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Determination of Ascorbic Acid Levels in Hibiscus Sabdariffa (Zobo Rod) and Other Tropical Leaves

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Abstract: ***Ascorbic acid level in zobo rod a multidimensional tropical leaf and other leaf samples were determined by titrimetric method using ammonium cerium (IV) sulphate and 2,6-dichlorophenolindophenol. It was discovered that leaves like zobo rod, orange, guava, lime, lemon, pawpaw and Icheku have high ascorbic acid content of 44.44mg/100g, 57.31mg/100g,40.80mgl/100g,41.35mg/100g, 41.35mg/100g,36.75mg/100g and 30.43mg/100g respectively while leaf of avocado pea and okra have low ascorbic acid level of 15.20mg/100g and 20.70mg/100g respectively. Results obtained using ammonium cerium (IV) sulphate showed higher level of vitamin C compared to that of 2,6-dichlorophenolindophenol though without large differences. Chemometrically the results obtained from the methods were compared and at 95% level of confidence was found to be significant.***

Keywords*:* Hibiscus sabdariffa, chemometrics, Ammonium cerium(IV) sulphate, 2,6-dichoropheneindophenol.,titrimetry

1. Introduction

The search for antiscorbutic agent which provided prophylaxis against the fatal avitaminosis (scurvy) mainly observed in humans and guinea pigs started in the early nineteenth century[1]. Even since then, there have been scholarly articles on the nature, method of determination, physical and chemical properties [2]-[3]-[4]-[6].Ascorbic acid commonly known as vitamin C is a water soluble vitamin that have wide range of biochemical role in the body owing to its anti oxidant and therapeutic properties. It is a vital, ubiquitous substance in the life process which can either be synthesized by living organisms, get it from exogenous sources or perish due to scurvy a result of uncorrected hypoascorbemia[6] .Studies[8] have shown that the Chemotherapeutic action of vitamin C is similar to those of sulfonamide compounds or mycelial antibiotics in acute infections as toxins such as those in snake bites, spider bites and insect stings are neutralized. It is germane in the formation and maintenance of collagen, absorption of iron from the intestine, metabolism of amino acids and prevention of tissue damage[5], in the treatment of diseases such as common cold, anaemia, scurvy, infertility and haemorrhage[6], maintenance of biochemical homeostasis under stress and in the treatment of intraocular pressure in the glaucomatous eye, diphtheria, streptococcus and staphylococcus infections [6].

Various analytical methods have been employed for the determination of ascorbic acid in different matrices. Some of the methods include titrimetric and voltammetric [7]-[12], liquid chromatography[7]-[15], complexometric[9], spectrophotometric, amperometric and enzymatic [9]-[16]-[17].

Titrimetric methods have been commonly used in the determination of vitamin C from fruit samples because it is simple, however difficulties are encountered with commonly used titrants and interferences often occur with coloured samples[7]. Zobo rod leaves are derived from a multidimensional plant. It is also referred to as Rodo leaves, sobo rod and sokpo rod in different parts of Nigeria. History has it that Zobo rod was used in the treatment of abominable disease in Kuta Niger State of Nigeria. Zobo rod is believed to contain micro and macro nutrients, vitamins and Iron. It is believed to act as prophylaxis for sore throat, mouth ulcer and a good regulator of heart beat. It is always recommended for hypertensive and diabetic patients[10].

No much work have been done on the determination of vitamin C from zobo rod leaves (dried petals and sepals) notwithstanding people's dependence on the deep red colored extract. This paper compares vitamin C content of zobo rod with other leaves commonly consumed in different areas and their determination using different methods. In this work, vitamin C have been effectively and efficiently determined from hibiscus sabdariffa using ammonium cerium(IV) sulphate and ferroin sulphate indicator. This method is fast, sensitive, selective and solves the problem of colour interference in vitamin C determination.

2. Experimental

All reagents were of analytical grade and used without further purification unless otherwise stated. 2,6-dichlorophenolindophenol (Merc,Germany) was standardized by titration with standard ascorbic acid solution. Ammonium Cerium (IV) sulphate (Merc,Germany) was standardized by the arsenic method[8]. Deionized water was used throughout the work.

2.1 Sample Analysed

The leaf samples analyzed are Tropical red sorrel, 'ICheku', guava, Avocado pea, orange, pawpaw, pineapple, lime, lemon and okra. Samples of leaves were obtained from a forest in Enugu State (Emene). Hibiscus sabdariffa (Zobo calyx) was purchased from Ogbete main market Enugu.

