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Estimation of capsaicin in seven cultivated varieties of *Capsicum Annuum* L.

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ABSTRACT

One of the most important traits of chillies is pungency. It is a desirable attribute in many foods which increases the acceptance of the insipid basic nutrient foods. Pungency is produced by the capsaicinoids (alkaloid compounds), that are formed only in the plant genus Capsicum. Among the different capsaicinoids in different Capsicum species, Capsaicin has been recognized as the major component. It makes upto 70% of the total capsaicinoids. Capsaicin is the major of the capsaicinoids which produce pungency in chillies. It is a stable and powerful alkaloid. Capsaicin content varies with the variety, climate, geographical location, maturity when harvested, and the methods for processing or preservation. The present study deals with the estimation of capsaicin in seven different varieties of Capsicum annuum and to ascertain the varieties which contain the most appreciable amounts of capsaicin. . Capsaicin was estimated at the turning red stage in all the seven varieties by the HPTLC technique. Capsaicin was detected and quantified in all the varieties studied. The amount of caspsaicin varied from 0.017% (cv Sanyogita special) to 0.199 % (cv Phule jyoti).The mean value recorded was 0.074%.

Keywords: Capsicum annuum, Capsaicin, Gibb's reagent and HPTLC.

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INTRODUCTION

Capsaicin is the major component of the capsaicinoids in chilli peppers. Various theories have been presented as regards the location, formation, accumulation and secretion of capsaicin. However, recent studies indicate that capsaicin is mostly located in the vesicles or vacuole like sub-cellular organelles of the epidermal cells of the placenta in the pod [1]. It is reported that capsaicin is not evenly distributed in pepper fruit. In general, the highest capsaicin concentrations are found in the ovary and in the lower flesh (tip) and the lowest capsaicin content can be found in the seeds [2]. Capsaicinoids are produced in glands on the placenta of the fruit [3]. The seeds are not the source of pungency but they occasionally absorb capsaicin because they are in close proximity to the placenta. No other plant part produces capsaicinoids.

Hot chilli peppers (genus Capsicum) are among the most heavily consumed spices throughout the world [4] . Besides its use as a food additive in various spicy cuisines, Capsicum (due to its capsaicin content) is currently used for various therapeutic purposes such as asthma, coughs, sore throats, to relieve toothaches, counter-irritant balm for external application, to alleviate pain, shingles, arthritis, diabetic nemopathy, etc.

The medicinal applications of capsaicinoids have brought innovative ideas for their use. The medicinal use of Capsicums has a long history, dating back to the Mayas who used to treat asthma, cough and sore throats. The Aztecs used chilli pungency to relieve toothaches. The popularity and familiarity of products containing Capsicum has led to rapidly growing economic significance in a wide array of food products, medicine, industry, law enforcement and pest control. Apparently, a market exists for the exploitation of chilli peppers for the medicinal properties of capsaicin. There is scope for regulating capsaicin biosynthesis in Capsicum genotypes to meet the demands of food, pharma and cosmetics industries.

Pungency in pepper pods is a consequence of accumulation of capsaicin and its analogs (capsaicinoids). They are produced as secondary metabolites in chilli peppers, probably as deterrents against herbivores. The chilli fruit develops greater pungency in tropical countries like India, Africa and Tropical America than in the cold regions.

Capsaicin is the main capsaicinoid in chilli peppers, followed by dihydrocapsaicin. These two compounds are also about twice as potent to the taste and nerves as the minor capsaicinoids nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin.

Fast and simple methods that do not require very modern equipment are needed for quantitative estimation and quality control [4].

Chromatographic methods, in particular TLC/HPTLC and HPLC are used extensively for material product identification, quantification and purification[5-7].

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TLC has retained favour as an analytical method primarily because of its simplicity, reliability, low cost and selectivity of detection. Of the many chromatographic methods presently available, TLC is widely used for rapid analysis of drugs and drug preparations since it offers several advantages [7-9].

In view of all these pharmaceutical applications, the aim of the present study was the quantitative determination of capsaicin in seven cultivated varieties of C. annuum (dried red pepper) by HPTLC.

MATERIAL AND METHODS

Seven cultivated varieties of C. annuum L. were selected for the present work. They were Achari (ACH), G4, Pusa Jwala (JW), Phule Jyoti (JY), Phule Kirti (KR), Pusa Sadabahar (SADA), and Sanyogita Special (SS). Each of the varieties were grown till full maturity. The fruits were picked when they just began to turn red. They were dried in shade for about two weeks. Fruits from 5 different plants of each cultivated variety were sampled together in duplicate. They were kept in a preset oven at 45 ±2 ˚C for four days till they were brittle. The chillies were then powdered and sieved minus their stalks. A fine powder was obtained which was stored in air tight containers. HPTLC was performed after appropriate modifications in the method reported by Wagner [8].

