sterol[3] (2, 60 mg), (iii) glycerol α-heptanoate α'-octanoate (3, 10 mg), (iv) β-sitosteryl-β-D-glucoside[3] (4, 50 mg) (v) sucrose[3] (5, 60 mg) and the eluates for these are (i) benzene: petroleum ether (1:19), (ii) benzene:petroleum ether (1:1), (iii) ethyl acetate:benezene (1:19), (iv) ethyl acetate:benzene (1:3), (v) methanol:ethyl acetate (1:4). The two glycerides are hitherto unreported compounds and their data is given hereunder.

Glycerol β-butanoate ∝,∝1-dipentanoate (1) was crystallised from benzene, m.p. 68-70°. Its R_f value was found to be 0.65 in benzene:petroleum ether (1:4). Found:C,61.77; H,9.05; $C_{17}H_{30}O_6$ required: C, 61.81; H, 9.09. ν_{max}(KBr, cm^{-1}): 725, 802, 864, 1034, 1096, 1173, 1258, 1373, 1466, 1682, 1736. ^1H NMR (δ, $CDCl_3$): 0.86 (9H, t, J 7.5 Hz, 3xMe), 1.25 (4H, m, $2xCOCH_2CH_2CH_2$), 1.61 (6H, m, $3xCOCH_2CH_2$), 2.29 (6H, t, J8.0Hz, $3xCOCH_2$), 4.05 (5H, m, $2xCH_2$, 1xCH).GCMS (m/z, rel. int.) : 333 (M^++3, 1.8), 332 (M^++ 2, 8.1), 331 (M^++1, 58.8), 275 (1.6), 271 (3.4), 229 (2.6), 213 (1.5), 212 (7.9), 211 (70.0), 169 (100.0), 127 (12.2), 109 (54.6).

Glycerol α-heptanoate ∝'octanoate (3) was crystallised from ethyl acetate, m.p. 86-88°. Its R_f value was found to be 0.80 in ethyl acetate:benzene (1:4) Found: C, 65.40; H, 10.27: $C_{18}H_{34}O_5$ required: C 65.45; H 10.30. ν_{max} (KBr, cm^{-1}) : 725, 802, 864, 1026, 1096 1380, 1466, 1736 and 3425.^1H NMR (δ $CDCl_3$): 0.87 (6H, J 7.5Hz, $2xCH_3$), 1.25 (14H, m, $7xCH_2$), 1.61 (4H, m, $2xCOCH_2CH_2$), 2.33 (4H, t, J 8.0 Hz, $2xCOCH_2$), 2.72 (1H, m, 1xCH), 4.15 (4H, d, J 7.5Hz, $2xCH_2$). GCMS (m/z, rel. int.): 332 (M^+.+2, 10.4), 331 (M^++1, 64.2), 218 (8.5), 212 (11.3), 211 (66.0), 169 (100.0), 127 (12.3), 109 (55.7).

ACKNOWLEDGEMENTS

We are thankful to Mr. Dubey, Landscape, HAU, Hisar, for the supply of the plant material.

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Spectrophotometric Determination of Hydroxy Citric Acid

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Accepted 23 July 1999
Received 2 July 1998

Spectrophotometric determination of hydroxy citric acid (HCA) present in *Garcinia cambogia* fruit is proposed here. This method is based upon, the colour complex formation (λmax: 467 nm) between hydroxy citric acid and sodium meta vanadate.

Hydroxy citric acid (HCA) is the major constituent of *Garcinia cambogia,* an exotic fruit grown in the southern parts of India[1]. *Garcinia cambogia,* commonly known as "Malabar Tamarind" is regarded recently as the best natural medicine for controlling obesity[2-4]. *Garcinia cambogia* extract as its calcium salt is a widely accepted OTC (over the counter) drug in the USA and Japan.

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The method proposed here by spectrophotometry is fast, accurate and specific for HCA. HCA is easily liable to form lactone[5] and lactones give negative results. In this estimation procedure, the HCA lactone was converted into the respective calcium salt followed by hydrolysis using dilute sulphuric acid and colour reaction with sodium meta vanadate solution. The absorbance was measured at 467 nm. The standard used was ehtylene

diamine salt of HCA. *Garcinia cambogia* rind was collected from Kottayam District in Kerala. All chemicals used are of AR grade. Ethylene diamine salt of HCA was purchased from Fluka chemical company (USA). Double beam spectrophotometer (Shimadzu 160A) and a pH meter were used for the analysis.

Dried and smoked *Garcinia cambogia* rind was used for analysis. One hundred grams of chopped and dried material having 15% moisture content was boiled thrice with 300 ml of water filtered and concentrated under vacuum to 50% moisture level. The thick concentrated liquid was filtered, the residue washed with small portions of water and combined with the mother liquid. It was neutralised with 4 N NaOH solution maintained at pH 7.5, followed by the addition of 50% solution of $CaCl_2$ and was well stirred. The precipitated residue was filtered through a Buchner funnel and was dried (moisture content-4%, Yield-25 g). About 0.2 g of the Calcium salt was accurately weighed and dissolved in 5 ml of 1 N H_2SO_4 and the solution was diluted to 25 ml with distilled water. The solution was decolourised with activated charcoal, filtered, washed with small portions of distilled water and then made up to 50 ml. Working standards were prepared using ethylene diamine salt of HCA [98% ED-HCA]. The salt equivalent to 0.042 g of the free acid was weighed accurately and dissolved in 5 ml of 1 N H_2SO_4 and approximately 25 ml of distilled water was added. It was Filtered and transferred into a 50 ml volumetric flask and was made up to the volume using distilled water. The resulting solution has a concentration of 828 µg/ml HCA.

One ml of the standard solution was pipetted out into a 100 ml volumetric flask and the volume was made up with distilled water. Two hundred microlitres of 5% sodium meta vanadate solution was then added. The yellow colour slightly changed to reddish orange after 20 min. The absorbance was measured at 467 nm against a blank which was prepared without sample solution. The same experiment was repeated using 1.5, 2, 2.5 and 3 ml of the standard solution. A calibration graph was plotted with the concentration of HCA against absorbance. This procedure was repeated using the sample solution and the percentage of HCA was calculated.

It was observed that colour complex formation between HCA and meta vanadate is unique and specific. Garcinia fruit contains no other organic acids in considerable amount except citric acid (3-5%). The amount of citric acid was estimated by Penta bromo acetone method. [A.O.A.C. - Association of Official Analytical Chemists][6]. When sodium meta vanadate solution is added to citric acid solution, the yellow colour remains unchanged. The average absorbance plotted against concentration of HCA obeys Lambert-Beer's law in a range 5-50 µg/ml HCA.

Stability of colour complex is time-dependent and gets stabilized after 20 min. The analysis of different samples of dried Garcinia fruits shows a positive correlation between the absorbance and concentration of HCA. In this method, all HCA lactones present in the fresh fruit of Garcinia are converted into pure acid by reacting with alkali and finally converted into calcium salt to get a stable form. Hence this method is very reliable in the estimation of HCA. The minimum specified limit of HCA in dietary food supplements in USA and Japan is 50%. By adopting this method the determination of HCA can be made rapidly and accurately.

ACKNOWLEDGEMENTS

The financial assistance from UGC, New Delhi is gratefully acknowledged.

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