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ORIGINAL ARTICLE

DETERMINATION OF THE ASCORBIC ACID CONTENT OF TWO MEDICINAL PLANTS IN NIGERIA

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The fresh and dried leaves of two edible plants, *Oldenlandia corymbosa* and *Dissotis rotundifolia* have been assayed for their ascorbic acid content. They were found to be rich sources of ascorbic acid (vitamin C) when compared with some common garden fruits and vegetables.
Students' t-test statistical analysis using INSTAT.EXE program for the results (mean ± SEM) shows that there was no significant difference for the fresh leaves of the individual plants and also there is no significant difference for the dried leaves (P = 0.05). However, there was significant difference between ascorbic acid content of the fresh and dried leaves of the same plant, obviously indicating that the fresh leaves contain more ascorbic acid than the dried leaves.

Keywords: Ascorbic acid content, *Oldenlandia corymbosa* (Linn), *Dissotis rotundifolia* (SM) Triana, 2,6-dichlorophenolindophenol and Iodimetric determinations.

INTRODUCTION

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.

In both plant and animal kingdoms, glucoronic acid is converted to ascorbic acid, but man, primates and guinea pigs are unable to bring about the conversion of L-gulonolactone to 2-keto-L-gulonolactone which is the last step in the synthesis of ascorbic acid (Stenlake, 1979).

Ascorbic acid is essential for the normal function of living cells and many enzymatic reactions in humans. (Marcus et. al, 2000; Gershoff, 1979; American Pharmaceutical Association, 1979) and is obtained from dietary sources (such as vegetables and fruits) and synthetic vitamin C. Vitamin C deficiency typically causes abnormalities in bones, teeth and scurvy. Apart from its role in nutrition, ascorbic acid acts as an antioxidant to protect the natural flavour and colour of many foods (Barbara, 1984; 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).

In this present study, the ascorbic acid content was determined titrimetrically using iodine (Olson and Hodges, 1987) and 2,6-dihydrophenolindophenol (Association of Official Analytical Chemists, 1984) solutions. These two analytical methods were employed for the purpose of comparison.

This work seeks to establish that the ascorbic acid content of *Oldenlandia corymbosa* (Linn) and *Dissotis rotundifolia* (SM) Triana are vital for the activity of the plant extracts, especially its antioxidant effect and that it could be responsible for the traditional usage or at least influence the bioavailability of active principles of the plant (Okeri et. al, 2004).

Oldenlandia corymbosa (Linn) is a diffusely branched annual herb used in African folk medicine as an oral oxytoxic agent (Olaniyi et. al, 1973) for snake bite. The decoction has also been given during labour to induce strong uterine contractions and short delivery time. The plant has also been used in treatment of intermittent fever and is recommended against nervous depression (Gill, 1992). *Oldenlandia corymbosa* (Linn) is known to also contain pentacyclic tritepene boswellic acid and other constituents have been characterized (Olaniyi and Ramstad, 1979).

On the other hand, *Dissotis rotundifolia* (SM) Triana is a pink-purple creeping herb that is easily cultivated in pots and have been used in African traditional medicine for the treatment of cough, bronchitis, sinusitis, conjunctivitis, circulatory troubles, rheumatism, venereal disease, painful swelling and healing of wounds (Dalziel, 1948; Oliver, 1959).

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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.

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, all of BDH England were obtained; l-ascorbic acid (Merck, Germany), starch (May & Baker, UK) were also obtained as well as powdered charcoal, deionised water and carbon dioxide-free distilled water from Chemistry Department, University of Benin.

Oldenlandia corymbosa (Linn) (Rubiaceae) and *Dissotis rotundifolia* (SM) Triana (Melastomataceae) as identified and authenticated by a Dr Abere, Pharmacognosy Department, University of Benin, Benin City, were collected in October 2003 at the University of Benin, Ugbowo campus, Benin City, Nigeria.

Method

Preparation of reagents

a For iodimetry 5% starch mucilage indicator and 0.07M of sodium thiosulphate pentahydrate solutions were prepared and the later was standardized using 50ml of 0.01M pure potassium iodate containing 2g of solid potassium iodide.

0.05M iodine solution was in turn standardized with the 0.07M standard sodium thiosulphate solution.

0.05M H_2SO_4 (28ml of concentrated sulphuric acid diluted to 1litre) and 0.03M H_2SO_4 (17ml of concentrated sulphuric acid diluted to 1litre) were also prepared.

b For indophenol method a 3.4moles/litre solution of 2,6-dichlorophenolindophenol sodium solution was prepared. 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.

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

Plant treatment

Parts of the plants suitable and most desirable for human consumption were sun-dried for one month and fresh ones were also obtained. This consisted of young, tender parts while discarding discoloured and insect-damaged portions. Most of the samples were collected just prior to or 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). All plants were collected within a 20 kilometer radius at the Ugbowo campus of the University of Benin, Benin City and taken directly to the laboratory and analysis done for the fresh leaves immediately upon arrival.

