CHEMICAL COMPOSITION OF *VITIS QUADRANGULARIS* (WALL)

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Vitis quadrangularis is a tender climber with fleshy quadrangular stems and is distributed all over the hotter parts of India. In Sanskrit it is known as *Asthisamhara*, in Hindi as *Nallar* and Telugu as *Nallern*. When cut and rubbed on the skin the stem produces a prickly sensation which lasts a considerable time.

The plant (stems, leaves) is commonly used as a green vegetable. The juice is added to pastry with a view to improve their texture and make them swell when fried. The ash is frequently employed in the place of baking powder. The fresh or dried stems, leaves and tender shoots hold a prominent place as household remedies mainly in connection with affections of the digestive system. In certain parts of India the tender shoots of the plant are ashed in closed vessels and administered in dyspepsia and indigestion. The drug seems also to find use in cases of asthma, scurvy and other ailments.[1] No chemical investigation appears to have been done on this drug till now.

The following results give a general idea of the composition of the air-dried drug :

	%
Moisture	13·1
Ash	18·2
Proteins	12·8
Carbohydrates	36·6
Fibre	15·6
Fat and Wax	1·0
Mucilages and Pectins	1·2

A preliminary test for alkaloids using Prollius's fluid showed that there was none. The following solvents were used in succession for extraction on a small scale and the extracts were examined :

		%	
1.	Petrol (60°–80°) ..	2·7	Wax and some chlorophyll.
2.	Ethyl ether	0·4	Chlorophyll and some resin.
3.	Carbon tetrachloride ..	0·2	Chlorophyll and some wax.
4.	Alcohol	2·1	Sugars and inorganic salts.
5.	Water	5·4	Gums, pectins and sugars.

Since the most considerable portions were extracted by petrol, alcohol and water several kilograms of the drug were extracted with these three solvents in succession and the extracts were studied in detail. The wax present in the petrol extract was isolated in the form of a yellow solid melting at about 80° and its general properties have been recorded. About 50 per cent. of the alcohol extract consisted of mineral matter comprising mostly of sodium and potassium salts (carbonates and chlorides). The remaining resinous portion contained some tartaric acid and a small quantity of yellow flavone pigments. The former was characterised as the acid potassium tartrate; the latter was too small to be isolated and studied. The final aqueous extract was found to undergo fermentation readily to give alcohol. Besides sugars it contained gums and pectins which could be thrown out by the addition of alcohol. Calcium was found to be present in the ash obtained from the precipitated solid.

An analysis of the sap of the green plant gave results somewhat similar to that of the aqueous extract. A microscopic examination of a section of the stem indicated the existence of plenty of small crystals of calcium oxalate. The irritating action of the freshly cut stem surface may be due to the penetration of these tiny crystals into the skin. The presence of this salt could be further established by extracting the air-dried material with dilute hydrochloric acid and neutralising the extract with ammonia.

The ash which formed 18·2 per cent. of the air-dried material was analysed in detail qualitatively and quantitatively. It was found to consist mostly of the carbonates and to a smaller extent of the phosphates of sodium, potassium, magnesium and calcium. The total absence of chlorides in the ash was remarkable since they were found to be present in the mineral matter that was isolated from the alcoholic extract. This is obviously due to the loss of the chlorides by volatilisation during the course of ashing. Such loss has been noted by other workers.[2]

From the above results there seems to be no organic compound in *Vitis quadrangularis* that could account for its medicinal properties. The high percentage of the ash in the plant and its composition may explain the beneficial effect in cases of dyspepsia and indigestion. Since the plant has been employed as a remedy against scurvy and is consumed to a large extent as a vegetable and since the sap and water extract have very high reducing power, experiments were made to estimate the amount of carotene and ascorbic acid (Vitamin C) present in the material. The value for carotene was 267*r* per 100 grams and hence in this respect this vegetable occupies a middle place. On the other hand the content of Vitamin C was remarkably high being about 400 mg. per 100 grams of the fresh material. The value

obtained for the freshly expressed sap was still higher and hence *Vitis quadrangularis* is one of the vegetable sources which top the list regarding this vitamin as shown in the following table :—

Vegetable Source	Vitamin C content in mg. per 100 grams of the material	Literature reference
Vitis quadrangularis (Shoots and leaves) ..	398	This paper
Do. Sap ..	479	Do.
Rose hips	308–416	Goldberg and Walsh[3]
Indian Gooseberry	413	Damodaran and Srinivasan[4]
Drumstick	216	
Cashew apple	203	
Guava	299	Ranganadhan[5]
Parsley	280	
Amaranth leaves	174·9	
Green chillies	143·0	
Cabbage	132·1	
Orange juice	102·0	
Cauliflower	65·8	
Spinach	36·9–63·7	

Experimental

The fresh plant was collected from the surroundings of Waltair, and after cleaning and drying in the sun ground into powder in a coffee mill. There was about 90 per cent. loss of weight on drying. The preliminary analysis for the proximate components was done by standard methods adopted by the A.O. A.C. ; the results are given in the introductory part.

