demonstrated by starter cultures (*Bacillus. subtilis*, *Staphylococcus xylosus*

Table 1: Sugar Fermentation on API fermentation Galleries by *Bacillus*, *Lactobacillus*, *Staphylococcus* and Yeast strains Isolated from Stored Soybean Daddawa

Substrate	Strains													
	Bsub	*Bcer*2	*Blic*	Bcoa	Lbre	Lrha	Llin	Sxyl	Sxyl2	Ssci	Catro	Crhum	Cafam	Crlau
Adonitol											95	0	95	60
Amidon	80	60	95	20	0	0	0							
Amygdaline	0	0	80	70	50	80	10							
Arbutine	80	40	95	50	60	60	0							
Calcium 2-cetogluconate											90	95	95	90
D-Adonitol	0	0	10	0	0	0	0							
D-Arabinose	0	0	5	0	0	0	0							
D-Arabitol	0	0	0	20	0	0	0							
D-Cellobiose	95	0	100	90	80	100	0				30	90	80	70
D-Fructose	95	90	80	100	100	100	90	90	100	95				
D-Fucose	0	0	0	0	0	0	0							
D-Galactose	30		70	95	100	95					90	100	95	90
D-Glucose	95	100	80	100	100	100	90	100	100	90	100	100	100	100
D-Lactose	10	0	10	30	60	80	0	20	20	70	0	60	0	95
D-Lyxose	0	0	0	0	0	0	0							
D-Maltose	95	90	100	95	80	95	95	80	85	90	95	100	100	95
D-Mannitol	95	0	90	95	40	90	0	90	90	95				
D-Mannose	90	0	90	95	90	100	0	90	90	95				
D-Melezitose	0	0	0	0	40	85	0				80	60	80	90
D-Melibiose	60	0	50	100	80	0	0	30	30	0				
D-Raffinose	60	0	50	65	50	0	0	50	50	0	0	90	80	95
D-ribose	95	70	95	60	80	80	0							
D-Saccharose	95	10	90	95	85	80	0	70	85	90	70	95	100	90
D-Sorbitol	80	0	90	85	30	90	0				95	95	95	60
D-Tagatose	0	0	40	0	10	95	0							
D-Trehalose	80	90	90	95	50	90	0	90	95	90	95	95	30	90
D-Turanose	50	0	20	40	20	0	0							
Dulcitol	0	0	20	0	0	0	0							
D-Xylitol								10	10	0				
D-Xylose	70	0	90	10	60	0	0	70	70	20	95	90	70	90
Erythritol	0	0	5	0	0	0	0							
Esculine	100	70	95	80	90	90	0							
Gentiobiose	60	0	70	65	0	80	0							
Glycerol	80	0	90	60	0	50	0							
Glycerol											20	80	0	0
Glycogene	90	60	90	30	85	0	0							
Inositol	20	0	50	0	0	0	0				0	100	0	80
Inuline	5	0	10	0	80	0	0							
L-Arabinose	90	0	95	50	80	0	0				20	95	70	90
L-Arabitol	0	0	0	0	0	0	0							
L-Arginine								0	0	0				
L-Rhamnose	5	0	20	40	0	40	0							
L-Sorbose	0	0	20	0	0	40	0							
L-Xylose	0	0	10	0	0	0	0							

Table continues overleaf

Substrate	Strains													
	Bsub	*Bcer2*	*Blic*	Bcoa	Lbre	Lrha	Llin	Sxyl	Sxyl2	Ssci	Catro	Crhum	Cafam	Crlau
MethylαD-Glucopyranoside	85	0	80	50	30	40	0	60	60	0				
MethylαD-Mannopyranoside	0	0	0	0	0	5	0							
Methyl-βD-Xylopyranoside	0	0	30	0	0	0	0				70	90	95	70
N-AcetylGlucosamine	40	90	20	20	80	80		80	80	60	90	85	95	90
Potassium2-Cetogluconate	0	0	0	0	0	0	0							
Potassium5-Cetogluconate	0	0	0	0	0	0	0							
Potassiun Gluconate	0	0	50	70	85	85	0							
Pottasium nitrate								80	90	80				
Salicine	90	30	100	80	95	100	0							
Sodium pyruvate								65	60	30				
Urea								90	90	0				
Xylitol	0	0	0	5	0	0	0				0	0	30	70
β-naphthyl phosphate								75	75	70				

