



## DIFFERENT METHODS OF EXTRACTION AND COMPARISON OF CAPSAICIN FROM CHILLI PEPPER VARIETIES

### Biological Science

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### ABSTRACT

Extracts of plants containing alkaloids were known and used due to their diverse activity by people of ages. Capsaicinoids are active constituents liable for the pungent and spicy flavor in chili peppers. They play many biological functions like powerful antioxidant, anti-mutagenic and antitumor properties. Several research works had therefore been extensively dispensed on the traditional way of extracting this active component called capsaicin. The article has discussed some common extraction methods of capsaicin from different chili varieties, to seek out the foremost suitable method, the optimum conditions required, and therefore the appropriate organic solvents. The various sorts of chili taken under review were Chili peppers (*C. frutescens*), dried red chili pepper (*C. frutescens*), dedo demo ca pepper (*c. baccatum L.*), Chiltepine peppers (*C. annum*), and Habenero peppers (*C. chinense*). The best content was found in Chiltepine pepper which was about 118.93 mg/g and therefore the most preferred method is HPTLC or HPLC.

### KEYWORDS

alkaloids, capsaicinoids, chili peppers, extraction, solvents

### INTRODUCTION

Alkaloids are huge groups of present organic compounds that are produced generally by many plant species, mainly by flowering plants and also by some animals. They're usually stored in many parts of the plant but in numerous amounts. Several extraction methods allow the recovery of alkaloids from an answer which may be separated from the mixture and be obtained in pure form. This paper mainly deals with Capsaicin, an alkaloid that belongs to the genus *Capsicum* within the nightshade potato family.

Chili peppers are extensively used over the years as a preservative and a spice to feature flavor to food preparations. Their spiciness is thanks to a chemical called capsaicin which could be a flavorless, odorless, and colorless compound found in varying amounts in peppers. Specifically, capsaicin occurs within the fruits of plants within the *Capsicum* family, including jalapeño peppers, cayenne peppers, and other chili peppers. They're expressed in terms of Scoville Heat Units (SHU). The SHU could be a method of describing what number drops of sugar water is required to dilute the warmth of any given pepper. Capsaicin may additionally stimulate the assembly of endorphins, which is why some people report experiencing a way of euphoria when eating spicy foods. Capsaicin has several non-culinary applications, including as a pain reliever and because the active ingredient in spray can. Although excessive exposure to capsaicinoids would be toxic causing irritation within the contact area or respiratory problems yet as some forms of cancers because of ingestion of high quantities, capsaicinoids present many biological activities, like powerful antioxidants, anti-mutagenic and antitumor properties [8]. They're also used traditionally for muscular pain and headache and to boost circulation and also commonly added to herbal formulations because it acts as a catalyst for other herbs and aids in their absorption. It's also said that it can suppress pain without necessarily inhibiting brain responses so it's considered as a possible replacement for traditional anesthesia which has several limitations just like the blockage within the nerve impulses, pharyngitis, vomiting after surgery, teeth damage, shivering, and other side effects [9]. Moreover, capsaicin and other capsaicinoids have shown strong evidence that has promising potential within the fight against many sorts of cancer [7]

Different forms of chili peppers possess different content of capsaicinoids in them. Up to now, there are different methods for the extraction of capsaicin from their chili varieties. In a Particular research, the extraction by GC and HPLC was conducted and also the detection limit was calculated [12], the comparison of extraction time, energy consumption, etc between the traditional technique Soxhlet with microwave-assisted extraction and PLE [13].

