

# Extraction, Characterization and Health Benefit of Dietary Chili Pepper (Capsaicin): A Review of Recent Progress

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**Abstract:** *Chili peppers are widely eaten spices across the globe, and capsaicin, the major source of the spicy taste, is said to contain a variety of biological functions such as pain relief, anti-inflammation, anticancer, cardio protection, as well as weight loss. In this review, we present the recent progress in various extraction and characterization methods of chili peppers.*

**Keywords:** Capsaicin, chili pepper, extraction, health benefits

## 1. Introduction

The Capsicum genus includes chili pepper, which is one of the world's most popular and oldest condiments, spice and vegetable. This genus contains roughly 25 species, with its origins in South and Central America, as well as Mexico (Zhang et al., 2021). Chili pepper has indeed been utilized as, natural colorant, vegetable, and in herbal medicine from prehistoric days, and it is one of the very first plants to be cultivated on the Mexican peninsula (M. Lu et al., 2017).

Natural ingredients remain one of the most important sources of therapeutic discoveries, offering an opportunity to research essential science. Capsaicin is an active ingredient in chili peppers that causes them to have an irritant and burning sensation (Alothman et al., 2012; Jang et al., 2008; Ng & Reuter, 2015; Seca & Pinto, 2018; Xiang et al., 2021). This volatile component in chili peppers was originally identified more than a century ago. Notwithstanding this early accomplishment, the exact chemical structure of capsaicin was not discovered until 1923, and finally, in 1930, Spath and Darling chemically synthesized capsaicin (Arora et al., 2021). S. Kosuge and Y. Inagaki, Japanese scientists, discovered compounds from chili pepper that was similar to capsaicin and termed them capsaicinoids in 1961 (Daoud et al., 2015). The most potent capsaicinoids are nordihydrocapsaicin, homocapsaicin, and dihydrocapsaicin, (Table.1) which have a composition of 1%, 7%, and 22%, respectively (M. Lu et al., 2017). Capsaicin accounts for around 68% of the entire chili pepper composition and is mainly accountable for the astringency of chili peppers (Patowary et al., 2017; Peña-Alvarez et al., 2012; Stoica et al., 2016). However, the precise proportion of other capsaicinoids and capsaicin vary based on the chili sources and method of extraction (Bajer et al., 2015; Gerardo F. Barbero et al., 2006b; Satya Prasad et al., 2004). Ever since its discovery, capsaicin has been the subject of intense investigation due to evidence of a wide range of biological effects. Capsaicin is now being studied in Phase 3 trials studies for the treatment of postoperative complications, rheumatism, musculoskeletal

problems, and chronic/acute nerve pain (Nagy et al., 2017; Xiang et al., 2021).

Despite its widespread usage, the intense spiciness limits the use of significant amounts of capsaicin in regular food and pharmaceuticals. Various researchers reported the following findings regarding the cytotoxicity of capsaicin in capsicum fruits: Capsaicin was shown to be mutagenic by Nagabhusan & Bhide, as evidenced by Ames and micronucleus tests (Nagabhusan & Bhide, 1986). Capsaicinoids were suspected of being a risk factor for, liver, gastric, gallbladder cancer, and duodenal stomach based on data from several animal experiments and case-control investigations (Agrawal et al., 1986; Lopez-Carrillo et al., 1994). Nevertheless, research is underway to alter the capsaicin compound in order to mitigate some of the negative consequences. Several published studies have also indicated serious respiratory issues and mortality in humans exposed to intense capsaicin aerosol formulations (Braga Ferreira et al., 2020; Nagy et al., 2017; Patowary et al., 2017). According to the research literature, capsaicin is fragrant, impairs temperature regulation, causes autonomic reflexes and gastrointestinal discomfort, and is poorly absorbed. The utilization of all of capsaicin qualities resulted in the invention of pepper spray, a strong contender for thwarting uncontrollable riots and apprehending errant offenders by law enforcement authorities (M. Lu et al., 2017).

