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Insights into capsaicin distribution in Andaman chilli peppers: A comprehensive regional analysis

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Abstract

This study investigates capsaicin levels in the Andaman Islands chilli peppers, emphasizing potential applications in pharmaceutical and phytomedicine. High performance liquid chromatography analyzed fifteen samples, representing South, North, and Middle Andaman, aiming to quantify capsaicin. Notable regional variations were found, with North Andaman's NAC-5 displaying the highest capsaicin levels. Methanol proved the most effective solvent for extraction. South Andaman's SAC-1 and North Andaman's NAC-3 stood out for their high capsaicin content. Scoville heat unit analysis highlighted spiciness diversity, notably in NAC-3. These findings offer practical implications for pharmaceutical and phytomedicine applications, suggesting chilli varieties can be selected based on pungency for potential medicinal uses. The study underscores the impact of genetic and environmental factors on capsaicin distribution, emphasizing careful selection in chilli cultivation and trade, providing valuable insights into optimizing quantification methods, and preserving unique chilli pepper varieties in the Andaman Islands.

1. Introduction

Chilli peppers, renowned for their fiery flavor and pungency, owe their spiciness to capsaicin, a naturally occurring compound (Govindarajan and Sathyanarayana, 1991; Bosland and Baral, 2007). The level of capsaicin within chilli peppers varies widely, not only among different species but also within the same species across various regions. The pursuit of understanding this variability and optimizing capsaicin quantification methods is essential, given the multifaceted applications of capsaicin in culinary, pharmaceutical, and scientific contexts (Wahyuni *et al*., 2011; Szolcsanyi, 2014; Pidigam *et al*., 2022).

Capsaicin, a lipophilic alkaloid is the primary bioactive compound responsible for the pungent taste and the heat associated with chilli peppers. It binds to receptors on the tongue and mucous membranes, stimulating the perception of heat and pain. The scoville heat unit (SHU) scale is widely used to measure the pungency of chilli peppers, with higher SHU values, indicating greater capsaicin content and spiciness (Popelka *et al*., 2017). Understanding capsaicin levels is not only of culinary interest but also has implications in the pharmaceutical industry, where capsaicin is used for pain relief, and in scientific research examining the potential health benefits of this compound (Fattori *et al*., 2016; Chan *et al*., 2020).

The Andaman Islands, located in the Bay of Bengal, host a unique environment for chilli pepper cultivation. The island's geographical diversity and distinct microclimates create conditions ideal for growing a wide variety of chilli peppers. However, little is known about the capsaicin content of chilli peppers across the different regions of the Andaman Islands. Furthermore, the choice of solvent for capsaicin extraction is a crucial factor that can significantly influence the accuracy and efficiency of capsaicin quantification (Thapa *et al*., 2009; Chinn *et al*., 2011). Thus, a systematic investigation into both capsaicin content and optimal extraction methods is necessary.

The primary objectives of this study encompass a comprehensive exploration of capsaicin in chilli peppers from the Andaman Islands. Firstly, the study aims to undertake a thorough capsaicin content analysis, investigating the chilli samples collected across diverse regions within the Andaman Islands, specifically South, North, and Middle Andaman. Secondly, the study aims to determine the pungency of these chilli samples through pungency evaluation, achieved by computing scoville heat unit (SHU) values, a widely accepted metric reliant on capsaicin concentration (Popelka *et al*., 2017). Thirdly, the research seeks to optimize the process of capsaicin extraction using systematic evaluation of three different solvents; methanol, ethanol, and acetone with the overarching objective of achieving maximal recovery rates, thus enhancing precision in capsaicin quantification. Finally, the study embarks on a journey of regional variation exploration to uncover potential disparities in capsaicin content among chilli samples originating from distinct regions of the Andaman Islands. This aspect of the research is poised to shed light on the influence of geographical factors on capsaicin distribution within the chilli peppers, providing a comprehensive understanding of regional chilli pepper diversity.

Understanding the capsaicin content in chilli peppers from the Andaman Islands not only contributes to our knowledge of regional chilli pepper diversity but also has practical implications. Culinary

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enthusiasts can use this information to select chilli peppers that suit their desired level of spiciness. In the pharmaceutical industry, accurate quantification of capsaicin is crucial for developing pain relief products. Additionally, scientific research exploring the health benefits of capsaicin relies on precise measurements.

