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Article

Tannins Concentrations in Three Citrus Fruits in Nigeria Using GC-MS

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Abstract

Tannins are polyphenolic compounds which are found in fruits and medicinal plants and are of importance. Although, tannins are nutritionally and medicinally important, little information is available on their presence in fruits commonly consumed in Rivers State, Nigeria. The current study was carried out to find the tannin content of Citrus sinensis (sweet orange), Vitis vinifera (grape) and Citrus limon (lemon) from the Choba market in Rivers State Nigeria. The fresh fruit samples were washed, blended and extracted by ethanol maceration method. The qualitative phytochemical screening was conducted using Ferric chloride and Lead acetate tests while quantitative determination was done using High-Performance Liquid Chromatography (HPLC). Gas Chromatography–Mass Spectrometry (GC–MS) was also used to identify the phytochemical content of Citrus sinensis and Citrus limon. The data collected were analyzed using Pearson correlation coefficient using Statistical Package for the Social Sciences (SPSS) software. All the three fruit samples gave positive result to qualitative phytochemical screening for the presence of tannins by observing the formation of cream or white precipitates in the lead acetate test and dark brown colour in the ferric chloride test. Citrus limon had the highest tannin content (13.59 ± 0.081 mg/L) followed by Citrus sinensis (12.28 ± 0.176 mg/L) and Vitis vinifera (4.20 ± 0.02 mg/L) respectively. Forty-five compounds were identified in Citrus limon and thirty-five compounds were identified in Citrus sinensis by using Gas Chromatography–Mass Spectrometry. In Citrus limon, 5-hydroxymethylfurfural (14.48%) was the predominant compound, while pentadecane, 2,6,10,14-tetramethyl (6.54%) was the main compound found in Citrus sinensis. Pearson's correlation analysis showed that there was a high negative but non-significant correlation between tannin content of Citrus sinensis and Vitis vinifera (–0.997, p = 0.052), a very low positive non-significant correlation between Citrus sinensis and Citrus limon (0.041, p = 0.974), and a low negative non-significant correlation between Vitis vinifera and Citrus limon (–0.123, p = 0.922). In conclusion, it is found that all these three fruits are source of natural tannins but the concentration of tannins is different in all the fruit. The study suggests the need for more extensive research on the distribution of tannins in various parts of the body, in different seasons, with larger sample sizes, and the use of more sophisticated chromatographic methods to gain further insight into tannin distribution and the implications for human nutrition and food functionalization.

Keywords: *Tannins, Citrus sinensis, Vitis vinifera, Citrus limon, High-Performance Liquid Chromatography, Gas Chromatography–Mass Spectrometry, phytochemicals, Rivers State, Nigeria.*

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Introduction

Medicinal plants are a source of therapeutic agents which have been of significance in the prevention and treatment of diseases for long. Thus, there has been a growing interest in recent years on naturally occurring bioactive compounds for their potential use in pharmaceuticals, nutraceuticals, food preservation and disease prevention. Tannins are one of these bioactive compounds, which have received a great deal of attention due to their antioxidant, antimicrobial, anti-inflammatory, antiviral and anticancer effects. These compounds are also an important part of the nutritional and medicinal properties of many edible fruits and vegetables [1]; [2].

Tannins are naturally occurring polyphenolic secondary metabolites produced by plants as a defense against herbivores, insects, fungi, bacteria and environmental stresses. Based on their chemical structure and hydrolytic properties, they can be divided into hydrolysable tannins and condensed tannins (proanthocyanidins). Their binding capacity to proteins and other macromolecules is related to their biological functions in plants and their therapeutic value in humans [3].

Cardiovascular diseases, diabetes mellitus, cancer, and neurodegenerative disorders are all linked to oxidative stress, which is combated by the remarkable antioxidant activity of dietary tannins, which help remove ROS. They also possess antimicrobial and anti-inflammatory properties and therefore are useful phytochemicals in functional foods and pharmaceutical formulations. But high amounts of tannins can lead to decreased protein and mineral (e.g., Fe and Zn) bioavailability due to complexation, which is why it is crucial to assess tannin levels in edible fruits.

Citrus sinensis (sweet orange), Vitis vinifera (grape) and Citrus limon (lemon) are among the most consumed fruits in the world due to their nutritional value and rich content in phytochemicals such as tannins, flavonoids, phenolic acids, vitamins and essential oils. These phytochemical compounds are responsible for their antioxidant, antimicrobial, anti-inflammatory and cardio protective effects. For this reason, these fruits are widely used in the food, pharmaceutical, cosmetic and nutraceutical industries [4].

Fruit compositions of phytochemicals depend on many environmental and agronomic factors such as soil characteristics, climatic conditions, rainfall, intensity of solar radiation, cultivar, maturity stage, storing, and post harvest handling. Thus, the concentration of tannin ranges in different geographical region. These differences warrant local research to develop the baseline phytochemical information of fruits eaten in the specific environment such as Aranda-Ledesma [5].