Although many experiments are done to extract capsaicinoids and

quantitatively identify them, we found that there's a requirement to look at the important methods identified overall, the foremost common methods that are being employed, and compare them. We discover the need to spot the best extract which is able to serve medical purposes. The main aim of the article was to check the various methods that were used for the extraction of capsaicin from different chili varieties supported the research papers and provides information about the efficient and better methods, conditions, and also the appropriate solvents required for the quantitative and qualitative extraction of capsaicin

### MATERIALS AND METHODS

#### I. SAMPLE PREPARATION

Chili peppers (*C. frutescens*) were produced in Sichuan province (China) and purchased from local markets was dissected into peduncle, seeds, and pericarp. Pericarp was dried in an exceedingly 65°C oven and so triturated to create homogenous samples. Ethanol, inhalation anesthetic, n-hexane, dimethyl sulfoxide, petroleum ether (60 to 90°C), and acetone are the reagents that have been employed by them for the extraction. [1]

- Samples they used were dried red chili pepper sorts of *Capsicum frutescens* purchased from the markets of Fier (Albania). The fruits of red chili peppers were ground into a fine powder with the assistance of a blender within the dimension of 25 µm and were stored in airtight containers. All extracts were obtained from ground peppers. Eight samples were prepared for TLC identification and 7 samples for HPLC analyses, with four different organic solvents: ethanol, acetonitrile, ether, and methanol. Ethanol extracts were prepared by three methods: Maceration, Soxhlet, and Ultrasound. Acetonitrile extracts used ultrasound, Soxhlet, and extraction method described by Collins et al. Standard capsaicin was obtained from Sigma Aldrich, India.

TLC; They prepared a stock solution of 1mg/ml by dissolving 10 milligrams of capsaicin in a very minimum quantity of methanol and increasing the mark in a 10 ml volumetric flask.

HPTLC; For HPTLC they prepared a stock solution of 1mg/ml by dissolving 10 mg capsaicin with methanol in a 10 ml volumetric flask. The working standard, 100 mg/L, was prepared from this stock solution. [2]

- In this experiment, they used pepper "dedo de moça" (*Capsicum baccatum L.*) obtained from a local market in Campinas/ SP, Brazil. Peppers were prepared in line with Aguiar et al [10]. Based on physical integrity they chose the fruits and sanitized them in a whitener solution (10 mL/L) for 20 min and washed them with water. First, they oven-dried the pepper samples at 70 ± 2 °C for twenty-four h and milled them in a knife mill to be homogenized and intensify the mass transfer

during the extractions. The dried and milled peppers were stored in a freezer at  $-18^{\circ}\text{C}$ . After 24 h of drying at  $70 \pm 2^{\circ}\text{C}$  they determined the moisture. The overall lipid concentration within the samples was obtained per the AOAC method 963.15 (1999), by Soxhlet with hexane. [3]

- Chiltepin (*Capsicum annuum* var. *glabriusculum*) peppers were employed in this experiment. They collected three different seed samples from the material and treated them under optimal growth conditions before germination. Samples were collected at two stages of maturity, green mature (fresh) and red ripening, then dried employing a cabinet-type convective dryer at  $65^{\circ}\text{C}$  by 24h and triturated. They used reagents, standards (capsaicin >95%), and high degree solvents that were purchased from Sigma-Aldrich (St. Louis, MO, USA). [4]

- Habanero pepper (*Capsicum chinense*) samples were dissected and separated into seeds and shells. An oven drying method ( $65^{\circ}\text{C}$ , until a relentless weight was achieved) was accustomed analyze the moisture content in them and also a temperature slightly below  $70^{\circ}\text{C}$  (recommended) to reduce the volatilization. Whole peppers and their parts were processed as fresh, oven-dried, and freeze-dried samples. Oven-dried preparations were dried in a very convection oven at  $65^{\circ}\text{C}$  for 24h to keep up quality. Freeze-dried preparations were placed in a freeze dryer for 4 days with a condenser refrigeration temperature of  $-80^{\circ}\text{F}$ , shelf temperature of  $+80^{\circ}\text{F}$ , and a chamber pressure of 500 atm. Ethanol, acetone, and acetonitrile are the solvents used for his or her experiment [5]

## INTERPRETATION.

Sample preparations of varied chili varieties from different papers are reviewed. While the dried red chili pepper forms of hot pepper purchased from the markets of Fier (Albania) were ground into a fine powder, all other preparations were done by either oven drying or convective drying. For plant samples, mostly oven-dried preparations are used which is an efficient way of removing over 80% of the moisture content. The effective temperature is  $65-70^{\circ}\text{C}$  may be used for twenty-four hours but less than 48 hours to avoid higher volatilization. Thus oven-dried preparations are recommended for the extraction of capsaicin from different sorts of chili.