2.2 Extraction Procedures for the Leaves and Analysis of Samples

Each of the leaves were weighed out and crushed to free the vitamin C. 1000 mL of deionized water was added and allowed for sometimes, squeezed and filtered. 1mL of the sample /extract acidified with dil. H_2S0_4 was titrated with 0.1M ammonium cerium (IV) sulphate using ferroin sulphate as the indicator. Dirty white precipitate which persisted for 10 seconds was obtained at the equivalence point [8].

2.3 Dichlorophenolindo phenol method

This method was used as described elsewhere [7].0.2g of the leaf samples were weighed, ground and 100mL of distilled water added. The solution is filtered and 10mL of the filtrate was pipetted into small conical flasks and 2.5mL acetone added. This is then titrated with 2,6-dichlorophenolindophenol to a faint pink colour which persisted for 15 sec.

3. Results and Discussion

Table 1: comparison of ascorbic acid content of tropical leave samples obtained by titration with Ammonium cerium(IV) sulphate (ACS) and 2,6-dichlorophenolindophenol (DCIP).

S/ NO	*SAMPLE* Common name	Botanical name	*Amount of (ACS) Mg/100g*	*Ascorbic Acid (DCIP) Mg/100g*	*Absolute Difference*	*Percentage Difference*
1	Zobo rod	Hibiscus sabdariffa	44.40	-	-	-
2	ICheku(Igbo)	Dalium guinensis	30.43	28.90	1.53	6.2
3	Guava	Psidium guajava	40.80	40.33	0.47	3.4
4	Avocado pea	Persea americana	20.72	19.00	1.72	6.0
5	Orange	Citrus sinensis	57.31	55.90	1.41	5.0
6	Pawpaw	Carica papaya	36.75	35.65	1.10	3.0
7	Pineapple	Ananas comosus	20.53	20.39	0.14	1.0
8	Lime	Citrus aurantifola	41.35	40.55	0.80	2.0
9	Lemon	Cucumis melo	40.96	37.00	3.96	20.0
10	Okra	Abelmuschus esculentus	15.20	10.79	4.41	23.0

The results obtained by the methods, titration with ammonium cerium (IV) sulphate and 2, 6-dichlorophenolindophenol is shown in table 1. The results are comparable within 6% in seven samples 'Icheku', guava, avocado pea, orange, pawpaw, pineapple and lime. Differences of about 20-23% were found in samples such as lemon and okra. Results obtained by titration with ammonium cerium(IV)sulphate are higher compared to those obtained with 2,6-dichlorophenolindophenol. The defect between the vitamin C contents of the leaves by the two methods could be due to interferences from substances such as tannins or Fe(II), Sn(II) and Cu(I) present in the leaf samples. Similar effects have been noted by Okei et al [7].

Ascorbic acid was difficult to be determined titrimetrically using 2,6-dichlorophenolindophenol from zobo rod. The end point could not be detected as the reagents have no effect on the deep reddish color of zobo rod. It has been noted[7]-[9]-[10] that although titrimetric determination of ascorbic acid using DCIP is the official method, however, it is not applicable to solutions containing Fe (II), Sn(II), Cu(I) and substances naturally present in fruits or biological materials such as tannis and betannins are oxidized by the dye. Reports [12] have also shown that the DCIP method is hindered by alkalinity of the sample and could only be applied when the concentration of dehydroascorbic acid is negligible. Consequently, the DCIP method is restricted to samples where there is absence of minerals. Owing to the fact that zobo rod contains micro and macro nutrients, vitamin C determination from this multidimensional leaf using the DCIP method seem unrealistic. The issue of overestimating or underestimating the level of ascorbic acid owing to the color which renders the end point difficult to judge accurately as noted by Okei et al [7] does not come since the titration does not give positive result.

The ascorbic acid content of zobo rod compares favourably with those of guava, orange, lime and lemon with the values of 44.4, 57.31, 41.35 and 40.96mg/100g respectively. People depend on zobo drink (boiled leaf extract) even though no much work has been done to characterize its content. However, treatment given to the leaf in the course of extracting the ascorbic acid may be a factor of interest. Producers of this drink subject it to temperature's sometimes above 60^0C in the preparation process. This high temperature denatures the vitamin C since vitamin C is not supposed to be dried or exposed to temperatures above 40^0C [7]-[13]-[14]-[15].

The vitamin C content of the leaves compares though with little differences with those obtained from fruits of the plants. Studies on the ascorbic acid levels in tropical fruit samples by Okei et al [7] revealed that fruits like orange, lime, pwapaw, guava, okra, pineapple, avocado pea and lemon have 64mg/cm^3, 56.57mg/100cm^3, 55.8mg/100cm^3, 51.02mg/100g, 25.22mg/100g, 35.20mg/100g, 30.00mg/100g and 49.00mg/100g respectively. This result compares favourably with the values obtained from the leaves of these plants even with different analytical methods. This is illustrated in table 2 below.

