ANTIMICROBIAL POTENTIAL OF *CYMBOPOGON CITRATUS* WATER AND METHANOLIC EXTRACT ON ISOLATES OF DATE FRUIT-TIGERNUT DRINKS SOLD IN DELTA STATE.

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ABSTRACT

Date fruit-Tiger nut drink is a highly nutritious traditional drink. Samples of tiger nut and date fruits were collected from some cities in the three senatorial districts of Delta State: Delta North (Asaba and Agbor); Delta South (Oleh and Warri); and Delta Central (Sapele and Abraka). Phytochemical analysis of lemon grass water extract gave the following results: glycoside, 11.78mg/g; phenols: 121.16mg/g; flavonoids, 43.16mg/g; tannins, 18.47mg/g; and terpenoids, 8.43mg/g; while it was: alkaloids, 31.00mg/g; steroids, 17.00mg/g; saponins, 28.43mg/g; flavonoids, 37.36mg/g; tannins, 14.47mg/g; terpernoids, 12.11mg/g; and phenol, 138mg/g for lemon grass methanolic extracts. The bacteria isolated from the drinks were identified as *Beta protoebacterium species*, *Klebsiella pneumoniae*, and *Erwinia chrysanthemi* strains by biochemical and molecular characterization. Lemon grass methanolic extract inhibited growth of organisms at 60 and 80(mg/ml) concentrations while lemon grass water extract did not inhibit the growth of the organisms at the various concentrations used. A proximate analysis of the Date fruit-Tiger nut drink at intervals of 48hours during treatment with lemon grass methanolic extract showed significant (P<0.05) reductions in carbohydrate (80.64 mg/ml $\pm$2.82 to 62.59 mg/ml $\pm$4.48), protein (13.46 mg/ml $\pm$0.57 to 4.17 mg/ml $\pm$0.97) , fat (3.30 mg/ml $\pm$0.98 to 1.00 mg/ml $\pm$0.40), Crude fibre (14.06 mg/ml $\pm$0.32 to 7.40 mg/ml $\pm$2.42) and Ash (5.18 mg/ml $\pm$0.04 to 5.03 mg/ml$\pm$0.05). Moisture contents significantly (P<0.05) increased from (4.2 mg/ml$\pm$0.19 to 4.94 mg/ml $\pm$0.09). Retarded deterioration of date fruit-tiger nut drink was indicated therefore, methanolic extract can be potentially useful for the preservation of date fruit-tiger nut drink.

Key words: Date fruit-Tigernut drink, Shelf- life, Lemon grass extracts, Antimicrobial Activity, Phytochemicals.

INTRODUCTION

Date fruit – tiger nut drink is a mixture of date fruits and tiger nuts in their processed form.The tree of dates or the palm date (*Phoenix dactylifera*L., Family Arecaceae) has played an important role as a food security crop in the Middle East, North Africa region (MENA) and Northern Nigeria by providing valuable food for people in for the last 5000 years (Ghnimi, 2017). In appreciation of its fruits, the date tree is referred to as the sacred tree the tree of life and the bread of the desert (Ghnimi, 2017). The Latin name of the tree is believed to have been derived from Greek Phoenix daktulos, which means purple or red finger (Ghnimi, 2017).

The major component of dates are carbohydrates (mainly the sugars, sucrose,

glucose and fructose), which may constitute about 70% (Aljaloud *et al.,* 2020). Date fruits are good source of fibre, as well as several important minerals and vitamins(Aljaloud *et al.,* 2020). The phenolic compounds (anthocyanims and flavonoids) and carotenoids are associated with antimutagenic and antioxidant activity (Aljaloud *et al.,* 2020). Date contain 6.5–11.5% total dietary fibers (84–94% insoluble and 6–16% soluble dietary fiber), 2% proteins, about 1% fat, 2% , and 2% ash. Date seeds, which contains about 10–15% of the whole fruit, is an important byproduct of date fruit processing industries it is also contains a high level of water-insoluble mannan fibers which can be useful in the enhancement of fiber content of some food products (Aljaloud,2020).

