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PRESERVATIVE ACTIVITIES OF AQUEOUS AND ETHANOL EXTRACTS OF *PARKIA BIGLOBOSA* (JACQ.) POD ON GINGER DRINK

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ABSTRACT
The study was aimed at determining the phytochemical, antimicrobial and preservative properties of aqueous (PAE) and ethanol (PEE) extracts of Parkia biglobosa pods on fresh ginger drink. Result of the phytochemical screening revealed the presence of phytochemicals in various combinations, while the antimicrobial assay revealed a broad spectrum of activity on the tested bacterial and fungal isolates at various concentrations. The GC-MS analysis of the two extracts (PAE and PEE) revealed the presence of compounds such as stearic acid in PAE and n-Dodecan-1-ol in PEE. The sensory evaluation of the preserved ginger drink using different concentrations of PAE and PEE indicates acceptance of the drink treated with 1% PEE most, with a percentage likeness of 70.0% compared to untreated drink with percentage likeness of 11.0% after 102 hours of storage. Sodium benzoate (a commercial preservative) had percentage likeness of 52.2% after 102 hours of storage, therefore, this study has demonstrated evidence of the prospects of aqueous and ethanol extracts of P. biglobosa as plant based food preservatives with higher degree of acceptability.
Keywords: Parkia biglobosa,Preservative, Ginger, Drink, Phytochemical

INTRODUCTION

The general idea of preserving food is to increase its shelf life, maintain its integrity and increase marketability (Bumpres, 2010). Recent interest on the preservative activity of the plant on foods has necessitated conduct of research on the topic. Some of the preliminary results include, the ability of *Parkia* pods aqueous extracts to sanitize the surfaces of fruits and vegetables as reported by Bukar and Magashi (2008). The phytochemical constituents and antimicrobial activity of the extract against some food-borne pathogens and spoilage microorganisms has also been reported by Bukar *et al.*(2010). Hence, the need to further determine its preservation potential in different food system. Ginger (*Zingiber officinale*) is a very important and highly valuable crop with a variety of applications in confectionaries, pharmaceuticals and beverage production (Van-Oss, 1970). The drink is composed of water, protein, fat, ash, fiber, volatile oil and resinous matter (David, 1970). Development in ginger processing shows that it can be processed into ginger soft drink using the fresh rhizome (Pigman and Horton, 1971; Ahammed *et al.*, 2014). The product was found to have an average shelf life of 24hrs (Ebewele, 1981), therefore, this study is aimed at determining the phytochemical, antimicrobial and preservative properties of aqueous and ethanol extracts of *P. biglobosa* pod on Ginger drink.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Pods of *Parkia biglobosa* were sourced from Gwarzo LGA, in Kano state.All samples were first identified in the field using standard keys and descriptions (Bukar, 2012*)*. Further confirmation and authentication of *P. biglobosa* pods (BUKAH/0262) was carried out at the Herbarium of the Department of Biological Sciences, Bayero University, Kano. All samples were processed according to methods prescribed by Mukhtar and Tukur (1999).

Extraction

The *Parkia* pods were grinded and sieved, and two portions (100g) each were subjected to ethanol and aqueous extraction in ratio 1g: 10ml (solute: solvent) according to method described by Fatope and Hamisu (1993).

Phytochemical Screening

Phytochemical analysis for the detection of alkaloids, saponins, tanins, flavonoid, phenolic compounds and reducing sugars were carried out

according to methods described by Ayoola *et al*. (2008); Ciulci(1994); Sofowara (1993); Trease and Evans, (2008).

Sources of Microorganisms

Staphylococcus aureus, Escherichia coli, Salmonella spp., *Shigella*spp., *Pseudomonas aeruginosa* and *Bacillus cereus* were bacteria isolates, while *Aspergillus fumigatus, Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp and *Mucor* spp were fungal isolates obtained from staple foods and drinks such as ginger drink, raw beef, boiled rice, bread, and fresh tomatoes.

Standardization of Innoculum

Density of bacterial suspension was adjusted to 0.5 McFarland's standard (Barium sulphate solution) as described by Cheesebrough (2002). Aloopful of fungal spores from an overgrown plate was taken and shaken thoroughly in 10ml of 20% Tween 80 solution to obtain a uniform spore suspension (Murugan *et al.,* 2007).