## II. EXTRACTION

The pericarp that was Oven-dried and titrated (0.5 g dry weight) was used to extract capsaicinoids by conventional (non-ultrasonic-assisted) and UAE methods. Both the preparation and solvent mixtures were homogenized in 10 ml conical glass tubes and placed in an ultrasonic water bath of 360 W coupled with a temperature controller. And they also evaluated different extraction parameters; including solvents (ethanol, ethyl ether, n-hexane, petroleum ether ( $60$  to  $90^{\circ}\text{C}$ ), and acetone), extraction temperature ( $20$  to  $50^{\circ}\text{C}$ ), and extraction time ( $0$  to  $120$  min). After extraction, the concentration of capsaicinoids was determined by measuring OD280 (optical density at  $280\text{ nm}$ ). [1]

- They spotted the capsaicin standard and all the samples of  $15\text{ }\mu\text{L}$  on pre-coated silica gel plates using a micro syringe. The employed mobile phase was Chloroform: Methanol: Acetic acid (9.5: 0.5: 0.1, v/v/v). The plates were developed up to  $80\text{ mm}$  in twin trough development chambers which were then dried and exposed to iodine vapor and the spots were visualized. Similarly for the HPTLC method of determination, all the extracts were first filtered by a micro filter of size  $0.2\text{ }\mu\text{m}$ . Capsaicin standard and the samples of ethanol, acetonitrile, and methanol extracts were spotted in  $3\text{ }\mu\text{L}$  on pre-coated silica gel GF254 plates, using CAMAG Linomat 5 sample applicator. The same mobile phase as used in TLC was employed in the same ratio. The plates were developed up to  $90\text{ mm}$  in CAMAG twin development chambers, after chamber saturation of 15 minutes. They dried the plate with the help of a heater. Densitometry scanning of the plates was performed at  $226\text{ nm}$ , using CAMAG TLC Scanner 3. The regression coefficient, relative standard deviation, slope, and intercept on the Y-axis were calculated by the software. The calibration graph was used to quantify the capsaicin content in each sample. WIN-CATS software was employed for the analysis [2].

- Their conventional method of extraction was Soxhlet to determine the total capsaicinoids in the extracts from dedo de moça pepper. They used four different solvents with different polarities in the procedure which includes: hexane, ethanol, methanol, and ethyl acetate. Each extraction was performed with  $150\text{ mL}$  of solvent and  $5\text{ g}$  of dried and

milled samples inside a filter paper. The reflux of boiling solvent was kept for 6 h. After extraction, the solvent was evaporated under vacuum (at  $25^{\circ}\text{C}$ ), the extracts were weighed and stored under freezing ( $-18^{\circ}\text{C}$ ). All experiments were performed in triplicates. [3]

- The capsaicinoids were extracted from the dried fruits which were then powdered and  $1\text{ g}$  was treated with  $10\text{ mL}$  of acetonitrile at  $65^{\circ}\text{C}$  along  $20\text{ min}$  under sonication, with a working frequency of  $35\text{ kHz}$ . The extracts were evaporated to dryness at  $60^{\circ}\text{C}$ , resuspended in  $0.5\text{ mL}$  of acetonitrile, and filtered through a  $0.45\text{ }\mu\text{m}$  cellulose acetate membrane filter. The absorbance of the unknown sample solutions was read using a one cm quartz cell in a UV-Vis spectrophotometer (HACH DR-5000). They made the standard stock solution of  $2\text{ mg/mL}$  by weighing pure capsaicin and dissolving it in acetonitrile up to  $2.0\text{ mL}$ . The solution was filtered with a  $0.45\text{ }\mu\text{m}$  syringe filter. The stock solution of capsaicin was diluted as required. Calibrate daily with at least six working standards over the range of  $10$  to  $200\text{ mg/L}$  for each standard. These dilutions were then scanned in the UV-Vis spectrometer and  $\lambda_{\text{max}}$  of capsaicin was selected as the wavelength of detection. The instrument was set at  $280\text{ nm}$ . The capsaicinoids content in the unknown extract solutions was calculated based on the absorbance values of known standard solutions [4]