Despite its negative consequences, capsaicin has been used to treat a wide variety of medical disorders. Topical capsaicin has been studied for the treatment of chronic pain disorders such as rheumatoid arthritis, neuropathic pain, hemodialysis-associated itching, diabetic neuropathy, psoriasis, post-mastectomy neuroma, and vestibulitis (Belvisi & Birrell, 2017). It's also been advised as a therapy for a variety of acute disorders because continuous topical administration of capsaicin particularly promotes the secretion and inhibition of neuropeptides in polymodal C-type, tiny myelinated A-delta-type cutaneous nerves and unmyelinated. Though capsaicin has been shown to be

cytotoxic (Nagabhushan & Bhide, 1986), however, topical research using high-purity capsaicin and established methods provide evidence that capsaicin's mutagenic and cancerous capabilities are relatively low and that purified capsaicin is very important (Shin et al., 2013). Capsaicin has also shown encouraging benefits in early studies for prostate cancer, blepharospasm, lung cancer, and many other different types of leukemia (M. Lu et al., 2017; Stoica et al., 2016). Even though capsaicin is detrimental to one's health in a variety of ways, it cannot be denied that capsaicin has numerous therapeutic implications. However, these implications have limited their applicability in other indications due to inconsistent findings. Capsaicin, for example, has been shown to have both chemotherapeutic and chemopreventive effects (Amantini et al., 2009; K. S. Chen et al., 2015). Furthermore, in vivo research supports capsaicin's anti-tumorigenic ability (H. F. Lu et al., 2010). Capsaicin, as a whole, is a potential medication applicant that might one day be used as a primary treatment therapy for several metabolic ailments. In this review, we provide an up-to-date extraction and analytical method of chili pepper.

## 2. Method of Extraction

Over the last few years, many extraction techniques for capsaicinoids from spicy peppers have been established. The first step in creating an extraction process is to choose a suitable solvent that will lead to a high output of the target component. The first step in creating an extraction process is to choose a suitable solvent that will lead to a high output of the target component (Renato et al., 2019). Methanol, acetonitrile, water, and ethanol are the most often used solvents for extracting capsaicinoids (Slack, 2016). In addition to the solvent selection technique, several other influential aspects must be addressed in order to attain maximum extraction yields, such as extraction duration, temperature, solvent volume, method reproducibility, and repeatability. Researchers have extensively used extraction methods such as magnetic stirring, maceration, enzymatic extraction, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid, and Soxhlet extraction.

### 2.1. Soxhlet extraction

A soxhlet operation is a classic approach that is commonly used to extract oil from organic matrices. It is utilized whenever the target molecule has minimal solubility in a solvent, whereas the contaminants are insoluble in this solvent (Arora et al., 2021; M. Lu et al., 2017). Bajer et al successfully isolated capsaicinoids from a variety of chili samples using the soxhlet technique with methanol as the main solvent over a timeframe of 2-hour (Bajer et al., 2015). In research conducted by Chuichulcherm et al., the same soxhlet approach was utilized, where a *Capsicum annuum* (1.0g) sample was extracted for 2 hours with 50 mL methanol (Chuichulcherm et al., 2013). Though soxhlet is the most commonly used extraction technique, it has drawbacks such as greater power consumption, lower capsaicinoids yields, and longer processing times when compared to other existing extraction techniques like PLE, UAE, and MAE. Table 2 displays these extraction results.

### 2.2. Enzymatic extraction

Enzymatic technologies have been developed to boost productivity and accuracy during the extraction of fruits (Wen et al., 2020). Several commonly available enzymes (olivex, viscozyme L, 5XAL, and peczyme) were utilized by Santamaría et al. in his research to loosen the tissues in Chili pepper and boost the extraction efficiency by 7%, with a final retrieval of 82% of capsaicinoids (Santamaría et al., 2000) (Santamaría et al., 2000). The processing did take place at 50°C, needed 7 hrs of agitation in a shaking incubator at 120 rpm, and had a chili powder-to-water ratio of 1: 50. L. Chen & Kang, used a similar technique that included energex (primarily glucanase) and extrazyme (mostly carbohydrases and pectinase), which improved capsaicinoid yield by 33% (L. Chen & Kang, 2013). The temperature, in this case, was kept at 43 C for a time frame of 12 hrs, while the ratio of chili dried powder-to-water was maintained at 1: 1. Relying on the treatment approaches described above, Salgado-Roman et al developed a noncommercial enzymatic treatment based on *Rhizopus nigricans* enzymatic extracts (Salgado-Roman et al., 2008). At 60°C, samples were extracted in a soxhlet apparatus together with tetrahydrofuran. Capsaicinoids had a greater yield of more than 86%, indicating a much more robust cellulosic ability of this non-commercial enzymatic extract to loosen the cell membranes and enable cell decomposition.