Most probable identity of strains based on Sugar fermentation on API galleries
Bsub= *Bacillus subtilis;* Bcer2= *Bacillus cereus* 2*;* Blic= *Bacillus licheniformis;* Bcoa= *Bacillus coagulans;* Lbre = *Lactobacillus brevis* 1; Lrha = *Lactobacillus rhamnosus*; Llin = *Lactobacillus lindneri*; Sxyl= *Staphylococcus xylosus*; Sxyl2= *Staphylococcus xylosus*; Ssci = *Staphylococcus sciuri* ; Catro= *Candida tropicalis* ; Crhum= *Cryptococcus humicola* ; Cafam= *Candida famata* ; Crlau= *Cryptococcus laurenti*

and *Leuconostoc mesenteroides ssp cremoris*) used for fermentation of soybean daddawa (Kolapo *et al.* 2019a). The only difference is in the higher level of esterase and esterase lipase activity by some *Candida* isolates. These enzymes are capable of bringing about various biochemical deteriorations which is a common experience in stored soybean daddawa. For instance, earlier studies (Kolapo and Sanni, 2007; Kolapo *et al.*, 2007a; Popoola *et al.,* 2007) have implicated lipid peroxidation as a key factor in soybean daddawa deterioration in addition to microbial deterioration (Kolapo and Sanni, 2006). The demonstration of high esterase and esterase lipase activity by some *Candida* isolates from the stored soybean daddawa in the present study is possibly giving credence to earlier mentioned proposition.

Increased production of volatile basic nitrogen by both acid and alkaline phosphatases has been implicated in the spoilage of raw muscle from horse mackerel (Kuda *et al.* 2002). Consequently, the high levels of activities of these enzymes in all the isolated microorganisms in the present study suggest their potential roles in soybean daddawa spoilage by increasing the production of volatile basic nitrogen. Leucine arylamidase is a proteolytic enzyme while trypsin and α-chymotrypsin are proteinases. The former had high level of activity in all the isolates while the later were detected in some isolated *Bacillus, Staphylococcus* and yeast strains. The roles of these three enzymes in food spoilage have been well documented (Venugopal, 1990).

Antimicrobial effect of garlic, onion and leek extracts on isolated strains of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeasts

The result of antimicrobial assay of garlic extract on different strains of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeasts isolated from stored soybean daddawa is presented in Table 3. A two way ANOVA test revealed that there was significant difference ($p<0.05$) between the control experiment and different concentrations of garlic extract used.

Table 2: Enzyme profile of microorganisms isolated from stored soybean daddawa

Assayed Enzymes	Bsub	lic	Bpum	Sxyl	Lbre	Lrha	Catro	Cafam	Crlau	Crhum
Alkaline phosphatase	4	4	5	4	3	4	2	5	4	5
Esterase(C4)	1	1	2	1	1	2	2	2	2	2
Esterase lipase(C8)	1	1	1	1	1	1	0	2	0	0
Lipase(C14)	0	0	0	0	0	0	0	0	0	0
Leucine arylamidase	3	4	5	5	5	5	5	4	0	5
Valine arylamidase	0	0	0	0	0	0	4	0	0	2
Cystine arylamidase	0	0	0	0	0	0	1	0	0	0
Trypsin,	0	0	4	4	0	0	0	0	0	4
α-chymotrypsin	0	0	0	0	0	0	0	4	0	2
Acid phosphatase	3	4	5	3	2	2	5	5	1	5
Naphthol-AS-BI-phosphohydrolase	2	1	2	2	4	3	2	1	1	4
α-galactosidase	0	0	0	0	0	0	5	0	0	0
β-galactosidase	0	0	0	0	0	0	5	0	0	0
β-glucuronidase	0	0	0	0	0	0	1	0	0	0
α-glucosidase	0	0	0	0	4	4	4	1	1	4
β-glucosidase	0	0	0	0	0	0	5	0	0	3
N-acetyl- β -glucosaminidase	0	0	0	0	0	0	0	0	0	1
α-mannosidase	0	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0	0

*0=Negative reaction (no activity); 1= Least Activity; 5=Maximum activity Bsub= *Bacillus subtilis* ; Blic= *Bacillus licheniformis*; Bpum= *Bacillus pumilus*; Sxyl= *Staphylococcus xylosus;* Lbre= *Lactobacillus brevis* 1; Lrha= *Lactobacillus rhamnosus*; Catro= *Candida tropicalis*; Cafam= *Candida famata*; Crlau= *Cryptococcus laurenti*; Crhum= *Cryptococcus humicola*