Citrus sinensis, Citrus limon and Vitis vinifera are popular fresh fruits or processed products in Rivers State, Nigeria. A few reports are available on the quantitative estimation of tannins present in these fruits that are commonly sold in the state but only few. The majority of published studies have been either general phytochemical screening or antioxidant activity, but with little quantitative information about the amount of tannin. With this view of this study aimed to determine the content of tannin in Citrus sinensis, Vitis vinifera and Citrus limon obtained from Rivers State, Nigeria for baseline information to nutritionists, food scientists, pharmacologists and public health researchers.

Methodology

Materials

The following items were used in this research work: Rotary evaporator, Filter paper, Measuring cylinder, Droppers, Glass funnel, Test tube, Test tube rack, Weighing balance, Spoon spatulas, Beakers, Stirring rod, Volumetric flask, Storage reagent bottles, Glass storage jars, Electric blender, Knife, Bowl, HPLC, 0.45μm syringe filter, GC-MS, Weighing boat, Magnetic stirring bar, Pipette filler, Electro photometer, Centrifuge, Pipette, Conical flask and Burette.

Reagents and chemicals

The chemicals used are ethanol (99 %), FeCl3 (ferric chloride, 5 %), lead acetate (5 %), distilled water, methanol, flavonoids, phenolic acids, xylene (filtered), sodium sulphate and sodium chloride.

Sample collection

The Citrus sinensis, Vitis vinifera and Citrus limon were bought from Choba market, Rivers State.

Sample preparation

Each Citrus sinensis, Vitis vinifera and Citrus limon were washed with distilled water and cut into small pieces and placed in separate beakers. The cut samples were each blended using an electric blender and transferred to separate glass storage jars and then labelled, FO, FG and FL.

Remove plants from the soil, using the maceration method.

The blended Citrus sinensis, Vitis vinifera and Citrus limon in separate glass storage jars were added 200ml ethanol and shaken vigorously and kept for two days with occasional shaking and filtered to get the filtrates. This ethanol was then evaporated using a rotary evaporator to get crude extract and stored in the reagent bottles and marked.

Qualitative Phytochemical Screening

The method of Pius [6] was adopted while qualitative phytochemical screening of Citrus sinensis, Vitis vinifera and Citrus limon was done to ascertain the presence of tannins.

Test for tannins

Ferric chloride test

1ml of the extracts of Citrus sinensis, Vitis vinifera and Citrus limon were collected using a measuring cylinder and transferred each to a different test tube and labeled. 2 drops of 5% ferric chloride solution were added to each of the test tubes using a dropper. Presence of tannins was found when a black or green-brown solution was formed.

Lead acetate test

Each 1ml of the extracts Citrus sinensis, Vitis vinifera and Citrus limon were taken using a measuring cylinder and placed in separate test tubes and labelled. Using a dropper, 3 drops of lead acetate solution was added to each of the test tubes. The cream gelatinous precipitate was formed, which showed the presence of tannin.

High performance liquid chromatography (HPLC) quantitative phytochemical screening:

The quantitative estimation of Tannins in the extract and filtrate of Citrus sinensis, Citrus limon and Vitis vinifera was done by the method of HPLC.

Preparation

10ml of methanol was used to dissolve 10mg of powdered sample to give the final concentration 1mg/ml which was then filtered through 0.45μm syringe filter (millipore) for sterilisation. Each standard was separately dissolved in 1ml of methanol, filtered through a 0.45μ syringe filter (millipore) and analyzed by HPLC.

Procedure

The prepared samples of extracts and standards were used for HPLC. Binary system equipped with PDA detector connected to system processor was used for analysis. The system used Empower software with standard certification for analysis of the results. The minimum pressure of 1500 psi and the maximum pressure of 2500 psi were maintained. The HPLC of solvents was run at 200 nm to 600 nm wavelength using reverse phase C-18 column. During the run, a flow rate 1ml/min was maintained using binary mode of gradient system. Various combinations of the solvents 20:80, 80:20, 60:20, 50:50 of methanol and water were used respectively. Finally, the experiments were carried out at 50:50 ratio of the solvent (methanol and water) to obtain the best resolution of peaks. Several standards of flavonoids (kaempferol and quercetin dihydrate) and phenolic acids (o-coumaric acid, p-coumaric acid, sinapinic acids, caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid and gallic acid) were used to identify the compounds. The peaks were determined by comparing the retention time (RT) of the standard compounds to the different peaks obtained.

The GC-MS analysis of Citrus limon and Citrus sinensis were determined.The citrus limon and Citrus sinensis were analyzed with GC-MS technique.