### 2.3. Pressurize liquid extraction (PLE)

The operation of PLE is frequently carried out at high pressure and temperature, allowing for hydrophilic properties of components in the solvents while maintaining the solvent below its boiling temperature, leading to high solvent penetration into the sample cell wall (Renato et al., 2019). Several studies have used the PLE technique to isolate capsaicinoid from spicy chilies (Bajer et al., 2015; Gerardo F. Barbero et al., 2006b; Chanthai et al., 2012). Barbero et al established a PLE process using ethanol, water, and methanol as extraction solvents at 200°C and 100 atm. HPLC-mass spectrometry was used to examine the results (Gerardo F. Barbero et al., 2006a). In a study performed by Chanthai et al the capsaicinoids, Capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were isolated from dried *C. annuum* specimens using the PLE technique with methanol at a temperature of 100 °C and a pressure of 1500 psi (Chanthai et al., 2012). Following the protocols described by Bajer et al, a pressurized hot water extraction technique was utilized to recover capsaicinoids from ten chili specimens. Water was chosen as the ecologically friendly medium in this experiment. HPLC-MS was used for the quantitative analysis. HPLC-MS was used for the quantitative analysis. They also examined the extraction yield of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin using several extraction procedures such as MAE, UAE, SOX, and PLE, and discovered that PLE produced the greatest yields (Bajer et al., 2015).

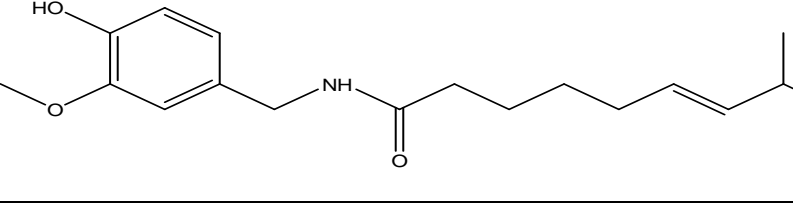
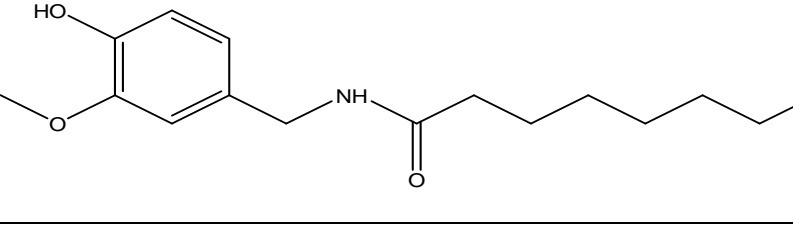
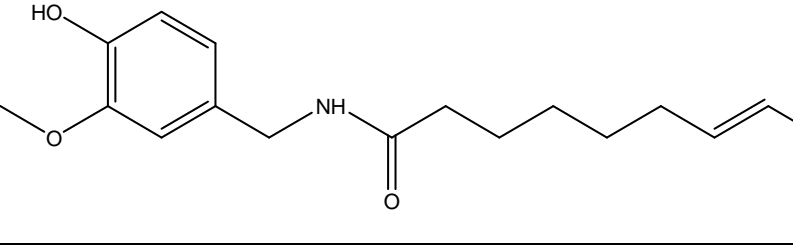
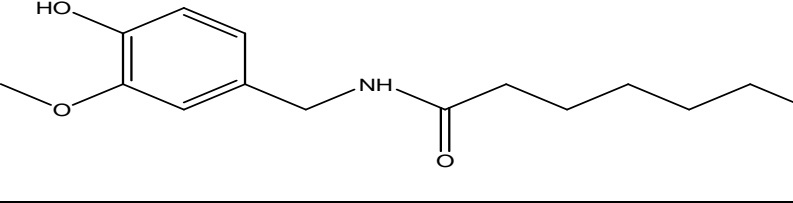
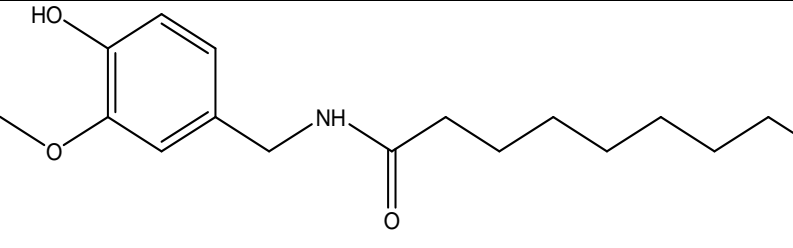
### 2.4. Microwave-assisted extraction (MAE).

