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## RESEARCH ARTICLE

### A Comparative Study on Cultivars of Capsicum: Critical Assessment based on Colour Values, Pungency and total Capsaicinoids Content

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#### Abstract:

#### Introduction:

Chillies or red peppers are consumed globally as a spice, condiment, culinary and as folk medicine because of their pungent taste, spicy flavour and therapeutic values. Colour, pungency and capsaicinoids are the potential characteristic attributes for assessing the quality of the dried chilli and are accountable for market price, trade and standardisation of the oleoresins.

#### Objective:

The present study was designed to prepare crude oleoresins from nineteen varieties of chilli and to enrich carotenoids contents in oleoresin paprika and capsaicinoids extractives in oleoresin capsicum separately for the evaluation and standardisation of colour, pungency and total capsaicinoids content.

#### Methods:

Crude oleoresins from dried fruits of chilli were prepared using conventional solvent extraction method, which were further enriched for the carotenoid and capsaicinoids extractives by fractionation techniques, and yielded oleoresin paprika and oleoresin capsicum separately. Oleoresins and their derivatives were subjected to analytical estimation of colour values, pungency and capsaicinoids content. The colour content was quantified using a UV Vis Spectrophotometer, whereas pungency was determined using HPLC and quantified as Million Scoville Heat Units. Total capsaicinoids contents were also quantified.

#### Results:

The highest extraction yield was found in the variety Teja (S-17) that is 8.82%, whereas enriched versions of oleoresin paprika and oleoresin capsicum were calculated higher in the varieties 273 and Teja (S-17) with the values 6.04 and 2.62%, respectively. The colour value of crude extract was the highest (3085.2 CU) in the variety 5531-IPM, whereas paprika was enriched high in the variety Lolly Chilli (3400.5 CU). The highest pungency (1.59 MSHU) was noted in the variety 5531-HPH. In the case of oleoresin capsicum, the pungency was highly enriched in the two varieties 4884 and Teja (S-17), with the MSHU values 6.11 and 5.5, respectively. Total capsaicinoids contents in the crude oleoresin were found maximum in the variety 5531-HPH (10.60%), whereas after purification (oleoresin capsicum), the maximum capsaicinoids contents were enriched in the varieties 4884 and Teja (S-17) with the values 40.73 and 36.67%, respectively.

#### Conclusion:

The outcome of our elaborated studies elucidates that Teja (S-17) 5531-HPH, 5531-IPM and 4884 varieties are better for extractive yield, pungency, and total capsaicinoids content, whereas 5531-IPM and Lolly Chilli varieties are better for colour extraction compared to all other varieties.

**Keywords:** Red Chilli, *Capsicum annum*, Oleoresin, Paprika, Pungency, Capsaicinoids.

#### Article History

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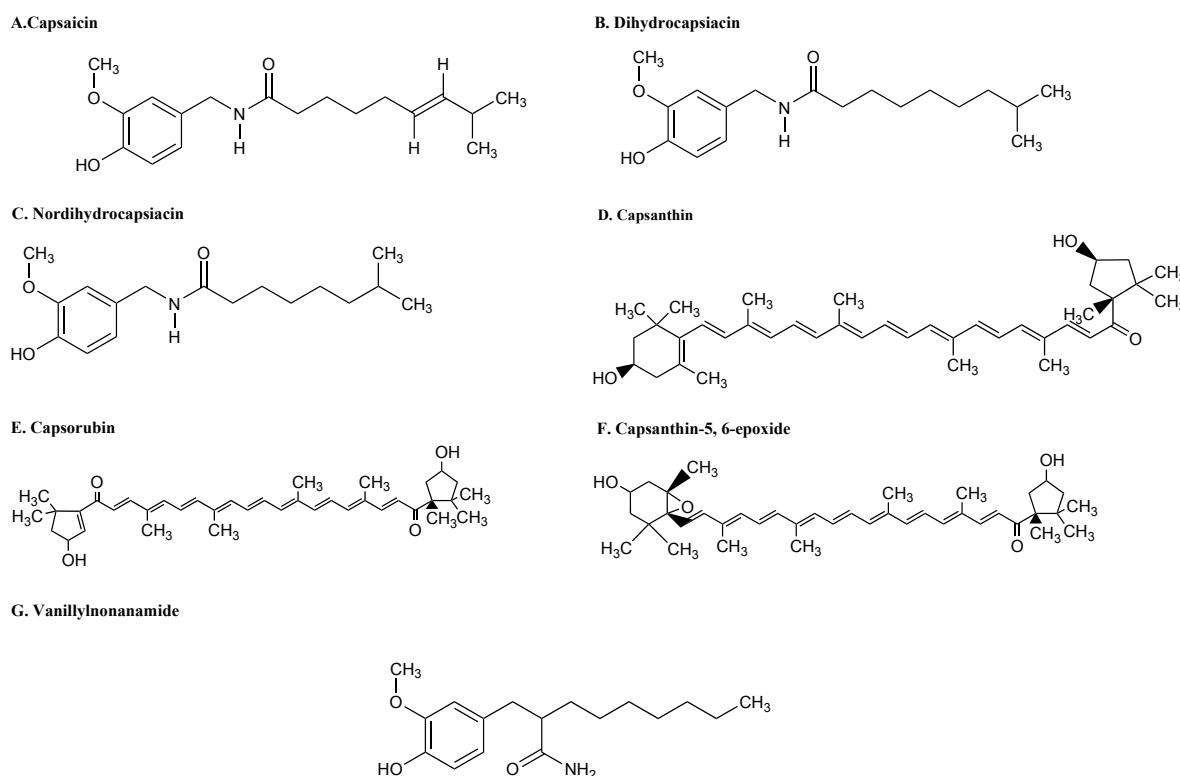
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## 1. INTRODUCTION