The samples were agitated very well and the volume was noted. All contents were transferred to separating funnel. Twenty five (25) ml of xylene was added to the sample container, rinsed properly and transferred into the separator funnel. The separating funnel was corked and shaken vigorously for about 30secs and allowed to separate. It was transferred into a 100 ml centrifuge tube by passing it through filter paper containing some amount of sodium sulphate. Another 25ml of xylene was used for the extraction. The xylene fraction was collected in the same centrifuge tube as the first extract. The extract was centrifuged for 15mins at 1500rpm. The extract was placed in a normal 10nm light path cuvette. The photometer or spectrophotometer was calibrated and the blue filter (peak transmission 425nm) was placed in the light path or set the wavelength at 425nm.

GC-MS analysis was performed with Agilent Technology (Little Falls, California, USA) 6890 series gas chromatography (GC) system, equipped with 5973 mass spectrometry (MS) detector and a 7683 series auto-injector was used. The compounds were separated by Rtx®-Wax (30 m × 0.25 mm, film thickness 0.25 µm; RESTEK, Pennsylvania, USA). The carrier gas used was helium (5N5 grade) and the split ratio was 60:1 while the flow rate of the carrier gas was 0.8 mL/min. The volume of sample injected was 1 µl and the temperature of the injector was 230°C. The column oven temperature was held at 70°C for 2 minutes, and then programmed to 130°C at 30°C/min and the gradient to 230°C with 10°C/ min. Finally, it was held at 230 °C for 6 minutes and the total run-time was 20 minutes. They detected using an electron ionization (EI) system that operates at an ionization energy of 70 eV. The ion source temperature was maintained at 230°C, interface temperature was maintained at 250°C and detector voltage was 2 kV. A mass spectrum was obtained in scan mode, at a scan rate of 0.98 scan/sec in the mass range 20-800 amu. The measurement was done twice for each sample with 2 min solvent delay.

Statistical analysis

Pearson correlation coefficient was used to carry out the statistical analysis on the platform of SPSS

RESULTS

Table 1. Result of Qualitative Phytochemical Screening.

Sample	Test	Observation	Inference
Citrus sinensis	Lead acetate test	Cream precipitate formed	Tannins Suspected
	Ferric chloride test	Dark brown solution	Tannins Confirmed
Vitis vinifera	Lead acetate test	White precipitate formed	Tannins Suspected
	Ferric chloride test	Dark brown solution	Tannins Confirmed
Citrus limon	Lead acetate test	White precipitate formed	Tannins Suspected
	Ferric chloride test	Dark brown solution	Tannins Confirmed

Table 2. Quantitative Tannins Concentration

Sample	Tannins Conc. (mg/l)			Mean±SD
Citrus sinensis	12.45	12.30	12.10	12.28±0.176
Vitis vinifera	4.18	4.20	4.22	4.20±0.02
Citrus limon	13.65	13.50	13.63	13.59±0.081

Mean Concentrations of Tannins (mg/L)

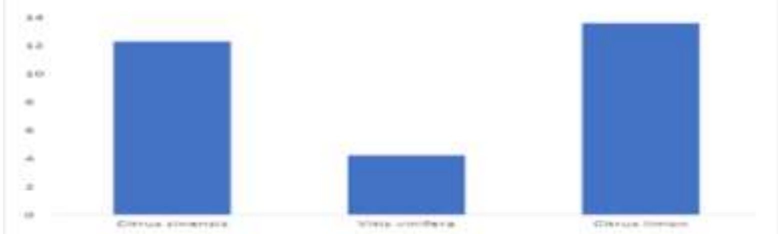

Figure 1. Mean Concentration of Tannins in Citrus sinensis, Vitis vinifera, Citrus limon

