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Research Article

Impact of Organic Solvents in the Extraction Efficiency of Therapeutic Analogue Capsaicin from *Capsicum chinense* Bhut Jolokia Fruits

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ABSTRACT

The aim of this study was to find out the efficient extraction of pharmacologically important analogue capsaicinoids in different organic solvents of *Capsicum chinense* Bhut Jolokia fruits. Non polar solvents (Hexane, Benzene, Chloroform and Diethyl ether), Polar Aprotic solvents (Ethyl acetate, Acetone, Acetonitrile and Dimethyl sulfoxide -DMSO) and Polar Protic solvents (n-Propanol, Ethanol, Methanol and Water) were used for the extraction. The crude extracts were subjected to TLC for the qualitative examination for the presence of capsaicinoids. The simple linear regression curve was plotted for standard capsaicin purchased from Sigma Chemical. For the quantitative estimation UV- visible spectrophotometer analysis and phosphomolybdic reduction method of total capsaicin were performed. The total phenol content in the extracts was estimated by Folin- Ciocalteu acid reagent method. The TLC profile with retention factor 0.078 corresponding to standard capsaicin was observed in all extracts except DMSO. Among the 12 tested solvents, acetone and acetonitrile showed high pungency level with 1,347,439 SHU and 1,266,250 SHU respectively, followed by ethanol with pungency level of 1,246,523 SHU. The non polar solvent (hexane, benzene, chloroform) and polar protic solvents (methanol and water) showed the value less than 1,000,000 SHU. In the comparative study on the UV, total phenol and total capsaicin estimation reveals that acetone and acetonitrile solvents were efficient to extract the high amount capsaicinoids. Our results with the solvents used for the extraction showed diverse solubility of capsaicinoid. Hence this study concludes that polar aprotic solvents acetonitrile and acetone were the best solvent system for the efficient extraction of capsaicinoids for pharmacological and biological application.

key words: *Capsicum chinense, organic solvents,* phosphomolybdic reduction, TLC, capsaicinoids and capsaicin

INTRODUCTION

Capsicum chinense Naga chilli or Bhut Jolokia is an erect, bushy, herbaceous and annual plant that belongs to the family *Solanaceae*. Naga chilli was reported as a variety of *Capsicum frutescens* (1). The molecular analysis with randomly amplified polymorphic (RAPD) DNA markers placed Naga chilli in a taxonomic position between *Capsicum chinense* and *Capsicum frutescens* with its clustering more closely with the *Capsicum chinense group* (**2**). Capsaicin and dihydrocapsaicin, the two major capsaicinoids, are accountable for up to 90 percentage of the total pungency of pepper fruits. The accumulation of capsaicin may also depend on fruit age and stage development (3). Capsaicinoids content was higher in the placenta than in other parts, but lowest in seeds as well as in green and red pepper fruit (4). Capsaicin a phenylpropanoid compound (trans-8-methyl-*N*-vanillyl-6-nonenamide) is a crystalline, lipophilic, colorless and odorless alkaloid with the molecular formula $C_{18}H_{27}NO_3$ (5). Capsaicin's molecular structure was resolved by Nelson and Dawson in 1919 (6). Growing interest in capsaicin has led to its characterization with methods such as spectrophotometer UV-VIS and chromatography. These have been modified over time to develop more sensitive, faster capsaicin characterization techniques. Thin-layer chromatography has been used to detect capsaicin on ground pepper (7). Multi-band thin-layer chromatography has been used to evaluate the Rf of capsaicin in different adsorbents and the same mobile phase under identical conditions (8).