Chillies or red peppers are a spice, consumed globally for their pungent taste, spicy flavour and therapeutic values. As

one of the oldest known spices to humankind, chilli finds its application in almost all cuisines across the globe [1, 2]. Chilli is categorised under the genus *Capsicum* and belongs to the family Solanaceae [3].



**Fig. (1).** Chemical constituents of Chilli and standard N-Vanillylnonanamide.

The cultivation of chilli originated from the Latin American region but now is widely distributed in all the tropical and subtropical countries, including India and China. In India, Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu are the major states cultivating a huge amount of varieties of chilli, as the South Asian climate is suited for cultivation [4, 5]. Even though chilli is majorly consumed as a culinary ingredient [6], it also finds extensive therapeutic applications, especially in pain management, cardiovascular and gastrointestinal wellness [2]. The pharmacological attributes of chillies are mainly due to the presence of a group of alkaloids known as capsaicinoids, which are secondary metabolites comprising molecules like capsaicin ( $C_{18}H_{27}NO_3$ ; (*E*)-*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide) dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ; *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnonanamide) and nordihydrocapsaicin ( $C_{17}H_{27}NO_3$ ; *N*-[(4-hydroxy-3-methoxyphenyl)methyl]-7-methyloctanamide) (Fig. 1A - C) [3, 6].

Colour, pungency and capsaicinoids contents are the potential characteristic attributes for assessing the quality of the dried chilli [7] and are accountable for market price, trade and standardisation of the extracts [8]. Additionally, morphological key characteristics like size and appearance also help to identify good quality dried fruits [9]. Some of the well-known varieties are cultivated mainly for carotenoid pigments, whereas; others are grown for their high pungency and taste. Carotenoid pigments are mainly responsible for contributing to

the colour of the fruits and are measured as ASTA colour units [7]. Carotenoids such as capsanthin, capsorubin capsanthin-5, 6-epoxide (Fig. 1D - F), capsorubin, zeaxanthin, lutein, kryptoxanthin alpha and beta-carotene are some of the phytochemicals results in the orange-red colour [10]. Capsaicinoids are responsible for up to 90% of total pungency, which are quantified in the Scoville Heat Unit (SHU) scale [11] and are accountable for the pungent taste of the fruit. It also aids in energy metabolism and thermogenesis [12]. The carotenoid and capsaicinoids contents in the fruits vary with several factors such as geographical region, climate, individual variety, cultivars or genotypes, method of cultivation, maturity levels and periods of collection [9, 13 - 15]. Many technologies like Supercritical Fluid Extraction, microwave-assisted extraction technique, enzyme pre-treatments, *etc.* are well explored for enriching carotenoids and capsaicinoids content [16 - 18], but they are expensive and comparably not robust enough. However, some conventional solvent extraction processes also exist and are well established in practice for easy extraction and rapid evaluation. The current study was performed to evaluate the colour and pungency properties (organoleptic characteristic) of different varieties of chillies, which are available in the southern part of India, by analysing the carotenoids and capsaicinoids contents. Carotenoids are hydrophilic in nature [19] and were extracted with a polar solvent system to arrive at a high purity extract, whereas capsaicinoids being non-polar molecules were extracted out with hydrophobic solvents [20].

In the present work, nineteen commercial varieties of chilli available in Guntur, India, were extracted to obtain crude oleoresins. Furthermore, these crude oleoresins were purified with a specific solvent ratio. The enriched and purified versions

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of oleoresins were subjected to the measurement of colour and pungency of the extractives. The colour content was quantified using a UV spectrophotometer, whereas pungency was determined by HPLC analysis. Apart from colour and pungency, total capsaicinoids content was also quantified in all the samples.

