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Original Article

Effects of Leaf Extracts of Selected Plants on Quality of Stored *Citrus sinensis* (Sweet Orange) Juice

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Abstract

Reduction in the quality of fruits during storage has been a concern to the consumers and the effect can be felt on the economy of developing countries. Leaves of plants such as *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellii* have been documented as food packaging materials in West Africa. Based on this, the quality of stored sweet orange juice was investigated using ethanolic extracts of leaves of *C. indica*, *M. macrostachyum* and *T. daniellii* to enhance the shelf life of the juice. The extracts were used to assess the quality of juice for 30 days using quantitative parameters such as total soluble solid, browning potential, pH, microbial analysis and turbidity at 4 °C and at room temperature (27-31 °C). The qualitative and quantitative phytochemical constituents of the extracts were determined. The extracts' toxicity was determined using Brine shrimp. The quality assessment evidently revealed that the freshly squeezed orange juice with the extracts possess tolerable activity to enhance the shelf life of orange juice. The leaf extract of *M. macrostachyum* had the highest preservation rate on the juice after 30 days. The qualitative phytochemical screening revealed the presence of alkaloid, tannin, saponins, flavonoids, steroids and terpenoids in the three plants tested. The quantitative phytochemical analysis of the most active extracts in the three plants revealed that *M. macrostachum* had the highest contents of alkaloids (107.48 mg/g) and flavonoids (56.92 mg/g).The study showed that the extracts were non-lethal on Brine shrimp. This study ascertained the potential preservative qualities of the test plants for enhancing the shelf-life of orange juice.

Keywords: leaf, orange juice, plant extracts, quality, shelf life

Introduction

Citrus sinensis is a member of the Citrus family, along with mandarins (tangerines), lemons, limes and grapefruit (Etebu and Nwauzoma, 2014). The fruit of *Citrus sinensis* forms a significant part of human diet and is usually regarded as a good source of food (Etebu and Nwauzoma, 2014). The fruits, which are the succulent part of *Citrus sinensis* are characterized by a sweet or acid taste and distinct flavour. The juice, which is directly extracted from the fruits of *C. sinensis* is of high nutritional value and of significant contribution to the health of humans (Bevilacqua *et al*., 2011).

Food products which are perishable, once introduced into the market, requires protection against spoilage during storage and distribution (Bhat *et al*., 2012). The longevity and extension of the shelf life of food involves the need to make it to be stable and safe for consumption through preservation (Brul and Coote, 1999; Adegunloye *et al*., 2006).

The reduction in quality of food might be ascribed to the activities of microorganisms. Preservation is a process by which enhancing agents are used to keep food from deteriorating. This enables the food to retain freshness, texture, colour, nutritional value and flavour (Gould, 1999).

Microbial spoilage of food decreases the shelf life of the food, which may result in substantial economic losses and potential health hazards to the consumers (Grillo and Lawal, 2010).

During production of food, it is crucial that proper measures are taken to ensure safety and stability of the product during its whole shelf-life (Brul and Coote, 1999). Food preservation is becoming an increasing issue to the survival and well-being of humans. The essence of preservation, which can be attached to food safety, is evidenced by the consumer preference for naturally

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occurring preservatives and this arose due to the toxicological problems that have prevailed due to the use of synthesized preservatives and additives.

The need for food products that are free from contamination and toxicological problem is driving a trend towards natural preservatives (Gould, 1999; Brul and Coote, 1999; Adegunloye *et al*., 2006; Dolf De Rovira, 2008; Bhat *et al*., 2012; Adeogun *et al*., 2016). Traditionally, many natural substances, such as sugar, salt, vinegar and alcohol have been used as natural preservatives. Many herbs and spices have also been screened for antimicrobial activity, but only few have been exploited as food preservatives on a commercial basis (Adekunle, 2000a; Adekunle, 2000b; Ogbulie *et al*., 2007; Tiwari *et al*., 2009; El-Mahmood, 2010).