Tigernuts *Cyperus esculentus* is an underutilized tuber from the family Cyperaceae, which produces rhizomes at the base of the tuber which is somewhat spherical (Djomond *etal*., 2020). It is a free growing tuber that is largely consumed in Nigeria, East Africa, other parts of west Africa, some parts of Europe especially Spain and also in Arabian Peninsula ref. In Nigeria, it is called "Aya" in Hausa, "Akiausa" in Igbo and "Ofio" in Yoruba, yellow, brown and black varieties of tigernuts are cultivated in Nigeria (Djomond *et al*., 2020). The yellow variety of tigernut is more preferred over the others due to its inherent properties which are, large size, fleshier nature and attractive colour (Djomond *et al*., 2020). It yields more milk when extracted, contains less anti nutritional factors polyphenols in particular, lower fat and high protein content (Djomond *et al*., 2020). Tigernuts produces sweet tubers which are eaten freshly or roasted. It can be processed as animal feed, and juiced for beverage production (Udeozor, 2012). Imam *et al*., (2013) reported that the phytochemicals present in tigernuts are flavonoids, steroids, alkaloids, saponins and tannins which are responsible for the several biochemical activities of the plant. This finding shows that tigernut contains important phytochemicals and it is save for consumption. Date fruit-tiger nut

drink can be prepared by sorting, washing and soaking date fruit and tigernut in clean water after which they are milled together and filtered, the filterate (date fruit-tigernut drink) is stored in clean vessels. Date fruit- tigernut drink is usually sold by hawkers mostly in the market. Date fruit-tiger nut drink is a popular drink consumed by many people. However it has a short-shelf life due to its nutritional contents which promotes the growth of microorgaisms, hence the need to preserve it in order to increase its shelf life. Lemon grass extracts has vital characteristics such as antimicrobial properties and antioxidant activities Vaziriana *et al*., (2012) which makes it a good natural preservative. The objective of this study is to extend the shelf life of date fruit-tigernut drinks by applying lemon grass water and methanolic extracts as preservatives.

MATERIALS AND METHODS

Sample Collection

Samples of Date- Tiger nut drinks were purchased from markets in some cities in the three senatorial districts of Delta State, Delta North (Asaba and Agbor), Delta South (Oleh and Warri) and Delta Central (Sapele and Abraka).

Microbiological Analysis

Nutrient agar (NA) and saboraud dextrose (SDA) agar plates were inoculated with 0.1ml of serially diluted date fruit-tigernut drinks for bacterial and fungal isolations respectively and incubated at 37^0C for 24/48 hours. Ketoconazole was introduced at 0.05mg/ml to inhibit fungal growth. The bacteria and fungal colonies that were developed were sub cultured and stored in slants at 4^0C for use in subsequent tests.

IDENTIFICATION OF ISOLATES

The fungal and bacterial isolates were all identified on the basis of morphological and biochemical characterization (Gram reaction, motility, catalase, citrate, indole, oxidase and carbohydrate fermentation tests). Molecular characterization was carried out using the Polymerase chain reaction (PCR).

EXTRACTION OF LEMON GRASS AND ANTIMICROBIAL TESTING

Aqueous and methanolic extractions of lemon grass water extract was by Soxhlet extraction method (Samson *et al.,* 2020). Different concentrations 20, 40, 60 and 80 (mg/ml) of the lemon grass water and methanolic extract were used during the experiment to determine the antimicrobial activity against the isolates by agar diffusion method in accordance to the method used by Clinical Laboratory Standards Institute (CLSI, 1940).

PHYTOCHEMICAL AND PROXIMATE ANALYSES

Qualitative and quantitative screening was also carried out to identify the presence and concentrations of phytochemical constituents present in the lemon grass water and methanolic extracts according to the procedure of (Shendurse *et al*.,2021).Proximate analysis for moisture content, crude fibre content, ash content, fat content, carbohydrates and protein contents in the Date fruit- tiger nut drinks treated with extracts were determined using AOAC (1990). pH of treated drinks were determined using millivoltmeter.