**2. MATERIALS AND METHODS**

**2.1. Chemicals and Reagents**

Spectrophotometric analysis was performed on Shimadzu UV1800 spectrophotometers (Kyoto, Japan) with 10 mm matched quartz cells. Pungency values were determined by a Shimadzu HPLC system (Prominence, LC-20AT), equipped with a variable wavelength PDA detector. Detailed analytical

conditions of HPLC are further specified in the respective section. Reference standard N-Vanillynonamide was purchased from Sigma Aldrich. All the reagents were of HPLC or analytical grade.

**2.2. Selection of Raw Materials (RMs)**

In the present study, nineteen commercial varieties of *Capsicum annuum* (Fig. 2) were collected from the Guntur Mirchi Yard-India, which is Asia's largest dried red chilli market. A threshold maximum of 5% moisture content was maintained for the selection of raw materials. Before the extraction, the stalks were removed and after that, seeds were separated from the dried fruits. Fruits (without seeds) were pulverised to a particle size of 20-30 mesh and were finally stored in an airtight container at 4 °C until use.

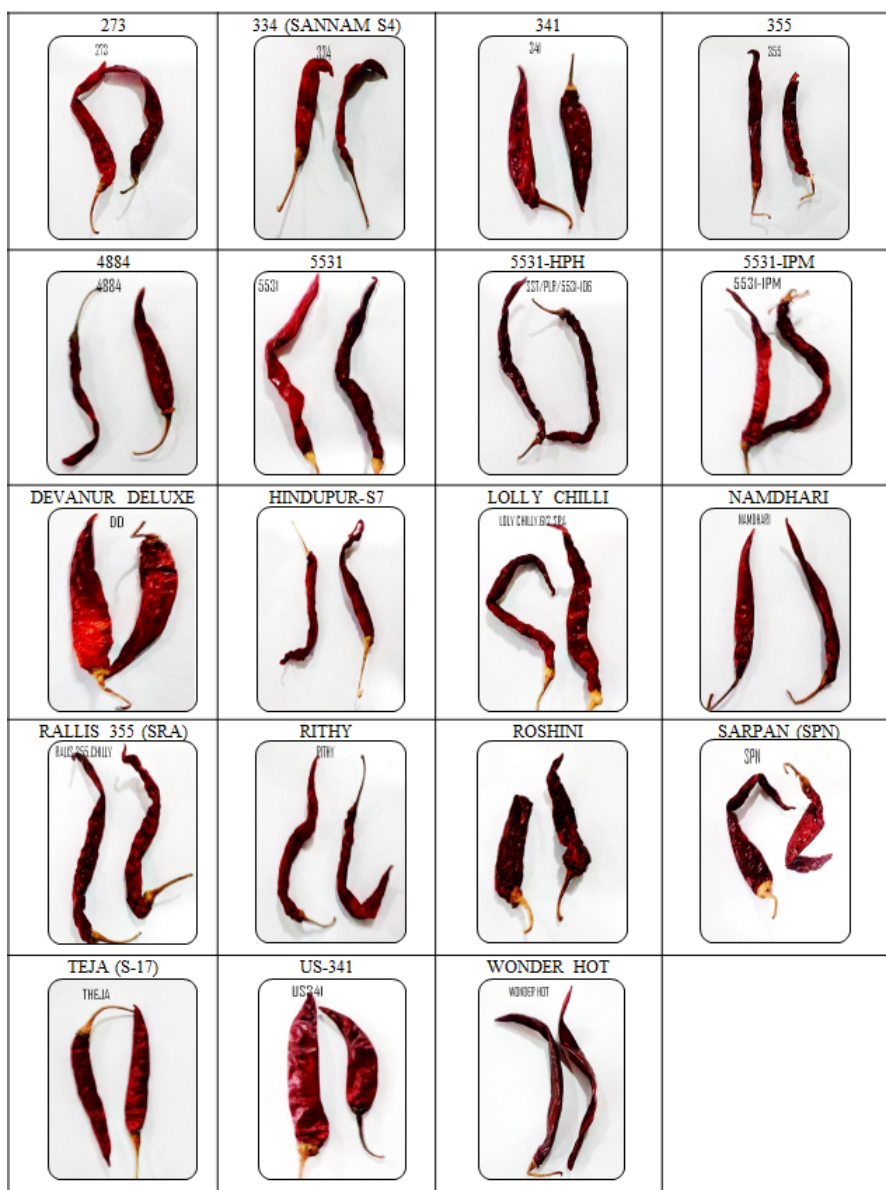


Fig. (2). Different varieties of chilli collected from Guntur, India.