The use of leaves to preserve food in Nigeria is quite popular and acceptable. It uses cut across the teeming populace in every geographical part of the country. Depending on location and locality, various types of plant leaves are used as wrappers, e.g. *Musa sapentium*, *Musa paradisiaca*, *Cola nitida*, *Cola acuminata*, *Piliostigma reticulatum*, *Theobromae cacao*, *Colocasia esculenta*, *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellii* leaves (Adegunloye *et al*., 2006), to package and present foods to clientele. The use of these leaves is ancient in various ways depending on the traditions of population; thus its basis cannot be easily ascertained, but a cursory look at these leaves reveal that they all have large surface areas, thus they can be used to hold/package/wrap large volumes of food (Ojekale *et al*., 2007).

Thaumatococcus daniellii (Benn.) Benth. belongs to family Marantaceae; it grows throughout the hot, humid tropical rain forest and coastal zone of West Africa. Its natural habitat is the undergrowth of forest trees. Large quantities of its fruit are consumed by the local people to sweeten over fermented palm wine and sour foods. From the aril of *T. danielliii*, an intensely sweet, non-toxic and heat stable protein – thaumatin - is extracted. This is used as sweetener or taste modifier in beverages, desserts, chewing gums and pet foods (Onwueme *et al*., 1979).

Megaphrynium macrostachyum (Benth.) Milne-Redh belongs to family Marantaceae. The leaf is papery and is much used as a roof-thatch. The leaf-petiole is often split and dyed for mat making in Nigeria (Milne-Redhead, 1952).

Canna indica Linn. belongs to the family Cannaceae. They are widely spread in tropical Africa, chiefly in the forest zone, near villages and along the roads. They are herbaceous plants, rhizomatous, forming small to large monotypic stands and the leaves are green. The leaves are used for wrapping food. The seed are made into necklaces and rosaries; children use them as shot in pop-guns (Dalziel, 1937).

The objective of the current study was to analyse the effect of ethanol extracts from leaves of *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellii* on quality of stored *Citrus sinensis* (sweet orange) juice.

Materials and Methods

Collection of the plant leaves

The leaves of *Canna indica*, *Megaphrynium macrostachyum* and *Thaumatococcus daniellli* were collected from the Botanical Garden, University of Lagos. The plants were authenticated at Lagos University Herbarium.

Milling of plant samples

The leaves of *C. indica*, *M. macrostachyum* and *T. daniellii* were shade-dried for ten days and ground using a specialized grinding machine at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos.

Extraction of plant extracts

Two hundred grams of ground leaves of *C. indica*, *M. macrostachyum* and *T. daniellii* were weighed differently and soaked with 400 ml of ethanol in different conical flasks. These were corked with adsorbent wool wrapped with aluminium foil and shook on Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 72 hrs. The extracts were filtered into sterilized conical flasks using Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure 40 °C using rotary evaporator (Cole Parmer SB 1100, Shangai, China). The solvent used was of high analytical grade (Merck Chemicals (PTY), Wadeville, South Africa).

Comparative study of the shelf-life of treated and untreated orange juice

The juice was prepared at room temperature in the ultra-violet room of the postgraduate research laboratory of Department of Botany, University of Lagos. Two millilitres of reconstituted extracts (*C. indica*, *M. macrostachyum* and *T. daniellii*) with Dimethyl sulfoxide (DMSO) was added to the prepared juice in a tube with lid. This was carried out in an aseptic condition to curtail the growth of contaminants that can accelerate spoilage. Preservative (1.25 mg/ml ascorbic acid) were added to another set of prepared juice. The juice alone was also prepared along with branded juice. These were aseptically and quickly opened and poured into tube with lids, all tubes were assessed via the following parametres: pH, total soluble solid, browning potential, turbidity, visual assessment and microbial load count using Martin-Diana *et al*. (2009) and Gil *et al*. (2006) as guide. The tubes were stored for 30 days at 4 °C and room temperature (27-31 °C); assessed at intervals (day 0, 7, 14 and 30). The samples for analyses were prepared in triplicates.

pH analysis

The pH of 20 mL orange juice was determined at room temperature and constant agitation using a pH-meter (model 420A, Orion, USA). It was expressed as the negative logarithm of the hydrogen ion concentration in a solution.