DATA ANALYSIS

Data from the proximate analysis was expressed as mean $\pm$ S.D. and analysed by one way Analysis of Variance (ANOVA) and post hoc comparison tests with Turkey and Fisher Least Significant Difference (LSD) tools. Also a non – parametric test (Wilcoxon test) was carried out, median values were determined and used to compare changes in organoleptic properties as perceived by respondents (taste, colour and aroma) at 48hrs and 96hrs storage. SPSS version 23.0 soft ware was used.

RESULTS

The identity of the organisms isolated can be seen in Table 1 based on phynotypic and biochemical tests while confirmation by molecular analysis is presented as *Beta proteobacterium* VE-7-2-3-2 with accession number AB748654, *Klesbsiella pneumonia* S-P-N-031.01 with accession number CP092809 and *Erwinia chrysanthemi* NZEC135 with accession number EF178670. Analysis of the composition of aqueous extract of lemon grass showed that it was predominantly pheolics and

flavonoids while the concentration of other components were markedly low as show in (Table 2). With respect to the methanolic extract, phenols had the highest concentration while glycosides which was present in the aqueous extract was not detected (Table 2). However, alkaloids, steroids, which were not detected in the aqueous extract were present in the methanolic extract (Table 2). The results of antimicrobial activity of lemon grass extracts against isolates are preseted in (Table 3). Based on zones of inhibition, the aqueous extract were markedly generally less inhibitory that than methanolic extract. Generally inhibition of the isolates increased with increasing concentrations.

Table 1: Identification of Isolates from Date Fruit-Tiger Nut Drinks

Cultural Morphology				Biochemical Characteristics					Identified organisms
Gram reaction	**Shape**	**Motility**	**Colony Morphology**	**Catalase**	**Oxidase**	**Indole**	**Carbohydrate Fermentation**	**Citrate**	
–	Rods	+/–	–	+	–	–	+	–	*Beta protoebacterium species*
–	Rods	–	Mucoid smooth margin	+	–	+	+	+	*Klebsiella pneumonia species*
+	Rod	+	_	+	–	–	+	–	*Erwinia chrysanthemi species*

Positive,+; Negative,– ; Motile/non-motile +/-

Table 2: Quantitative Analysis of lemon grass Methanolic and Water extracts

Phytochemicals	**Lemon grass Water extracts**		**Lemon Grass Methanolic extract**	
	Concentration (mg/g)	**Concentration classification**	**Concentration (mg/g)**	**Concentration classification**
Glycoside	11.78	+	–	–
Phenols	121.16	+++	138	+++
Flavonoids	43.16	++	37.36	++
Tannin	18.47	+	14.47	+
Terpenoids	8.43	+	12.11	+
Alkaloids	–	–	31.00	+
Steroids	–	–	17.00	+
Saponins	–	–	28.43	+

Low,+; ++; Moderate; +++, High.

Table 3: Zones of Inhibition due to Antimicrobial Activities of Lemon grass Methanolic and water Extract on Isolates of Date fruit- Tiger nut drinks (mm)

Lemon grass Extracts	Conc.(mg/ml)	*Beta proteobacterium*[a]	*Beta proteobacterium*[b]	*Klebsiella pneumoniae*[c]	*Klebsiella pneumoniae*[d]	*Erwinia chrysanthemi*[e]	*Erwinia chrysanthemi*[f]
Water	20	0.20	0.20	0.40	0.40	0.50	0.50
	40	0.60	0.60	0.70	0.70	0.80	0.80
	60	2.00	2.00	1.80	1.80	2.30	2.30
	80	4.20	4.20	3.40	3.40	4.60	4.60
Methanol	20	0.80	0.80	1.50	1.50	1.00	1.00
	40	3.20	3.20	4.50	4.50	4.20	4.20
	60	7.50	7.50	8.00	8.00	8.60	8.60
	80	10.40	10.40	11.60	11.60	12.10	12.10

Agbor, a; Asaba, b; Oleh, c; Abraka, d; Warri, e; Sapele

There were reductions in the nutrients of the drink during storage after preservative treatment with lemon grass extracts as shown in (Table 4). The reductions increased with storage time and with the exception of moisture content, the differences were not marked (Table 4).Compare to control pH of the drink declined with storage time (Figure 1). Figure 2 presents organoleptic tests, it shows the trend of scores for taste, colour and aroma arising from the respondents between 48 and 96hrs of treatment with lemon grass methanolic extract. Refrigerated treated drinks were rated better in taste, colour and aroma when compared to non- refrigerated drinks (Figure 2).