**2.3. Development of Extracts and Purified Version of Oleoresins**

The crude oleoresins in the form of extract were prepared by the conventional solvent extraction method, which is very simple, easy to perform, sensitive and time-efficient to get the desired extractives. The pre-treated raw materials were subjected to maceration in a solvent mixture of acetone and hexane in a specific ratio of 70:30. Occasional stirring was maintained for 24 h at room temperature. The filtrates were subjected to desolventisation at a controlled temperature of 70 °C under reduced pressure to obtain concentrated extracts termed as crude oleoresins. After calculating the yield, crude oleoresins were subjected to analytical estimation of colour and pungent values. All the crude oleoresins were further purified to get an enriched colour and pungent extractives.

In brief, the crude oleoresins were mixed with 80% MeOH and were stirred well for 10 min. This solution was partitioned in a separating funnel into two fractions, an upper layer of pungent extractives, which was further concentrated to yield oleoresin capsicum. In contrast, the bottom layer comprised of carotenoids extractives, which were further concentrated to yield oleoresin paprika. Both the enriched oleoresins were kept separately in an airtight container at a cold temperature for further analysis. Total nineteen crude extracts and thirty-eight enriched oleoresins were prepared for the evaluation.

**2.4. Evaluations**

Crude oleoresins along with enriched extracts (oleoresin paprika and oleoresin capsicum) were analysed for colour and pungent extractives as standardisation is much required to control the product quality [21].

**2.4.1. Analysis of the Colour Values (Paprika) by UV Spectroscopy**

The extractable colour of crude oleoresin, oleoresin

paprika and oleoresin capsicum (Fig. 3) was quantified by following the protocol described by the American Spice Trade Association (ASTA 20.1) [22, 23]. Stock solutions of all the oleoresins (0.1%) were prepared by dissolving in acetone separately. Furthermore, the solutions were diluted to 0.01% for measuring the absorbance via spectrophotometer (Shimadzu UV-1800) at the wavelength of 460 nm, using acetone as a blank substrate at an absorbance range of 0.2-0.8 Au. The colour value of the oleoresins in terms of ASTA 20.1 was calculated as per the following formula [24].

$$\text{ASTA colour value for oleoresin} = \frac{\{A_{\text{extract at 460nm}}\} \times (164 I_f)}{\text{g sample}}$$

Where A: absorbance; I<sub>f</sub>: Instrument Correction Factor = (NIST Absorbance for glass filter at 465 nm/Measured Absorbance of glass filter at 465 nm); 164: Conversion factors of American Spice Trade Association (ASTA).

**2.4.2. Pungency by HPLC Method**

The pungency of all the crude extracts and purified oleoresin capsicums was estimated on the Shimadzu HPLC system (Prominence, LC 20AT), equipped with a solvent delivery pump, column oven, with 20 µl loop and variable wavelength PDA detector. A designed program controlled the system, and data were analysed by LabSolutions™ software. The pungency analysis was performed on the Shiseido C-18 column (250 mm × 4.6 mm i.d., 5 µm particle size) referring AOAC, 995.03 and ASTA, 21.3 methods [25, 26].

In brief, the isocratic mobile phase consisting of acetonitrile and DI - H<sub>2</sub>O with 1% v/v acetic acid solution (40:60, v/v) was eluted through the column at 25 °C temperature with a flow rate of 1.5 mL/min. Solutions were filtered through a 0.45 µm nylon membrane before HPLC injection. The injection volume was 20 µl.

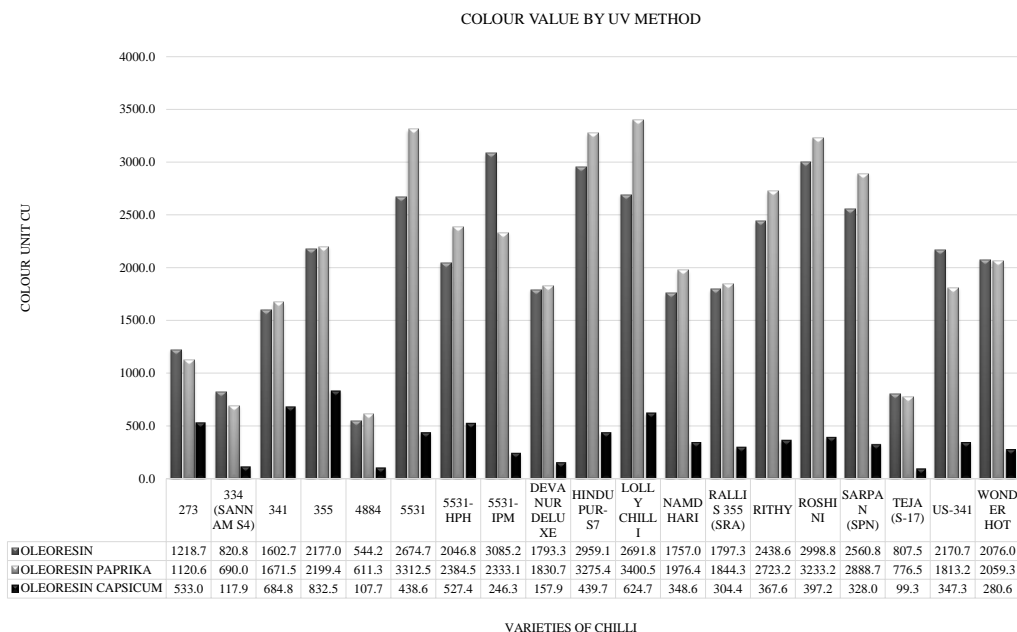


Fig. (3). Colour values of the samples.