The antibacterial activity of the extract was concentration-dependent, with the highest extract concentration exhibiting the most significant inhibitory activity on the proliferation of the investigated bacteria. However, there was no significant difference ($p > 0.05$) between the antibacterial activity of the garlic extract against the individual *Bacillus*, *Staphylococcus* and *Lactobacillus* strains. Furthermore, there was no significant difference ($p > 0.05$) between the inhibitory effects of both aqueous and ethanolic extracts on the different strains of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeast strains except for *L. rhamnosus* and *Cryptococcus laurentii that* were more sensitive to ethanolic extract.

Garlic has long been recognized for its antibacterial and antifungal effects, and recent search for natural preservatives has led to interests in its potential for preventing microbial contamination in foods. It has been reported to inhibit *Aerobacter*, *Aeromonas*,

Table 3. Viable counts (log_{10}cfu.ml^{-1}) of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeast strains following incubation* with different concentrations of aqueous and ethanolic extracts of garlic

Garlic Extract Concentration	Isolates												
	Bsub	Blic	Bpum	Bcere2	Sxyl	Ssci	Lbrev	Lrha	Llin	Catro	Crhum	Cafam	Crlau
7%	7.78^{c}	8.29^{c}	8.03^{c}	8.39^{c}	8.53^{c}	8.45^{c}	8.03^{c}	8.31^{d}	8.01^{c}	7.00^{d}	7.00^{c}	7.00^{d}	7.30^{c}
	(8.28^{bc})	(8.65^{bc})	(8.11^{c})	(8.05^{a})	(8.22^{b})	(8.09^{cd})	(5.13^{c})	(5.77^{c})	(5.15^{d})	(7.48^{c})	(7.78^{c})	(7.70^{c})	(7.45^{c})
5%	8.63^{b}	8.46^{c}	8.37^{c}	9.18^{b}	8.89^{bc}	9.15^{b}	8.36^{b}	8.44^{c}	8.62^{b}	8.67^{b}	7.40^{b}	8.18^{b}	7.78^{c}
	(8.99^{b})	(9.38^{b})	(8.93^{b})	(8.17^{a})	(8.16^{b})	(8.79^{bc})	(5.36^{b})	(5.84^{c})	(5.47^{c})	(8.30^{b})	(7.85^{b})	(7.70^{c})	(7.48^{c})
3%	9.41^{a}	9.39^{b}	8.96^{ab}	9.44^{ab}	9.46^{a}	9.16^{b}	8.56^{b}	8.70^{b}	8.64^{b}	8.70^{b}	7.48^{c}	8.23^{b}	8.30^{b}
	(9.89^{ab})	(9.49^{b})	(9.17^{b})	(8.28^{a})	(8.47^{b})	(9.13^{b})	(5.66^{b})	(7.15^{b})	(5.71^{b})	(8.73^{b})	(7.90^{b})	(7.95^{b})	(7.78^{c})
0%	9.50^{a}	10.05^{a}	9.59^{a}	9.87^{a}	9.68^{a}	10.01^{a}	10.19^{a}	9.64^{a}	10.48^{a}	10.94^{a}	10.75^{a}	10.09^{a}	10.01^{a}
	(11.01^{a})	(10.84^{a})	(10.57^{a})	(9.55^{a})	(10.88^{a})	(9.98^{a})	(6.50^{a})	(8.86^{a})	(6.52^{a})	(9.78^{a})	(10.18^{a})	(10.88^{a})	(11.05)^{a}
Initial bacterial suspension	7.00^{d}	7.32^{d}	6.66^{d}	7.28^{d}	7.20^{d}	7.00^{d}	7.18^{d}	7.09^{e}	7.20^{d}	7.30^{c}	8.60^{b}	8.08^{c}	7.70^{c}
	(7.44^{c})	(7.61^{c})	(7.54^{c})	(7.75^{a})	(7.41^{c})	(7.66^{c})	(4.18^{d})	(5.09^{d})	(4.20^{d})	(7.48^{c})	(8.00^{b})	(8.38^{b})	(8.28^{b})

