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A Simple and Efficient Method for Extraction and Quantification of Capsaicin from Pepper Fruits through High Performance Thin Layer Chromatography.

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ABSTRACT

A simple and efficient method for extraction and quantification of capsaicin using high performance thin layer chromatographic (HPTLC) was developed and validated from seven different types of *Capsicum annuum* fruit. The crude extracts were subjected to TLC for the qualitative and quantitative examination for the capsaicin. The chromatographic separation was carried out on precoated silica gel plate using Chloroform: Methanol: Acetic acid: Hexane (2.85: 0.15: 0.15: 1, v/v/v) as mobile phase and densitometric analysis was carried out in absorbance mode at 282 nm. The mobile phase gave well defined peaks of capsaicin at R_f value of 0.78. The linear regression analysis data for the calibration plots using capsaicin standard showed good linear relationship with regression coefficient (R^2) of 0.985 for height and 0.995 for area, respectively; in the concentration range of 1-5 µg/spot. Among the fourteen different solvent, the ethanol produce maximum extraction efficiency of capsaicin. The proposed HPTLC method can be applied for robust identification and quantitative determination of capsaicin from different types of pepper fruits.

Keywords: *Capsicum annuum,* HPTLC, capsaicin

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INTRODUCTION

Capsicum annuum is a non-climacteric bell-pepper fruit and belong to the family Solanaceae. They are used for culinary purposes, natural colouring agent and pharmaceutical ingredient. *Capsicum* is the only genus having the potential to produce capsaicinoids, with capsaicin and dihydrocapsaicin accounting for up to 90 percentage of the total pungency of pepper fruits[1].Capsaicin a phenyl propanoid compound (trans-8-methyl-*N*-vanillyl-6-nonenamide) is a crystalline and lipophilic alkaloid with the molecular formula $C_{18}H_{27}NO_3$.The degree of pungency in pepper fruits is regulated by the amount of produced capsaicin which is governed by various biotic and abiotic factors viz. genetical, environmental and by genotype–environment interaction. Therefore, high intra and inter genotype variations of pungency level is found [2-4]. The variation is attributable to pungency being a polygenic trait [5]. Capsaicin production and as a result pungency increases in case of water stress, high temperature and soil fertility[6-11].The accumulation of capsaicin may also depend on fruit age and stage development [12]. Capsaicin is the major constituent accounts for the pharmaceutical properties of pepper. It has analgesic effects and is used against arthritis pain and inflammation [13]. It also showed anticancer activity, protective effects against high cholesterol levels and obesity and activity against neurogenic inflammation [14-19]. Because of the increasing use in medicine and pharmacy it has become important to establish a sensitive, accurate and simple technique for extraction of capsaicin from pepper fruits.

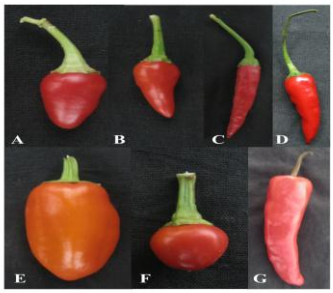
High performance thin-layer chromatography (HPTLC), a contemporary version of conventional thin layer chromatography is a rapid, inexpensive and accurate method for detection and phytochemical evaluation of the herbal [20, 21]. It does not require detailed sample clean up and crude extracts can be directly used for HPTLC analysis. This study is focused on effective separation, identification and quantification of the capsaicin extracted using different solvents from *Capsicum annum* using HPTLC technique.

MATERIALS AND METHODS

Plant Material

Seven varieties with distinct morphological characters of pepper fruits were collected from local market and seeds were harvested. The seeds were germinated and maintained at the experimental garden of RKMVC College, Rahara, Kolkata. These plants were identified at Botanical Survey of India, Howrah under the voucher specimen number RKMVCC-CP-3 toRKMVCC-CP-10. All the herbarium of the plants was deposited at the Central National Herbarium (Howrah, India).The mature fruits of the plants were collected and sun dried (Fig. 1). The dried fruits were ground to powder and stored at 4°C in airtight containers until use.

Figure 1: The seven different types of pepper fruits used for capsaicin extraction.



Chemical list

The standard capsaicin (8-methyl-N-vanillyltrans-6-nonenamide) was purchased from Sigma Chemical Co, St. Louis, MO, USA. All solvents (HPLC grade) used for capsaicin extraction and analysis were purchased from Merck India.