Table 3: GC-MS result for *Citrus limon*

S/n	Retention Time	Area Percent	Name	Chemical formula
1	2.17	0.38	1H-Indene, octahydro-, cis	C_9H_{16}
2	2.548	4.26	Decane	$C_{10}H_{22}$
3	2.828	0.9	Mesitylene	C_9H_{12}
4	3.01	1.49	Hexadecyl octyl ether	$C_{24}H_{50}O$
5	3.308	3.62	Undecane	$C_{11}H_{24}$
6	3.908	4.62	Benzene, 1-methyl-3-(1-methylethenyl)-	$C_{10}H_{12}$
7	4.176	5.67	Dodecane	$C_{12}H_{26}2$
8	4.62	14.48	5-Hydroxymethylfurfural	$C_6H_6O_3$
9	4.822	2.04	Sulfurous acid, 2-ethylhexyl tetradecyl ester	$C_{22}H_{46}O_3S$
10	5.091	3.94	Tridecane	$C_{13}H_{28}$
11	5.239	0.25	Tetradecane	$C_{14}H_{30}$
12	5.377	1.31	3-Methyl-2-(2-methyl-2-butenyl)-furan	$C_{10}H_{14}O$
13	5.582	1.28	Cyclopentane, (2-methylpropyl)-	C_9H_{18}
14	5.771	2.45	Dodecane, 2,6,10-trimethyl	$C_{15}H_{32}$
15	6	6.16	Tetradecane	$C_{14}H_{30}$
16	6.331	0.88	Naphthalene, 2,7-dimethyl	$C_{12}H_{12}$
17	6.525	4.63	2,6,10-Trimethyltridecane	$C_{16}H_{34}$
18	6.885	4.25	Pentadecane	$C_{15}H_{32}$
19	7.263	0.47	Benzene, 1-(1-formylethyl)-4-(1-buten-3-yl)-	$C_{13}H_{16}O$
20	7.423	1.84	Naphthalene, 2,3,6-trimethyl	$C_{13}H_{14}$
21	7.6	0.75	Naphthalene, 2,3,6-trimethyl	$C_{13}H_{14}$
22	7.731	4.13	Hexadecane	$C_{16}H_{34}$
23	7.926	0.43	Naphthalene, 1,4,6-trimethyl	$C_{13}H_{14}$
24	8.097	2.9	Pentadecane, 2,6,10-trimethyl	$C_{18}H_{38}$
25	8.543	5.96	Pentadecane, 2,6,10,14-tetramethyl	$C_{19}H_{40}$
26	8.863	0.42	1-Hydroxymethyl-7,7-dimethyl-bicyclo[2.2.1]heptane-2,3-dione	$C_{10}H_{14}O_3$
27	9.103	0.67	Decane, 2-cyclohexyl	$C_{16}H_{32}$
28	9.303	3.63	Octadecane	$C_{18}H_{38}$

29	10.035	2.01	Heptadecane	$C_{17}H_{36}$
30	10.475	0.42	Phenol, 3-methyl	C_7H_8O
31	10.732	1.41	Eicosane	$C_{20}H_{42}$
32	10.955	0.5	N-Benzhydryl-1,1,1-trimethylsilanamine	$C_{16}H_{21}NSi$
33	11.395	1.17	Heneicosane	$C_{21}H_{44}$
34	12.035	1	Docosane	$C_{22}H_{46}$
35	12.641	0.84	Docosane	$C_{22}H_{46}$
36	13.018	0.28	3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl)phenol	$C_{23}H_{36}O$
37	13.229	0.64	Eicosane	$C_{20}H_{42}$
38	13.384	0.31	3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl)phenol	$C_{23}H_{36}O$
39	13.87	5.41	(Z)-3-(Heptadec-10-en-1-yl)phenol	$C_{23}H_{38}O$
40	14.47	0.44	Bicyclo[3.1.0]hex-3-en-2-one, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{14}O$
41	15.207	0.24	meso-Hydrobenzoin	$C_{14}H_{14}O_2$
42	16.07	0.29	Cresol purple	$C_{21}H_{18}O_5S$
43	16.396	0.42	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	$C_9H_9N_3OS$
44	17.07	0.25	Phenol, 2-methyl	C_7H_8O
45	22.81	0.58	Propanamide, N-(4-methoxyphenyl)-2,2-dimethyl	$C_{12}H_{17}NO_2$

Table 4: GC-MS result for *Citrus sinensis*

S/n	Retention Time	Area Percent	Name	Chemical formula
1	1.965	0.57	Nonane	C_9H_{20}
2	2.176	0.61	1H-Indene, octahydro-, cis-	C_9H_{16}
3	2.553	2.71	Decane	$C_{10}H_{22}$
4	2.833	0.9	Benzene, 1,2,3-trimethyl-	C_9H_{12}
5	3.016	1.28	Decane, 2-methyl-	$C_{11}H_{24}$
6	3.313	6.49	Undecane	$C_{10}H_{14}$
7	3.913	3.85	Benzene, 1,2,3,5-tetramethyl-	$C_{10}H_{14}$
8	4.182	6.39	Dodecane	$C_{10}H_{26}$
9	4.634	5.93	5-Hydroxymethylfurfural	$C_6H_6O_3$
10	4.822	1.85	Nonane, 3-methyl-	$C_{10}H_{22}$