100 g. of the powdered drug were extracted with Prollius's fluid for 24 hours in the cold and the extract examined for the presence of alkaloids. The test was negative. The same weight of the drug was extracted in a

soxhlet apparatus successively with various solvents. The summary of the results of this extraction has been given in the introductory part.

The Petroleum extract: *Wax*.—For purposes of a detailed study of the important fractions several kilograms of the dry powder were extracted first with light petroleum (b.p. 60°–80°) in a continuous extractor for 12 hours. After recovering the solvent from the extract, a greenish yellow viscid mass was obtained. When treated with ether or hot benzene most of the green colouring matter could be removed and a yellow wax was left behind. The most convenient method of obtaining the wax pure was to boil the viscous solid with alcohol for 15 minutes, filter hot and allow the solution to cool. The yellowish solid that was deposited looked clean; it was subsequently filtered and washed with small quantities of alcohol. The process was once again repeated for further purification (yield 1 per cent.). The product had all the properties of a wax. It was greasy to the touch and left a grease spot when rubbed on paper. It was insoluble in water and cold dilute alcohol and floated on water. But it dissolved easily in benzene, carbon tetrachloride, chloroform and ether. Its properties were examined by the standard methods and they are recorded below:—

Colour	Light yellow
Melting point	78°–80°
Acid value	27·5
Saponification value ..	121·8
Iodine value	11·2
Unsaponifiable matter ..	49·6

The brown amorphous unsaponifiable matter gave rise to a faint yellow product melting at 83°–86° when crystallised from alcohol. It contained some sterols also since it gave a violet colour when to a chloroform solution acetic anhydride and sulphuric acid were added.

Alcohol extract: *Mineral matter*.—The residue left after the extraction with petrol was subsequently extracted in the same apparatus with methylated spirit for 12 hours. When the extract was concentrated to a small bulk it deposited a colourless crystalline solid which could be separated pure by filtering and washing with small quantities of alcohol. It was inorganic in nature and consisted of the carbonates and chlorides of potassium and sodium. It amounted to nearly 50 per cent. of the alcoholic extract or one per cent. of the air-dried drug. The remainder of this fraction obtained on completely evaporating the solvent was a resinous mass which could not be made to crystallise. After extracting it with hot water, the water-insoluble resin was found to possess neither taste nor smell. It dissolved in alkali and was partly soluble in sulphuric acid to give a green solution. The aqueous

extract was acidic to litmus, gave strong tests for sugars (glucose as osazone) and gave indications for the presence of flavone pigments.

The hot water extract obtained above was treated with neutral lead acetate solution and the precipitated lead salt was isolated and decomposed in aqueous suspension with hydrogen sulphide. After filtering off the sulphide of lead the aqueous solution was concentrated to a small bulk. A small quantity of an amorphous gummy solid was thus obtained and though this gave tests for flavones no crystalline solid could be isolated and the quantity was too little to enable further investigation to be carried out.

After removing the neutral lead acetate precipitate the aqueous mother liquor was treated with excess of basic lead acetate and the yellow precipitate thereby obtained was decomposed with hydrogen sulphide as before. When the final aqueous solution was concentrated *in vacuo* and treated with an aqueous solution of potassium acetate and subsequently with alcohol a colourless solid was obtained. This was identified as potassium hydrogen tartrate from its properties and reactions.

The final mother liquor was deleaded by passing hydrogen sulphide and concentrated *in vacuo*. No crystalline substance could be isolated, but the amorphous residue was saccharine in nature and gave rise to a good yield of glucosazone on being treated with phenylhydrazine hydrochloride, sodium acetate and acetic acid.

Hence the alcoholic extract was found to contain a good amount of mineral salts, some alkali soluble resin, sugars, some tartaric acid and a trace of flavone pigments.