Table 4: Effect of extract treatment on Proximate Composition of Date fruit-Tigernut Drink

Proximate Composition	0Hr	48Hrs	96Hrs	F(*p*)
Carbohydrates	80.64±2.82[a]	72.72±4.48[b]	62.588±5.81[ab]	17.98(0.000)
Protein	13.46±0.58[ab]	4.84±1.07[b]	4.17±0.98[a]	16.92(0.000)
Fat	3.30±0.98[ab]	1.16±0.37[a]	1.00±0.40[ab]	16.93(0.000)
Crude fibre	14.06±0.32[ab]	10.28±2.91[a]	7.40±2.42[ab]	11.56(0.002)
Moisture	4.42±0.19[ab]	4.76±0.19[a]	4.94±0.09[ab]	11.82(0.001)
Ash	5.18+0.04[ab]	5.07±0.61[a]	5.03±0.05[ab]	9.98(0.003)

Significant difference: Values are presented in mean ± standard deviation (S.D). Values with the same superscripts across the rows differ significantly ($P<0.05$).

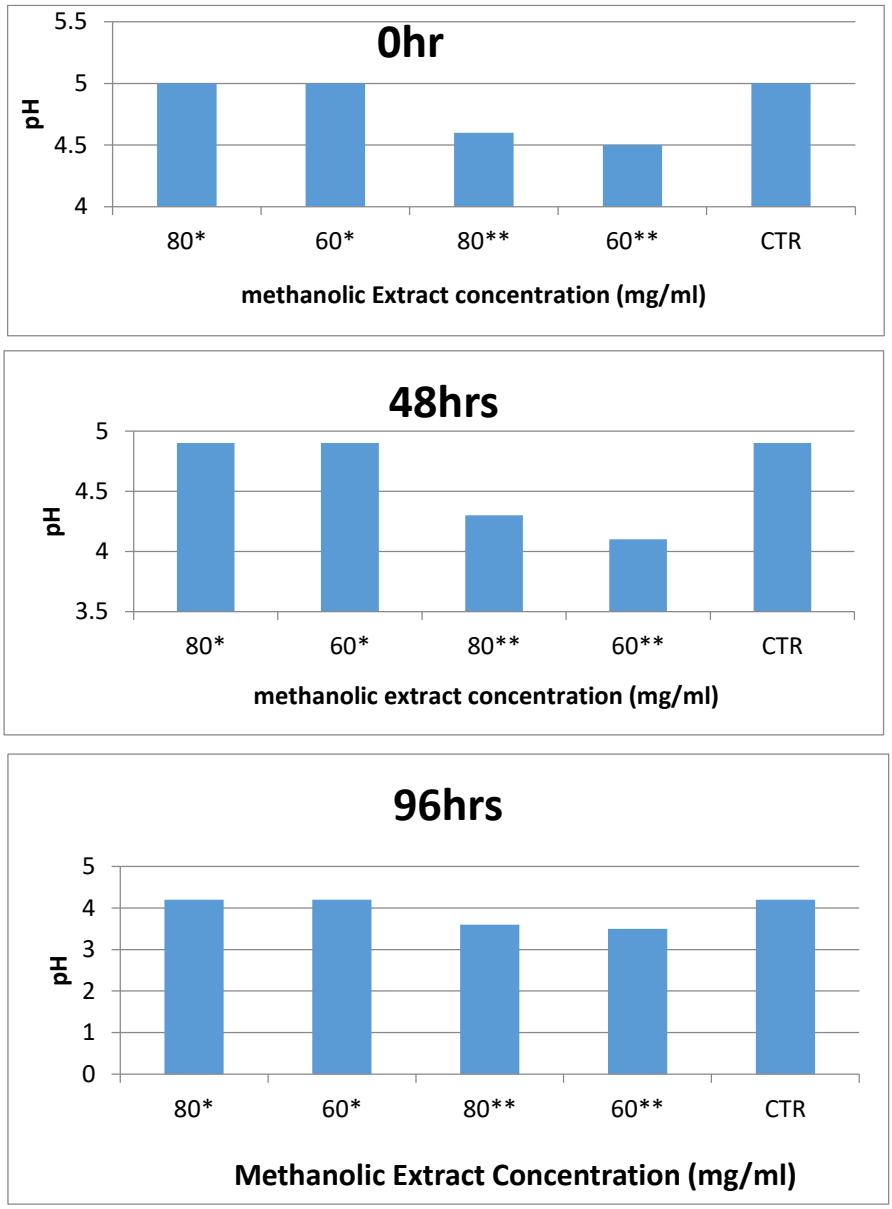

Fig.1 : Changes in pH of Date fruit-Tiger nut drink after treatment with lemon grass methanolic extract at different hours of storage (*, Refrigerated; **, Non-refrigerated)