Total soluble solids 'TSS' (° Brix)

The Brix was determined by measurement of the refraction index with a refractometer (Bellingham and Stanley, England) at room temperature. Refractive index

was recorded and expressed as °Brix. Measurements were performed at room temperature.

Browning potential

Ten millilitres of fresh orange juice were treated with ethanol for 60 min and then centrifuged at 4,830 ×g at 10 °C for 10 min, retaining the supernatants. After, a further amount of ethanol was added to bring the final volume to 25 mL. Absorbance at 320 nm of aliquots of these extracts was measured. The results were expressed as absorbance units (AU) mL^{-1} fresh orange juice.

Turbidity

The turbidity of each sample of juice was measured using a direct reading spectrophotometer (model DR/2000, Hach, USA). The wavelength of the instrument was brought to 810 nm and deionised water used as a blank. The measurements of the samples of orange juice were done in triplicate with a solution of 1:25 (juice/water), to work within the detectable range. The results were given in milligrams of suspended solids per litre of solution.

Microbial load counting

Microbial analyses were carried out on the samples at regular intervals through the storage period. One millilitre of decimal dilution of orange juice samples was pipetted to petri dishes (25 ml of fresh orange juice was homogenised in 225 ml of 0.1% (w/v) sterile peptone water with a homogenizer). The total number of viable cells in the suspension was calculated by the methylene blue method of Lee *et al*. (1981) using a hemocytometre. A drop of the suspension was placed on the ruled area of a clean Neubauer counting chamber. A cover slip was placed first and the cells were permitted to run underneath by capillary action from Pasteur pipette tip. The counting cell was allowed to stand for 10 min to permit the viable cells to settle into the same focal plane as much as possible. Using a light microscope, the fungal spores were counted with 10x ocular and 5 mm objective and this was done for the total grid in triplicate. The cell count was calculated thus:

Viable cells/ml = Number of cells in total grid × dilution factor × 10^{-4}.

Cytotoxicity test using brine shrimp

The brine shrimps cytotoxicity test using the larvae of brine shrimp nauplii, *Artemia salina* L., was carried out using the method adopted by Adeogun *et al*. (2016). 1 mg ml^{-1} concentration of reconstituted extracts was prepared from the original stock solution of *C. indica*, *M. macrostachyum* and *T. daniellii* that was used for comparative shelf life study. The oils were further diluted to final concentrations of 20, 40, 60, 80 and 100 $\mu g ml^{-1}$ in different vials using DMSO. Ten nauplii were transferred into each vial using Pasteur pipettes and were not given food because hatched brine shrimp can survive up to 48 hours without food, as they still feed on their yolk during this period. The control vials contained normal saline solution and dimethyl sulfoxide (DMSO) separately. The experiments were done in triplicate. After 24 hours of incubation, the content of each vial was transferred into 65 mm Petri dish and examined; the number of surviving larvae was counted and the percentage of mortality was evaluated. Larvae were considered dead if they did not exhibit any form of movement during several seconds of observation. Extracts are regarded as non-toxic if its LC50 is greater than 100 $\mu g ml^{-1}$ in brine shrimp lethality assay.

Qualitative and quantitative phytochemical screening

Ethanol extracts of *C. indica*, *M. macrostachyum* and *T. daniellii* were subjected to qualitative preliminary phytochemical screening and quantitative analysis of the phytochemical constituents by adopting the methods described by Adeogun *et al*. (2016).

Statistical analysis

The data obtained from pH, total soluble solid, browning potential, turbidity and microbial load count analysis were analysed using SPSS 20.0 (Leech *et al*., 2008).