Values are means of triplicate determinations. Data in bracket are values for ethanolic extract. Along column, values with different superscripts differ significantly ($p < 0.05$)

Bsub= *Bacillus subtilis;* Blic= *Bacillus licheniformis;* Bpum= *Bacillus pumiluss;* Bcer2= *Bacillus cereus* 2*;* Sxyl= *Staphylococcus xylosus*; Ssci = *Staphylococcus sciuri* ; Lbre = *Lactobacillus brevis* 1; Lrha = *Lactobacillus rhamnosus*; Llin = *Lactobacillus lindneri*; Catro= *Candida tropicalis* ; Crhum= *Cryptococcus humicola* ; Cafam= *Candida famata* ; Crlau= *Cryptococcus laurenti*

**Bacillus* and *Staphylococcus* strains were incubated at 37 °C for 24 h while *Lactobacillus* strains were incubated at 33 °C for 48 h. Yeast starins were incubated at 28°C for 48 h.

Bacillus, *Citrella*, *Citrobacter*, *Clostridium*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Mycobacterium*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Vibrio* (Sivam 2001). This author further stated that previous studies have shown that it has the potential of protecting against *Helicobacter pylori* infections, and it was postulated that this effect could be responsible for the inverse association between *Allium* species consumption and gastric cancer, which is linked to *H. pylori* infection.

Another study found that allicin showed promise in preventing and treating malaria (Coppi *et al.,* 2006). Various organosulfur components, particularly allicin derivatives, have been shown to have an important role in the antimicrobial activity of garlic. However, polyphenol extracts from garlic were also demonstrated to have high inhibitory effects against the bacteria: *Staphylococcus aureus* and *Salmonella enteriditis,* and against three fungi, *Aspergillus niger, Penicillium cyclopium* and *Fusarium oxysprorum* (Hedger and Lister, 2007).

Table 4 shows the result of antimicrobial assay of onion extracts on different strains of isolated *Bacillus*, *Staphylococcus, Lactobacillus* and yeasts. Both aqueous and ethanolic extracts exhibited significant ($p <$

Table 4. Viable counts (log_{10}cfu.ml^{-1}) of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeast strains following incubation* with different concentrations of aqueous and ethanolic extracts of onion.

Onion Extract Concentration	Isolates												
	Bsub	Blic	Bpum	Bcere2	Sxyl	Ssci	Lbrev	Lrha	Llin	Catro	Crhum	Cafam	Crlau
7%	8.21^{d}	8.58^{b}	8.22^{c}	8.06^{b}	8.53^{a}	8.90^{b}	5.17^{d}	7.57^{c}	8.15^{c}	7.00^{d}	7.00^{c}	7.12^{c}	7.00^{c}
	(9.05^{b})	(8.83^{b})	(8.62^{c})	(8.29^{c})	(9.38^{b})	(8.70^{b})	(4.73^{d})	(6.13^{c})	(5.32^{d})	(7.60^{b})	(7.30^{c})	(7.70^{c})	(8.60^{b})
5%	8.75^{c}	9.15^{b}	8.89^{b}	8.13^{b}	8.59^{a}	9.44^{a}	5.29^{d}	7.72^{c}	8.27^{b}	7.60^{c}	7.37^{c}	7.30^{c}	7.70^{c}
	(9.11^{b})	(9.02^{b})	(8.84^{bc})	(8.51^{c})	(9.49^{b})	(8.78^{b})	(4.89^{d})	(5.99^{c})	(5.59^{d})	(7.58^{b})	(7.60^{c})	(7.90^{c})	(8.61^{b})
3%	9.19^{b}	9.18^{b}	9.08^{b}	8.54^{b}	9.09^{a}	9.47^{a}	6.16^{c}	8.55^{b}	8.64^{b}	8.23^{b}	8.41^{b}	7.60^{bc}	8.03^{b}
	(9.58^{b})	(9.55^{b})	(9.36^{b})	(9.44^{b})	(9.48^{b})	(8.91^{b})	(5.06^{c})	(7.15^{b})	(6.47^{c})	(7.60^{b})	(8.23^{b})	(8.04^{bc})	(8.74^{b})
0%	10.20^{a}	10.05a	9.50^{a}	11.04^{a}	9.59^{a}	9.65^{a}	8.14^{a}	9.62^{a}	10.47^{a}	10.94^{a}	10.75^{a}	10.09^{a}	10.01^{a}
	(10.71^{a})	(10.73^{a})	(10.57^{a})	(10.73^{a})	(10.69^{a})	(9.98^{a})	(8.14^{a})	(9.62^{a})	(10.47)	(9.78^{a})	(10.18^{a})	(10.88^{a})	(11.05^{a})
Initial bacterial suspension	6.70^{e}	7.33^{c}	7.00^{d}	7.02^{c}	6.67^{b}	7.20^{c}	7.18^{b}	7.09^{d}	7.20^{d}	7.30^{c}	8.60^{b}	8.08^{b}	7.70^{c}
	(7.43^{c})	(7.75^{c})	(7.31^{d})	(7.74^{c})	(7.31^{c})	(7.63^{c})	(7.18^{b})	(7.09^{b})	(7.20^{b})	(7.48^{c})	(8.00^{b})	(8.38^{b})	(8.28^{c})