### 2.4.3. Standard Preparation

Accurately weighed 75 mg N-vanillylnonanamide (Fig. 1.G) and transferred it into 500 mL volumetric flask. Diluted to volume with ethanol, and mixed.

### 2.4.4. Sample Preparation

Accurately weighed 1-2 g oleoresin into a 50 mL volumetric flask. 5 mL acetone was added into the flask and swirled contents of flask until the test sample was completely dispersed. Contents of the flask were diluted to volume with ethanol and mixed well and further diluted (5 ml to 25 ml) with ethanol to prepare the ideal concentration for injection.

The pungency was quantified by observing specific peaks of capsaicinoids at relevant retention times in a detection wavelength of 280 nm by UV detectors.

The pungency of red pepper oleoresins was calculated as follows:

Nordihydrocapsaicin [N]:  $[(P_n/P_s) \times (C_s/W_t) \times (250/0.98) \times 9300]$

Capsaicin [C]:  $[(P_c/P_s) \times (C_s/W_t) \times (250/0.88) \times 16100]$

Dihydrocapsaicin [D]:  $[(P_d/P_s) \times (C_s/W_t) \times (250/0.93) \times 16100]$

Where, P<sub>n</sub>: Area of Nordihydrocapsaicin in the sample; P<sub>c</sub>: Area of Capsaicin in the sample; P<sub>d</sub>: Area of Dihydrocapsaicin in the sample; P<sub>s</sub>: Average peak area of standard solution N-vanillylnonanamide; C<sub>s</sub>: Concentration of standard in mg/ml; W<sub>t</sub>: Weight of the sample in g;

### 2.5. Accepted Heat Factors and Response Factors:

Nordihydrocapsaicin [N] - H<sub>N</sub> = 9300; R<sub>N</sub> = 0.98

Capsaicin [C] - H<sub>C</sub> = 16100; R<sub>C</sub> = 0.89

Dihydrocapsaicin [D] - H<sub>D</sub> = 16100; R<sub>D</sub> = 0.93

H<sub>N</sub>, H<sub>C</sub>, and H<sub>D</sub> = heat factors for respective capsaicinoids; R<sub>N</sub>, R<sub>C</sub>, and R<sub>D</sub> = response factors of respective capsaicinoids relative to standard

Total pungency was calculated as the sum of these compounds.

Total pungency in Scoville Heat Unit (SHU) = Nordihydrocapsaicin [N] + Capsaicin [C] + Dihydrocapsaicin [D].

Furthermore, total capsaicinoids contents were also calculated by using factor as 1 microgram total capsaicinoids/g = ca 15 SHU].

## 3. RESULTS

### 3.1. Extraction

The nineteen varieties of chilli were collected from Guntur and were extracted using acetone and hexane 70:30 ratio. The crude oleoresins were subjected to a second extraction process to enrich and purify paprika and capsaicin. The percentage yield of crude oleoresins along with enriched extracts (oleoresin paprika and oleoresin capsicum) is mentioned in Table 1. In the case of crude oleoresin, maximum yield (8.82%) was found in the variety Teja (S-17), followed by the varieties 273 (8.16%) and 341 (7.68%). Minimum yield was found in Wonder Hot variety, which was only 3.2%. Similarly, in the case of oleoresin paprika, the highest yield from raw materials (RMs) was found in the variety 273 (6.04%), followed by Teja (S-17) and 334 (Sannam S4) as 5.76% and 5.20% respectively, whereas Lolly Chilli yielded the lowest amount (1.51%). In the case of oleoresin capsicum, the highest yield was found in the variety Teja (S-17), 2.63%, followed by 341 and Namdhari, whereas the lowest yield was recorded in the variety Devanur Deluxe (0.52%).