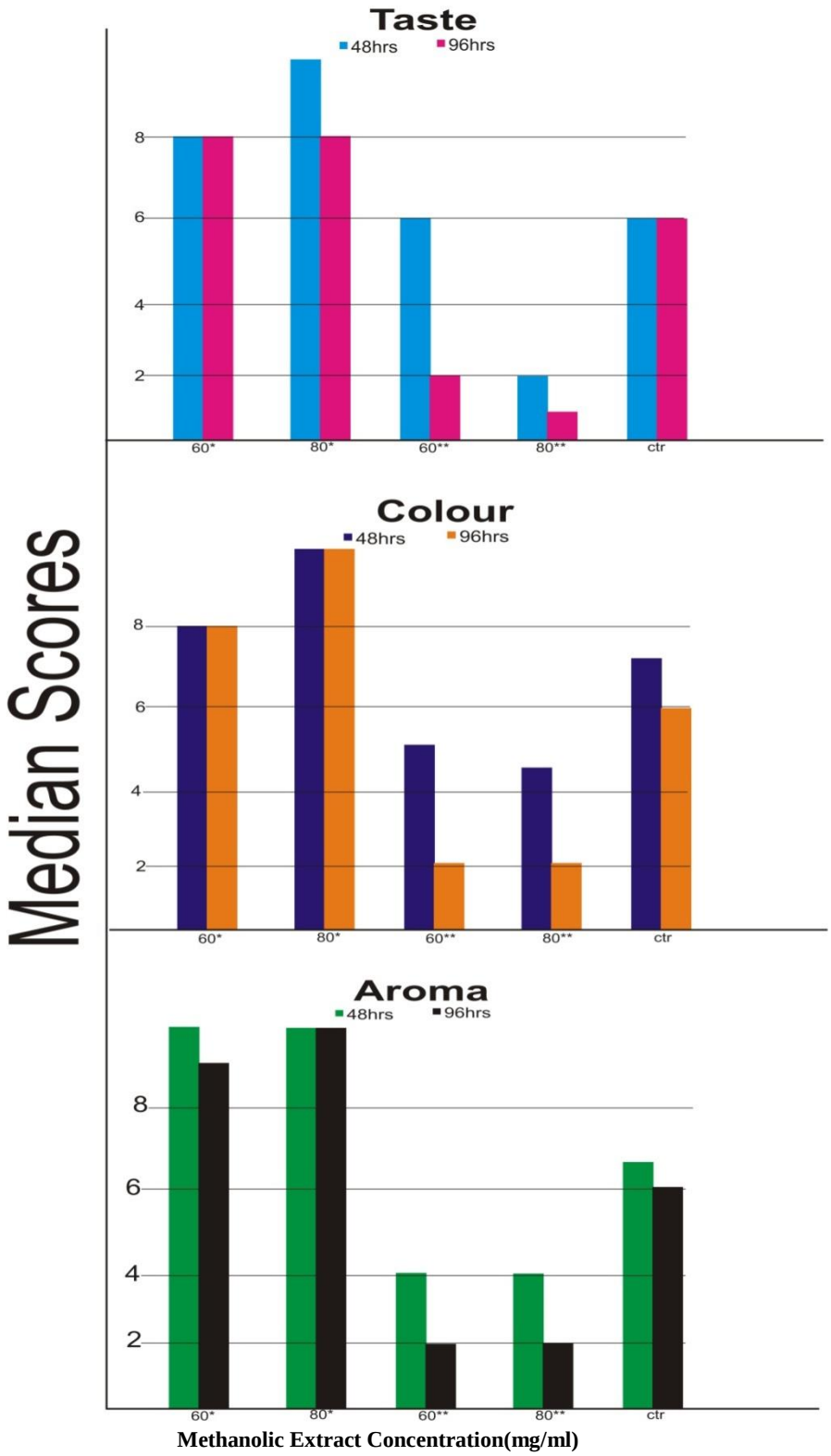

Fig:2 Organoleptic testing of date fruit- tiger nut drinks after treatment with lemon grass methanolic extract at different hours (, Refrigerated; **, Non-refrigerated)**

DISCUSSION

The three organisms isolated and identified from date fruit- tigernut drinks sold in towns across

Delta state were *Beta proteobacterium*VE-7-2-3-2with accession number AB748654 (Agbor, Asaba), *Klesbsiella pneumonia*S-P-N-031.01 with accession number CP092809 (Oleh, Abraka) and *Erwinia chrysanthemi* NZEC135 with accession number EF178670 (Warri, Sapele). *Beta proteobacterium* is a Gram negative, rod shaped, motile and non-motile, facultative anaerobe, catalase positive, oxidase, citrate and indole negative bacteria, (Maheshwari *et al*., 2023). It is one of the most ubiquitous and opportunistic bacteria genera that is found in drinking water, (Aniriban *et al*., 2020). This organism could have gained access to the date-tigernut drink from the water used during the preparation of the drink. *Klebsiella pneumoniae* is a Gram negative, rod shaped, mucoid non- motile, facultative anaerobe, oxidase negative, catalase, indole and citrate negative bacteria, (John *et al.*, 2023). It is mostly found in the soil, surface water, drinking water and industrial effluents. The organisms could have come from the tiger nut, Tiger nuts have contact with the soil since it's a root plant, and the organism may not be removed if not properly