The choice of solvent for capsaicin extraction is a pivotal aspect of this study. By determining the most effective solvent, researchers and analysts can enhance the accuracy of capsaicin quantification, ultimately improving the quality of data in various applications (Chinn *et al*., 2011; Srinivasan *et al*., 2018).

This research also addresses the broader question of how environmental and geographical factors influence the chemical composition of chilli peppers. It offers insights into the adaptability and diversity of chilli pepper varieties in distinct regions, which can be valuable for future agricultural practices and conservation efforts.

The present study endeavors to bridge the knowledge gap regarding capsaicin content in chilli peppers from the Andaman Islands, providing valuable insights for both practical applications and scientific understanding.

2. Materials and Methods

2.1 Sample collection

Chilli pepper samples were collected from diverse regions of the Andaman Islands, specifically South, North, and Middle Andaman. The detail of the collection site with the geographical location is shown in Table 1. A total of 15 chilli samples, with five samples from each region, were carefully chosen to represent the variations in chilli cultivars across these areas. The samples were harvested at their peak ripeness to ensure an accurate representation of capsaicin content.

Table 1: Detail of chilli samples collected with geographical location

Sample code	Sample detail	Collection site GPS coordinates
SAC-1	South Andaman	11.66510°N 92.65633°E
SAC-2	South Andaman	11.57156°N 92.65335°E
SAC-3	South Andaman	11.56876°N 92.71939°E
SAC-4	South Andaman	11.64684°N 92.65305°E
SAC-5	South Andaman	11.56822°N 92.64906°E
MAC-1	Middle Andaman	12.35304°N 92.77648°E
MAC-2	Middle Andaman	12.52374°N 92.81502°E
MAC-3	Middle Andaman	12.50571°N 92.90715°E
MAC-4	Middle Andaman	12.61431°N 92.85753°E
MAC-5	Middle Andaman	12.58744°N 92.79821°E
NAC-1	North Andaman	13.25275°N 92.97987°E
NAC-2	North Andaman	13.24635°N 92.96680°E
NAC-3	North Andaman	13.25470°N 92.92008°E
NAC-4	North Andaman	13.26050°N 92.98285°E
NAC-5	North Andaman	13.26050°N 92.98285°E

2.2 Capsaicin extraction

Capsaicin extraction from the chilli samples was performed using three different solvents: methanol, ethanol, and acetone. For each solvent, a subsample of approximately 10 g from each chilli sample was homogenized to ensure uniformity. The extraction process involved incubation for 24 h under controlled dark conditions to prevent degradation. After extraction, the samples were filtered and the resulting extracts were concentrated using a rotary evaporator under reduced pressure (Amruthraj *et al*., 2014).

2.3 High performance liquid chromatography (HPLC) analysis

Quantification of capsaicin levels was conducted using a high performance liquid chromatography (HPLC) system equipped with a UV visible detector. A reverse phase C18 column was employed for the separation of capsaicin. The mobile phase, consisting of acetonitrile and water, was utilized in a gradient elution program. Detection of capsaicin was performed at a wavelength of 280 nm (Zamora *et al*., 2015).

2.4 Scoville heat unit (SHU) determination

The scoville heat unit (SHU) was calculated based on the capsaicin content determined by HPLC analysis (Popelka *et al*., 2017). The capsaicin concentration (µg/g) in each chilli sample was calculated using the calibration curve generated from the capsaicin standard. A conversion factor specific to the HPLC method and capsaicin standard was applied to convert capsaicin concentration to SHU. The formula used for SHU calculation was:

SHU = capsaicin concentration (µg/g) × 18 (conversion factor)

3. Results

3.1 HPLC analysis of capsaicin content in chilli samples from different regions of Andaman

Capsaicin, the active compound responsible for the spicy heat in chilli peppers, plays a significant role in determining the pungency level of chilli varieties. In this study, HPLC analysis was conducted to quantify capsaicin content in chilli samples collected from three distinct regions of the Andaman Islands including South Andaman, Middle Andaman and North Andaman and determined their scoville heat unit (SHU) values as a measure of pungency. The extraction and quantification procedures were thoroughly executed for each sample. In the establishment of the calibration curve, periodic injections of standard solutions were systematically interleaved with sample injections to validate and ascertain the consistency of retention times. The chromatographic profiles presented in Figure 1(a) and (b) depicted the elution patterns corresponding to a standard and a sample respectively. Notably, these profiles certainly revealed that capsaicin compounds were eluted at a retention time of 6.12 min.

