EVALUATION OF THE ASCORBIC ACID CONTENT OF THE LEAVES OF SCOPARIA DULCIS.

***Okeri, H. A.[1], Alonge, P. O.[1] and Abere, T. A.[2].**
1. Department of Pharmaceutical Chemistry,
2. Department of Pharmacognosy,
[1&2] Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

**Correspondence: Okeri H.A. Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City. hokeri1@yahoo.com*
08023112394

ABSTRACT
The ascorbic acid content of the fresh and dried leaves of *Scoparia dulcis* (Linn) has been determined titrimetrically after extraction with metaphosphoric acid. The results show that the fresh leaves are rich in ascorbic acid (vitamin C) when compared with ascorbic acid content of the dried leaves and some common garden fruits and vegetables. Students' t-test statistical analysis using INSTAT.EXE program for the results (mean ± SD) shows that there was no significant difference for the fresh leaves and also there is no significant difference for the dried leaves (P = 0.05) with the two analytical methods used. However, there was significant difference between ascorbic acid content of the fresh and dried leaves obviously indicating that the fresh leaves contain more ascorbic acid than the dried leaves. The possible reasons for this difference are highlighted and it is submitted that the fresh leaves of *Scoparia dulcis* can be used as a source of vitamin C in the form of vegetables.

Key Words: *Scoparia dulcis (Linn), ascorbic acid content, 2,6-Dichlorophenolindophenol and iodometric determinations.*

INTRODUCTION

Ascorbic acid or vitamin C (antiscorbutic vitamin) is essential for the normal function of living cells and many enzymatic reactions in humans. It is vital in the formation of collagen, normal metabolism of the amino acid tyrosine and cholesterol, synthesis of bile acids, hormones and neurotransmitters like serotonin and norepinephrine, utilization of amino acid tryptophan and mineral iron, contribution to the health of teeth and gums, healing of broken bones, wounds and bruises, reduction of diseases like cancer and heart diseases, helping to build up the immune system to ward off cold and viruses etc. (American Pharmaceutical Association, 1979; Lewine, 1986; Marcus *et. al.,* 2001).

Prolonged deficiency of this vitamin which typically causes scurvy (characterized by abnormalities in bones, teeth and gums) was first characterized in sailors in the eighteenth century and were eliminated by compelling sailors to eat limes, a source of vitamin C (Bean, 1975; Williams, 1989).

Ascorbic acid is the only vitamin that is not a co-enzyme and is obtained from dietary sources (such as vegetables and fruits) although it can also be chemically synthesized. Man and animals cannot synthesize their own ascorbic acid because of the lack of the enzyme L-gluconalactonase (Stenlake, 1979) but many vegetables have since been found to contain large quantities of vitamin C, for example the rosehips and the search for other anti-scorbutic agents have continued.

Apart from its role in nutrition, ascorbic acid acts as an antioxidant to protect the natural flavour and colour of many foods (e.g. processed fruits, vegetables and diary products) (Williams, 1989). Ascorbic acid is a powerful

reducing agent that is readily oxidized in solution, so that the natural vitamin is often destroyed in the cooking and freezing of fruits and vegetables (Williams, 1989).

Recommended dietary allowances for infants, women, men, pregnant and lactating women as well as dietary reference intakes for vitamin C are well documented (Food and Nutrition Board, National Research Council, 2000; Institute of Medicine, 2000). The National Academy of Sciences recommends the consumption of 60mg of ascorbic acid per day.

There is a renewed awareness of the value of natural resources, and this utilization has led to experimentation of plants as food and medicinal supplements. These plants could be useful components of the diet, especially for rural families since the plant is found in abundance and collection for food would be a relatively easy task. Since foods and vegetables are among the most common consumer items that are regularly taken, it would therefore be useful to analyze some of these to determine their vitamin C content and show the relationship between it and their traditional usage (Okeri and Alonge, 2006).

Scoparia dulcis (Linn) Scrophulariaceae is a well distributed herb in northern and southern Nigeria. When chewed it is first bitter but latter becomes sweet. The leaves and other parts of the plant has been utilized in traditional medicine for the treatment of many disease conditions such as upper respiratory tract problems, menstrual disorders, fever, gonorrhea, constipation, sore throat, ear ache, conjunctivitis etc. The properties of *Scoparia dulcis* that has been investigated include analgesic, antibacterial, antidiabetic, antifungal, antiherpetic, anti-inflammatory, diuretic, antiseptic, antispasmodic, antiviral, antoneoplastic, emmemagogue, emollient, expectorant, febrifuge, hypotensive, hypocholesterolemic, refrigerant and antiscikling properties (Dalziel, 1956; Gill, 1992; Institute of Medicine, 2000; http://www.rain-tree.com/vassourinha.htm).
The active principles that have been characterized from *Scoparia dulcis* include scopadullic acids A and B, scopadiol, scopadulciol, scopadullin, scoparic acids A, B and C, amelin, triterpenoids, diterpenoids, sparteine saponins, tannins, betulinic acid and other alkaloids (Bep-Oliver, 1960; Ayensu, 1987; Bep-Oliver, 1986; Alonge, 2000; http://www.rain-tree.com/vassourinha.htm).