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Extraction of capsaicin

The extraction and quantification of capsaicin in different solvents was performed according to Collins et al. (1995) with minor modifications [22]. This method was described as general method. Briefly, the dried powder was weighed and mixed with the different solvents given in Figure2 in the ratio 1:10 (gram: milliliter) and extracted at 60°C for 24 hours. The extracts were filtered and concentrated to a final volume of 2ml using lyophillizer (Model No. CTFD-12PT, Creatrust, China).In the modified method, capsaicin was extracted using Soxhlet apparatus (Borosil glass, India). One gram powder was taken and extracted with ethanol as solvent using Soxhlet apparatus. The extraction was performed until the tissue was decolorized(about 8 hours) at 60°C. The extracts obtained were concentrated to a final volume of 2mlusing lyophillizer.

Instrumntation and chromatographic conditions

Five microliters of the ethanolic extract (500 mg/mL) was applied (band length –5.0 mm; distance between bands –14.5 mm; distance from left edge - 20.0 mm; distance from lower edge - 10.0 mm) on a precoated TLC aluminum sheets of silica gel G60 F254 of 200 µm thickness plate- 20x10cm (Merck, Mumbai) using Linomat 5 automated TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-µL syringe (Hamilton, Nevada, USA). The standard capsaicin at concentration of 0.1mg/ml was spotted as a reference on the TLC plate. Prior application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Chloroform: Methanol: Acetic acid: Hexane (2.85: 0.15: 0.15: 1, v/v/v) in a Camag HPTLC twin-trough chamber (20x10cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed upto85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode at 282 nm operated by WinCATS software (version 1.4.8). Quantitative analysis of the extracts was done by comparative densitometric analysis via height and area with the standards.

Scoville Heat Unit Conversions

The capsaicin contents obtained from different fruits were converted to Scoville heat units (SHU) in order to classify them according to their various pungency levels. This conversion to SHU was done by multiplying the capsaicin content by the coefficient corresponding to the heat value for pure capsaicin that is 1.6×10^7 [23].

RESULT AND DISCUSSION

Method optimization

The compositions of the mobile phase were optimized to establish a suitable and precise HPTLC method for analysis of capsaicin. The mobile phase composed of Chloroform: Methanol: Acetic acid: Hexane (2.85: 0.15: 0.15: 1, v/v/v) resulted in a sharp, symmetrical and well resolved peak at R_f value of 0.78 for capsaicin (Fig. 2A). The UV spectra measured for the peak of capsaicin showed maximum absorbance at approximate 282 nm (Fig. 2B)

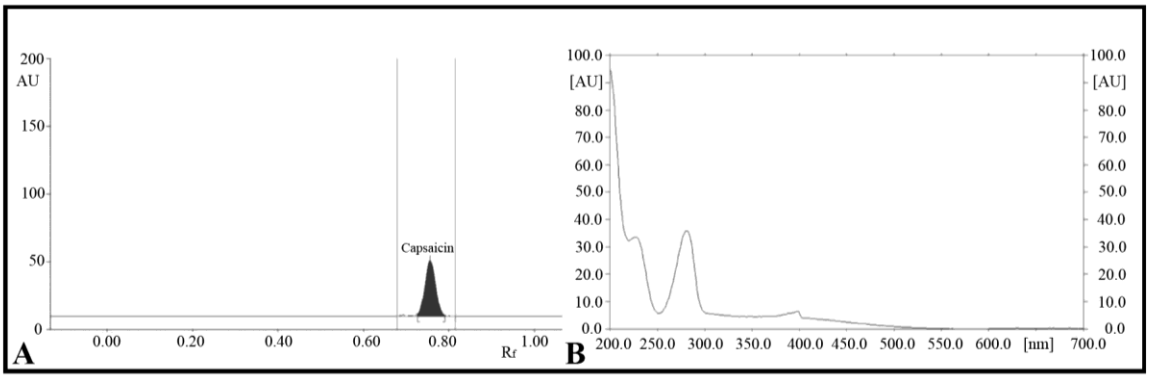

Figure 2: HPTLC chromatogram of standard capsaicin (A); UV absorption spectrum of the standard capsaicin (B).