Table 2: Comparison of the ascorbic content of selected tropical leaves/ fruits determined using different analytical methods ACS= ammonium cerium(IV) sulphate, DCIP= 2,6-dichlorophenolindophenol, CV=Cyclic voltammetry and NBS= N-bromosuccinimide.

S/N	Ascorbic acid level	Leaf		Fruits	
		ACSmg/ 100g	DCIPmg/ 100g	Mg/ 100g CV	NBSmg/ 100g
1	Zobo rod	44.40	-	-	-
2	‘ ICheku’	30.43	28.90	-	-
3	Guava	40.80	40.33	51.02	50.3
4	Avacado pea	20.72	19.00	30.00	28.24
5	Orange	37.31	35.90	64.00mg/ $100cm^3$	61.36mg/ $100cm^3$
6	Pawpaw	36.75	35.65	55.24	43.33
7	Pineapple	20.53	20.39	24.93	24.84
8	Lime	41.35	40.55	56.57mg/ $100cm^3$	48.61mg/ $100cm^3$
9	Lemon	40.96	37.00	49.00mg/ $100cm^3$	49.53mg/ $100cm^3$
10	Okra	15.20	10.79	25.22	19.65

Most people depend on the leaves of these plants as part of food (vegetable) and also as drug. This research has revealed that the consumption of the leaves is almost equivalent to the consumption of the fruits themselves since the ascorbic acid contents are not significantly different.

The differences between the results as noted in table 2 above may be attributed to the various factors that affect the levels of ascorbic acid in leaves and fruits. As noted[16] factors such as variety of plants, size, amount of sunshine, degree of maturity and duration of storage affect vitamin C content of leaves and fruits. Okei, et al[7] noted that the ascorbic acid content of a fruit is determined by the level of nitrogen fertilizer used in growing the plant, the more nitrogenous fertilizer added, the lower the ascorbic acid content of the plant. Studies[7]-[10]-[13] have shown that Climatic Conditions such as light and temperature affect the chemical composition of horticultural crops. The more sunlight a plant receives, the higher the amount of vitamin C. This is in conformity with Stone [2] who maintained that the more stress an organism is exposed to, the more vitamin C it produces.

Statistical comparison of the various methods used in vitamin c determination has been done and presented in tables 3-5.

Table 3: Result of ANOVA test for the different methods ACS and DCIP

Source of variation	SS	MS	df	f	sig
Between group	17.64	179.64	1	0.816	0.378
Within group	3964.25	220.264	18		
Total	4144.4		19		

Table.4: Descriptive statistics of the methods ACS and DCIP

Method	Mean	N	S.D	Standard Error mean
ACP	34.845	10	13.015	4.115
DCIP	28.851	10	16.465	5.206

Table 5: Paired sample test and correlations for ACS and DCIP

Pair	N	df	t	sig	Mean	S.D	Correlation	SEM	95%CI
ACP and DCIP Pair	10	9	1.37	0.196	5.99	13.56	0.598	4.29	15.699 (upper) 3.711lower

At 95% confidence interval, 2-tailed.SEM=standard error mean, S.D=standard deviation.

The f and t values from table 3 and 5 are 0.816 and 1.397 respectively reflecting a significant relationship between the two methods at 95%level of confidence. Also, the two methods are positively correlated as shown in table 5 with mean difference of 5.994 and standard deviation of 13.567.The result at 95% level of confidence cannot be rejected since the two methods do not differ significantly.

This study has shown that vitamin C can be determined from deeply coloured samples using Ammonium cerium (IV) sulphate. The method is simple, cheap and does not require expensive equipment.

4. Conclusion

In Nigeria and other parts of Africa, scurvy is still prevalent because people are not properly informed on the need for dietary requirement of vitamin C. moreover, even when they consume these leaves that give vitamin C, the treatment and storage method before consumption denatures the vitamin C. Specifically, zobo rod is grown in the Savannah middle belt area of Nigeria. It is always harvested, dried and stored. Most people depend on the dried leaf which is known to have decreased vitamin C content. The storage method should be improved to ensure quantitative retention of vitamin C which is important in the treatment of common cold, anaemia, Scurvy, heart diseases, diabates, glaucoma, stroke, cancer, diphtheria and other toxins. Ascorbic acid can be determined and quantified by chemical laboratories using ammonium cerium (IV) sulphate.

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