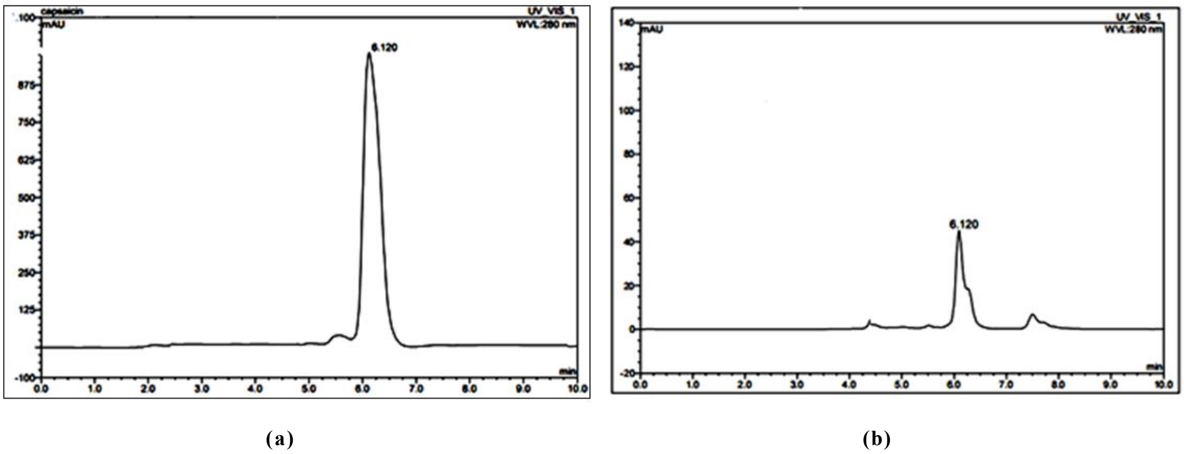

Figure 1: (a) HPLC chromatogram of capsaicin standard. (b) capsaicin in sample.

3.2 Comparative analysis of capsaicin content in Andaman Islands chilli peppers using different solvents

The capsaicin content was analyzed using three different solvents including methanol, ethanol and acetone. The peak areas (mAU - milliabsorbance units) obtained from the HPLC analysis for capsaicin in each sample and solvent combination are presented in (Figure 2). The HPLC analysis revealed significant variations in capsaicin content among the chilli samples collected from different regions of the Andaman Islands. Notably, North Andaman exhibited the highest capsaicin content in all three solvents, with sample NAC-5 registering the highest values. South Andaman and Middle Andaman, on the other hand, showed comparatively lower capsaicin content. The choice of solvent had a noticeable impact on capsaicin extraction. Methanol generally resulted in the highest peak areas, indicating better capsaicin extraction efficiency, while acetone produced intermediate results, and ethanol yielded the lowest peak areas.