Agar Well Diffusion Method

Antimicrobial and antifungal activity for *Parkiabiglobosa* aqueous and ethanol extracts was determined by the well diffusion method as described by Esimone*et al*. (2012). Four concentrations were prepared from the stock solution such that 0.01ml was placed in each well equivalent to 4000µg/ml, 6000 µg/ml, 8000 µg/ml and 10,000 µg/ml respectively. The assessment of antibacterial and antifungal activity was based on measurement of the diameter of the inhibition zones formed around the wells (Murugan *et al.,* 2007).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the active concentrations were determined using the tube dilution technique described by Akinyemi *et al*. (2005); Lima *et al*.(1993); Shamsuddeen *et al,*.(2014).

Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC)

Sterile Mueller-Hinton agar plates were inoculated with samples from the MIC tubes that showed no visible bacterial growth.The lowest concentration in which no growth occurred on the medium was considered as the MBC (Lima *et al*., 1993)

Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis

GC-MS analysis was conducted on the aqueous and ethanol extracts of *P. biglobosa* to determine the constituent compounds.

Preparation of Ginger Drink

The preparation of ginger drink was carried out according to method described by Abdulkareem *et al*. (2011), where fresh ginger rhizomes were properly sorted to remove undesirable particles. It was then washed in clean water and 10g of the fresh rhizome was weighed and crushed in pulverizer with water added for easy crushing. Muslin cloth was used for filtration, while the raffinate is discarded. The filtrate was allowed to stand for 2hrs to enable the starch settle (sediment). Mother liquor of the crushed ginger rhizomes were decanted from the sediment starch. The pure ginger extract was then blended with 100g of sugar and two (2) fresh lime. No flavor was added.

Preservation of Ginger Drink

Organoleptic parameters were assessed by a panel of ten (10) judges and scores were graded on a Hedonic scale (Peryam, 1998; Bukar, 2012). The panellists were selected from Lecturers, students and lab attendants of the department of Microbiology, Bayero University, kano.

A set of four 20 ml capacity sterile bottles were filled with 10ml of the ginger drink each. Four different treatments were carried out as follows:

A – Untreated ginger drink with no additive.

B - Treatment with 0.5% w/v of Extracts

C – Treatment with 1.0% w/v of Extracts

D - Treatment with 0.5% w/v sodium benzoate (conventional preservative for drinks).

Number of days taken for each treatment to spoil was recorded. Organoleptic parameters namely, taste, texture, odor, and color were assessed by a panel of judges. The score were graded on a Hedonic scale and after six (6) days, the treatment most preferred by the judges was determined. The preservation test was carried out on commercially prepared ginger drink and both *Parkia biglobosa* Aqueous Extract (PWE) and *Parkia biglobosa* Ethanol Extract (PEE) were used for this test (Bukar, 2012).

Determination of pH and Temperature of Ginger.

Five milliliters (5ml) of each sample A, B, C, D were dispensed into separate clean sterile beakers. pH and temperature were recorded daily using a portable Jenway digital pH meter and a thermometer was used to measure the temperature of the study area daily (Bukar, 2012).

Statistical Analysis

Data generated from the sanitizing activity tests and the scores generated based on the assessment of judges (from the preservative experiments) using Hedonic scale, were statistically analysed using Analysis of Variance (ANOVA) at 5% probability level using the software package developed by Microsoft corporation. Where significant difference was observed, the means separation was carried out using least significant difference (LSD).

RESULTS AND DISCUSSION

The aqueous extract of *P. biglobosa* yielded more extract (39.2g) than the ethanol extract (33.8g). The two extracts of *P.biglobosa* were observed to be dark brown in colour with the aqueous extract (PAE) being astringent, while the ethanol extract (PEE) was slightly astringent respectively (Table 1). It could be observed that distilled water, being a more polar solvent yeilded more extracts of *P.biglobosa* as compared to ethanol. This is in line with the findings of Udobi and Onaolapo (2009); Bukar *et al*., (2009) whose report showed that water recovered more extracts of *P. biglobosa* than ethanol.

The result of the phytochemical screening of PAE and PEE are presented in Table 2. From the results, saponnins, flavonoids, reducing sugars, Phenolic compounds and tannins were detected in PAE and PEE, while alkaloids were only detected in PAE. These findings were in accordance to the work carried out by Ajaiyeoba (2002) and Bukar *et al*. (2010). Phytochemicals in plants and plant parts have been extensively reported to possess antimicrobial activities both *in vitro* and *in vivo*. Alkaloids, saponins, tanins, and phenolic compounds etc. have been found to have bactericidal and fungicidal effect by inhibiting vital metabolic processes such as DNA synthesis and cell wall synthesis inhibition among others (Haslam, 1996; Rahman *et al*, 1995; Tsuchiya *et al.,* 1996).