Figure 1 Capsicum chinense Bhut Jolokia – Entire dry fruit

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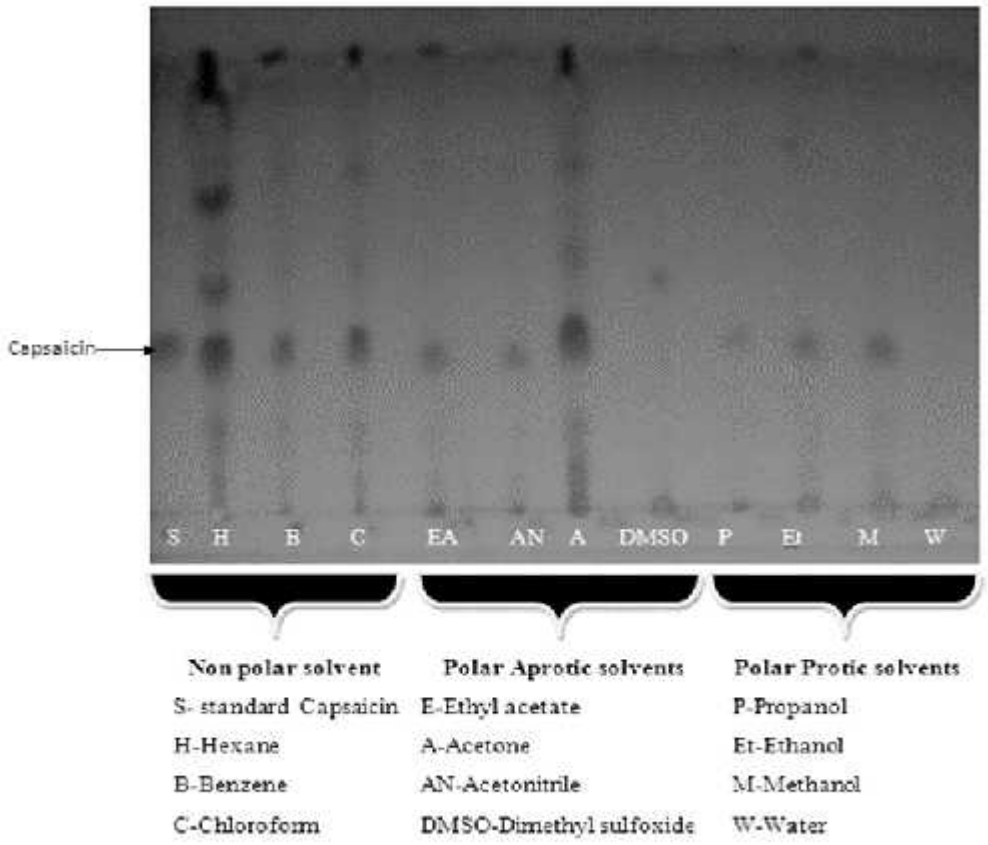

Figure 3 Thin layer Chromatogram of capsaicinoid extracted from different solvent

Tabel 1 Amount of capsaicinoid content in the solvents estimated by UV spectrophotometer, Phosphomolybdic acid reduction and Folin- Ciocalteu methods

Solvents	UV-Visible spectrophotometer		Phosphomolybdic acid Reduction method		Folin- Ciocalteu reagent method	
	µg/ml Mean ± SE	SHU	µg/ml Mean ± SE	SHU	µg/ml Mean ± SE	SHU
Hexane	31.99 ± 0.20	511,980	196.70 ± 2.77	3,147,219	49.01 ± 2.69	784,215
Benzene	46.29 ± 0.29	740,582	234.73 ± 1.3	3,755,772	75.15 ± 4.37	1,202,417
Chlorofrom	61.84 ± 1.09	989,490	272.29 ± 0.29	4,356,675	25.37 ± 1.46	405,966
Ethyl acetate	63.66 ± 0.64	1,018,501	224.74 ± 0.24	3,595,984	74.99 ± 4.38	1,199,970
Acetonitrile	79.14 ± 0.06	1,266,250	236.38 ± 0.09	3,782,120	49.05 ± 2.94	784,799
Acetone	84.22 ± 0.02	1,347,439	293.37 ± 2.78	4,694,074	59.52±0.85	952,420
DMSO	121.4 ± 0.13	1,942,191	229.21 ± 0.33	3,667,378	6.50 ± 0.26	104,000
Propanol	72.36 ± 0.04	1,157,751	253.91 ± 0.09	4,062,598	45.98 ± 2.73	735,689
Ethanol	77.91 ± 0.07	1,246,523	217.52 ± 0.19	3,480,393	9.244 ± 0.64	147,916
Methanol	55.86 ±0 .25	893,756	221.29 ± 0.38	3,540,738	66.54 ± 4.31	1,064,674
Water	25.51 ± 0.27	408,123	285.30 ± 0.33	4,564,908	1.03 ± 0.41	16,480