Sample preparation:

One gram of each red chilli sample was extracted by heating under reflux for 10mins in 10ml of methanol, and was filtered through Whatman No. 41. The filtrate was evaporated to dryness in a water bath and reconstituted in 5ml methanol. This extract was used for HPTLC. The extracts of ACH and JY were diluted (1:9) and SADA diluted (1:1) using methanol. The rest i.e. G4, JW, KR and SS were applied without dilution. Application volumes for each of the varieties were standardized as follows: ACH, JW and JY (2µl each); G4, KR and SADA (3µl each); and SS (4µl).

Standard preparation:

Standard capsaicin (purity > 99.0% HPLC) was procured from Sigma Aldrich (St. Louis, U.S.A.). Ten milligrams of capsaicin was dissolved in minimum quantity of A. R. Grade methanol, and diluted upto mark in a 10ml volumetric flask. This formed the 1000ppm stock solution. The 100 ppm working standard solution was prepared from the stock solution.

Preparation of Gibb's reagent:

Gibb's reagent, or 2,6-dichloroquinone-4-chloroimide (assay 99%), was procured from Loba Chemie Ltd., Mumbai. Reagent for derivatization was prepared by dissolving 500mg of Gibb's reagent in 100ml methanol.

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Capsaicin standard, as well as the samples were spotted on pre-coated silica gel $60F_{254}$ plates (E.Merck), using CAMAG Linomat V sample applicator. The mobile phase employed was Chloroform: Methanol: Acetic acid (9.5: 0.5: 0.1, v/v/v). The plates were developed upto 80mm in CAMAG twin trough development chambers (20x10), post chamber saturation of 15 minutes. The plate was allowed to dry in air, dipped in Gibb's reagent, air dried again and later exposed to ammonia vapours in a twin trough chamber. Blue violet zones were visible due to the spontaneous reaction which remained for 2-5mins. Densitometric scanning of the plates was performed at 576nm [10], using CAMAG TLC Scanner 3. The values of the areas obtained for the standards were plotted as a function of the concentrations of the standard applied on each track. The regression coefficient, relative standard deviation, slope and intercept on the Y-axis were calculated by the software. Based on the value of the relative standard deviation, the Limit of Detection (LOD) and the Limit of Quantitation (LOQ) were then calculated. The calibration graph so obtained was used to quantify the capsaicin content in each sample. The software employed for the analysis was WINCATS.

RESULTS

The HPTLC plate developed for analysis of the extracts of all the seven cultivated varieties of C. annuum L. is shown in **Plate 1**. Volumes loaded, amount fractions spotted, R_f values, and areas obtained for each track of standard spotted on the plate are shown in **Table 1**. The plot of the areas of the peaks on each track versus the amount fraction per track gave rise to a calibration plot as shown in **Figure 1**. The **regression coefficient (r)** was calculated as **0.9989**, **relative standard deviation** as **3.61**, **Limit of Detection (LOD)** as **0.51ng**, and **Limit of Quantification (LOQ)** as **1.56ng**. The capsaicin content of all the selected varieties was calculated to be 0.109% in ACH, 0.032% in G4, 0.048% in JW, 0.199% in JY, 0.019% in KR, 0.096% in SADA, and 0.017% in SS. A bar graph representation of the same has been depicted in **Figure 2**. The Genotypic Coefficient of Variance (GCV) and Phenotypic Coefficient of Variance (PCV) were both calculated to be about 90.56%.