All the isolates were found to be sensitive at the tested concentrations of (10,000µg/ml, 8,000µg/ml, 6,000µg/ml and 4,000µg/ml). This corroborates the findings of Bukar *et al*. (2010), however, their findings indicated the sensitivity of foodborne microorganisms at lower concentrations of 4000µg/ml, 2000µg/ml, 1000µg/ml and 500µg/ml respectively. This could be ascribed to the difference in geographical location of the *Parkia* tree, seasonal variation, maturity which could consequently affect some of the primary and secondary metabolites, pathological or diseased condition of the plant as well as mutation (Sofowora, 1993; Cowan, 1999).

The GC-MS analysis of the aqueous and ethanolic extract of *P. biglobosa* revealed compounds such as Decanoic acid (Capric acid) in PEE, which occurs naturally especially in plant seed oil, and used in the manufacture of artificial fruit flavors and perfumes, food additives and pharmaceuticals as described by Mezaki *et al*. (2000). Palmitic acid (n- Hexadecanoic acid), Stearic acid (Octadecanoic acid), pentadecanoic acid were all identified as constituents of PAE (Table 5). Palmitic acid is used as a natural additive in foods as it improves its texture (Seidall, 1952).

From the result, the untreated ginger drink was rejected after 2 days of storage. This is in line with the findings of Zvaigzne *et al*. (2009) who reported that the content of vitamin C in most fruits and juices substantially decreased after 1 day of storage at both refrigerated and ambient conditions. The different treatments of the ginger drink took at least 4 days to spoil. This is in line with study conducted by Ahammed *et al*. (2014), who reported changes in sensory and organoleptic parameters of ginger drink using chemical preservatives. From the results (Table 6) the judge's preferred ginger drink treated with 1.0% PEE, whose shelf life was extended to 5 days (102hrs) with percentage likeness of 70% while 0.5% PEE recorded 67.7%. Treatment with 1% PAE was also liked by the judges with 63.3%, however 0.5% was rejected after 72hrs of storage

Table 1: Physical Characteristics of the Extracts and Oils

S/N	Physical Parameters	PAE	PEE
1	Weight of extract/leaves (g)	100	100
2	Weight of extract recovered (g)	39.2g	33.8g
3	Colour of extract	Dark brown	Dark brown
4	Texture	Sticky	Sticky
5	Taste	Astringent	Slightly astringent

Key: PAE= *P. biglobosa* pod aqueous extract, PEE=*P. biglobosa* pod ethanol extract

Table 2: Phytochemical Constituents of Extracts of *Parkia biglobosa*

S/N	Phytochemicals	PAE	PEE
1	Saponnins	+	+
2	Reducing sugars	+	+
3	Alkaloids	+	-
4	Phenolic Compounds	+	+
5	Tannins	+	+
6	Flavonoids	+	+

Key: PAE= *P. biglobosa* pod aqueous extract, PEE= *P. biglobosa* pod ethanol extract. + = Present, - = Absent

Table 3: Zone Diameter of Inhibition (mm) of *Parkia biglobosa* Aqueous extract against Bacterial and Fungal Isolates

Organism	PAE Concentration (µg/ml)					
Bacteria	**10000**	**8000**	**6000**	**4000**	**MIC**	**MBC**
S. aureus	14.25±0.50	12.25±1.25	11.25±1.25	8.25±1.25	2000	2000
Salmonella spp.	15.75±1.25	13.00±0.81	11.00±0.81	9.00±0.81	2000	2000
Shigella spp.	13.25±0.95	11.25±0.50	10.00±0.81	8.75±0.50	1000	2000
E. coli	12.75±0.50	11.25±0.50	9.25±0.50	8.25±0.50	2000	2000
P. aeruginosa	15.75±1.25	13.00±0.81	11.00±0.81	9.00±0.81	2000	2000
B. cereus	13.75±0.50	11.25±95	9.25±0.95	7.25±0.95	1000	4000
Fungi					**MIC**	**MFC**
A. flavus	16.00±0.81	13.00±0.81	11.25±0.50	9.75±0.50	4000	4000
Pencillium spp	17.75±0.50	13.75±1.25	12.75±0.50	10.75±0.50	1000	2000
A. fumigatus	13.75±1.25	13.00±0.81	10.75±0.95	8.75±1.25	1000	2000
Mucor spp	14.25±3.30	12.25±0.50	10.75±0.95	9.00±1.14	4000	-
A. niger	18.75±0.95	16.00±0.81	13.75±0.50	10.25±0.50	1000	2000