Capsaicin is also considered as an active principle which accounts for the pharmaceutical properties of peppers. It has been used as an analgesic against arthritis pain and inflammation (9), anticancer activity (10), active against neurogenic inflammation (11) and showed protective effects against high cholesterol levels and obesity (12). Capsaicin and it's analogue has large number of physiological and pharmacological effects on the gastrointestinal tract, the cardiovascular and respiratory system as well as the sensory and thermoregulation systems and is use to treat some peripheral painful states, such as rheumatoid arthritis (13 – 20). Because of the increasing use in medicine and pharmacy, it has become important to establish a sensitive, accurate and simple technique for extraction of capsaicin and its analogues. This study highly focused on effective separation, identification and quantification of the capsaicinoids extracted using different organic solvents from *Capsicum chinense* Bhut Jolokia

MATERIALS AND METHODS

Sample processing: *Capsicum chinense* fruits were obtained from Manipur, North India Figure 1. The morphology of the fruit shape, colour, seed colour and size of the *C. chinense* were examined following Moscone (21) and Dias (23) .The dry fruits were dried by traditional method i.e. sun dried for a day, ground, sieved through 20–30 mesh and kept in air tight containers until further process. The standard Capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide) was purchased from Sigma Chemical Co, St. Louis, MO, USA. All solvents used for capsaicinoids analysis were purchased from Merck.

Extraction: The extraction and quantification of capsaicinoids in different solvents was performed according to Collins (24) with little modifications. The chili powder was mixed with following 12 different solvents (Figure 2) in the ratio of 1:10 (gram: milliliter).

Thin layer Chromatography: Qualitative analysis of the extracts was done by thin layer chromatography. TLC method provides the finger print of plant extracts (25). The presence of capsaicinoid in the 12 different solvents was separated and identified using thin layer chromatography. It was performed on TLC silica gel 60 F254 aluminum sheets (Merck, Mumbai). The standard capsaicin at concentration of 1mg/ml was spotted as a reference on the TLC plate. Ten-µL aliquots of all the samples were applied onto the plates. The plates were dried in a hood for 10 min before the development. The following ratio (Petroleum ether: Chloroform: Acetonitrile – 40: 45: 15) was used as mobile phase. The chromatogram was detected in iodine chamber and also viewed under the UV light at 308nm. The standard capsaicin Rf value was compared with different extracts.

Quantification by UV spectrophotometer: The simple linear regression curve was plotted using standard capsaicin purchased from Sigma Chemical. A stock solution of one milligram capsaicin per milliliter of ethanol was dissolved and different concentrations were prepared from the stock solution 10µg to 100 µg. The optical density was recorded at 280 nm. The linear regression equation was generated using the online Statistics and forecasting software (www. wessa.net). The capsaicinoid extracted from solvents were estimated by UV visible spectrophotometer (Hitachi- U1800). The crude extract was diluted to 300X using respective solvent. The optical density was recorded at 280 nm. The capsaicinoid concentrations in samples were calculated using capsaicin linear regression equation and it was expressed as microgram of capsaicin per millilitre and finally converted to Scoville Heat Unit.