MAE is a technology created by combining microwave and conventional solvent extraction that uses the energy

produced by microwave radiation to heat the solvents and accelerate the speed of separation. When compared to conventional reflux and shaking flask extraction techniques, Paduano et al. found that the MAE approach tripled the capsaicinoids yield and greatly reduced the duration of extraction (Paduano et al., 2014). Barbero et al. enhanced MAE settings for extracting capsaicinoids from raw pepper specimens. The extraction settings of 125°C, power of 500 W, for 5 minutes of extraction time, and 0.5 g of pepper dissolved in 25 mL of ethanol were determined to be optimal in this investigation (Gerardo F. Barbero et al., 2006b). The experts also examined the effectiveness of

regularly used techniques, like magnetic stirring, and found that MAE is a quicker process. Chuichulcherm et al. compared three distinct extraction procedures: Soxhlet, UAE, and MAE, as seen on the findings confirmed that the MAE approach produced the most capsaicinoids for an extraction period of 20 minutes with moderate power consumption, whereas the soxhlet technique produced the most capsaicinoids over an extraction timeframe of 300 minutes with high power consumption. Among the three methods, the UAE approach exhibited the lowest energy usage per capsaicinoids and the lowest time of extraction (Chuichulcherm et al., 2013).

**Table 1:** Chemical structures of capsaicinoids

capsaicinoid name	Abbreviation	Molecular formula	Chemical structure
capsaicin	C	$C_{18}H_{27}NO_3$	
dihydrocapsaicin	DC	$C_{18}H_{29}NO_3$	
homocapsaicin	HC	$C_{19}H_{29}NO_3$	
Nordihydrocapsaicin	N-DC	$C_{17}H_{27}NO_3$	
Homodihydrocapsaicin	H-DC	$C_{19}H_{31}NO_3$	

## 2.4 Supercritical fluid extraction (SFE)

Based on the principle of supercritical fluid extraction, pressure and temperature are elevated above their critical points by taking advantage of gas and liquid properties (Ciko et al., 2018). In this extraction method, carbon dioxide is a popular solvent because it's non-toxic, cheap, and safe to use (Lefebvre et al., 2021). During the

extraction process, supercritical carbon dioxide can only extract nonpolar or components of low polarity, but it can still remove polar solvents when polar co-solvents (e. g., methanol and ethanol) are employed. It is the combination of temperature and pressure that determines the selectivity of the targeted bioactive molecule in supercritical fluid during extraction (Heffernan et al., 2016). The low critical pressure and temperature associated with carbon dioxide

allow the preservation of bioactive molecules with little or no degradation (da Silva et al., 2016).

Santos et al recovered capsaicinoids from *Capsicum Frutescens* l. using supercritical fluid extraction aided with carbon dioxide and ultrasound as the solvents at temperatures, pressures, and flow rates of 40 °C, 15 MPa, and 1.774x10<sup>-3</sup> kg/s, respectively. The ultrasonic power was set at 360 W for 60 minutes (Santos et al., 2015) . The Supercritical fluid extraction rate significantly increased. Dias et al conducted a comparable supercritical fluid extraction test on dedo de moca pepper with (80 min, 40C, 25 MPa, and 600W) but without (40C, 25 MPa) ultrasound treatment. The flow rate of carbon dioxide as held constant at 1.774x10<sup>-3</sup> kg/s. The findings revealed that the overall production of supercritical fluid extraction was substantially

improved (Dias et al., 2016) . In conclusion, the use of ultrasound may enhance the yield of capsaicinoids from supercritical fluid extraction, which could be used as an alternative to the existing extraction procedures that employ dangerous chemical solvents.

#### 2.4. Ultrasound-assisted extraction

The UAE method is successful owing to the cavitation phenomena that occurs whenever an acoustic wave passes through the extraction liquid, creating power to accelerate the blending and diffusion of the liquid into the sample cell wall (Alves Filho et al., 2020) . The use of UAE has several benefits, including the temperature, decrease of solvents, and extraction time, which is critical for the recovery of heat-sensitive and volatile compounds.