Table 4: Zone Diameter of Inhibition (mm) of *Parkia biglobosa* ethanol extract against Bacterial and Fungal Isolates

Organism	PEE Concentration (µg/ml)					
Bacteria	**10000**	**8000**	**6000**	**4000**	**MIC**	**MBC**
S. aureus	13.75±0.50	11.75±1.25	10.00±0.81	8.75±0.95	2000	2000
Salmonella spp.	15.00±0.81	11.75±1.25	10.25±0.50	9.75±0.50	2000	4000
Shigella spp.	13.25±0.95	11.75±0.95	10.00±0.81	8.25±0.50	2000	2000
E. coli	13.75±1.25	12.25±0.50	10.25±0.95	8.75±0.95	2000	2000
P. aeruginosa	11.25±0.50	9.75±0.50	8.75±0.50	7.75±0.50	2000	4000
B. cereus	13.75±0.50	11.25±0.50	9.25±0.50	7.75±0.95	2000	4000
Fungi					**MIC**	**MFC**
A. flavus	19.00±0.80	16.00±0.81	12.00±0.81	9.00±1.00	2000	4000
Pencillium spp	14.75±0.50	12.25±0.95	11.75±0.50	10.00±0.81	4000	-
A. fumigatus	16.00±0.81	13.75±0.50	12.25±0.50	10.00±0.81	2000	4000
Mucor spp	16.00±0.81	12.75±0.95	10.00±00	7.75±0.50	4000	-
A. niger	14.00±0.80	11.75±1.25	9.75±0.50	7.75±0.95	2000	2000

Key: PAE= *P. biglobosa* aqueous extract, PEE= *P. biglobosa* ethanol extract MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, MFC=Minimum Fungicidal Concentration. Values are mean ± SE and each value is mean of three determinations

Table 5: Some of the constituents of aqueous extract of *P. biglobosa* sourced through GC-MS analysis

	Molecular weight (g/mol)	IUPAC Name	Molecular formula	Chemical structure
1	186	n-Dodecan-1-ol	$C_{12}H_{26}O$	
2	206	Phenol, 3,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	
3	284	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	
4	256	n-Hexadecoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	

Table 6: General acceptability (percentage likeness) of preserved Ginger drink by Judges.

Number of Days	A	B	C	D	E	F
0hr	8.00±0.26 (88.8)	8.00±0.26 (88.8)	8.00±0.26 (88.8)	8.00±0.26 (88.8)	8.00±0.26 (88.8)	8.00±0.26 (88.8)
1	5.30±0.21 (58.8)	8.6±0.27 (95.5)	8.00±0.21 (88.8)	8.90±0.1 (98.8)	8.30±0.15 (92.2)	8.60±0.16 (95.5)
2	3.40±0.16 (37.7)	7.60±0.16 (84.4)	7.80±0.13 (86.6)	8.00±0.15 (88.8)	8.10±0.1 (90.0)	6.80±0.35 (75.5)
3	2.20±0.133 (24.4)	6.0±0.21 (66.6)	7.30±0.15 (81.1)	7.90±0.1 (87.7)	7.90±0.1 (87.7)	6.10±0.18 (67.7)
4	1.10±0.1 (12.2)	2.70±0.21 (30.0)	5.90±0.17 (65.5)	6.90±0.17 (76.6)	6.90±0.17 (76.6)	4.90±0.17 (54.4)
5	1.00±0.00 (11.0)	3.00±0.39 (33.3)	5.70±0.15 (63.3)	6.10±0.17 (67.7)	6.30±0.15 (70.0)	4.7±0.21 (52.0)

Key: A= untreated food/drink (control), B= 0.5% *P. biglobosa Aqueous* extract (PAE), C= 1.0% *P. biglobosa Aqueous* extract (PAE), D= 0.5% *P. biglobosa* ethanol extract (PEE), E= 1.0% *P. biglobosa* ethanol extract (PEE), F= 0.5% Sodium benzoate.
General acceptability: Scale 0 - 9

CONCLUSION

It can be concluded that extracts of *P. biglobosa* pod possess antimicrobial and preservative effect on ginger drink stored for up to six days.

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