Extraction of vitamin C and analysis

a For iodimetry 10g each of fresh and dried leaves were weighed 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 (USP method, 1980).

b For indophenol method: 10g each of fresh and dried leaves were weighed into two separate mortars and 48ml metaphosphoric acetic acid and 2ml of sodium citrate solution were added, respectively. The mixtures were stirred for about 20 minutes and rapidly filtered using a suction pump and Buchner funnel. 10 ml of the filtrates were quickly titrated to the end-point (change from blue to a permanent pink colour) with the standardized 2,6-dichlorophenolin-dophenol solution.

The titrations were repeated in triplicates and blank determinations were also carried out following the above procedure but using 10 ml of metaphosphoric acetic acid instead of the filtrate (Association of Official Analytical Chemists 1984).

RESULTS

Factor of 0.05M iodine solution used was calculated to be 0.9874 (0.9992 x 25.00/25.30); 1ml of 0.05M iodine is equivalent to 0.008806g (8.806mg) of ascorbic acid.

1ml of the 2,6-dichlorophenolindophenol is equivalent to 0.00013g (0.13mg) of ascorbic acid.

Value of the blank determination was 0.1ml in all the cases. The results obtained for 10g of plant material were extrapolated for 100 g of plant material. The results obtained in this study are shown in table 1.

Calculations

Factor of iodine

$$(FI_2) = \frac{\text{Factor of } Na_2S_2O_3 \text{ x Volume of } Na_2S_2O_3}{\text{Titre volume of iodine}}$$

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

STATISTICAL ANALYSES

Results are expressed as Mean ± SEM.

Statistical (Student's t-tests) analysis of all data was done using INSTAT package at a 95% probability level. 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.

DISCUSSIONS

Of the various methods available for the analysis of vitamin C, titrimetric techniques are the most frequently used. The determination of the ascorbic acid content is based upon the quantitative oxidation of ascorbic acid to dehydroascorbic acid with iodine (USP, 1980) and 2,6-dihydrophenolin-dophenol (Association of Official Analytical Chemists, 1984). Both methods are oxidation-reduction (redox) reactions that measure only the reduced ascorbic acid which is the dehydro – form. They are preferable to an acid-base titration because a number of other species in the plant materials and can act as acids, but relatively few interfere with the oxidation of ascorbic acid. Two assay methods and two extracting solvents were used for the purpose of comparison. CO_2-free distilled water was used to prevent the chemical oxidation of ascorbic acid.

In this study, the two assay methods (iodimetric and indophenol determination of ascorbic acid) used compared well and there is no significant difference between the values of ascorbic acid obtained in both cases. Oxalic acid was added in iodimetry 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.

Oldenlandia corymbosa (Linn) was found to contain quantities of ascorbic acid that are as high as an average of 86.00mg/100g of fresh leaves and 48.58mg/100g of dried leaves while *Dissotis rotundifolia* (SM) Triana was found to contain 90.00mg/100g of fresh leaves and 41.25mg/100g of dried leaves, respectively. This is as a result of loss and decomposition during drying. Since the amount of vitamin C is high, it means that the plant contains some ascorbic acid stabilizing factors. Depending on the season, 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 plants 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) (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 43.60% of the initial ascorbic acid content of leaves of *Oldenlandia corymbosa* (Linn) and 55.20% for *Dissotis rotundifolia* (SM) Triana were 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

Table
Ascorbic acid content of fresh and dried leaves of *Oldenlandia corymbosa* (Linn) and *Dissotis rotundifolia* (SM) Triana

	Ascorbic acid content in mg per 100g of leaves	
Fresh leaves of *Oldenlandia corymbosa*	86.20 ± 0.71	85.80 ± 0.38
Dried leaves of *Oldenlandia corymbosa*	49.27 ± 1.45	47.88 ± 0.43
Fresh leaves of *Dissotis rotundifolia*	90.40 ± 0.38	89.60 ± 0.22
Dried leaves of *Dissotis rotundifolia*	41.00 ± 0.35	41.50 ± 0.15

Key: Average of three readings. Values are reported as Mean ± SEM

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. Also because of the antioxidant properties of ascorbic acid (vitamin C), the active principles of the plants that are used therapeutically are protected against oxidation. Vitamin C can also improve the absorption of these active principles.

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 the human body 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. 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).

CONCLUSION

Oldenlandia corymbosa (Linn) and *Dissotis rotundifolia* (SM) Triana are a rich sources of vitamin C and are recommended for use as dietary sources of vitamin C. The ascorbic acid contents of these plants are comparable to those of the citrus fruits (Stenlake, 1977; Rodale, 1957). In the traditional use of the plants, it should be kept in air-tight containers and protected from heat, light and air so as to prevent the decomposition of its ascorbic acid content. Our best sources of vitamin C are still fresh foods and green vegetables and a careful food selection can provide consumers with several hundred milligrams of antioxidants daily.

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