- Fresh, oven-dried, and freeze-dried preparations ( $0.5$  dry weights) were extracted using biomass: solvent loading of  $15\%$  (w/w) based on the initial moisture content of the pepper samples/parts. Sample and solvent mixtures were homogenized in  $50\text{ mL}$  conical glass tubes and placed in a shaking water bath ( $50^{\circ}\text{C}$ ). Samples were taken every  $20\text{ min}$  for a period of  $1\text{ h}$ . Samples were processed by vacuum filtration (Whatman GF/A glass fiber filters,  $1.6\text{ }\mu\text{m}$ ). It can be stored at  $-20^{\circ}\text{C}$  until the analysis is done using HPLC. Capsaicin standards were diluted to concentrations of  $10$ ,  $30$ , and  $50\text{ mg/L}$  to prepare the calibration curve. H C18 Column equipped with an automated sampler, a gradient pump, and a UV/vis detector at a wavelength of  $280\text{ nm}$  was used to quantify samples. Samples were eluted at a flow rate of  $1\text{ mL/min}$  with a mobile phase of  $40\%$  acetonitrile and  $60\%$  deionized water adjusted to pH 3 with acetic acid at  $30^{\circ}\text{C}$  [5]

## INTERPRETATION

The extraction procedure involves five different methods which include the UAE (Ultrasonic assisted extraction) method, TLC and HPTLC, Soxhlet method, spectrophotometric analysis, and HPLC method. The UAE method appears as an alternative to conventional extraction since it works at lower temperatures and shorter time. The dried red chili peppers from Albania were studied using both TLC and HPTLC methods. Although the TLC method takes a shorter duration than HPTLC, it mainly gives only the qualitative identification of the capsaicin from the extracts whereas the HPTLC method gives the quantitative identification. The de dodo demo ca pepper was analyzed using the Soxhlet method which is the most convenient method and the most feasible method applied. But the use of UAE and several other advanced techniques have replaced the conventional extraction techniques although higher ultrasonic intensities can also reduce their yields. The Chiltepine peppers were analyzed using the spectrophotometric method. This method was found to be cost-effective, valid, and feasible but it was not accurate as HPLC or HPTLC. Although the conventional methods are easier for the extraction of the capsaicinoids, the HPTLC and the HPLC might be the appropriate method giving good yield, TLC, spectrophotometer, and Soxhlet being the most feasible and cost-effective method

## RESULTS

- To evaluate the effect of the ratio of solvent to mass on the extraction yield of capsaicinoids, they administered a series of ratios ( $4$ ,  $6$ ,  $8$ ,  $10$ , and  $12\text{ mL/g}$ ) with different volumes of solvent while maintaining a relentless quantity of triturated hot Chili peppers of about  $0.5\text{ g}$ . The rest extraction conditions were: the temperature at  $20^{\circ}\text{C}$ ,  $30\text{ min}$  of extraction, and acetone as solvent. All assays were performed in triplicate, and it is observed that the extraction yield of capsaicinoids was enhanced to the critical value ( $3.89 \pm 0.05\text{ mg/g}$ ) at the ratio of  $10\text{ mL/g}$ , so increased in a very mild slope with a ratio of solvent to mass increasing. Thus, they decided to use the ratio of the amount of solvent (mL) to mass (g) at  $10\text{ mL/g}$ . Similarly for the evaluation of extraction temperature for the yield of capsaicinoids the results obtained were; at temperatures not up to  $30^{\circ}\text{C}$ , the extraction yield of capsaicinoids was increased with increasing extraction temperature which can result in the slower extraction kinetics at a lower temperature. Therefore,  $30^{\circ}\text{C}$  was used because of the critical extraction temperature. And for the