Quantification of *Total* capsaicin*:* Capsaicin is a protoalkaloid which is responsible for the pungency and the quality of the chilli fruit. Extracts of oleoresin were determined by the capsaicin content. The phenolic group in capsaicin reduces the phosphomolybdic acid to lower acids of molybdenum. The resulting compound appeared blue in colour which was directly proportional to the concentration of capsaicin and was read at 650nm. Standard capsaicin solution was diluted to range of 100 µg, 80 µg, 60 µg, 40 µg and 20 µg and linear regression curve was generated using the online Statistics and forecasting software (www. wessa.net). 0.5 g of dry chilli powders were weighed into a glass stopper test tube and 10ml of dry acetone (25g anhydrous sodium sulphate were added into 500ml acetone of analytical grade at least 1 day before use) were added and kept in shaker for an hour. The content was centrifuged at 10000 rpm for 10 min. One ml of the clear supernatant was pipetted out and was dried in hot water bath. The residue was dissolved in 5 ml of 0.4% sodium hydroxide solution and 3 ml of Phosphomolybdic acid were added to it and was kept for an hour. The solution was centrifuged to remove the floating debris. The coloured solution was directly read at 650 nm. The amount of capsaicin was expressed in microgram per millilitre and finally converted to Scoville Heat Unit.

Quantification of Total phenolics: Total phenolic contents of hot peppers were analyzed using the modified Folin-Ciocalteu reagent method. One ml of 1/10 dilution Folin-Ciocalteu reagent (Fisher) and 2ml of 7.5% (w/v) Na_2CO_3 were added to 0.1ml of solvent extract. After vortexing for 10 seconds, the mixture was incubated at 45ºC in water bath for 15 min. Samples were allowed to cool at room temperature before reading the absorbance at 765 nm using HITACHI UV-Vis spectrophotometer. A blank was prepared by excluding the extract. Capsaicin standard

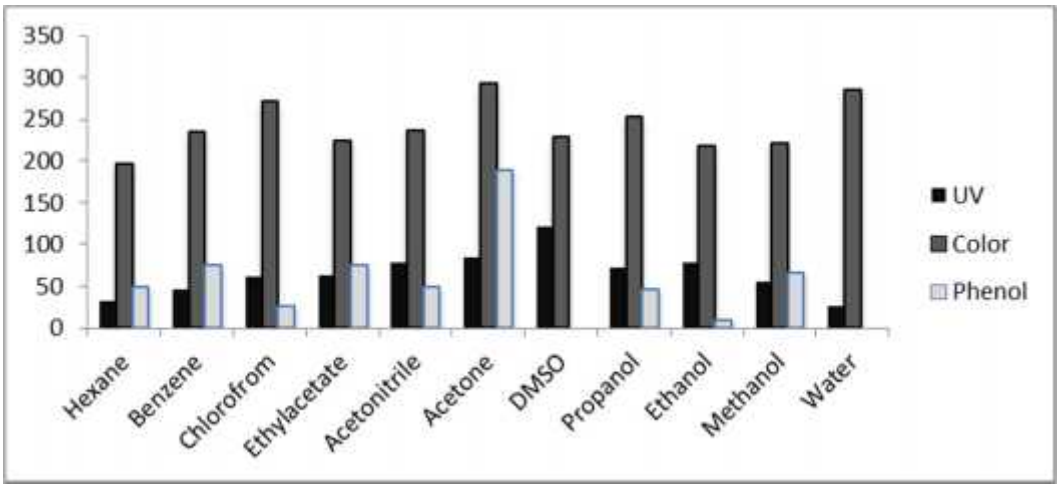

Figure.4 Comparative chart on the capsaicinoid content in the solvents estimated by UV spectrophotometer, Phosphomolybdic acid reduction and Folin- Ciocalteu methods