11	5.097	3.95	Tridecane	$C_{13}H_{28}$
12	5.342	1.34	Naphthalene, 2-methyl-	$C_{11}H_{10}$
13	5.771	4.16	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$
14	6.005	6.4	Tetradecane	$C_{14}H_{30}$
15	6.525	6.25	2,6,10-Trimethyltridecane	$C_{16}H_{34}$
16	6.891	4.07	Pentadecane	$C_{15}H_{32}$
17	7.263	0.56	trans-Calamenene	$C_{15}H_{22}$
18	7.423	1.14	Naphthalene, 1,6,7-trimethyl-	$C_{13}H_{14}$
19	7.554	0.75	Naphthalene, 1,6,7-trimethyl-	$C_{13}H_{14}$
20	7.731	4.01	Hexadecane	$C_{16}H_{34}$
21	8.097	3.62	Decane, 2,6,8-trimethyl-	$C_{13}H_{28}$
22	8.543	6.54	Pentadecane, 2,6,10,14-tetramethyl	$C_{19}H_{40}$
23	9.303	3.57	Octadecane	$C_{18}H_{38}$
24	10.035	1.74	Nonadecane	$C_{19}H_{40}$
25	10.732	1.58	Eicosane	$C_{20}H_{42}$
26	11.401	1.26	Heneicosane	$C_{21}H_{44}$
27	12.035	0.78	Docosane	$C_{22}H_{46}$
28	12.646	1.49	Tricosane	$C_{23}H_{48}$
29	13.229	4.85	Tetratriacontane	$C_{34}H_{70}$
30	13.87	4.7	(Z)-3-(pentadec-8-en-1-yl)phenol	$C_{21}H_{34}O$
31	14.19	2.06	Tetracosane	$C_{24}H_{50}$
32	14.47	0.87	Tetracosane	$C_{24}H_{50}$
33	17.39	1.13	2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane	$C_{13}H_{18}$
34	21.231	1.12	1-Propanone, 3-(2-hydroxyphenyl)-1,3-diphenyl-	$C_{21}H_{18}O_2$
35	23.671	1.48	p-Acetoacetanisidide	$C_{11}H_{13}NO_3$

Table 5: Pearson's Correlation on the Relationship Between the Concentration of Tannin in Three Fruits.

		Citrus sinensis	*Vitis vinifera*	*Citrus limon*
Citrus sinensis	Pearson Correlation	1		
	p-value			
Vitis vinifera	Pearson Correlation	-.997	1	

	p-value	.052		
Citrus limon	Pearson Correlation	.041	-.123	1
	p-value	.974	.922	
	N	3	3	3

Discussion

Qualitative Phytochemical Screening of Tannins in Selected Samples

Table 1 shows the qualitative phytochemical screening results of tannins in Citrus sinensis (sweet orange), Vitis vinifera (grape) and Citrus limon (lemon) using lead acetate and ferric chloride tests. The results showed that all the fruit samples were positive for tannins. The lead acetate test gave cream or white precipitates in all samples that showed presence of probable tannin content and feferric chloride test gave dark brown color in all samples showing presence of tannins in each fruit sample.

The lead acetate test is a classical qualitative test used for preliminary testing of tannins. Lead acetate reacts with polyphenolic compounds to produce insoluble lead–tannin complexes, resulting in the formation of cream or white precipitates. This is only presumptive evidence but it is accepted as a quick test for plant material with tannin content. The method of confirmation by ferric chloride is suggested because ferric ions react with tannin hydroxyl groups to form coloured complexes, which are dark green, blue-black or brown depending on the class and concentration of tannins. Thus, the agreement between the two tests used in the present study is very good and gives strong qualitative evidence for the presence of tannins in all the fruit samples investigated [7]; [8]; [9].

The sweet orange, Citrus sinensis, presented a positive result, indicating that sweet orange also has a significant amount of tannins, besides other phytochemicals that are already known, including flavonoids, vitamin C, limonoids, carotenoids and phenolic acids. Tannins are responsible for the mild astringency of citrus fruits and improve the antioxidant properties by removing free radicals and reducing oxidative stress. Thus, their ubiquitous presence might partially account for the general use of sweet orange for the promotion of immune function and decreased the risk of chronic diseases associated with oxidative damage [10]; [11].

In the same way, Vitis Vinifera gave positive responses in both qualitative tests which proved the presence of tannins. This discovery aligns with the known phytochemical profile of grapes, which are one of the most condensed tannin (proanthocyanidin) rich foods in the diet. The main site of tannin accumulation in grapes is the seeds, skins and stems, providing defence against pests, colour stability, taste and antioxidant activity. Grapes are known as cardiovascular-protective, anti-inflammatory, antimicrobial, and reducing oxidative stress due to high content of tannin. The current results thus confirmed the previous ones, which have determined grape fruits as reservoirs with high amounts of tannins and polyphenolic compounds [12].

The qualitative detection of the tannins in Citrus limon is also consistent with previous phytochemical studies which have shown that lemons have marked amounts of phenolic compounds, such as tannins, flavonoids, hesperidin, eriocitrin and phenolic acids. These compounds play a significant role in the antioxidant, antimicrobial, hepatoprotective and anti-inflammatory effects of lemon. The occurrence of positive ferric chloride reaction in the present study suggests that the fruit samples used in the study are rich in phenolic compounds which can form iron complexes [13]; [14].

The results from this study are in agreement with Kumar, who analyzed the phytochemical constituents in selected citrus fruits and verified the presence of tannins by ferric chloride and lead acetate tests. Similarly, Hussain found that sweet orange and lemon fruits taken from different agro ecological regions yielded positive qualitative test results for tannins. Likewise, Zhang found high levels of condensed tannins in grape fruit and that these compounds accounted for much of the antioxidant activity that they measured from grape extracts. The results of the present study are consistent with the earlier studies, which enhances the validity of the qualitative methods used.