**Table 2:** Quantification of various capsaicinoids extracted from pepper

Extraction Method	Solvent	Condition	Pepper	Cap	DHC	N-DHC	HC	H-DHC	unit	Reference
Soxlet	Methanol	Time: 2h Pressure : 1atm	Trinidadscorpion	42.83±0.403	18.09±0.18	0.42±0.03	x	x	g/kg of dried sample	(Bajer et al., 2015)
			Moruga fruit				x	x		
			Yellow bedder fruit	2.49±0.03	2.53±0.09	0.29±0.09	x	x		
			Ring fire fruit	1.74±0.06	1.72±0.05	0.51±0.02	x	x		
			Jamaican hot red fruit	2.08±0.08	1.17±0.06	0.20±0.02	x	x		
			Yellow habanero fruit	0.54±0.03	0.41±0.02	0.027±0.01	x	x		
			Tabasco fruit	3.19±0.02	2.50±0.09	0.95±0.01	x	x		
			Bhut jolokia spice	8.53±0.07	5.73±0.05	0.17±0.02	x	x		
			Fatalii red spice	10.64±0.09	3.06±0.09	0.10±0.01	x	x		
UAE	Ethyl acetate	Time: 6h Temp: 25° C	Malagueta pepper	2.16 ± 0.2	1.20±0.08	0.10±0.01	x	0.03±0.03	g/kg of dried sample	(Santos et al., 2015)
			dichloromethane	2.27±0.03	1.22±0.17	0.09±0.15	x	0.04±0.06		
			Ethyl ether	1.76±0.12	0.97±0.07	0.06±0.03	x	0.03±0.07		
			Hexane	1.88±0.17	1.05±0.09	0.09±0.01	x	0.03±0.04		
UAE	Methanol	Temp: 50 °C Time: 10min Pressure : 1atm	Cayenne	448±28	265±17	94±6	30±1	47±2	µmol/kg of fresh pepper	(G. F. Barbero et al., 2008)
			Bolilla rehondo pepper	370±24	190±11	40±3	x	20±1		
			Bollila largo pepper	275±17	124±7	24±3	x	14±1		
SFE	Carbon dioxide	Temp: 40 °C Pressure : 25Mpa	Dedo de moca pepper	0.88±0.11	0.37±0.05	0.06±0.02	0.04±0.01	0.01±0.00	g/kg of raw material	(Dias et al., 2016)
			Biquinho pepper	0.30±0.01	0.075±0.007	x	x	x	g/kg of dried sample	(de Aguiar et al., 2014)

		Temp: 50°C Pressure : 15Mpa	Biquinho pepper	0.22±0.001	0.042±0.002	x	x	x	g/kg of powdered sample	
PLE	Methanol, ethanol; water	Temp: 40°C Pressure : 10atm	Long marble pepper	369.8±23.3	190.1±10.8	40.3±2.7	x	19.7±0.9	µmol/kg of fresh pepper	(Gerardo F. Barbero et al., 2006b)
			Round marble pepper	275±17.5	122.6±7	25.3±1.8	x	15±0.7		
	Methanol	Temp: 40°C Pressure: 1500psi	Capsicum annum sampls	0.75	0.34	0.13	x	x	g/kg of dried pepper	(Chanthai et al., 2012)
				1.80	0.68	0.14	x	x		
				0.73	0.32	0.064	x	x		
				0.59	0.24	0.047	x	x		
				1.49	0.89	0.19	x	x		
	Water	Temp: 40°C Pressure : 20Mpa	Trinidad scorpion	46.45±0.16	15.54±0.16	0.30±0.02	x	x	g/kg of dried sample	(Bajer et al., 2015)
			Yellow bedder fruit	3.96±0.09	3.1±0.09	0.5±0.03	x	x		
			Ring fire fruit	1.86±0.06	1.83±0.04	0.61±0.03	x	x		
			Jamaican hot red fruit	2.56±0.08	1.35±0.05	0.267±0.01	x	x		
			Tabasco fruit	3.94±0.04	2.70±0.09	1.07±0.05	x	x		
			Chiltepin fruit	0.31±0.02	0.22±0.02	0.08±0.01	x	x		
			Bhut jolokia spice	9.15±0.08	4.93±0.08	0.22±0.02	x	x		
			Trinidad scorpion	20.26±0.21	10.57±0.1	0.53±0.03	x	X		
MAE	Ethanol	Temp: 40°C	Cayenne	451.5±32.8	265.4±18.1	93.8±6.6	29.6±3.9	44.9±2.4		(M. Lu et al., 2017)
		Time: 5 min	Long marble pepper	378.6±24.3	185.5±10.3	40.3±2.7	x	18.9±0.8	µmol/kg of fresh pepper	
		Pressure : 1atm	Round marble pepper	265.2±16.8	132.4±8.0	23.2±14	x	15.4±0.6		
SFE+US	Carbon dioxide	Ultrasound power: 600W Temp: 40 oC Time: 80min Pressure : 25Mpa	Dedo de moc,a pepper	0.94 ± 0.09	0.39 ± 0.04	0.06 ± 0.00	0.04 ± 0.00	0.01±0.00	g/kg of raw material	(Dias et al., 2016)
	Carbon dioxide	ultrasound Power: 360W Temp: 40 oC Time: 60min Pressure	Malagueta pepper	1.98±0.05	1.01±0.03	0.07±0.02	x	0.03±0.003	k/kg of raw material	(Santos et al., 2015)