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Comparative analysis of extraction method

In general method, the extraction of capsaicin from pepper fruits was performed using fourteen solvent. The obtained crude extract from theses solvents were subjected to TLC analysis. The capsaicin extracted from the 14 different solvents resolved on TLC plate were analyzed under the UV light at 282 nm (Fig. 3). It was observed that the R_f value (0.78) corresponding to standard capsaicin were found in all extracts except water. The peak purity of capsaicin was assessed by comparing the spectra at peak start, peak apex and peak end positions of all the spots. From the HPTLC fingerprint it was observed that among 14 extracts the TLC profile of ethanol has capsaicin spot with less of other impurities. The comparative densitometric analysis of height and peak area of the capsaicin from these 14 solvent were performed using WinCATs software and it was found that ethanolic extract produce maximum values. The height and area of capsaicin extracted by ethanol was found to be 180.877 ± 2.024 and 5203.667 ± 26.038, respectively (Fig 4). The minimum values of height and area was found to be 38.857 ± 1.552 and 579.790 ± 31.227, respectively in case of acetonitrile.

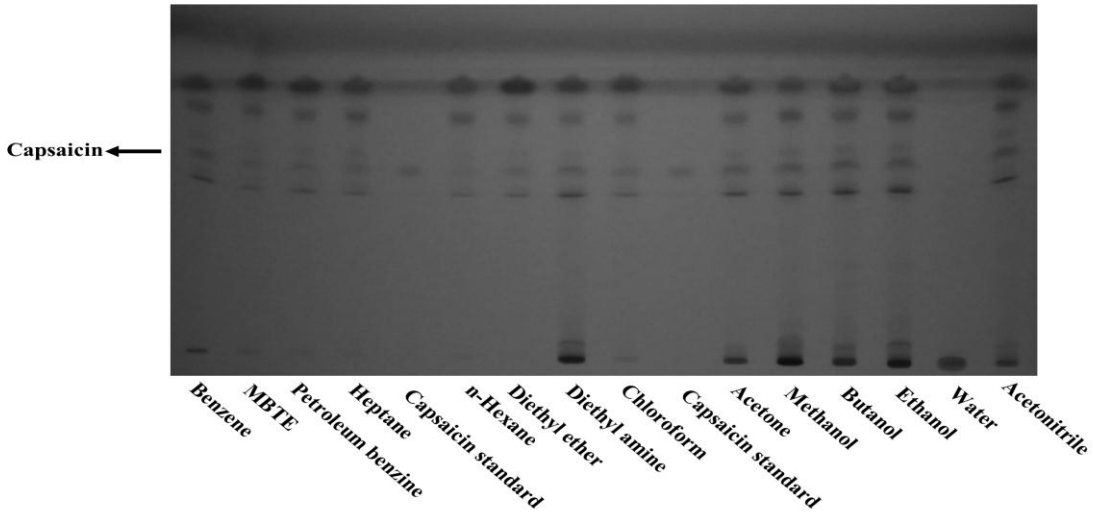

Figure 3: HPTLC fingerprints of pepper fruit extracts using different solvent.

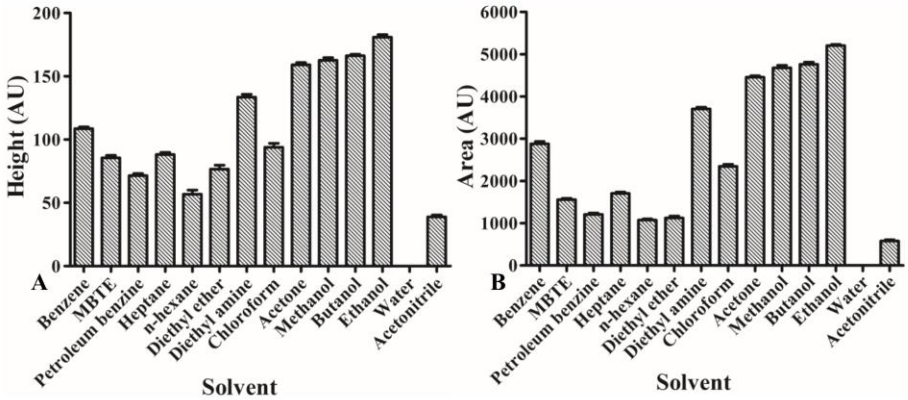

Figure 4: HPTLC-densitometric analysis of capsaicin extracted from different solvent; Mean height (A) and area (B).

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Among the fourteen different solvent, the extraction of capsaicin was performed using ethanol in modified method. The comparative analysis of extraction efficiency was performed using both general and modified method using ethanol as solvent. The amount of capsaicin extracted using modified method was found to be 1.55 and 1.692–fold higher with respect to height and area, respectively compared to the general method (Table 1).In our study we have confirmed ethanol will be the best solvent for the extraction of capsaicin from pepper fruits.