The similarities observed could be due to the common physiological function of tannins in plants. The tannins act as secondary metabolites to guard fruits from insects, herbivores, microbial pathogens, UV- ray and environmental stress. It was expected that tannins would be detected in all the three species since they are all fruit plants which have to develop chemical defence mechanisms during growth and maturation. However, qualitative analysis does not give any information about the concentration of tannins. Therefore it is necessary to use quantitative analysis to identify if there are significant differences between the fruit species.

The presence of tannins in all samples, is an important nutritional and public health implication. Middle doses of tannins have been linked to decreased oxidative stress, better heart health, suppression of harmful microorganisms, regulation of inflammation, and a reduction in certain types of cancer. Tannins, however, can form insoluble complexes with proteins and metal ions, thus decreasing protein digestibility and interfering with the absorption of essential minerals like iron and zinc, if they are consumed in large quantities. Hence, knowledge of the tannin content in fruits that are commonly consumed is crucial for determining the nutritional benefits and potential antinutritional effects [15]; [16].

The qualitative phytochemical screening has validated the results, hence all three species of Citrus sinensis, Vitis vinifera and Citrus limon are natural tannin sources. The uniformity of the results obtained by both the lead acetate and ferric chloride tests gives confidence in the analytical results and gives a good basis for carrying out the quantitative determination of the concentrations of tannins that are presented in the following parts of this study.

Quantitative Tannin Concentration in Selected Samples

The quantitative concentrations of tannins in Citrus sinensis (sweet orange), Vitis vinifera (grape) and Citrus limon (lemon) are given in Table 2. The results indicated that there were a significant difference between the tannin content of the fruits investigated. Citrus limon had the highest mean tannin content (13.59 ± 0.081 mg/L), followed closely by Citrus sinensis (12.28 ± 0.176 mg/L) and Vitis vinifera had the lowest mean tannin content (4.20 ± 0.020 mg/L). The relatively low standard deviations for all samples show good analytical precision, and high reproducibility of the experimental measurements, suggesting low variation among the triplicate determinations.

The higher tannin content found in Citrus limon compared to other citrus species may be due to the high phenolic content. The lemon fruits are rich in hydrolysable tannins, flavonoids, phenolic acids and other polyphenolic compounds, which are responsible for the astringent and high antioxidant activity of lemon fruits. A number of factors, such as species, cultivar, climatic conditions, soil composition, fruit maturity, irrigation practices, post-harvest handling and extraction procedures, affect the biosynthesis and accumulation of tannins in citrus fruits. The increased tannin content found in Citrus limon, thus, represents its natural phytochemical composition and corresponds with the report that lemons are one of the citrus fruits with relatively high content of phenolic antioxidants.

The tannin concentration in citrus sinensis was also relatively high (12.28 ± 0.176 mg/L), therefore sweet orange is also an important source of tannins in the diet. Sweet oranges are known for their rich vitamin C and flavonoid content, but they also contain significant amounts of tannins, which have antioxidant properties and may help prevent oxidative stress. The sweet oranges have been reported to have antibacterial and anti-inflammatory properties, while the tannins have been reported to inhibit the formation of free radicals and reduce lipid peroxidation. The current discovery aligns with the increasing awareness of the sweet orange as a functional food that contains more phytochemical benefits than its vitamin C content.

Vitis Vinifera, on the other hand, had the minimum tannin level (4.20 ± 0.020 mg/L). Grapes are generally known to contain large amounts of tannins though the lower amount in the present study can be attributed to various reasons. The first is that tannins are mainly found in the seeds and skins and not in the pulp of the grapes. Significantly lower levels would be predicted if only the edible pulp or peeled fruit were analysed. Secondly, the tannin concentrations vary greatly among grape cultivars; the red grapes tend to have much higher levels of tannin than green or white grapes. Other factors such as the environmental conditions, geographical origin, ripeness, agricultural practices, storage conditions, and extraction solvents also affect the tannin recovery during the laboratory analysis.

The differences among the three species of fruit may reflect the roles of tannins as secondary metabolites produced by the phenylpropanoid pathway. The amount of tannins in plants is dependent on their genetic makeup and environmental adaptations. They help to protect against environmental stresses, herbivores, insects, and microbial pathogens and increase resistance to UV radiation. Hence, the differences in tannin content between different species are likely to be observed even when grown under the same environmental conditions.

The amounts of phenolic compounds found in the fruits of this study are on par with the results presented by González-Molina who reported that citrus fruits have higher concentrations of phenolic compounds than many commonly consumed fruits owing to the presence of abundant hydrolysable tannins and flavonoids. Likewise, Abbas found that lemons showed relatively higher values of total phenolics and tannins than the sweet oranges which were harvested from various tropical environments, which they attributed to the differences as the result of the species characteristics and the environment.