Results

Authenticated plant samples

The accession numbers of deposited test plant leaves are LUH 6087, 6088 and 6089 for *T. danielli*, *M. macrostachyum* and *C. indica* respectively.

Comparative study of the shelf-life

The effects of the parameters of orange juice prepared with extracts of *T. daniellii*, *C. indica* and *M. macrostachyum* in separate tubes, prepared juice with preservative (ascorbic acid), prepared orange juice only and branded juice on days of storage and temperature ranges (4 °C and 27-31 °C) are depicted in Table 1. The results showed that there was variation in values as the day of storage increased from day 0 to day 30 taking into cognizance the temperature used to store the samples. The pH values of the juice stored at 4 °C with *T. daniellii* were 4.25 ± 0.003 at day 0 and 4.44 ± 0.003 at day 30, while the pH values at room temperature were 4.65 ± 0.018 at day 0, while 4.75 ± 0.013 for day 30. The values of browning potential of the juice stored at 4 °C with *C. indica* leaf extract were 0.98 ± 0.003 at day 0 and 1.65 ± 0.02 at day 30, while the browning potential values at room temperature were 1.22 ± 0.001 at day 0, while 1.43 ± 0.002 for day 30. The values of the turbidity state of stored juice with *M. macrostachyum* at 4 °C were 0.28 ± 0.003 at day 0 and 0.24 ± 0.002 at day 30, while the turbidity values at room temperature were 0.36 ± 0.100 at day 0 and 0.24 ± 0.002 for day 30.

Qualitative phytochemical screening and quantitative analysis determination

The qualitative phytochemical constituents of *C. indica*, *M. macrostachyum* and *T. daniellii* leaf extracts are shown in Table 2a signified the presence of alkaloids in M. macrostachyum and *T. daniellii*, tannins in *C. indica* and *T. daniellii* and saponins in the 3 extracts.

The results of the quantitative phytochemical constituents of extracts of *C. indica*, *M. macrostachyum* and *T. daniellii* leaves is depicted in Table 2b.