optimum extraction time they found that with increasing extraction time from 0 to 30 min, the extraction yield of capsaicinoids increased from low to high till at 30 min to maximum, so increased slightly after 30 min in these extraction conditions.

Trial number	Factor				Results of extraction yield, $\bar{Y}_i$ (mg/g)
	Ratio of solvent to mass, A (ml/g)	Extraction time, B (min)	Extraction temperature, C ( $^{\circ}$ C)	Experimental error, E	
1	6	20	25	1	3.512
2	6	30	30	2	3.462
3	6	40	35	3	3.582
4	8	20	30	3	3.566
5	8	30	35	1	3.492
6	8	40	25	2	3.726
7	10	20	35	2	3.702
8	10	30	25	3	3.762
9	10	40	30	1	3.786
$\bar{K}_1$	10.556	10.78	11	10.79	$\bar{Y}_T = \sum_{i=1}^9 \bar{Y}_i = 32.59$
$\bar{K}_2$	10.784	10.716	10.814	10.89	
$\bar{K}_3$	11.25	11.094	10.776	10.91	
$\bar{K}_4$	3.519	3.593	3.667	3.597	
$\bar{K}_{12}$	3.595	3.572	3.605	3.63	Order of significant factors Optimal levels
$\bar{K}_{13}$	3.75	3.698	3.592	3.637	
$\bar{R}_j$	0.231	0.126	0.075	0.04	
				A>B>C	
				A <sub>1</sub> B <sub>1</sub> C <sub>1</sub>	

FIGURE-1; extraction yield of capsaicinoids [1]

So finally with the assistance of an orthogonal array experiment, they concluded that the very best yield of extraction obtained was 3.786 with the ratio of solvent to mass being 10ml/gm, the extraction time being 40 minutes and by maintaining the extraction temperature of 25 to 30  $^{\circ}$ C [1]

• For the event of the TLC method, they used two different compositions of mobile phase: Water: Methanol (2:8) which failed to give any separation, and Chloroform: Methanol: ethanoic acid (9.5: 0.5: 0.1) was found to be best because it gave good resolution for capsaicin. The capsaicin spots were identified by the quality spot or by the worth of the retention coefficient. The identification of capsaicin is finished by the quality sample and also the  $R_f$  values calculated are respectively 0.562 and 0.66 for ether and methanol samples. When acetonitrile was used as a solvent by using three different methods, Ultrasound extraction the measured  $R_f$  was 0.52; Extraction by maceration the measured  $R_f$  was 0.52, extraction by Collins method [22] the measured  $R_f$  was 0.52.

For quantitative determination of capsaicin from these extracts using HPTLC and it was detected to be 90 ng/ $\mu$ L and also the lowest amount of capsaicin which may be quantified was found to be 186.61 ng/ $\mu$ L. The HPTLC analyses were realized 3 times in 15.20C temperature and 42% humidity.

Sample	Capsaicin ng $\pm$ SD	Capsaicin %*
<b>Ethanol extracts</b>		
Maceration	925.3 $\pm$ 59.94	0.0308 %
Soxhlet extraction	1559 $\pm$ 143.68	0.0519 %
Ultrasound	< 270	0.0090 %
<b>Acetonitrile extracts</b>		
Maceration	782.0 $\pm$ 45.82	0.0260 %
Ultrasound	< 270	0.0090 %
Collin's method	559.83 $\pm$ 99.40	0.0186 %
<b>Methanol extracts</b>	1077 $\pm$ 29.14	0.0359 %