		: 15Mpa							
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Cap: capsaicin, DHC: dihydrocapsaicin, HC: homocapsaicin, H-DHC: homodihydrocapsaicin, MAE: microwave-assisted extraction, N-DHC: nordihydrocapsaicin, SFE: supercritical fluid extraction, PLE: pressurized liquids extraction, US: ultrasound: ultrasound-assisted extraction: UAE; X: not detected.

### 3. Analytical Methods

Isolation and characterization of bioactive compounds from the pepper is a technique that has shown significant advancement in the past few years. Due to the complicated molecular structure of some compounds, little is still understood about the analysis of these antioxidants. To gain a deeper understanding of these antioxidants, robust analytical techniques are required to map the structure and size of biological compounds. Due to this, current research has focused on developing new optimization-based techniques. Choosing an appropriate quantification method will lead to more accurate findings. One of the most commonly used is liquid chromatography-mass spectrometry (LC-MS) for mass-to-charge ratio (m/z)-based detection. For detection based on a maximal absorbance peak, high-performance liquid chromatography (HPLC) linked to ultraviolet (UV) or diode-array detector (DAD) is used. Alternatively, nuclear magnetic resonance (NMR) can be used to determine its structure.

Gahungu et al used column chromatography on silica gel to extract capsaicinoids from a cultivar of chili, which they subsequently quantified using reverse phase-high performance liquid chromatography/photodiode array detection (RP-HPLC/PAD). The most abundant capsaicinoids detected were 48.632 mg/g of capsaicin and 24.162 mg/g of dihydrocapsaicin (Gahungu et al., 2011). Al Othman et al Used HPLC to separate, identify, and quantify capsaicin and dihydrocapsaicin in red chilies, hot chilies, green peppers, green chilies, yellow peppers, and red peppers. Hot chilies had the greatest proportion of capsaicin (423 g/g) and the most pungency, while green chilies had the lowest measured content (1 g/g), and red, yellow, and green peppers were non-pungent (Al Othman et al., 2011). Furthermore, L. Chen *et al* utilized HPLC-UV to determine the existence of capsaicin in an extract of red pepper. The concentration of Capsaicin was found to be 9.48 mg/g, according to the HPLC-UV chromatogram (L. Chen & Kang, 2013). In addition, Santos et al used HPLC-MS to identify the five primary capsaicinoids found in peppers in their investigation (Santos et al., 2015). Daood et al collected and studied several Capsicum fruits utilizing HPLC. The optimal analytical conditions were achieved with a reversed-phase apparatus, a mobile phase of-acetic acid-acetonitrile, a detector wavelength of 280 nm utilizing a UV detector, and a flow rate of 1.0 ml/min (Daood et al., 2015). In place of HPLC, González-Zamora et al investigated direct spectrophotometric detection of various capsaicinoids concentrations in Chiltepin pepper. The findings revealed that comparison results obtained by HPLC and spectrophotometrically ranged from 30 to 128 mg of capsaicinoids per g (González-Zamora et al., 2015). As a result, the spectrophotometric approach may be frequently used for total capsaicinoid analysis and quality assurance in pharmaceutical preparations, with a correlation of 0.9.

### 4. Conclusion

This paper presents an overview of several extraction parameters which may aid in the standardisation of the operation, which is especially significant at the commercial scale. The modification of the extraction method allows for maximum yield while lowering the cost of production associated with solvent and energy consumption, hence guiding the decision for a sustainable and efficient method of producing isolates high in capsinoids. Thus, future research on effective, fast, and quick extraction technologies may hasten the process of commercialization.

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