Table 1. Quality parameters of stored fresh orange juice for 30 days

Juice Type	Day	pH		Browning Potential		Turbidity		Total Soluble Solid	
		4 °C	RT (27 -31 °C)	4 °C	RT (27-31°C)	4 °C	RT (27-31°C)	4 °C	RT (27-31°C)
TDL + Juice	Day 0	4.25±0.003	4.65±0.018	1.32±0.003	1.62±0.019	0.29±0.002	0.30±0.027	10.100	10.100
	Day 7	4.28±0.004	4.50±0.033	1.42±0.006	1.74±0.005	0.30±0.000	0.31±0.003	10.100	10.100
	Day 14	4.32±0.002	4.77±0.028	1.47±0.005	1.65±0.003	0.29±0.001	0.34±0.003	10.800	10.800
	Day 30	4.44±0.003	4.75±0.013	1.59±0.002	1.64±0.001	0.29±0.003	0.29±0.003	10.800	10.700
CIL + Juice	Day 0	4.34±0.000	4.64±0.012	0.98±0.003	1.65±0.02	0.34±0.003	0.34±0.070	10.100	10.100
	Day 7	4.39±0.003	4.59±0.004	1.11±0.002	1.65±0.03	0.29±0.001	0.32±0.002	10.100	10.100
	Day 14	4.29±0.12	4.82±0.010	1.21±0.003	1.76±0.004	0.36±0.000	0.36±0.002	10.900	10.700
	Day 30	4.43±0.02	4.67±0.02	1.22±0.001	1.43±0.002	0.35±0.002	0.35±0.002	10.900	10.700
MML + Juice	Day 0	4.28±0.003	4.47±0.002	1.06±0.003	1.65±0.006	0.28±0.003	0.36±0.100	10.100	10.100
	Day 7	4.31±0.003	4.25±0.009	1.34±0.004	1.77±0.004	0.21±0.002	0.31±0.003	10.100	10.100
	Day 14	4.37±0.003	4.70±0.001	1.48±0.004	1.79±0.000	0.26±0.003	0.34±0.002	10.800	10.700
	Day 30	4.44±0.002	4.63±0.05	1.62±0.004	1.63±0.001	0.24±0.002	0.24±0.002	10.800	10.700
Ascorbic Acid	Day 0	4.29±0.003	4.66±0.04	0.77±0.003	1.49±0.029	0.32±0.003	0.42±0.030	10.100	10.100
	Day 7	4.33±0.003	4.66±0.006	0.84±0.01	1.33±0.004	0.38±0.003	0.30±0.003	10.100	10.100
	Day 14	4.54±0.003	4.44±0.01	0.84±0.003	1.64±0.02	0.34±0.002	0.33±0.002	10.060	10.030
	Day 30	4.72±0.004	4.28±0.02	1.92±0.006	1.28±0.001	0.37±0.003	0.17±0.002	10.050	10.030
Fresh Orange Juice	Day 0	4.19±0.003	4.66±0.02	1.18±0.003	1.54±0.04	0.12±0.003	0.20±0.120	10.050	10.050
	Day 7	4.29±0.003	4.59±0.003	1.64±0.003	1.36±0.003	0.22±0.003	0.10±0.002	10.050	10.040
	Day 14	4.42±0.003	4.40±0.02	1.69±0.001	1.60±0.002	0.22±0.000	0.15±0.005	10.020	10.030
	Day 30	4.67±0.002	4.47±0.03	1.78±0.01	1.24±0.001	0.34±0.002	0.19±0.003	9.400	10.000
Branded Juice	Day 0	3.71±0.003	0.27±0.001	0.27±0.001	0.95±0.10	0.14±0.003	0.36±0.035	10.100	10.100
	Day 7	3.99±0.003	3.71±0.02	0.74±0.002	0.74±0.03	0.22±0.003	0.24±0.003	10.100	10.090
	Day 14	4.44±0.003	3.72±0.03	0.76±0.004	0.68±0.004	0.27±0.003	0.15±0.002	10.060	10.080
	Day 30	4.65±0.003	4.53±0.03	0.82±0.002	0.65±0.002	0.29±0.002[e]	0.02±0.002[f]	10.060	10.070

TDL: *Thaumatococcus daniellii* Leaf, CIL: *Canna indica* Leaf, MML: *Megaphrynium macrostachyum* Leaf

Table 2a. Qualitative phytochemical screening of leaf extracts of test plants

S/No.	Phytoconstituent	Leaf extract		
		Canna indica	*Megaphrynium macrostachyum*	*Thaumatococcus daniellii*
1	Alkaloid	-	+	+
2	Tannins	+	-	+
3	Phlobatannins	-	-	-
4	Saponins	+	+	+
5	Flavonoids	-	+	+
6	Steroids	-	-	-
7	Terpenoids	-	-	-
8	Cardiac glycosides	-	-	-

Table 2b. Quantitative phytochemical determination of leaf extracts of test plants

Leaf extract	Phytoconstituents (mg/100 g)			
	Alkaloids	Tannins	Saponins	Flavonoids
Canna indica	-	17.81	17.55	-
Megaphrynium macrostachyum	107.48	-	7.66	56.92
Thaumatococcus daniellii	98.96	81.77	22.92	35.03

Table 3. Cytotoxicity assay of leaf extracts of test plants on Brine shrimp

Conc. (µg/ml)	Total No.	Control		Ethanol extracts		
		DMSO	Saline water	*C. indica*	*M. megaphrynium*	*T. daniellii*
20	10	0	0	2	1	1
40	10	0	0	2	1	3
60	10	0	0	1	1	2
80	10	0	0	3	3	2
100	10	0	0	3	5	4
LC_{50}				233.03 µg/ml	107.21 µg/ml	281.12 µg/ml

Cytotoxicity activities of extracts of C. indica, M. megaphrynium and T. danielli

The lethality concentration of extracts required to kill fifty percent of population of Brine Shrimps is depicted in Table 3. It shows that the ethanol extracts of *C. indica*, *M. megaphrynium* and *T. daniellii* of 233 µg/ml, 107.21 µg/ml and 281.12 µg/ml respectively were not toxic because they were above general toxicity test agreement of LD50. A plant is assumed to be safe when its LD50 is above the general toxicity agreement of 100 µg/ml.