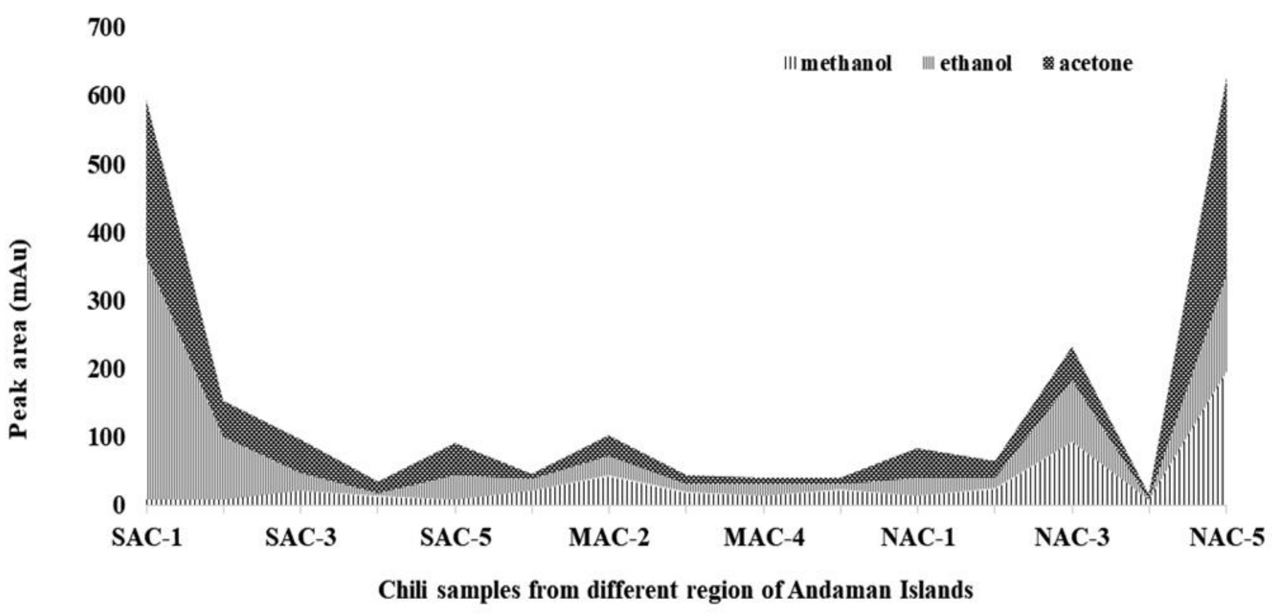

Figure 2: Variations in capsaicin content peak areas with different solvent based extractions.

The capsaicin content, expressed in µg/g, for each sample and solvent combination, is depicted in (Table 2) and quantified using the standard capsaicin concentration as determined by the standard curve (Figure 3). The HPLC analysis revealed significant variations in capsaicin content among the chilli samples collected from different regions of the Andaman Islands. Notably, North Andaman exhibited the highest capsaicin content in all three solvents, with sample NAC-5 registering the highest values. South Andaman and Middle Andaman, on the other hand, showed comparatively lower capsaicin content. The choice of solvent had a noticeable impact on capsaicin extraction. Methanol generally resulted in the highest peak areas, indicating better capsaicin extraction efficiency, while acetone produced intermediate results, and ethanol yielded the lowest peak areas. The selection of an appropriate solvent is crucial when quantifying capsaicin content, as it affects the accuracy of the analysis.

The HPLC analysis reveals a substantial range of capsaicin content among the chilli samples from the Andaman Islands. The capsaicin content varies significantly not only between regions but also within the same region. Chilli samples from South Andaman generally exhibit moderate to high capsaicin content, with variations depending on the solvent used. Sample SAC-1, in particular, showed a high capsaicin content in all three solvents. Chilli samples from Middle Andaman display a wide range of capsaicin content. Samples MAC-2 and MAC-5 showed the highest capsaicin content, while others vary considerably. North Andaman chillies, specifically sample NAC-3, stand out with exceptionally high capsaicin content across all three solvents, indicating their potential as a valuable source of capsaicin. The choice of solvent plays a crucial role in capsaicin extraction. Methanol generally yields the highest capsaicin content followed by acetone, while ethanol results in the lowest extraction efficiency.