**Table 1. Extractive yields of nineteen varieties of chilli.**

S. No.	Varieties	Oleoresin Yield in %	Oleoresin Paprika RMs Yield in %	Oleoresin Capsicum RMs Yield in %
1.	273	8.16	6.04	1.89
2.	334 (SANNAM S4)	7.32	5.20	1.55
3.	341	7.68	4.76	2.44
4.	355	5.58	2.97	1.54
5.	4884	5.09	3.61	1.03
6.	5531	6.91	4.82	2.07
7.	5531-HPH	4.39	1.84	1.63
8.	5531-IPM	4.36	2.57	1.50
9.	DEVANUR DELUXE	4.99	4.24	0.52
10.	HINDUPUR-S7	5.67	3.29	1.22
11.	LOLLY CHILLI	4.31	1.51	1.30
12.	NAMDHARI	7.41	4.77	2.18
13.	RALLIS 355 (SRA)	5.36	4.14	1.14
14.	RITHY	5.71	3.50	1.17
15.	ROSHINI	6.33	4.21	1.09
16.	SARPAN (SPN)	5.51	3.18	1.41
17.	TEJA (S-17)	8.82	5.74	2.62

(Table 1) contd....

S. No.	Varieties	Oleoresin Yield in %	Oleoresin Paprika RMs Yield in %	Oleoresin Capsicum RMs Yield in %
18.	US-341	3.85	2.46	1.30
19.	WONDER HOT	3.32	1.83	0.58

### 3.2. Colouring Principles in Oleoresins

Colour units (CU) were calculated as per ASTA protocol in all the three samples, namely oleoresin, oleoresin paprika and oleoresin capsicum. The extractable CU was estimated through absorbance, measured via UV spectrophotometer. The higher the ASTA colour value, the greater the effect on the brightness or richness of the final product. In the case of crude oleoresin, the highest colour value was found in the variety 5531-IPM (3085.2 CU), followed by Roshini (2998.8), whereas the lowest value was found in the variety 4884 with a measured CU value of 544.2. After the first extraction, paprika was enriched high in the variety Lolly Chilli, where the colour value was found to be 3400.5. Second-highest CU was obtained in the variety 5531, which was 3312.5. The lowest value was found as 611.2 CU in the variety 4884. In the case of oleoresin capsicum, the colour value was found to be highest in the variety 355, followed by 341, whereas the lowest value was recorded in the variety Teja (S-17), which was only 99.3 CU value.

### 3.3. The Pungency of Oleoresins and Total Capsaicinoids

The pungency was quantified by analysing the HPLC chromatogram of reference standard N-Vanillynonamide and three major peak areas of corresponding capsaicinoids at specific retention times in detection wavelength 280 nm. The reference standard, N-Vanillynonamide, exhibited a specific peak at retention time 23.102 min (Fig. 4A). Three major peak areas of corresponding capsaicinoids in the sample were obtained at retention time 20.58, 23.03 and 36.34 min (Fig. 4B). The area percentages of capsaicin, dihydrocapsaicin and nordihydrocapsaicin were calculated separately against a reference standard and combined to calculate total pungency in Scoville Heat Unit (SHU); finally were presented in Million Scoville Heat Unit (MSHU). The concluded data is presented in Fig. (5). The highest pungency (1.59 MSHU) in the crude

oleoresin was observed in the variety 5531-HPH, whereas the second-highest pungency was estimated in the variety 5531-IPM. In the case of oleoresin capsicum, pungency was highly enriched in the two varieties 4884 and Teja (S-17), with the pungency value, 6.11 and 5.5 MSHU, respectively. The lowest value was found in the variety Hindupur-S7, which was only 1.01 MSHU.

Total capsaicinoids were calculated from the pungency values. Pungency value is directly proportional to the capsaicinoids contents present in the sample. The total capsaicinoids content in oleoresin and oleoresin capsicum followed the same pattern as pungency data, which is presented in Fig. (6). The highest value of capsaicinoids content was observed in the variety 5531-HPH followed by the variety 5531-IPM. After the purification step, the maximum capsaicinoids content in oleoresin capsicum was found to be 40.73% in the variety 4884. Further capsaicinoids contents were found to be second highest in the variety Teja (S-17) with calculated values of 36.67%. The Hindupur-S7 variety exhibited the lowest capsaicinoids contents after the enrichment process.

## 4. DISCUSSION

In the food processing industry, the extracts of chilli pepper are of immense commercial importance when compared to raw chilli [27]. Extensive researches have already been carried out for developing and optimising the process of extraction, purification and isolation of active principles [28]. A large number of varieties are cultivated; however, there is limited knowledge regarding the quality of each variety concerning the comprising extractives, colouring and pungent values. Our study was designed to focus on the evaluation of the most commonly cultivated varieties and to generate scientifically relevant data useful for commercial benefits and selecting the appropriate variety based on customised requirements.