washed. It may have come from the water used in the preparation of the drink. *Erwiniachrysanthemi* is a Gram positive, rod shaped, motile, catalase positive, oxidase, indole and citrate negative bacteria, (Vasundhara *et al.,* 2017).It is a plant pathogenic (phytopathogen) enterobacterium responsible for soft rot diseases in a wide range of plant species, (Vasundhara *et al.,* 2017) . Improper sorting of either the date fruits or tiger nuts used for the production of the drink may have enabled the organisms enter the drink. *Klebsiellae pneumonia* was reported by Nuru *et al*., (2022) as a spoilage organism in tiger nut milk, also *Erwinia chrysanthemi* has been reported to cause soft rot disease in the tissues of date fruits, (Ziedan *et al.,*2020). The absence of some of the identified

organisms in some of the locations can be attributed to variations in hygienic habits especially when washings fail to dislodge bacteria already adapted to the preparation vessels. Locations where the drinks are prepared could be unsanitary thereby leading to microbial contamination of the drinks via files and bioaerosols from nearby dumpsites or drainages. For example Adamu *et al* (2017) reported the presence of *Salmonella spp, E. coli, Staphylococcus spp, Proteus spp, Shigella spp and Klebsiella spp* in hawked Zobo drinks (a non-alcoholic local beverage) in Konduga town Borno State Nigeria which he attributed to poor hygienic habits of the producers.

