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Determination of capsaicin content in organically and conventionally grown chili fruits

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Abstract
Capsicum annuum L. (chili) is widely consumed for its pungency and is commonly used as a condiment, while also boasting numerous pharmaceutical and medicinal properties. Capsaicin, an odorless alkaloid with an intense, often irritating flavor, is the primary bioactive compound in chili, accompanied by other capsaicinoids like dihydrocapsaicin and nordihydrocapsaicin, which offer high nutritional value. Chromatographic techniques were employed to identify and quantify a broad range of capsaicin and related compounds in four chili fruit samples of Kharpuri variety from both conventional and organic cultivation. Using the HPTLC method, capsaicin content was found to be significantly higher in organic chili fruits grown in Rajasthan, India (0.3635 mg/g), compared to conventionally grown fruits (0.1368 mg/g). The difference in capsaicin levels highlights the impact of cultivation methods, with conventional farming relying on chemical fertilizers and pesticides, while organic farming uses eco-friendly fertilizers and biopesticides. This comparative study underscores the nutritional and bioactive advantages of organic chili fruits grown under natural conditions.

Keywords: Chili, Capsaicin, HPTLC, Capsaicinoids, Organic farming

Introduction

Capsaicin plays a crucial role in determining the pungency and commercial quality of chili fruits globally. Pungency, the sensory perception of heat in chili, is primarily due to alkaloids known as capsaicinoids. The two predominant capsaicinoids, (i) capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and (ii) dihydrocapsaicin (N[(4-Hydroxy-3-methoxyphenyl) methyl]-8-methylnonanamide), account for approximately 80-90% of total capsaicinoid content, with capsaicin alone constituting around 71% in typically pungent varieties (Barbero *et al*., 2014; Kumar *et al*., 2024) [2, 14]. Capsaicin is not only a key factor in chili pungency but also exhibits significant pharmacological, neurological, and dietary benefits (Gurnani *et al*., 2016) [13], including anti-cancer properties (Anandakumar *et al*., 2013; Dawan *et al*., 2017) [6, 11]. Research has demonstrated that capsaicin has chemopreventive properties, effectively reducing the malignant growth of cancers such as lung, colorectal, gastric, pancreatic, breast, and prostate (Pramanik and Srivastava, 2013) [13]. It has also been shown to shrink tumors by inhibiting cancer cell progression (Szallasi, 2023) [16].

Capsaicin was found to be predominantly distributed in the placenta of the chili fruit, compared to the pericarp and seed (Taira *et al*., 2012) [15]. The amount of capsaicin in a specific variety can differ, based on the light intensity, temperature, and the age of the fruit.

With growing consumer demand for vegetables and fruits rich in nutritional content and quality, the development of superior pepper varieties through crop improvement programs has become increasingly important. Understanding the variation in bioactive compounds, such as capsaicin, across different pepper germplasms is essential for this process. This study aimed to quantify capsaicin, a key determinant of pungency and a compound with significant medicinal properties, in chili samples using High-Performance Thin-Layer Chromatography (HPTLC) for its isolation and identification. Capsaicin, known for its intense pungency, is colorless and highly soluble in oils, fats, ethanol, methanol, and ethyl alkali (Ekwere & Udon, 2016) [12].

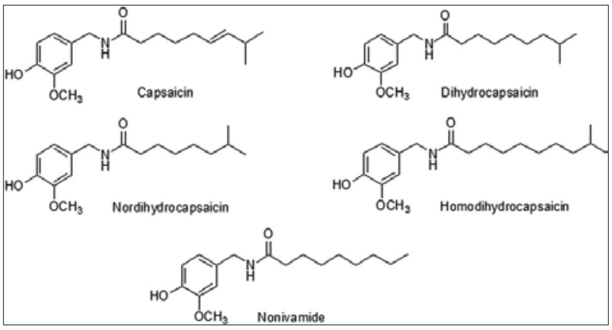

Fig 1: Schematic representation of various capsaicinoids

Previous studies have shown that ethanol extracts yield a high concentration of capsaicin.