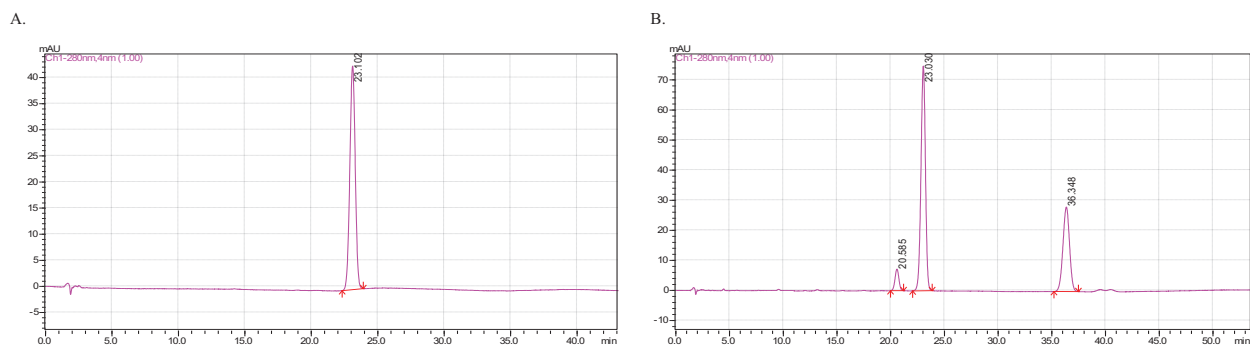


Fig. (4). HPLC Chromatograms A. Reference standard; B. Capsaicinoids representation in one sample.

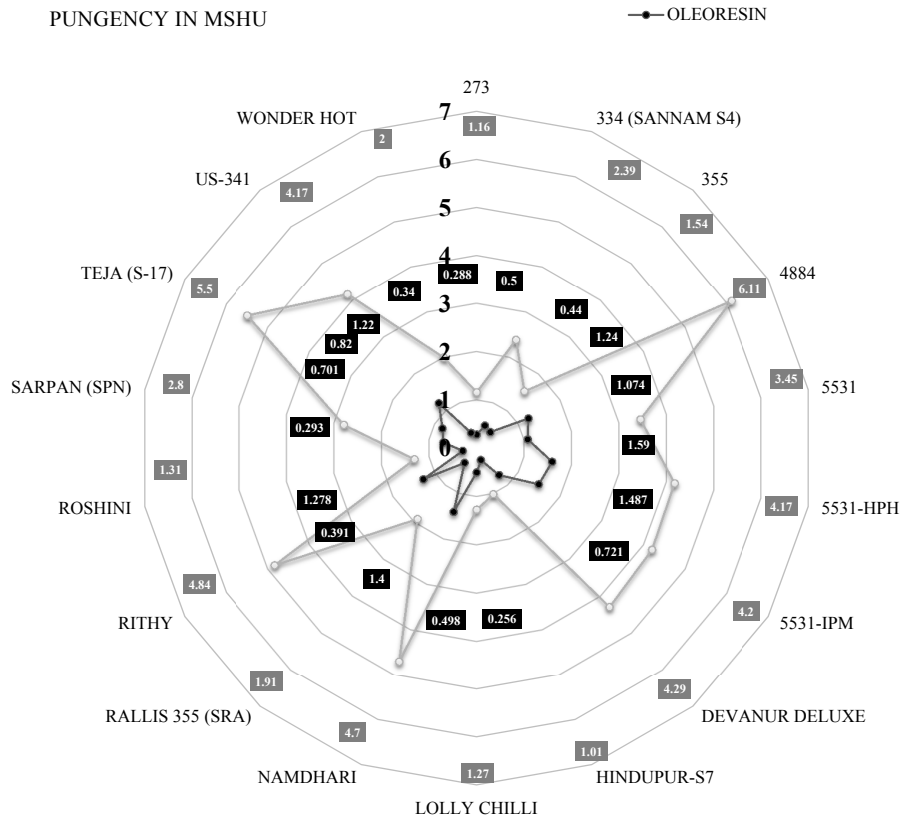


Fig. (5). The pungency of all crude oleoresins and enriched oleoresin capsicums.