Figure-2; The Mean Value Of Capsaicin Content [2]

The above table shows the mean of capsaicin content for every extract expressed in nanograms  $\pm$  variance (ng  $\pm$  SD, n = 3) and in percentage (w/v). From the results obtained they also calculated the content of capsaicin within the amount of dried drug (g) used for every extract which is shown below;

Sample	Capsaicin %*
<b>Ethanol extracts</b>	
Maceration	0.3084 %
Soxhlet extraction	0.2078 %
Ultrasound	0.0450 %
<b>Acetonitrile extracts</b>	
Maceration	0.2606 %
Ultrasound	0.0450 %
Collin's method	0.1864 %
<b>Methanol extracts</b>	0.3590 %

Figure-3; Different Solvents And Their Extraction Methods [2]

From these values, they will conclude within the superiority of extraction capsaicin by using ethanol and methanol (0.3084% and 0.3590%). Also, the tactic of maceration with two solvents (ethanol and acetonitrile) gives the most effective result with the very best values of percentage. [2]

• Extraction from Soxhlet using hexane and ester resulted in higher capsaicinoid yields of  $1.44 \pm 0.07$  and  $1.77 \pm 0.21$  (mean  $\pm$  standard deviation of the analyses) in comparison to methanol and ethanol. This can be mainly due to the affinity of the non-polar characteristic of capsaicinoids to low polarity solvents thereby increasing their yield. Also, the force per unit area of hexane and ester explains the upper Capsaicinoid yields. Moreover, the lower diffusivity and better viscosity of ethanol and methanol can reduce the efficiency of those solvents to extract capsaicinoids. [3].

• As the capsaicinoids exhibit absorption in the UV region of the spectrum between 200 and 350nm and because they obey the Beer-Lambert law, their absorbance measurement was used to quantify the concentration of a pure (standard) capsaicinoid or to estimate the total capsaicinoid concentration in a mixture or extract of capsaicinoids in a sample. The capsaicin standard was purchased commercially and dissolved in acetonitrile and the spectrum was scanned to evaluate their fine structure.

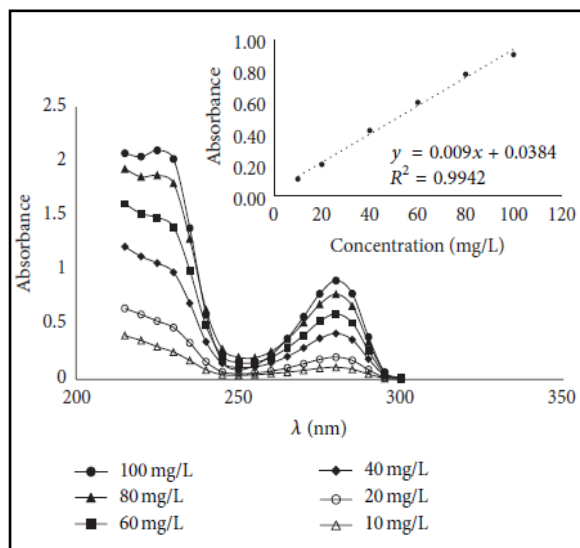


Figure -4 Uv-vis Spectra Of Different Concentrations Of Capsaicin [4]

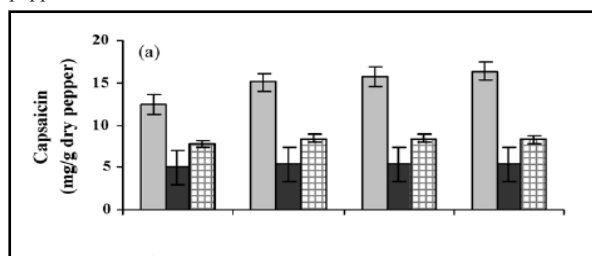
Using the absorbance measured at its maximum wavelength (280 nm) and taking into consideration the dilution factor, purity degree, and molecular mass (capsaicin 305.41 g/mol), the molar absorption was calculated. The molar absorptivity coefficient of capsaicin is ( $\epsilon_{280} = 3410 \text{ M}^{-1} \text{ cm}^{-1}$ ) and its LOD and LOQ were found to be 0.070 and 0.213 g/mL. The results indicated excellent recoveries ranging from 98.5 to 99.3% for capsaicin and reliability of the method.