Method	Height (Mean ±SD)	Fold change	Area (Mean ±SD)	Fold change
Modified	71.483±2.560	1.550	1855.963±61.657	1.629
General	46.110±2.290		1139.380±45.965	

Table 1: Comparative analysis of height and area capsaicin extracted from pepper fruits.

Quantification of capsaicin from different pepper fruits

The capsaicin of seven different pepper fruits was extracted using modified method and subjected to HPTLC densitometric analysis (Fig. 5). The linear regression analysis data for the calibration plots using capsaicin standard showed good linear relationship with regression coefficient (R^2) of 0.985 for height and 0.995 for area in the concentration range of 1-5 µg/spot. The capsaicin content of the analyzed samples were calculated using capsaicin linear regression equation and it was expressed as microgram of capsaicin per gram tissue as well as the pungency expressed in Scoville heat units (SHU)were represented in Table 2.The highest pungency level evaluated in SHU was observed with sample D while sample E showed lowest value. The results obtained exhibited that the amount of capsaicin in the peppers used for this study varied in the 2–4.5 mg/g range. The contents of capsaicin found in the present study for the different pepper varieties are in good agreement with those found by other authors [24-26].

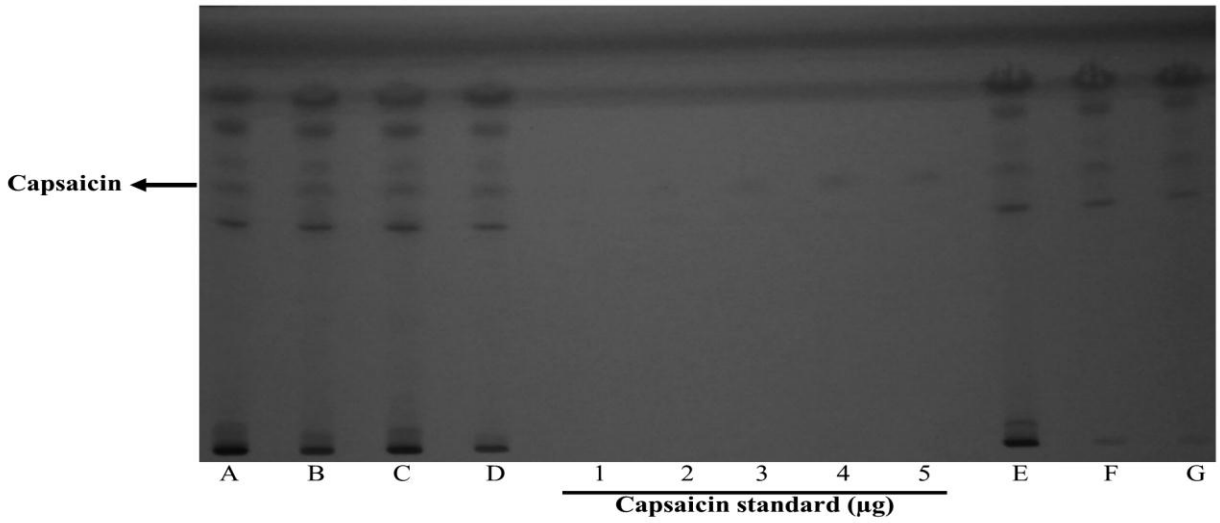

Figure 5: HPTLC fingerprints of capsaicin standard and seven pepper fruit extracts using ethanol as a solvent.

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Sl No.	Sample ID	Amount of capsaicin (mg / g) (Mean ±SD)	SHU
1	A	3.958 ± 0.160	63321.431
2	B	3.242 ± 0.045	51875.391
3	C	3.704 ± 0.084	59267.002
4	D	4.485 ± 0.101	71762.397
5	E	2.087 ± 0.060	33397.459
6	F	3.563 ± 0.084	57013.142
7	G	2.714 ± 0.104	43430.810

Table 2: Comparative analysis of capsaicin content and SHU of seven*Capsicum annuum* fruits

CONCLUSION

The present study reported an efficient extraction, identification and quantification method of capsaicin from *C. annuum* fruits by HPTLC. The method is reproducible and precise for the analysis of capsaicin with additional benefits of shorter extraction time, nominal sample preparation, in addition to the minimal cost. The developed HPTLC method can be applied for robust quantitative determination of capsaicin from different types of pepper fruits for biological and pharmacological application.

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