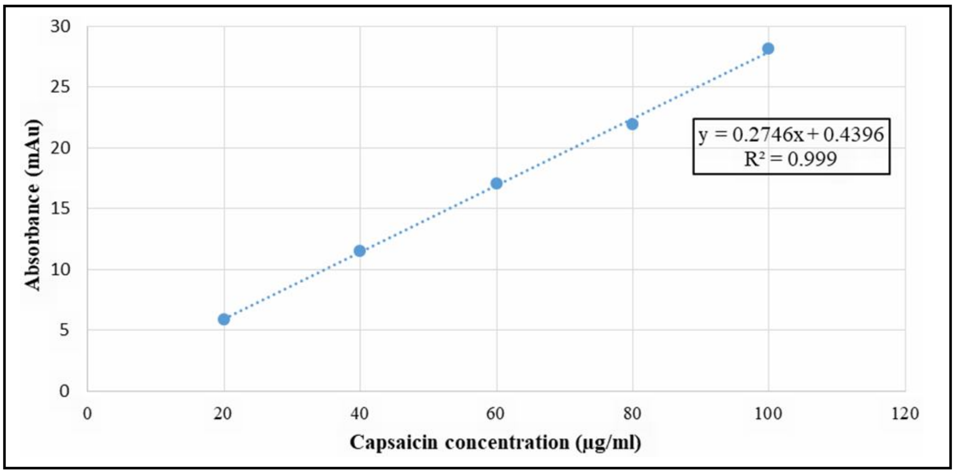

Figure 3: Capsaicin standard curve for quantitative analysis.

Table 2: Concentration of capsaicin content (µg/g) in analyzed samples

Sample code	Sample detail	Methanol (µg/g)	Ethanol (µg/g)	Acetone (µg/g)
SAC-1	South Andaman	311.01 ± 0.27	12900.49 ± 0.61	8353.26 ± 0.80
SAC-2	South Andaman	349.14 ± 0.50	3253.07 ± 0.31	1917.93 ± 0.50
SAC-3	South Andaman	741.09 ± 0.50	976.09 ± 0.95	1724.81 ± 0.20
SAC-4	South Andaman	440.69 ± 0.50	158.50 ± 0.12	630.64 ± 0.70
SAC-5	South Andaman	290.40 ± 0.60	1262.58 ± 0.50	1714.76 ± 0.09
MAC-1	Middle Andaman	739.45 ± 0.50	625.76 ± 0.28	273.21 ± 0.40
MAC-2	Middle Andaman	1541.2 ± 0.40	1026.23 ± 0.10	1083.48 ± 0.20
MAC-3	Middle Andaman	794.48 ± 0.50	318.04 ± 0.08	290.66 ± 0.50
MAC-4	Middle Andaman	502.24 ± 0.82	898.38 ± 0.56	1558.46 ± 0.20
MAC-5	Middle Andaman	861.49 ± 0.50	547.65 ± 0.06	890.15 ± 0.50
NAC-1	North Andaman	3352.93 ± 0.50	3304.28 ± 0.20	1714.76 ± 0.20
NAC-2	North Andaman	256.31 ± 0.50	14.51 ± 0.60	256.57 ± 0.08
NAC-3	North Andaman	7143.20 ± 0.50	5195.72 ± 0.08	10462.65 ± 0.70
NAC-4	North Andaman	652.82 ± 0.50	418.04 ± 0.04	460.54 ± 0.06
NAC-5	North Andaman	464.07 ± 0.60	617.42 ± 0.30	349.80 ± 0.10

3.3 Scoville heat units (SHU) analysis of chilli samples from Andaman Islands

In the present investigation, an extensive analysis was undertaken to ascertain the scoville heat units (SHU) of chilli samples collected from diverse regions of the Andaman Islands, specifically South Andaman, Middle Andaman and North Andaman. SHU is a measurement used to quantify the spiciness or pungency of chilli peppers. It quantifies the concentration of capsaicin, the compound responsible for the heat in chilli peppers. In this study, the SHU values vary widely among the chilli samples, indicating significant differences in spiciness levels. The evaluation of spiciness or pungency in the chilli samples was grounded in SHU values derived post capsaicin extraction using three distinct solvents including methanol, ethanol, and acetone. The SHU values for each chilli sample and solvent combination were meticulously documented in (Table 3) offering a comprehensive overview of the spiciness levels prevalent in the Andaman chilli varieties.

The results unveiled substantial variations in SHU values across the chilli samples, underscoring pronounced differences in spiciness levels within and between the regions of South Andaman, Middle Andaman, and North Andaman.

In the context of South Andaman, the chilli samples exhibited a wide spectrum of spiciness levels. Notably, SAC-3 emerged as the most pungent, demonstrating the highest SHU values across all three solvents, while SAC-2 exhibited relatively milder spiciness. These variations suggest a diverse array of chilli varieties within the same geographical region, contributing to the overall richness of chilli biodiversity.