The outcomes also indicate that Vitis Vinifera has been found to be an important natural source of condensed tannins, consistent with the findings of Zhang. Unlike the present study, in which grapes had the least concentration of tannin, Zhang found significantly more tannin, as they analysed the seeds and skins of grapes, which have the largest amount of tannin in grapes. This apparent difference is therefore probably not a contradiction in the phytochemical composition of grapes but due to differences in sample preparation, analytical methodology, grape variety, geographical origin and the grape tissues analysed.

Similarly, Kumar noted that there were variations in tannin content among edible fruits from various parts of Asia and concluded that the phytochemical composition can be heavily affected by climatic factors, maturity stage, extraction solvents and laboratory techniques. Their results corroborate the differences found between fruit samples analysed in the present study.

In terms of nutritional value, the relatively high concentration of tannin found in Citrus limon and Citrus sinensis indicate that they have the potential to greatly increase the antioxidant intake of the diet in small amounts. The major biological activities of tannins are anti-oxidant and anti-microbial; anti-viral, anti-inflammatory, cardioprotective and also anti-carcinogenic, which make them a valuable part of functional food. However, the high consumption of tannins can make proteins less digestible and decrease the absorption of minerals like iron, calcium and zinc due to their tendency to form complexes with proteins and metal ions. Thus, moderate intake is still crucial to obtain optimal health benefits while avoiding the potential for anti-nutrient effects.

The quantitative analysis on all three species showed that all are sources of tannins, but with varying amounts of tannins present. The order of the tannin concentration found in the present study was:

Citrus limon is better than Citrus sinensis which is better than Vitis vinifera.

This variation shows the differences in the phytochemical composition of species and highlights the need to consider the selection of the fruit sources for nutritional, pharmaceutical, and functional food applications. The results also give baseline data for Rivers state, Nigeria as well as make a contribution to the increasing body of literature on the phytochemical characterization of commonly eaten fruits.

GC-MS result for Citrus limon

The number of compounds identified by GC-MS from Citrus limon were observed to be 45. The chromatograms can be found in appendix 1. The chromatograms of the extractions revealed a number of high peaks and a number of lower peaks. Propanamide, N-(4-methoxyphenyl)-2,2-dimethyl showed the longest retention time of 22.81 while 1H-Indene, octahydro-,cis showed the shortest retention time of 2.17.The most predominant compound of Citrus limon was the 5-hydroxymethylfurfural accounting for the % area of 14.48 and followed compounds were pentadecane, 2,6,10,14-tetramethyl and dodecane with 5.96 % and 5.67%, respectively. 5-hydroxymethylfurfural which is the most predominant is very useful as intermediate for the production of the biofuel dimethylfuran (DMF) and other molecules. It is considered as a primary renewable building block for the synthesis of fine chemicals and materials, with a focus on medicinal chemistry due to its rich chemistry and potential availability from carbohydrates such as fructose, glucose, sucrose, cellulose and inulin. Furans derived from biomass are increasingly being used for various chemical applications to make the paradigm shift towards sustainable chemical industry more rapid. There has been significant interest in the recent years to convert carbohydrates to 5-hydroxymethylfurfural. It also serves as intermediate for the production of important molecules such as levulinic acid, 2,5-furandicarboxylic acid (FDA), 2,5-diformylfuran (DFF), dihydroxymethyl furan and 5-hydroxy-4-keto-2-pentenoic acid. pentadecane, 2,6,10,14-tetramethyl which is the second predominant compound found in Citrus limon is a major fatty acid. It is a hydrocarbon that is more recalcitrant to biodegradation than other hydrocarbons. Methyl branching can block the L-oxidation pathway increasing the resistance of the hydrocarbon to biodegradation. Dodecane, the third major compound, can be used as an inorganic diluent to make the organic phase with the extractant tri-n-octylamine and the active organic diluent decanol. Can be used as food additive, flavoring agent in food processing, industrial applications and alternative fuel to kerosene based fuels (Jet-A) in the aviation industry. It has even been used as a lubricant and lubricant additive. Retention time (RI), the name of the compound, formula and % area for all the identified compounds of Citrus limon in this study were summarized in Table 4.1.3. The chemical compounds and concentration from GC-MS obtained in this study was not similar to that reported by Lota et al., (2002) because of natural occurrence of the various chemical compounds in the different plant materials and soil composition.