HPTLC, an advanced form of Thin-Layer Chromatography, is a robust analytical technique that operates on the principles of adsorption and partition, depending on the absorbent materials used on the plates and the solvent system for the mobile phase (Bairy, 2015) [9]. To assess pungency, the Scoville test method and advanced instrumental technologies were employed to determine capsaicin content in the samples.

Material and Methods

Chili fruits of Kharpuri variety were collected from different fields employing conventional and organic farming practices. Fields using chemical fertilizers and pesticides represented conventional farming, while organic fields were free of chemical fertilizers and pesticides. The fruits were thoroughly washed with tap water to remove dust and air-dried in the shade. The chili samples were categorized into four groups: Con (conventional farming), Org I, Org II, and Org III. The organic samples were collected from fields practicing chemical-free farming for varying durations: Org I (2-3 years), Org II (4-5 years), and Org III (8-9 years).

- **Extraction Method:** Capsaicin extraction and quantification followed the method of Collins *et al.* (1995) [10] with minor modifications. Dried chili powder was mixed with solvents in a 1:10 ratio (g) and extracted at 60 °C for 24 hours using a Soxhlet apparatus. For the process, 4 grams of chili powder and 300 mL of ethanol were used. The extraction continued until the tissue was decolorized (approximately 16 hours).
- **Standard preparation:** 10 mg standard capsaicin was dissolved in 10 mL of Methanol. Further, 1 ml of the prepared solution was diluted with 10 mL of methanol to get 100 µg/mL solution. This solution was applied on a TLC plate.
- **Capsaicin identification:** Capsaicin peak identification in the extract was done by matching the retention time with that of standard capsaicin, which was further confirmed by co-injecting standard capsaicin together with the extract for quantitative analysis of capsaicin in chosen samples of Capsicum fruits in this study.
- **Instrumentation and chromatographic conditions:** Using a Linomat 5 automated TLC applicator, five microliters of ethanolic extract (500 mg/mL) were applied to pre-coated TLC silica gel plates. A standard capsaicin solution (100 µg/mL) was used as a reference. The TLC plates were pre-washed with methanol, dried, and developed using a mobile phase in a CAMAG HPTLC chamber. After plate saturation and equilibrium, the plates were developed to 85 mm thickness and dried. HPTLC densitometric scanning at 282 nm was performed to measure the separated bands. Comparative densitometric analysis of peak height and area allowed quantitative analysis of the extracts.

Observations and results

Capsaicin was extracted from chili fruits grown under conventional (Con) and organic (Org I, Org II, and Org III) cultivation methods (Figure 1), followed by densitometric HPTLC analysis. Capsaicin identification was confirmed by comparing band heights of the extracts with external standards, using retention factor (Rf) values for validation. Linear regression analysis of the capsaicin standard demonstrated a strong linear relationship, with a regression coefficient (R^2) of 0.985 for band height and 0.995 for peak area, within the concentration range of 1-5 µg/spot (Figure 2; A).

Capsaicin isolated from conventional and organic (Org I, Org II, Org III) chili samples was analyzed by comparing their Rf values with a standard capsaicin reference (Figure 2;B). The recorded Rf values were 0.527 for Org I, 0.523 for Org II, 0.515 for Org III, and 0.523 for the conventional sample, compared to the standard capsaicin Rf of 0.517. The mobile phase of methanol provided optimal resolution for capsaicin, with an Rf value around 0.52, enabling simultaneous quantification. The peak area for standard capsaicin was 0.00379, while the peak areas for Org I, Org II, Org III, and conventional samples were 0.00644, 0.00537, 0.00589, and 0.00392, respectively (Figure 3).

Capsaicin content was quantified using the HPTLC method, revealing that organic chili fruit samples contained higher amounts of capsaicin compared to conventional samples. The highest capsaicin concentration was observed in organic I chili fruits (0.3635 mg/g), while the lowest was found in conventional chili fruits (0.1368 mg/g). The detailed results are presented in Table 1 and Figure 4.