Water extract : gums and pectins.—The plant material left after extraction with alcohol was subsequently boiled up with water and filtered hot. A brownish, highly viscous extract was thereby obtained and this was found to ferment rapidly on keeping to give rise to alcohol. When twice the volume of alcohol was added to it a white gummy solid separated out. This amorphous substance underwent hydrolysis easily to give products which reduced Fehlings solution and on incineration left an ash containing calcium, showing thereby that pectins were present. The solution left after the removal of the gums and pectins gave good tests for glucose (osazone) and on concentration and incineration yielded inorganic matter.

Examination of the ash.—The ashing was effected in large platinum basins. A qualitative analysis revealed the following as major components : calcium, magnesium, potassium and sodium, carbonates, phosphates and silica. Iron, aluminium and sulphate were found in traces. For the quantitative estimation the residue insoluble in dilute hydrochloric acid was taken

as silica. Phosphoric acid was estimated by the volumetric method through phosphomolybdate. The carbonate was determined gravimetrically by decomposing the ash with excess of acid and absorbing the carbon dioxide evolved with soda lime. Lime was estimated volumetrically as the oxalate and magnesia by precipitation with 8-hydroxyquinoline in ammoniacal solution. Potassium and sodium were weighed as chlorides and after estimating the former as the perchlorate the latter was obtained by difference. The following table gives the average of several analyses :—

	%
SiO_2	3·00
P_2O_5	4·13
CO_2	23·95
CaO	18·44
MgO	15·79
K_2O	17·54
Na_2O	13·16

Isolation of calcium oxalate.—200 g. of the powdered drug were extracted with petrol and then with alcohol and the residue boiled with 1 per cent. hydrochloric acid for 2 hours. The acid extract was concentrated to a small bulk on a water-bath and twice its volume of alcohol added so as to precipitate gums and pectins. After filtration, the filtrate was rendered slightly alkaline with ammonia and then acidified with acetic acid. On heating the solution for half an hour on a water-bath a copious white precipitate settled down. It responded to all tests for calcium and oxalic acid and was identified as calcium oxalate.

Estimation of Carotene

About 10 grams of the fresh material were boiled for an hour with 20 per cent. alcoholic potash. After filtration the residue was soaked with aqueous ether and extracted with acetone repeatedly till the extract was colourless. The combined extracts were concentrated and the residue taken up in petroleum ether. Xanthophyll was removed by shaking the above solution with small quantities of 85 to 90 per cent. methyl alcohol. The petroleum solution of carotene was then made up to a known volume and compared with a 0·2 per cent. solution of potassium dichromate in a comparator. The amount of carotene was obtained from a standard curve.[6] The average value from several determinations was 267 γ of carotene for 100 grams of the plant material.

Vitamin C.[7]—10 Grams of the freshly cut material were ground up with 20 per cent. trichloroacetic acid (6·5 c.c.) and specially purified sand and filtered through muslin. The extraction was repeated 3 times with fresh

portions of the acid solution. The combined extract was made up to 100 c.c. with water and filtered through a filter paper. The concentration of trichloroacetic acid in this solution was thus kept at about 5 per cent. This solution was titrated against 1 to 5 c.c. of standardised 2 : 6 dichlorophenol-indophenol solution within 1 to 2 minutes. The dye solution was prepared by dissolving 0·05 g. in 100 c.c. of water and standardised against a solution of ascorbic acid whose purity had been tested with a standard solution of iodine. The average of several determinations using the tender portions of the stem and using 1 to 5 c.c. of the dye solution was 398 mg. per 100 grams of the plant. The fibrous bottom portions of the stem gave a lower value, 232 mg. The freshly expressed sap was diluted with water and sufficient trichloroacetic acid so as to make its concentration 5 per cent. and then titrated against the dye solution. In another set of experiments trichloroacetic acid was omitted. In both cases, however, the same values for ascorbic acid were obtained : 479 mg. per 100 grams. The quality of the sap deteriorated rapidly on keeping. After about 24 hours the loss was found to be about 25 per cent.

Summary

A detailed chemical examination of the stems and leaves of *Vitis quadrangularis* for the organic components and mineral matter has been made. Besides gums and pectins, a yellow wax and tartaric acid as the acid potassium salt were isolated. The presence of calcium oxalate crystals may account for the irritating action of the freshly cut stem on the skin. The plant contains a high percentage of mineral matter and the ash consists mostly of the carbonates of magnesium, calcium and the alkali metals. As a source of carotene it occupies a middle place and it contains a remarkably high percentage of ascorbic acid (Vitamin C).

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