The ascorbic acid content was determined titrimetrically using iodine (United States Pharmacopoeia, 1980) and 2,6-dihydrophenolindophenol (Association of Official Analytical Chemists, 1984) solutions. These two analytical methods were employed for the purpose of comparison.

The determination of ascorbic acid content of vegetables like this is expedient since it could be related to the healing functions of the plant materials by acting synergistically with the active principles of the plant.

MATERIALS AND METHODS

Materials: Analytical grades of 2,6-dichlorophenolindophenol, glacial acetic acid, potassium iodate, potassium iodide, sodium thiosulphate pentahydrate, oxalic acid, sodium citrate, soluble starch, metaphosphoric acid and sulphuric acid, were all obtained from BDH England; l-ascorbic acid (Merck, Germany), starch (May & Baker, UK) were obtained from the sources indicated as well as deionised water and carbon dioxide-free distilled water from Department of Chemistry, Faculty of Science, University of Benin.

Scoparia dulcis leaves were collected in October 2005 at the University of Benin, Ugbowo campus, Benin City, identified and authenticated by a plant systemist in Pharmacognosy Department of the University of Benin, Benin City, Nigeria.

Method

Preparation of Reagents:

For iodometry: Starch mucilage (5%) indicator was prepared by first making a paste with 5g of soluble starch in 50ml of distilled water, making up to 500ml with boiling water and boiling until it was clear.

Sodium thiosulphate pentahydrate solution (8.7g of $Na_2S_2O_3.5H_2O$ dissolved in 500ml of

freshly boiled water) which gave a 0.07M solution was prepared and standardized using 50ml of 0.01M pure potassium iodate containing 2g of solid potassium iodide.

Iodine solution (0.05M) was prepared by dissolving 3.2g of iodine crystals in potassium iodide solution and distilled water. This solution was in turn standardized with the 0.07M standard sodium thiosulphate solution.

H_2SO_4 (28ml of concentrated sulphuric acid diluted to 1litre) to produce a 0.05M solution and 0.03M H_2SO_4 (17ml of concentrated sulphuric acid diluted to 1litre) were prepared.
For indophenol method: 2,6-dichlorophenolindophenol sodium (100mg) was weighed and dissolved in 100ml of deionised water to produce a 3.4moles/litre solution. 5ml of the above solution was then diluted to 50ml with deionised water warmed, filtered into an amber-coloured bottle and then standardized with 0.8mg/100ml ascorbic acid dissolved in metaphosphoric acetic acid.

Mepaphosphoric acetic acid (0.38moles/litre) was prepared by dissolving 3g reagent grade mepaphosphoric acid containing 35% HPO_3 in 10ml of 5% glacial acetic acid and adding water to make 100ml.

Plant treatment:
All plants parts were collected within a 20kilometer radius at the Ugbowo campus of the University of Benin, Benin City and taken directly to the laboratory. The young and tender leaves of the plants which are most suitable and desirable for human consumption were the ones collected. The samples were collected just during the flowering period because it was expected that the vitamin content would be at its highest level at that time (Zennie and Ogzewalla, 1977; Institute of Medicine, 2000).

Some of the fresh leaves were immediately treated and their ascorbic acid content determined upon arrival. The other part were sun-dried for one month before laboratory analysis was carried out on them..

Extraction of vitamin C and analysis:

(a) For iodometry: Fresh and dried leaves each weighing 10g were put into separate mortars and 30ml of 0.03M H_2SO_4, 20ml CO_2-free distilled water and 0.5g of oxalic acid were added. The mixtures were stirred for about 20minutes and rapidly filtered using a suction pump and Buchner funnel. 10ml of the filtrates were quickly titrated to the end-point with the standardized 0.05M iodine solution using 5% starch indicator.

The titrations were repeated in triplicates and blank determinations were also carried out followed the above procedure but using 10ml of CO_2-free distilled water instead of the filtrate. This is the United States Pharmacopoeia (USP) method (1980).

(b) For indophenol method: Fresh and dried leaves each weighing 10g were put into two separate mortars and 48ml mepaphosphoric acetic acid and 2ml of sodium citrate solution were added, respectively. The mixtures were stirred for about 20minutes and rapidly filtered using a suction pump and Buchner funnel. 10ml of the filtrates were quickly titrated to the end-point (change from blue to a permanent pink colour) with the standardized 2,6-dichlorophenolindophenol solution.
The titrations were repeated in triplicates and blank determinations were also carried out followed the above procedure but using 10ml of mepaphosphoric acetic acid instead of the filtrate. This is the method described by the Association of Official Analytical Chemists (1984).

RESULTS
The factor of the freshly prepared 0.05M iodine solution was 0.9874 and 1ml of 0.05M Iodine is equivalent to 0.008806g (8.806mg) of ascorbic acid.
1ml of the 2,6-dichlorophenolindophenol is equivalent to 0.00015g (0.15mg) of ascorbic acid.
The value of the blank determination in all the

cases was 0.13ml.
The results obtained for 10g of plant material were extrapolated for 100g of plant material. The results obtained in this study are shown in the Table below.