The capsaicinoid content, as determined by the spectrophotometric method, was generally higher in GM ripe fruit than red fruit. The content varied from 20.18–97.73mg/g for RR samples to 59.47–118.93mg/g for the GM stage. Samples showed a considerable percentage of decrease in capsaicinoid levels depending on the stage of maturation from GM to RR.

Besides, their results indicate that, in contrast to other studies where capsaicin content was generally higher in ripe fruit than green fruit, Chiltepine red ripening fruits showed lower levels of capsaicin than green mature fruits in all samples. The correlation coefficient was 0.91 ( $< 0.001$ ) for capsaicinoids in Chiltepine pepper fruit, indicating a strong positive association between these values. Thus simple spectrophotometric procedure proved to be a valid, efficient, and cost-effective method for the quantification of total capsaicinoids in these conditions, which adequately represent total capsaicinoids content in hot chili pepper, extracts [4]

• They used Analysis of variance (ANOVA) results to describe the overall fixed effects of the whole plot and split-plot factors on capsaicin yields. Capsaicin recovery from the different pepper parts overtime was greatest and significant for seed extractions. Times 20, 40, and 60 min all showed statistically greater capsaicin yields than time zero. Approximately 16mg capsaicin/dry g was recovered from the seeds across all solvents investigated. Recovery from whole peppers was 8mg capsaicin/dry g, followed by 5 mg/dry g from the pepper shells.



**Figure-5 [5]**

Solvent and preparation interactions were statistically significant for whole peppers but it did show noticeable trends for the seed and shell parts.

**Seed sample:** among the fresh seed samples, capsaicin recovery using acetonitrile and ethanol was 27% greater than recovery using acetone. Fresh sample extractions with ethanol and acetonitrile were also 23–29% greater than extractions with those solvents on freeze-dried and oven-dried seeds ( $p < 0.05$ ). Significantly more capsaicin (39%) was recovered from oven-dried seeds than freeze-dried seeds using acetone ( $p < 0.05$ ), yet statistically similar amounts were extracted from fresh and oven-dried preparations.

**Shells:** Capsaicin recovered from the shells across time was approximately three times less than that from seeds per dry gram of part. Yet on average, more capsaicin was recovered from freeze-dried and oven-dried preparations than fresh preparations for all solvents. Among the dried samples, on average acetone seemed to be more effective solvent, yielding 8–10% more capsaicin within the freeze-dried samples and 2–12% more within the oven-dried samples.

**Whole peppers:** among the fresh whole pepper samples, capsaicin recovery using acetonitrile and ethanol was approximately 30% greater than recovery using acetone ( $p < 0.05$ ). Freeze-dried whole pepper extractions using ethanol resulted in 55% greater recovery than oven-dried extraction using ethanol ( $p < 0.05$ ). Significantly more capsaicin was recovered from oven-dried whole peppers using acetone as opposed to ethanol, indicating again that acetone was a better solvent for dried pepper parts. Also, acetone performed best on freeze-dried whole peppers, which resulted in 66% more capsaicin than fresh whole pepper samples ( $p < 0.05$ ).