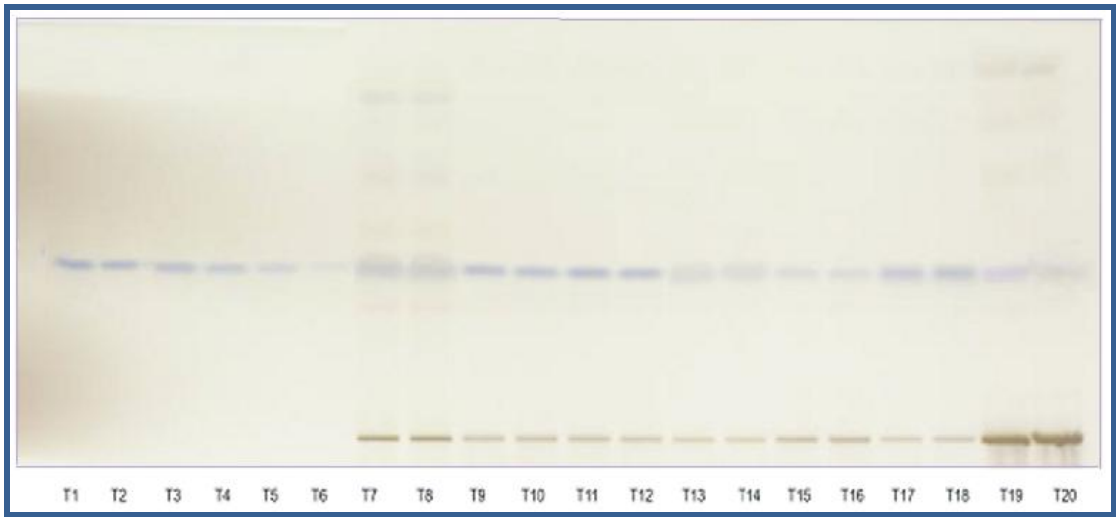

Plate 1

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HPTLC plate of analysis of standard capsaicin and the extracts of the seven cultivated varieties of Capsicum annuum L. after derivatisation : T1 180 ng std; T2 150 ng std; T3 120 ng std; T4 90 ng std; T5 60 ng std and T6 30 ng std; T7 & T8 ACH; T9 & T10 G4; T11 & T12 JW; T13 & T14 JY; T15 & T16 KR; T17 & T18 SADA and T 19 & T20 SS.

Table 1: Multilevel standard calibration curve details for linearity using capsaicin reference standard

Track	Standard level	Application Volume(µl)	Amount fraction per track (ng)	Rf	Area
1	Standard level 1	3.0	30.00	0.42	724.53
2	Standard level 2	6.0	60.00	0.42	1442.31
3	Standard level 3	9.0	90.00	0.44	2263.10
4	Standard level 4	12.0	120.00	0.44	3008.60
5	Standard level 5	15.0	150.00	0.45	3664.40
6	Standard level 6	18.0	180.00	0.45	4514.20

Figure 1: Calibration graph for standard Capsaicin

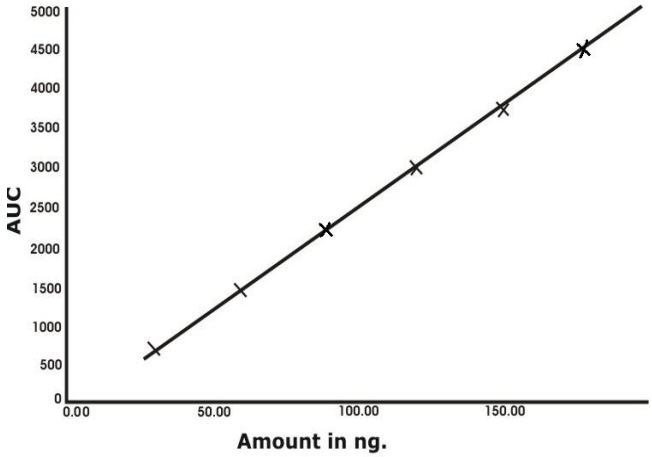
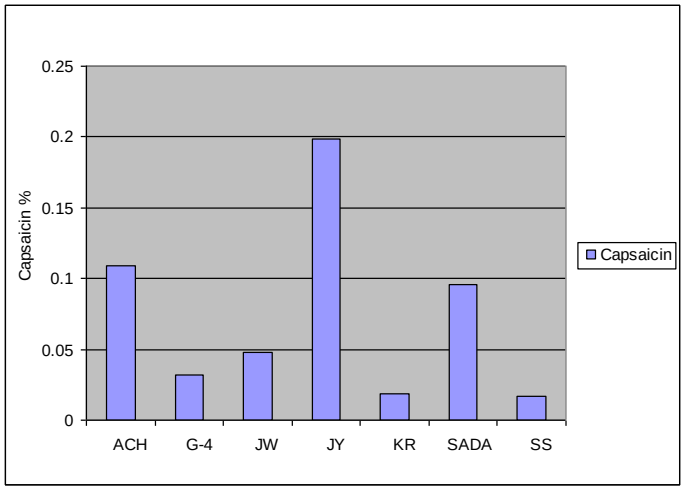


Figure 2: Bar graph representation of calculated capsaicin content in the selected varieties of Capsicum annuum L.



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DISCUSSION

The amount of capsaicin among the seven varieties varied from 0.017%-0.199%. The mean value recorded was 0.074%. The variety Sanyogita special had the lowest capsaicin content (0.017%) and Phule jyoti had the highest capsaicin content (0.199%).

According to Scheffe's test, the seven different genotypes were clubbed into six different subsets. KR and SS were placed together in one subset showing 0.019% and 0.017% capsaicin content respectively. ACH, G-4, JW, JY and SADA were each placed in other subsets because their capsaicin content varied considerably amongst each other. The Genotypic efficient of Variance and Phenotypic Coefficient of Variance are found to have same values for capsaicin content. This indicates that the genotype has a highly significant effect on the phenotypic expression, with hardly any effect of the environment. High heritability estimates (100%) resulting from high GCV (90.56%) indicates that this trait can be improved by selection. Similar results were reported in C. chinense Jacq. with high heritability (99.62%) with respect to capsaicin content [10,11].

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