GC-MS result for Citrus sinensis

The number of compounds identified by GC-MS from Citrus sinensis were observed to be 35. The chromatograms are shown in appendix 2. The chromatograms of the extractions revealed high peaks, and low peaks. p-Acetoacetanisidide was observed to have the highest retention time of 23.671, and Nonane was observed to have the lowest retention time of 1.965. The major compound of Citrus sinensis was found to be 2,6,10,14-tetramethyl pentadecane ($C_{15}H_{27}$) with % area 6.54 % and other compounds present were Undecane, $C_{11}H_{24}$ (6.49 %) and Tetradecane, $C_{14}H_{30}$ (6.4 %). Pentadecane, 2,6,10,14-tetramethyl which is the most predominant in Citrus sinensis is a major fatty acid. It is a hydrocarbon which is more resistant to biodegradation than other hydrocarbons. Increased resistance to biodegradation by blocking the L-oxidation pathway may be due to methyl branching. The second major component of Citrus sinensis is undecane which could prove beneficial in the prevention and/or treatment of inflammatory diseases of the skin such as atopic dermatitis and other allergic diseases. Additionally, it is used as a mild sex attractant for a number of moths and cockroaches and an alert pheromone for a number of ants. Undecane can also serve as an internal standard in GC, if using other hydrocarbons. The boiling point of undecane (196 °C) is well known and thus can be used as a reference for the retention time of molecules whose structure has just been determined. Tetradecane which is the third predominant compound in Citrus sinensis is an alkane hydrocarbon containing 14 carbon atoms. It is majorly used for organic synthesis, and used as a kind of organic solvent. Mixtures with hexadecane are binary phase change materials which can be used as cool storage materials in district heating cooling systems for refrigeration and air-conditioning. Plants are rich sources of untapped chemical scaffolds which could possess important biological and economic functions. Plants have established its significance as various compounds of economic value are purified and synthesized on a large scale in every corner of the world. Table 4.1.4 summarized retention time (RI), compound name, formula and % area for all the identified compounds of Citrus sinensis found in this study. The level of concentration and chemical compounds of Citrus sinensis in this study obtained from the GC-MS result was not similar and found to contain more compounds when compared with the result obtained by Cholke could be attributed to the natural occurrence of the phytochemicals in plant materials, and the origin of the plants and soil composition.

Pearson Correlation Result of Tannins Concentration

The Pearson's correlation results on the relationship between the concentrations of tannins in the three fruits showed that the concentration of Citrus sinensis and Vitis vinifera had strong but inverse relationship ($r=-0.997$, $p=0.05$) whereas the concentration of tannin in Citrus sinensis and

Citrus limon were weakly related (r=0.041, p=0.974), and the relationship between the concentration of tannin in Vitis vinifera and Citrus limon was weak and inverse (r=-0.123, p=0.922).

Conclusion and Recommendations

The samples analysed revealed that tannins were suspected in Citrus sinensis, Vitis vinifera and Citrus limon, when lead acetate was used to test for the presence of tannins. Ferric chloride test revealed dark brown colouration in Citrus sinensis, Vitis vinifera and Citrus limon which are indicative of the presence of tannins. The HPLC result shows mean concentrations of tannins of 12.28±0.176, 4.20±0.02 and 13.59±0.081 mg/l for Citrus sinensis, Vitis vinifera and Citrus limon respectively, indicating that the fruits contain tannins. The chemical constituents detected in Citrus limon by GC-MS were 45 and in Citrus sinensis 35 compounds were detected which shows that these plants have some biological properties which can be utilized to cure different types of ailments when consumed. Isolation of the tannins identified and then further developed in the formulation of drugs. The moderate consumption of tannin rich fruits should be encouraged by health professionals, nutritionists and public health practitioners because of the antioxidant, antimicrobial, anti-inflammatory and disease-preventative properties of the fruits. It is important to highlight the health benefits as well as the balanced consumption through public awareness campaigns. The compound identified by GCMS result should be extracted and used as intermediates for other productions, an example is the very important compound 5-hydroxymethylfurfural, present in Citrus limon as the most predominant compound and can be used as intermediates in the production of biofuel dimethylfuran (DMF) and other molecules. The GC-MS compounds identified in Citrus sinensis should be used as intermediates to synthesize medicines that can be used by man, for instance, undecane which is the second most abundant compound identified in Citrus sinensis and could be used to prevent or treat inflammatory disorders of the skin like atopic dermatitis and other allergic diseases.

Contributions to knowledge

During this dissertation it has been revealed that Citrus sinensis, Vitis vinifera and Citrus limon contain the presence of tannins.

1) . They are scientific findings that add to the existing knowledge by establishing the relative abundance of tannins in the samples tested, and further evidence that can be used by nutritionists, dietitians, food scientists, and public health professionals to identify food sources in nature for useful polyphenolic compounds.

3. It has been confirmed by this research that Citrus limon contains 45 compounds that can be utilized by man and an example is 5-hydroxymethylfurfural found in Citrus limon which is the most predominant and very useful as intermediate for the production of biofuel dimethylfuran (DMF) and other molecules.

4. It has been made known that Citrus sinensis contains 35 compounds that will be useful to human health and an example is undecane which is the second predominant compound found in Citrus sinensis and may be useful for the prevention or treatment of skin inflammatory disorders, such as atopic dermatitis, and other allergic diseases.

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