And from their experiment, they find that ethanol and acetonitrile to be the better solvents for capsaicin extraction from fresh samples while acetone was better for dried pepper parts. Also, it can be said that although the seeds per unit dry matter possessed more capsaicinoids than whole peppers and shells, whole peppers would likely serve as a more viable processing choice for capsaicinoid extraction and also

from the perspective of large scale processing and economics, whole peppers are more practical to use for capsaicinoid production. [5]

## INTERPRETATION

The extraction or the yield of capsaicin varied depending on the variety of chili used, the extraction time, the solvents used, the parts of chili under study, and the method of extraction. The chili pepper (*C. frutescens*) from china has a yield of about 3.786mg/g by using ultrasonic-assisted extraction. The dried red chili pepper (*C. frutescens*) from Albania has given the highest extraction of 90 to 180ng/ul by using the HPTLC method. Dedo de Moca pepper (*C. baccatum*) from Brazil yield of extracts  $1.77 \pm 0.21$  by using conventional Soxhlet method. A Chiltepine pepper (*C. annum*) from Mexico has given a yield up to 97.73mg/g in red fruit and 118.93mg/g in green mature ripe fruit. The habanero pepper (*C. chinense*) from the USA has given 16mg/g for the seeds, 8mg/g for whole peppers, and 5mg/g for pepper shells. From the study, we can interpret that Chiltepine peppers (*C. annum*) especially in their green mature form have given the highest yield of capsaicin of 118.93 mg/g.

## CONCLUSION & DISCUSSION

When comparing all the methods used for the extraction procedure it's said that spectrophotometer is incredibly feasible. This method is incredibly useful for the precise determination of total capsaicinoids in pepper fruit samples and yields an in depth estimation although it can't be as accurate as HPTLC or HPLC. The tactic is an efficient means for internal control and may be a viable alternative to the prevailing analytical methods for routine analyses, allowing a rapid and accessible quantitation of capsaicinoids in fruit samples with none time-consuming sample separation. However, the foremost accurate results may be obtained using the HPTLC method. It's selected to be used for the quantitative determination of capsaicin because the extracts don't need purification before injection like the HPLC method. Thus it's recommended for herbal analyses by many authors (Patra et al, Subramanian et al). Similarly, HPLC is additionally preferred for several secondary metabolites extraction due to its simplicity, versatility, and scope of the reversed-phase method because it can handle compounds of diverse polarity and molecular mass. On the other hand, UAE appears to be an alternative to traditional extraction, since it works at lower temperatures and shorter time but higher ultrasonic intensities may reduce their yields. Although Soxhlet is a simple method in terms of sample preparation and feasibility, they require high solvent consumption and/or long extraction times. The extraction time is additionally an obstacle because it's associated with high energy consumption and might severely decrease the sample throughput; thus, it's a drawback in terms of business applicability. When comparing Soxhlet to UAE, the Soxhlet method provides a better yield of capsaicinoids.

In terms of the solvent, their contact time for effective extraction yield and merchandise quality will possibly occur within the primary 20 min of the processing; however, it'll be important to spot suitable conditions supporting the chosen pepper fruit, preparation, solvent, and scale of operation. When chromatographic techniques are used for the extraction, the samples preferred are ethanol and methanol whereas when the Soxhlet method is employed the very best extraction is obtained using hexane and ester. Acetonitrile is additionally preferred as an acceptable solvent in capsaicin extraction. In general, the solvent that may be used depends on the aim of the experiment. Among all solvents that are used for extracting capsaicinoids, methanol, ethanol, acetonitrile, and water are the foremost common. If we would like to live the quantity in pepper, for instance using UV-vis Spectro, we will extract with acetonitrile. Otherwise, we are able to use some food base solvent, like ethanol.

Capsaicin recovery from the various pepper parts overtime was greatest and significant for seed extractions [5]. The pepper they needed taken understudy was habanero peppers (*C. Chinense*). In contrast when put next to a different paper which also says that capsaicin isn't evenly distributed in pepper fruit but normally, the best capsaicin concentrations are found within the ovary and therefore the lower flesh and therefore the lowest capsaicin content are often found in seeds. The best content of capsaicin (227 mg per 100 g of fresh weight) was found within the 'Takanotsume' cultivar. [11]. Therefore the amount of capsaicin in numerous parts of peppers and might vary and depend upon the variables taken under study.

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