Discussion

The concentration of capsaicin and dihydrocapsaicin is a major determinant of pepper pungency (Sweat *et al*., 2016) [5,]. These two capsaicinoids are responsible for the majority of the heat sensation in chili peppers, with capsaicin typically contributing the most-were analysed using GCMS (Choudhary *et al*., 2022) [4]. The growing system (organic farming), ripening stage, and genotype are key factors that contribute to the variation in capsaicinoid content in chili fruits (Guijarro-Real *et al*., 2023) [1]. Bicikliski *et al*., 2017 [8] observed capsaicin levels in peppers grown organically tended to have higher capsaicin levels compared to those grown conventionally, across all six genotypes in Republic of Macedonia. However, the difference in capsaicin content between the two cultivation methods was not statistically significant, indicating that while organic farming may impact capsaicin levels, the variation is not enough to clearly distinguish between organic and conventional methods. A mesocosm experiment found that using a combination of organic and mineral fertilizers (OMF) significantly increased capsaicin content in *Capsicum chinense* cv Trinidad Scorpion compared to mineral fertilizer alone using HPLC. Capsaicin levels were five times higher with the OMF treatment, highlighting the effectiveness of this combined fertilization approach (Pampuro *et al*., 2017) [7].

Table 1: Chromatographic data for HPTLC for capsaicin of conventional and organic chili fruits

S. No.	Sample	Rf	Maximum Height	Area	Content µg/gram dry weight
1	Standard capsaicin	0.517	0.216	0.00379	14.545
2	Conventional chili fruit	0.523	0.119	0.00392	0.1368
3	Organic I chili fruit	0.527	0.180	0.00644	0.3635
4	Organic II chili fruit	0.523	0.153	0.00537	0.2672
5	Organic III chili fruit	0.515	0.148	0.00589	0.3135