Values are means of triplicate determinations. Data in bracket are values for ethanolic extract. Along column, values with different superscripts differ significantly ($p < 0.05$)

Bsub= *Bacillus subtilis;* Blic= *Bacillus licheniformis;* Bpum= *Bacillus pumiluss;* Bcer2= *Bacillus cereus* 2*;* Sxyl= *Staphylococcus xylosus*; Ssci = *Staphylococcus sciuri* ; Lbre = *Lactobacillus brevis* 1; Lrha = *Lactobacillus rhamnosus*; Llin = *Lactobacillus lindneri*; Catro= *Candida tropicalis* ; Crhum= *Cryptococcus humicola* ; Cafam= *Candida famata* ; Crlau= *Cryptococcus laurenti*

**Bacillus* and *Staphylococcus* strains were incubated at 37 °C for 24 h while *Lactobacillus* strains were incubated at 33 °C for 48 h. Yeast starins were incubated at 28 °C for 48 h.

0.05) inhibition on the growth of the tested bacteria, however, there was no significant difference ($p > 0.05$) between their anti-staphylococcal and anti-bacilli activity. At the tested concentrations, there were no significant differences ($p > 0.05$) between different preservative concentrations of both aqueous and ethanolic extracts on the different strains of *Bacillus* and *Staphylococcus* isolated from stored soybean daddawa. Of all the tested *Bacillus* and *Staphylococcus* strains, *S. xylosus* was less sensitive to both aqueous and ethanolic aqueous extracts of onion. The exhibited anti-lactobacilli and anti-yeast activities of both aqueous and ethanolic extracts of onion were both dependent on preservative concentration and the types of solvent used for extraction. Of the three tested LAB, the proliferation of *Lactobacillus brevis* 1 was more significantly ($p < 0.05$) inhibited.

The result of antimicrobial assay of leek extract on different strains of isolated *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeasts is presented in Table 5. A two-way ANOVA test of activity of aqueous leek extract reveals a concentration dependent, non-organism specific inhibitory activity on the assayed *Bacillus* and *Staphylococcus* strains. In a similar trend, ethanol extract of leek exerted a concentration dependent inhibitory activity on the tested *Bacillus* and *Staphylococcus* strains. However, there was no significant difference ($p > 0.05$) between the sensitivity of all *Bacillus* and *Staphylo-*

Table 5. Viable counts (log_{10}cfu.ml^{-1}) of *Bacillus*, *Staphylococcus*, *Lactobacillus* and yeast strains following incubation* with different concentrations of aqueous and ethanolic extracts of leek.