Moving on to Middle Andaman, the chilli samples also displayed considerable variability in spiciness levels. MAC-2 was recorded with the highest SHU values across all solvents, indicating the presence of exceptionally spicy chilli varieties in this region. The uniqueness in spiciness levels among the Middle Andaman samples further emphasizes the regional diversity in chilli characteristics.

In North Andaman, the chilli samples presented remarkable results, with NAC-3 revealing higher SHU values across all three solvents. These particular chilli samples suggest the existence of extremely spicy chilli varieties in North Andaman, contributing to the overall regional spiciness report. The wide range of SHU values observed in North Andaman highlights the potential for cultivating chilli varieties with varying levels of pungency.

Table 3: Solvent based SHU values in different region of Andaman Islands

Sample code	Sample detail	SHU-methanol	SHU-ethanol	SHU-acetone
SAC-1	South Andaman	5598.22	232208.78	150358.60
SAC-2	South Andaman	6284.53	58555.32	34522.77
SAC-3	South Andaman	13339.66	17569.60	31046.66
SAC-4	South Andaman	7932.45	2852.99	11351.54
SAC-5	South Andaman	5227.21	22726.41	30865.74
MAC-1	Middle Andaman	13310.17	11263.70	4917.82
MAC-2	Middle Andaman	27741.63	18472.22	19502.67
MAC-3	Middle Andaman	14300.63	5724.73	5231.80
MAC-4	Middle Andaman	9040.25	16170.76	28052.34
MAC-5	Middle Andaman	15506.74	9857.65	16022.62
NAC-1	North Andaman	60352.70	59476.96	30865.74
NAC-2	North Andaman	4613.66	261.15	4618.25
NAC-3	North Andaman	128577.68	93522.91	188327.72
NAC-4	North Andaman	11750.74	7524.73	8289.70
NAC-5	North Andaman	8353.28	11113.59	6296.33

4. Discussion

Capsaicinoids consist of a group of related alkaloid compounds that only exist in the Capsicum genus. This group of compounds is produced as secondary metabolites by chillies (Chan *et al.,* 2020). The biosynthesis of capsaicinoids starts by condensation of fatty acids and vanillylamine where the placenta of pepper is the primary site for capsaicinoids biosynthesis (Thiele *et al*., 2008). It is the variation in the acyl group that determines the quantity of the burning sensation of chillies. The group of capsaicinoids includes unique components such as capsaicin, dihydrocapsaicin, nordihydrocap-saicin, and nonivamide that are found in the raw form of chillies. The total capsaicinoid level in the extract of fresh chillies is reflective of the relative hotness of the chilli (Meghvansi *et al*., 2010). The pharmaceu-tical properties of capsaicin were reported in several studies. Prabhakar (2022) thoroughly explored the realm of capsaicinoids, emphasizing the therapeutic efficacy of capsaicin and dihydrocapsaicin in conditions like rheumatoid arthritis and diabetic neuropathy, contributing valuable insights for diverse pharmaceutical applications. Also, capsaicin has been reported to have anti-hypertensive and antidiabetic effects (Ahuja *et al.,* 2006; Komori *et al*., 2007). In the review conducted by Soni *et al*. (2022), the anti-inflammatory properties of capsaicin were thoroughly elucidated, and the findings were incorporated from both *in vitro* and *in vivo* animal investigations. The comprehensive examination highlighted the emergent requirement of capsaicin and a potent analgesic agent. Furthermore, the review underscored capsaicin's potential as a primary therapeutic intervention for neuralgia associated with herpes. The review conducted by Fattori *et al.* (2016) revealed the versatile role of capsaicin in pain modulation, emphasizing its analgesic effects and exploring the underlying mechanisms. The review not only improves our understanding of capsaicin's potential as a pain management agent but also broadens its clinical application. Beyond traditional pain relief, the study revealed the potential benefits of capsaicin in diverse medical conditions, including diabetes, obesity, cardiovascular diseases, cancer, airway ailments, itch, gastric disorders, and urological issues.