Table 1: Determination of Ascorbic Acid Content of Fresh and Dried Leaves of *Scoparia dulcis* (Linn).

	Ascorbic acid Content in mg per 100g of Leaves	
	Iodometric method	Indophenol method
Fresh Leaves	89.37 ± 2.54	87.58 ± 1.04
Dried Leaves	48.60 ± 0.79	47.80 ± 0.36

Calculations:
Amount of ascorbic acid/10g =
(Sample titre – Blank) x Factor x Dilution Factor x Equivalent weight of ascorbic acid.

Statistical analyses using INSTAT package.
Results are expressed as Mean ± SD.
Statistical analysis of all data was done at a 95% probability level using Student's t-tests.
Results with $p<0.05$ were considered to be significant

There was no significant difference between the values obtained using the two different assay methods for the two samples. However, there was significant difference when the values for the fresh leaves were considered against those of the dried leaves.

DISCUSSION AND CONCLUSION

The vitamin C content was done determined by titrimetric methods based on the oxidation-reduction (redox) reactions that measure only the reduced ascorbic acid which is the dehydro – form of the vitamin. The determination of the ascorbic acid content is based upon the quantitative oxidation of ascorbic acid to dehydroascorbic acid with iodine (United States Pharmacopoeia, 1980) and 2,6-dihydrophenolindophenol (Association of Official Analytical Chemists, 1984). Unlike acid -base titrations, the redox titrations are preferable because there is relatively few interference with the oxidation of ascorbic acid.

Two assay methods and two extracting solvents were used for the purpose of comparison. CO_2-free water was used to prevent the chemical oxidation of ascorbic acid. The two assay methods (iodometric and indophenol determination of ascorbic acid) used in this study compared well and there is no significant difference between the values of ascorbic acid obtained in both cases. The oxalic acid was added in iodometry before filtration so as to stabilize the ascorbic acid which otherwise be oxidized by air or during filtration process. Activated charcoal is added to decolourize the solution. In the indophenol method, sodium citrate and glacial acetic acid help to prevent the oxidation of vitamin C.

Scoparia dulcis (Linn) was found to contain quantities of ascorbic acid that are as high as an average of 88.44mg/100g of fresh leaves and 47.78mg/100g of dried leaves. Since the amount of vitamin C is high, it means that the plant contains some ascorbic acid stabilizing factors. The values compare well with those of raw green pepper contains about 128mg/100g, orange juice contains about 50mg/100g, grape contains 38mg/100g onions contain 15mg/100g and raw tomatoes/sweet potato contains 20 – 23mg/100g of ascorbic acid (Rodale, 1957). The values obtained in this study shows that the plant could provide more than a daily dietary allowance of Vitamin C in a 100g sample and when compared to oranges on a weight basis the plants had higher values of vitamin C of the food for an average man or for a woman during pregnancy and lactation (75mg and above, as the case may be) (Adams and Richardson, 1981; Olson and Hodges, 1987; Marcus and Coulston, 2000).

The value obtained for the fresh leaves are higher because of the loss of considerable amount of ascorbic acid due to oxidative decomposition catalysed by heat when the leaves were subjected to drying. About 54% of the initial ascorbic acid content of leaves of *Scoparia dulcis* (Linn) was lost due to drying. Heat, light, alkalies, oxidative enzymes etc are other conditions that easily oxidize ascorbic acid, and because of its relative instability and high aqueous solubility, it is readily lost during cooking and when large amounts of cooking water are discarded. Preferably, the plant should be consumed prior to wilting or aging so that the palatability and vitamin content would be high.

The traditional use of these plants as a remedy for cough and other medicinal applications such as healing of wounds could be attributed to the vitamin C content of the plant or possible augmentation of the activity of the active principles of the plant. Vitamin C can also improve the absorption of these active principles.

Because of the antioxidant properties of ascorbic acid (vitamin C), the active therapeutic principles of plants are naturally protected against oxidation. Adaikpoh et al. (2007) tested for the possible antioxidant properties of aqueous extracts of *Scoparia dulcis* and the results showed that there was significant antioxidant activity sufficient to mitigate against free radical induced oxidative stress in experimental cadmium intoxicated rats. From the results of this study, the antioxidant activity of the aqueous extract of *Scoparia dulcis* could be due to its ascorbic acid content.

There may be a word of caution to chemists who isolate beneficial substances and leave the rest of the plants behind. The other constituents of the plants may have an inherent balancing or modifying mechanism that exerts control over the active principles.

Since man cannot synthesize ascorbic acid and need both synthetic and dietary sources, it is pertinent that vegetables and herbs that contain high ascorbic acid be recommended. And although synthetic ascorbic acid (vitamin C) is a more economical source, biflavanoids that often accompany ascorbic acid are present in fruits and vegetable (Zennie and Ogzewalla, 1977). The plant should be well stored in airtight containers and protected from heat, light and air so as to prevent the decomposition of its ascorbic acid content and hence affect some other biological activities of the plant material that are dependent on ascorbic acid.

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