Leek Extract Concentration	Isolates												
	Bsub	Blic	Bpum	Bcere2	Sxyl	Ssci	Lbrev	Lrha	Llin	Catro	Crhum	Cafam	Crlau
7%	7.39^{c}	7.72^{c}	7.63^{c}	7.35^{cd}	8.02^{d}	7.24^{b}	7.87^{c}	8.31^{b}	8.01^{c}	7.30^{c}	7.00^{c}	7.48^{c}	7.00^{c}
	(7.45^{c})	(8.83^{c})	(8.06^{c})	(8.24^{c})	(8.21^{d})	(8.33^{b})	(4.90^{d})	(5.77^{c})	(5.15^{c})	(7.60^{c})	(7.00^{c})	(7.30^{c})	(7.70^{c})
5%	7.54^{c}	8.39^{b}	8.34^{bc}	8.13^{bc}	8.77^{c}	8.03^{b}	8.34^{b}	8.44^{b}	8.62^{b}	7.70^{c}	7.87^{c}	8.36^{b}	7.30^{c}
	(7.64b^{c})	(9.35^{b})	(8.22^{c})	(8.62^{b})	(8.61^{c})	(8.45^{b})	(5.32^{cd})	(5.85^{c})	(5.47^{c})	(7.90^{bc})	(7.48^{c})	(7.78^{c})	(7.85^{c})
3%	8.48^{bc}	8.54^{b}	9.02^{c}	8.72^{b}	9.04^{b}	8.61^{ab}	8.56^{b}	8.70^{b}	8.64^{b}	8.23^{b}	8.11^{b}	8.48^{b}	7.70^{b}
	(7.77^{b})	(9.38^{b})	(9.09^{b})	(8.67^{b})	(8.85^{b})	(8.45^{ab})	(5.65^{c})	(5.86^{c})	(5.71^{c})	(8.00^{b})	(7.60^{c})	(8.60^{b})	(8.08^{b})
0%	9.90^{a}	10.37^{a}	10.47^{a}	10.26^{a}	10.73^{a}	10.46^{a}	10.19^{a}	9.64^{a}	10.48^{a}	10.94^{a}	10.75^{a}	10.09^{a}	10.01^{a}
	(11.04^{a})	(11.38^{a})	(11.33^{a})	(11.22^{a})	(11.20^{a})	(11.11^{a})	(8.14^{a})	(9.62^{a})	(10.47^{a})	(9.78^{a})	(10.18)^{a}	(10.88^{a})	(11.05^{a})
Initial bacterial suspension	7.27^{c}	7.18^{d}	7.53^{c}	6.93^{d}	7.18^{e}	7.02^{b}	7.18^{b}	7.09^{d}	7.20^{d}	7.30^{c}	8.60^{b}	8.08^{bc}	7.70^{b}
	(7.44^{c})	(7.61^{d})	(7.54^{c})	(7.75^{d})	(7.41^{e})	(7.66^{c})	(7.18^{b})	(7.09^{b})	(7.20^{b})	(7. 48^{c})	(8.00^{b})	(8.38^{b})	(8.28^{b})

Values are means of triplicate determinations. Data in bracket are values for ethanolic extract. Along column, values with different superscripts differ significantly ($p < 0.05$)

Bsub= *Bacillus subtilis;* Blic= *Bacillus licheniformis;* Bpum= *Bacillus pumiluss;* Bcer2= *Bacillus cereus* 2*;* Sxyl= *Staphylococcus xylosus*; Ssci = *Staphylococcus sciuri* ; Lbre = *Lactobacillus brevis* 1; Lrha = *Lactobacillus rhamnosus*; Llin = *Lactobacillus lindneri*; Catro= *Candida tropicalis* ; Crhum= *Cryptococcus humicola* ; Cafam= *Candida famata* ; Crlau= *Cryptococcus laurenti*

**Bacillus* and *Staphylococcus* strains were incubated at 37 °C for 24 h while *Lactobacillus* strains were incubated at 33 °C for 48 h. Yeast starins were incubated at 28 °C for 48 h.

coccus strains to ethanol extract except *Bacillus subtilis*, whose growth was more significantly ($p < 0.05$) inhibited compared to other organisms.

The proliferation of the *Lactobacillus* strains was significantly inhibited ($p < 0.05$) by both aqueous and ethanolic extracts of leek. However, they significantly differed in their susceptibilities to these extracts. For instance, the inhibition of *L. lindneri*, and *L. brevis* 1 by aqueous extract was concentration dependent while the sensitivity of *L. rhamnosus* to aqueous extract was not significantly different ($P > 0.05$) at the three tested concentration.

On the contrast, the susceptibility of the three investigated LAB to the ethanolic extract of leek was neither organism specific nor concentration dependent. This is a possible indication that the anti-LAB activity of ethanolic extract of leek is significantly higher than its aqueous counterpart. The inhibitory activities of both aqueous and ethanolic extracts of leek on all the tested yeasts were not significantly different ($p > 0.05$). The exhibited anti-yeast activities of both aqueous and ethanolic extracts of leek were dependent on preservative concentration and non-organism specific.