Date fruit- tiger nut drink is highly nutritious, it contains several minerals, macronutrients and healthy elements Nikita, (2023). The nutrients serves as food for microorganisms, and leads to spoilage of the drink and reduction in shelf life. This necessitated the need for preservative treatment in order to extend the shelf life, hence lemon grass water and methanolic extracts were tested as potential preservatives. It was observed that lemon grass methanolic extract had more phytochemicals with higher concentrations than the water extract which helped to inhibit the growth of the isolates found in the date fruit- tiger nut drink. On the other hand the aqueous extract could not inhibit the microorganisms isolated from the drinks because the water failed to extract more active antimicrobial ingredients from the lemon grass. This phytochemical analysis agrees with the analysis by Hafiza *et al.*,(2022) on phytochemical composition and pharmacological potential of lemon grass. They reported that lemon grass extract was used to modulate the gut ecosystem by generating antimicrobial, anti-mutagenic and anti-oxidant responses, thereby increasing the optimum nutrient absorption in the gut system. The result of the study showed that lemon grass methanolic extract limited the deterioration of the drink, although there were variations in the preservative potential of the extracts. Higher concentrations of extracts tended to

limit growth of organisms better than with lower concentrations of extract. Higher concentrations of methanolic extract indicated larger zones of inhibition. This was also observed to vary with the bacteria species responsible for spoilage, and these variation can be attributed to species length of lag phase or approach to stationary phase . Lemon grass extracts has been used over the years in the preservation of foods, Salisu *et al*., (2022) reported that lemon grass oil was used in the preservation of yoghurt. According to Alwani *et al*., (2019) lemon grass water extract was used to preserve a Chinese food Tofu (bean curd) and it was able to increase its shelf life by four days.

There were changes in the nutritional content with increase in time during proximate analysis, this agrees with the report of (Adejuyitan *et al*.., 2018). The residents organisms metabolized the nutrients for energy and growth. .All reductions of nutrients were generally due to metabolic activities of the organisms in the drink (Adejuyitan *et al*., 2018).

The pH for all samples decreased with increase in storage time due to fermentation by microorganisms in the Date-tiger nut drink. A similar observation was reported by Ndukwe *et al*., (2019) on fermentation of Tigernut – milk drink by Lactic Acid Bacteria. Kiky *et al*., (2019) also reported decrease in pH values of banana and red guava juices with increase in time of preservation.. The organoleptic tests carried out showed that taste, colour and aroma of the drinks were generally acceptable by consumers when preserved with lemon grass methanolic extract and refrigeration. Treated date fruit- tiger nut drinks supported by refrigeration gave better results and were more preferred than the non- refrigerated treated drinks. This suggests that refrigeration can be used as a second hurdle in the preservation of the drink. Refrigeration reduces metabolic activities of microorganisms because it slows down the growth of microbes, (Sylvester *et al*., 2016). The addition of refrigeration is in line with hurdle technology which involves the combination

of more than a single approach in food preservation. Sylvester *et al.,* (2016) used ginger extract, in combination with sugar and refrigeration to preserve and extend the shelf life of Zobo drinks(a non-alcoholic local beverage).

The findings indicated that date fruit- tiger nut drink is better preserved by the combination of two hurdles, lemon grass methanolic extract and low temperature. This combined preservative measure extended the shelf life from less than 24hours to 96hours.

CONCLUSION

The results of this research showed that organisms such as *Betaproteoacterium species*, *Klebsiella pneumoniae* and *Erwinia chrysanthemi* strains were found in date fruit -tiger nut drinks sold in Delta state. They were associated with the deterioration of the drink. Glycosides, phenols, tannins, steroids, terpernoids, alkaloids, saponins and flavonoids were the phytochemicals found in lemon grass water and methanolic extract which tested for inhibitory activities against the isolated bacteria. However, the methanolic extract was found to be more inhibitory which indeed limited the growth of the isolates. The inhibitory activities were indicated by the extent of loss of nutrients. The losses did not make the drink unacceptable by respondents based on taste, colour and aroma. Refrigeration enhanced preservation when it was combined with the methanolic extract, reminiscent of hurdle technology because two hurdles (extract and low temperature) were used. In conclusion, lemon grass methanolic extract in combination with refrigeration extended the shelf life of date fruit- tiger nut drink from less than 24hours to 96hours.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests**.**

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