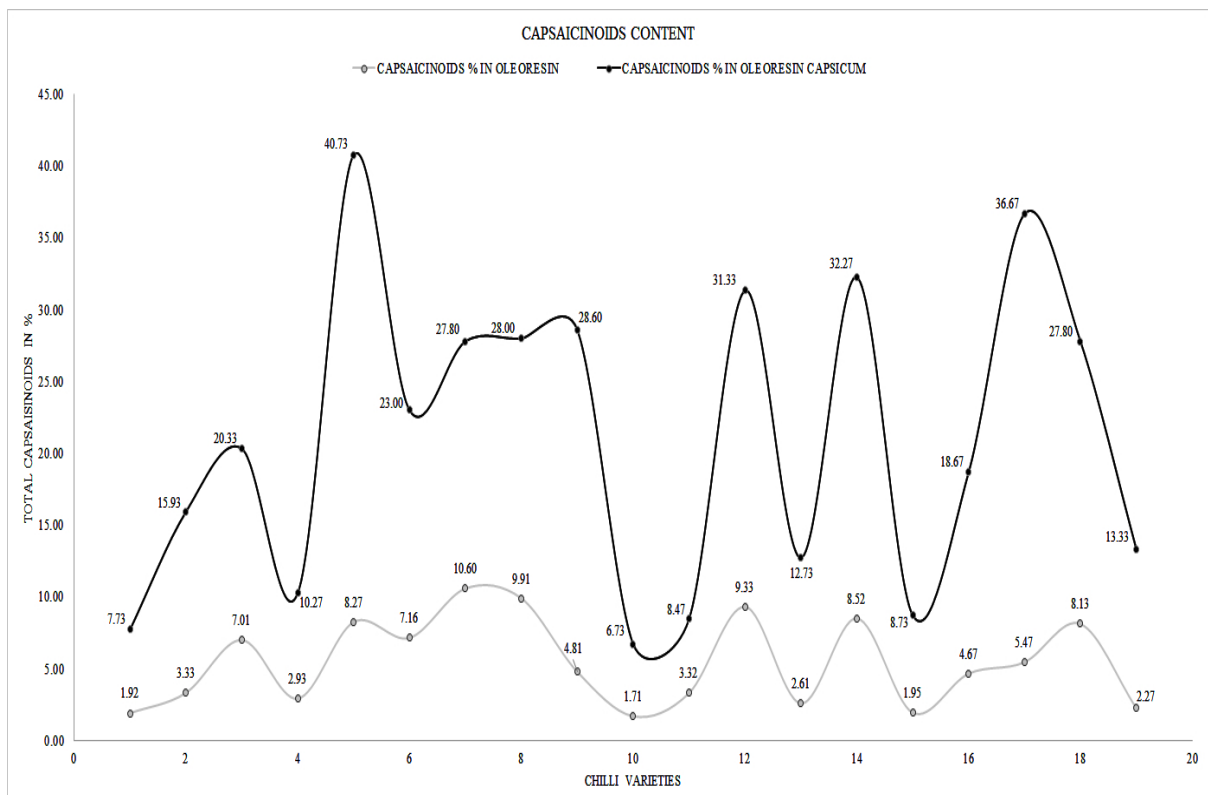


Fig. (6). Total capsaicinoids in all crude oleoresins and enriched oleoresin capsicums.

Different varieties have different yield because of their unique phytochemical distribution, cultivation, harvesting, collection, climate and chemical background of soil and water [29]. Similarly, the colour value was also different based on carotenoids concentration enriched in the crude extracts and all the purified versions. Therefore, pungency and total capsaicinoids content in individual varieties were also analysed, and data were compared to get the answer to the question, “which are the good variety for commercial extraction and oleoresins trade”?

Considering all the evaluated varieties, extract yield was found to be highest 8.82% in the variety Teja (S-17), whereas extract yield in the oleoresin paprika and oleoresin capsicum was calculated higher in the varieties 273 and Teja (S-17 with their value 6.04% and 2.62%, respectively). Colour value in crude oleoresin was found to be highest (3085.2 CU) in the variety 5531-IPM whereas, in the case of oleoresin paprika, the colour value was enriched high in the variety Lolly Chilli (3400.5 CU). In the case of oleoresin capsicum, CU value was found highest in the variety 355.

Once pungency analysis was performed on crude oleoresin, the highest pungency (1.59 MSHU) was observed in the variety 5531-HPH. In the case of oleoresin capsicum, pungency was highly enriched in the two varieties 4884 and Teja (S-17) as 6.11 and 5.5 MSHU, respectively. Total capsaicinoids contents in crude oleoresin were found to be maximum in the variety 5531-HPH (10.60%). In contrast, after enrichment, the maximum capsaicinoids contents in oleoresin capsicum was enriched in the varieties 4884 and Teja (S-17) with the values 40.73% and 36.67%, respectively, which were also supported by the other finding [7, 30].

## CONCLUSION

The outcome of our elaborated studies elucidates that Teja (S-17), 5531-HPH, 5531-IPM and 4884, varieties are better for extractive yield, pungency and total capsaicinoids contents, whereas 5531-IPM and Lolly Chilli varieties are better for colour extraction compared to all other varieties. The obtained data of all the nineteen varieties of red chilli shall be helpful to the scientific community, food processing industries and agricultural communities. Moreover, the data furnished may provide a clear idea of identifying the suited variety for diverse applications. The resulting outcome may also provide insights to the agronomists to develop superior cultivars, from the existing varieties. Separate and advanced analytical studies need to be performed to confirm individual claims for selected varieties.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

Not applicable.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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