Discussion

The increase in consumption of fruit juices has significant positive influence on third world economy and this can be affected by microbial attacks, which reduce their availability in the markets (Aneja *et al*., 2014). The need to enhance the shelf-life of orange juice and ward off the influence of microbes raised the need for preservation. The synthetic antimicrobial agents and chemically synthesized food preservatives have been used since antiquity as an effective method for controlling food spoilage. Nowadays, consumers concern toward chemical preservatives paves way for an increasing interest on some natural antimicrobials that can be obtained from plant extracts (Perricone *et al*., 2015).

The ethanol extract of individual test plant assayed for quality assessment of the orange juice at two different storage conditions: 4 °C and 27-31 °C showed that the juice depreciated in quality. There was disparity in the level of deterioration of the juice treated with the extracts compared with the juice without the extracts (fresh juice only, branded juice and fresh juice plus ascorbic acid). There was higher pH values in the juice treated with the extracts compared with the untreated juice and this can be ascribed to the addition of the extracts. The increase was for 30 days of storage. The increase in pH values of juice without extracts compared with the one with the extract can be ascribed to the capacity of the extracts to reduce fruit acidity based on its acid-binding properties (Cortes *et al*., 2008). The observation also supported by previous work by Martin-Diana *et al*. (2009) even though the study dwelt on chitosan as agent of preservation. Abd and Niamah (2012) reported that the increase in pH values of orange juice treated with chitosan compared with untreated orange juice might be due to the capacity of chitosan to reduce fruit juice acidity based on its acid-binding properties. They also pointed out that when the pH is lower than 6.5, chitosan carries a positive charge along its backbone which implies a decrease in the buffering property of the juice. Such effect was not observed in the hereby study, as all samples showed a similar pH increase over storage time. The current study recorded higher values in pH range compared with previous works of Martin Diana *et al*. (2009) and Abd and Niamah (2012).

The activity of the extracts with juice compared to fresh juice only can be ascribed to the ability of the positive charged polysaccharides in the extracts to coagulate suspended solids and this increased the flocculation capacity of the extracts, which aids the binding of the negatively charged sugar (Martin-Diana *et al*., 2009). The Brix values of the total soluble solids of the juice observed in the current study were higher than those reported by several authors on fruit juices enhanced with natural antimicrobials (Cortes *et al*., 2008; Martin-Diana *et al*., 2009; Abd and Niamah, 2012). This might be attributed to different factors such as nutritional composition of the fruit juices, genetics, growing environment, management practises and maturity time of the fruits.

The results of the study also showed that browning potential of the juice without the addition of the extract rapidly reduced, while the browning potential of juice with individual extract had slow reduction. Higher increase of the browning potential values of fresh juice compared with the one with the extract can be attributed to enzymatic properties of the extracts and the control of browning could be associated with the capacity to coagulate solids to which browning-related enzymes are bound (Abd and Niamah, 2012; Martin-Diana *et al*., 2009). Martin-Diana *et al*. (2009) explained that browning reduction in oranges clarified with chitosan could be as a result of antioxidant capacity of chitosan which is similar to capacity associated with phenolic compounds. The similarity with phenolic compounds could explain the anti-oxidative property.

The increase in turbidity values of the juice with extract as the days of storage increased is at variance with the increase in orange juice without treatment, which had higher increase than the juice with the extract. Fresh orange juice is usually cloudy and this is an acceptable and desirable component of the juice (Sin *et al*., 2006). The turbidity in the juice is mainly caused by the polysaccharides present in the juice (Grassin and Fauquembergue, 1996). The increase in turbidity values of the juice with extract during storage disagree with earlier works of Abd and Niamah (2012) and Martin-Diana *et al*. (2009) that reported the reduction in turbidity values during storage using chitosan.