Fig 1: A-Organic farming of chilli; B- Fruits

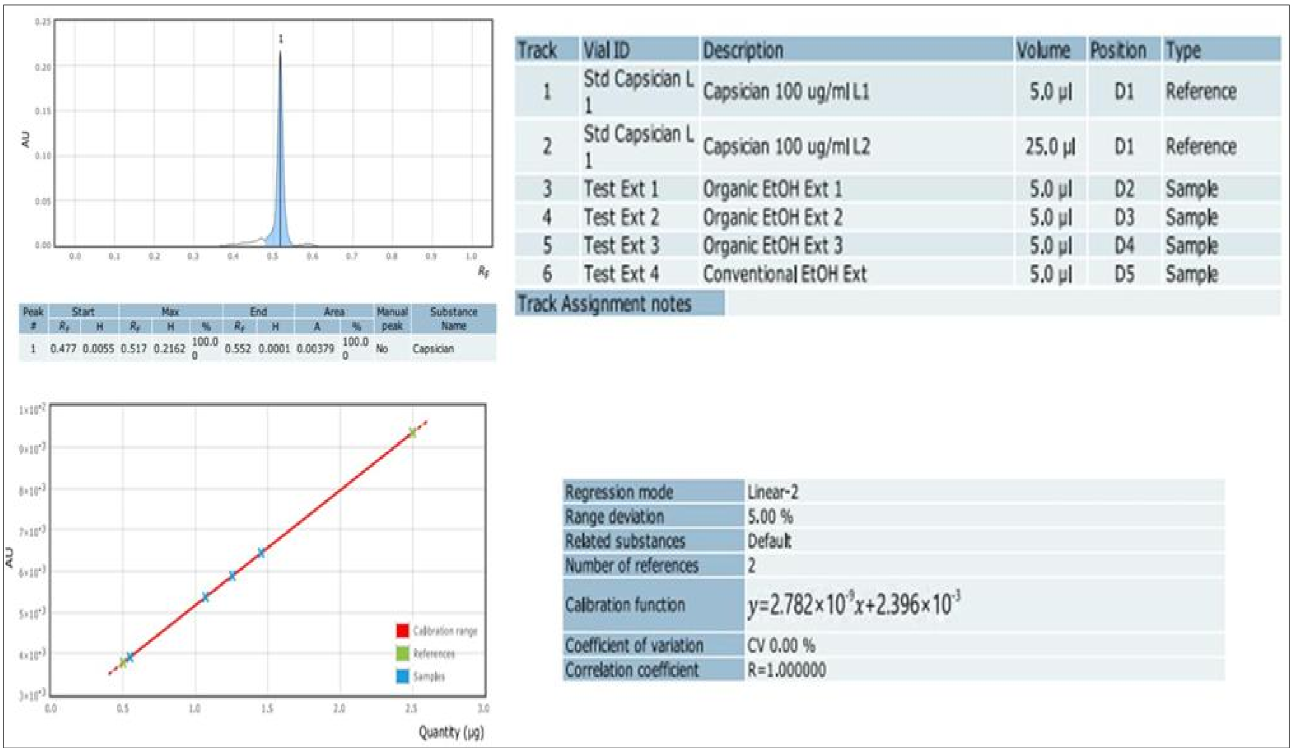

Fig 2: HPTLC chromatogram of Standard Capsaicin and Calibration curve

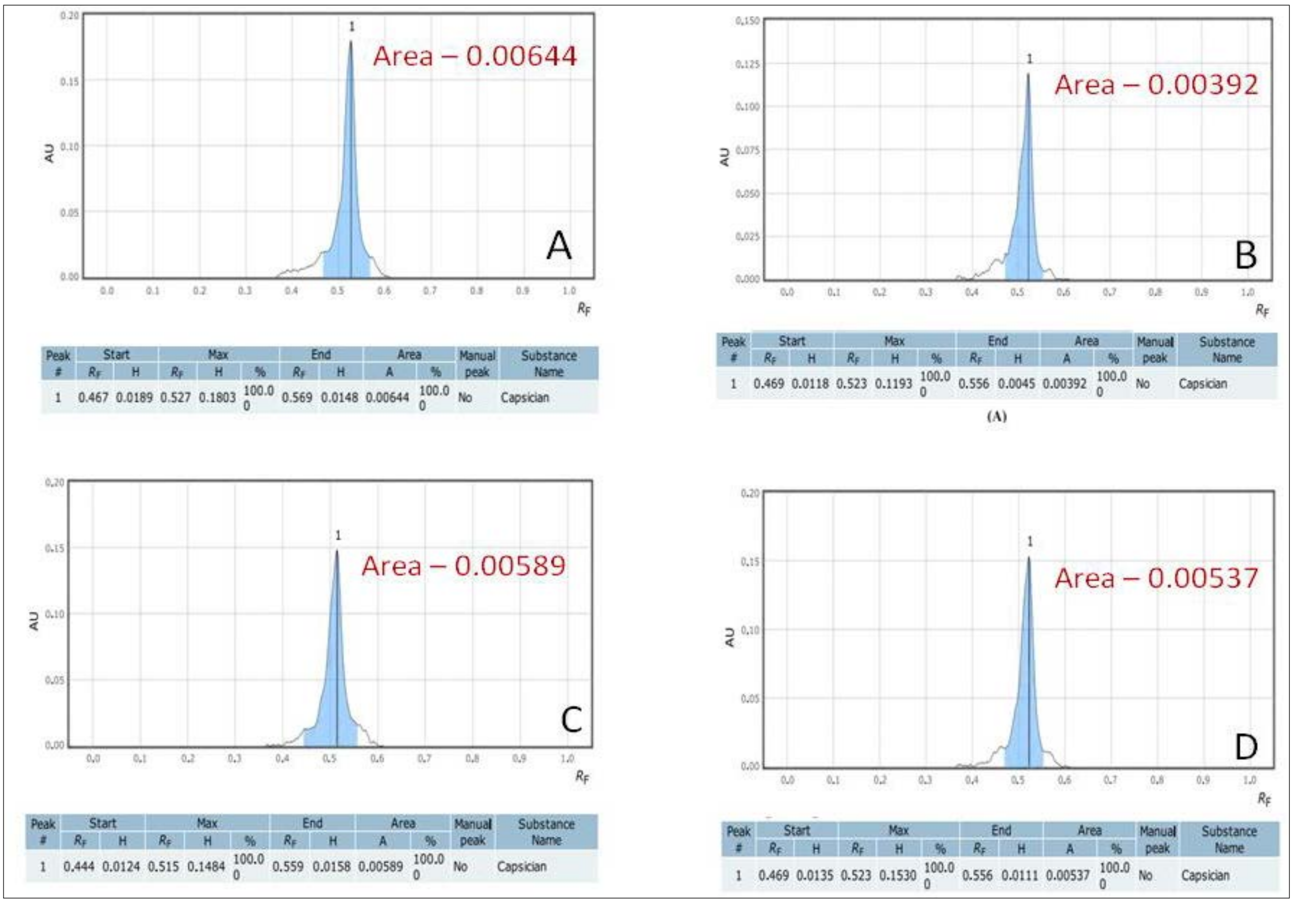

Fig 3: HPTLC chromatogram of Organic and Conventionally grown chilli fruits: A- ORG I; B- ORG II; C- ORG III; D- CON.

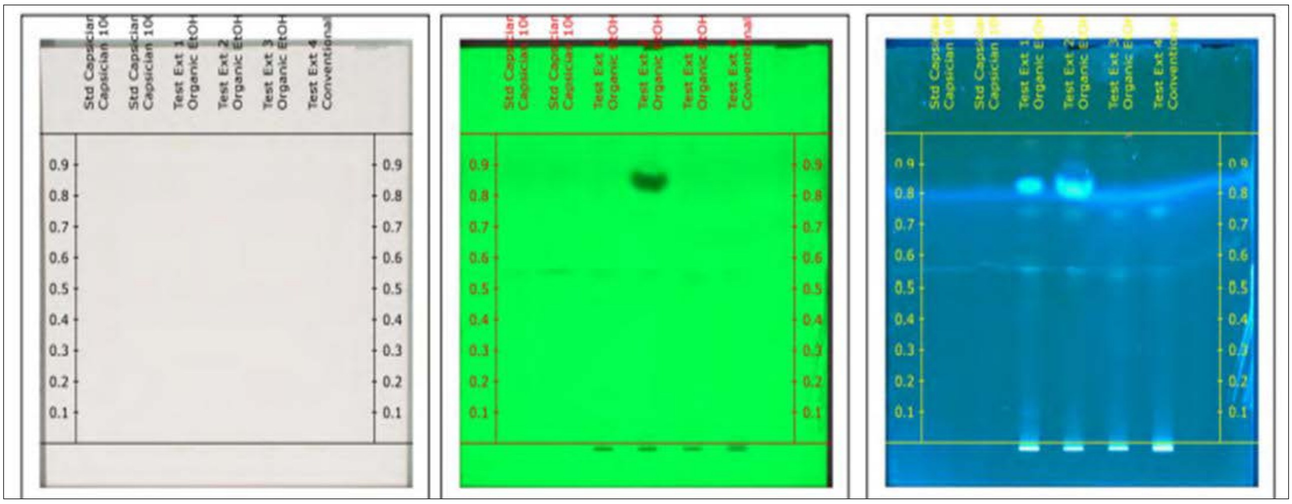

Fig 4: HPTLC Fingerprints of capsaicin under 282 nm and band for growing under ORG I, II, III and CONV (Track 1 & 2- Standard; Track 3- ORG I; Track 4- ORG II; Track 5- ORG III; Track 6- CON)

Conclusion

Quantification of capsaicin and associated capsaicinoids using HPTLC to compare organic and conventional fruits is first of its kind. The HPTLC proved to be a quick and easy approach for the analysis and quantification of capsaicin in conventional and organically grown chili fruits. Organic samples of Kharpuri variety of chili showed much higher capsaicin in comparison to conventionally grown chili fruits of same variety.

Declarations

- **Ethics approval and consent to participate:** Not required
- **Consent for publication:** Yes
- **Availability of data and material:** Yes
- **Competing interests:** None
- **Funding:** None

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