Antifungal effects of onion and garlic essential oils have been investigated. *A. niger* is less inhibited by low concentrations of essential oils of green and yellow onions but red onion and garlic essential oils show stronger inhibitory effects (Benkeblia, 2004). Fistulosin, an antifungal compound isolates from onions, shows antifungal activities against several fungal species (Phay *et al.,* 1999). Research on antifungal effects of leek is very limited, but in a study carried out by Kivanc and Kunduhoglu (1997), leek was found to have the lowest inhibitory activity against yeasts compared to onion, cabbage, radish and garlic. In various research studies on antimicrobial activities of *Allium* spp., the effectiveness of the observed inhibition was related to the solvent used in the extraction (Sharma *et al.,* 1979; Pruthi, 1980; Tsao and Yin, 2001; Irkin and Korukluoglu, 2007). Extracts obtained with the use of solvents such as ethanol and ether showed leek having higher antimicrobial activities compared with the acetone and water extracts.

A one way ANOVA test to determine the comparative effect of garlic, onion and leek extracts on the inhibition of spoilage microorganisms depicted that the degree of observed growth inhibition was microorganism and solvent of extraction specific. The aqueous leek extract exhibited the highest significant ($p < 0.05$) growth inhibitory activity on *B. subtilis*, *B. licheniformis*, *Staphylococcus sciuri* and *Cryptococcus laurentii.* The aqueous extract of onion also had the highest significant ($p < 0.05$) antimicrobial effect on *Lactobacillus rhamnosus*, *L. brevis* and *Candida famata.* There was so significant difference ($p > 0.05$) between the inhibitory activities of aqueous extracts of the three investigated *Alliums* on *B. pumilus*, *B. cereus2*, *S.xylosus*, *L.lindneri*, *Candida tropicalis* and *Cryptococcus humicola*. Similarly, there was so significant difference ($p > 0.05$) between the inhibitory activities of ethanolic extracts of the three investigated *Alliums* on *B. pumilus*, *B. licheniformis*, *B. cereus2*, *L.lindneri*, *L.brevis*, *L.rhamnosus*, *Candida tropicalis* and *C. famata*. Out of the ethanolic extracts of the three *Alliums*, garlic extract exhibited highest significant ($p < 0.05$) reduction for *S.xylosus* and *Cryptococcus laurentii* while leek extract most significantly ($p < 0.05$) reduced the growth of *B. subtilis* and *Crytococoocus humicola*.

Irkin and Korukluoglu (2007) had demonstrated the ability of both aqueous and ethanolic extracts of garlic, onion and leek to inhibit the growth of *Aspergillus niger*. Similarly, Abdel-Salam *et al.* (2014) reported antimicrobial abilities of aqueous and ethanolic extracts and essential oils of these three *Alliums* on *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Aspergillus niger* and *Fusarium oxysporum*. The results of antimicrobial activities of the various extracts obtained in the present study compare favourably with the earlier observations in terms of the kinds of *Alliums*, the solvent used in the extraction process and the extract concentration. Results from the present study seem to suggest that the ethanolic extracts of the three investigated *Alliums* is more effective than water extracts.

Today *Alliums* are used for their flavour, aroma and taste; being prepared domestically or as a raw material in a variety of food manufacturing processes (Stajner and Varga, 2003). On the other hand, therapeutic and medicinal values of garlic and onions have become the objectives of many research efforts. Different clinical studies have shown their beneficial effects in the reduction of cardiovascular disease risk by inducing lowering of serum cholesterol and blood pressure (Steiner and Linm, 1994). Garlic and onions have liver protective (Dion and Miler, 1996), immune enhancement and anti-infection (Lau, 1989), anti-stress and anti-

fatigue (Kawashima, 1986), anti-cancer and cancer preventive effects (Dion and Miler, 1997; Pinto *et. al.,* 1997), brain and neurotrophic (Moriguchi, 1996) and other pharmacological effects (Yeh, 1996).

Conclusions

Results of this study demonstrated that extracts of garlic, onion and leek have significant *in vitro* antimicrobial activity against microorganisms that are associated with the deterioration of soybean daddawa. However, the degree of observed growth inhibition was microorganism and solvent of extraction specific. Owing to the exhibited antimicrobial activity and their widely reported antioxidant activity, therapeutic and medicinal uses, their use, could be exploited in the preservation of soybean daddawa. However, there is a need for further investigation on the effect of *Allium* extracts on the sensorial quality of soybean daddawa preserved with these extracts.

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