The microbial count of juice stored for a period of 30 days indicated that the extracts also delayed growth of microbes in orange juice compared to orange juice without the extracts portending a positive effect for the extension of the shelf-life. The reduction in load of microbes in juice with the extract compared with untreated juice without extract suggested an active antimicrobial effect of the extracts occurring over storage time. The mode of action of the extracts on the growth of fungi might be due to the interaction of extracts with membranes or cell wall components, the mechanism underlying the inhibition of bacterial growth is thought to be cationically charged amino-group present in the extracts which may suppress fungal growth by impairing the exchanges with the medium, chelating transition meal ions and inhibiting enzymes due to the positive charge, resulting in increased permeability of the membranes and leakage of cell material from tissue or due to water binding capacity and inhibition of various constituents within the extracts (Jung *et al*., 1999; Liman *et al*., 2011). The presence of the extract might inhibit the formation of cell wall resulting in the death of the fungi (Adekunle *et al*., 2005; Adekunle and Ikumapayi, 2006).

The pH, turbidity, total soluble solids, browning potential and microbial load values of the juice when stored at 4 °C had higher delay compared to storage at room temperature (27 °C-31 °C) which agrees with Marcilla *et al*. (2006) that noticed lowest off-flavour value of orange. The activity of the *Megaphrynium macrostachyum* extract was the highest on the parameters assayed.

There is a general toxicity test agreement that LC50 required to kill 50% of the population of brine shrimp above 100 µg/ml is non-toxic, while below 100 µg/ ml is indicative of toxicity (Adeogun *et al*., 2016). This study showed that the ethanol extracts from *C. indica*, *M. macrostachyum* and *T. daniellii* leaves displayed non-lethality activity against Brine shrimp nauplii with LC50 of 233.03 µg/ml, 107.21 µg/ml and 281.12 µg/ml respectively. The non-lethality of the leaf extracts of the three plants indicated that there were less harmful compounds presence in the ethanol extracts of leaves of *C. indica*, *M. macrostachyum* and *T. daniellii*. The results on cytotoxicity test on these plants validate earlier reports of Moshi *et al*. (2010) and Adeogun *et al*. (2016) and on the non-lethality of these plants. They reported that the activity of these extracts were non-lethal.

The hereby study was able to establish that the bioactive compounds present in leaf extracts of the test plants might be responsible for the enhancement of shelf life of test orange juice. The activity of the extracts showed that the test plants' leaves possess preservative properties. Qualitative phytochemical screening of the extracts revealed the presence of alkaloids, tannins, saponins and flavonoids. The quantitative phytochemical determination of the ethanol extracts of the three plants revealed high yield of tannins and saponins in *C. indica*, alkaloids and flavonoids in *M. macrostachum* and alkaloids, flavonoids, saponins and tannins in *T. daniellii*. The activity of the test plant' leaves confirmed and laid credence to several works that have identified the role of plant constituents as agents of preservation in the inhibition of food spoilage fungi (Rasooli, 2007; Corsetti and Settanmi, 2008; Efterpi *et al*., 2012; Lucera *et al*., 2012; Adeogun *et al*., 2016).

Conclusions

The study reported the potential of leaf extracts of *C. indica*, *M. macrostachyum* and *T. daniellii* as preservative agents. The in-vivo assessment of leaf extracts of the three plants on orange juice showed that they have the capability to enhance the quality of orange juice. The phyto-constituents in the extracts might be responsible for the micro-activity. There will be need to purify and elucidate the extracts to ascertain the active compounds that contribute to the enhancement of shelf life of orange juice. The current study also contribute to the drive towards the use of natural antimicrobials as preservation agents and also cement the notion that some plants are non-toxic compared to acknowledged side effects of synthetic additives.

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