Lalitha *et al.* (2022) expounded on the noteworthy efficacy of capsaicin in their review, emphasizing its effectiveness in reducing gallstones. The thorough analysis sheds light on capsaicin's potential as a valuable intervention for addressing concerns related to gallstone formation, providing valuable contributions to the literature on gallstone management. In the review authored by Rani *et al.* (2023) emphasis was placed on the utilization of capsaicin, derived from chilli, as a digestive stimulant aimed at augmenting the hepatic synthesis of bile acids. These bile acids play a crucial role in the breakdown of fats during the digestive process.

Given the significant medical and health benefits attributed to capsaicin, it becomes imperative to establish a standardized and efficient solvent extraction system. This ensures the optimal recovery of these valuable secondary metabolites from chilli samples. Standardization in the extraction process is essential for obtaining reliable and consistent results, promoting accuracy in the assessment of capsaicin content. This systematic approach not only enhances the reliability of research outcomes but also facilitates a more thorough understanding of the potential therapeutic applications of capsaicin in various health related contexts.

In the present study, the pungency of chilli peppers, as indicated by capsaicin content, appears to follow a regional trend. North Andaman chillies consistently exhibited higher pungency compared to South Andaman and Middle Andaman. This regional variation may be influenced by factors such as climate, soil composition, and chilli pepper variety.

The observed variations in capsaicin content among chilli samples from different regions of the Andaman Islands, as evidenced by HPLC analysis, align with similar studies (Zamora *et al.*, 2015). Notably, the elevated capsaicin levels in North Andaman chillies, particularly sample NAC-5, resonate with findings in diverse chilli varieties worldwide, where geographical factors contribute significantly to the spiciness of peppers (Islam *et al.*, 2015). This regional trend may be attributed to a combination of environmental factors, such as temperature, humidity, and soil composition, as well as genetic factors inherent to chilli pepper cultivars (Gurung *et al.*, 2011).

The impact of solvent choice on capsaicin extraction efficiency, with methanol yielding the highest results, corroborates with established methodologies in capsaicin analysis (Srinivasan *et al.*, 2018). The preference for methanol is often attributed to its ability to efficiently extract capsaicinoids while minimizing interference from other compounds. However, the variations in solvent efficiency highlight the importance of standardizing extraction protocols to ensure the reliability and comparability of results across studies (Chinn *et al.*, 2011; Amruthraj *et al.*, 2014). The pungency of chilli peppers, as indicated by capsaicin content, appears to follow a regional trend. North Andaman chillies consistently exhibited higher pungency compared to South Andaman and Middle Andaman. This regional variation may be influenced by factors such as climate, soil composition, and chilli pepper variety. Researchers and chilli processors must consider these solvent-dependent variations when extracting capsaicin for commercial purposes. Scoville heat units (SHU) serve as a pivotal metric for quantifying the intensity of spiciness or pungency in chilli peppers, directly correlating with the concentration of capsaicin, the bioactive compound responsible for the characteristic heat in chilli peppers (Al-Othman *et al.*, 2011; Popelka *et al.*, 2017).

5. Conclusion

The results of this study can be valuable for chilli farmers and the food industry in Andaman, helping them select chilli varieties for different culinary and commercial purposes based on their pungency requirements. Apart from the immediate applications in food and agriculture production, the present investigation opens doors to broader exploration in the realms of pharmaceuticals and phytomedicine. The medicinal properties of capsaicin present in chilli peppers provide promising opportunities for research in pain management, anti-inflammatory treatments, and potential solutions for conditions like arthritis and obesity. Additionally, the pharmaceutical industry could benefit from the development of new drugs and formulations from the natural properties of capsaicin.

Further investigations into the genetic basis of regional variations and their implications for chilli cultivation and trade will contribute to the sustainable development of the chilli industry in the Andaman Islands. Simultaneously, the investigation of chilli peppers as a source of medicinal compounds aligns with the global trend towards imposing natural products for pharmaceutical purposes. This present research approach not only enhances our understanding of regional agricultural practices but also promotes the integration of traditional crops into modern healthcare solutions. The study catalyzes future research endeavors that connect the gap between agriculture, traditional knowledge and scientific applications. The collaboration between chilli farmers, the food industry, the pharmaceutical industry and the scientific community holds immense potential not only in optimizing the chilli cultivation practices in the Andaman Islands but also in contributing to the development of innovative solutions in the fields of phytomedicine and pharmaceuticals.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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