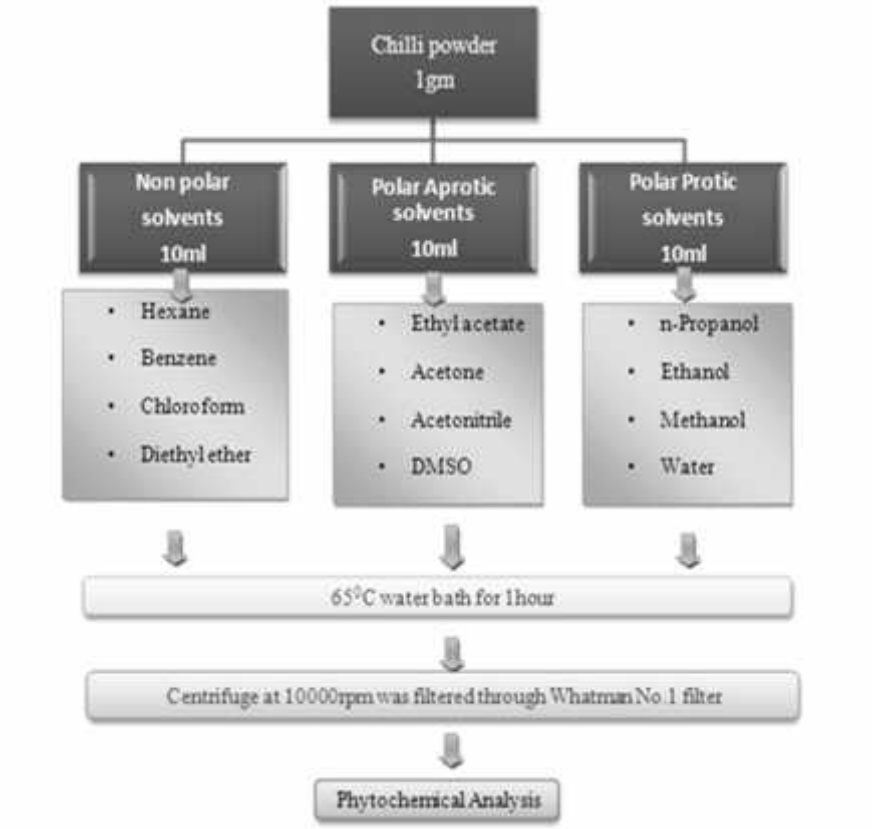

Figure .2 Schematic presentation of phytochemical for extraction of capsaicinoids

curve was prepared from a freshly made 1 mg/ml of capsaicin (sigma) stock solution and simple linear regression equation was generated using the online Statistics and forecasting software (www. wessa.net).

Scoville Heat Unit Conversions: SHU was calculated by converting the capsaicin content expressed in grams of capsaicin per gram of pepper. This conversion to Scoville heat units was done by multiplying the capsaicin content in pepper dry weight by the coefficient corresponding to the heat value for pure capsaicin, which is 1.6×10^7 (26).

RESULTS AND DISCUSSION

Capsicum chinense Bhut Jolokia is the most pungent chilli peppers known to date. Pungency is an exceptional characteristic feature that distinguishes the chilli pepper from other vegetable species. The most pungent cultivars were Orange criolle Habanero, Red Savina Habanero and Bhut Jolokia, all belongs to the species *Capsicum chinense*. Among which Bhut Jolokia was significantly more pungent with 1,001,304 SHU (2). It is generally accepted that capsaicinoids are produced solely in pepper, although the biosynthesis and accumulation of these alkaloids was localized within the fruit. In addition to food additive in our daily diet, the medical applications of capsaicin make this compound very popular. It is currently available as various topical pharmaceutical formulations such as ointments, high-dose dermal patches, creams, large bandages (20). Because of the increasing demand by consumers for hot and spicy foods and also the increasing use in medicine and pharmacy, it has become important to establish a sensitive, accurate and simple technique for extraction of capsaicin and its analogues. In extraction technique, it is important to examine capsaicinoid yield and factors such as composition and structural heterogeneity of the *Capsicum* fruits that contribute to successful product recovery (2). *C. chinense* Bhut Jolokia with high capsaicinoid content draw our attention towards this plant for its fruits. However, from the perspective of large scale processing and economics, *C. chinense* is more convenient to use for the extraction of high yield to meet the growing demands.

The capsaicinoid extracted from the 12 different solvents resolved on TLC plate were viewed under the UV light at 308 nm. The Rf value corresponding to standard capsaicin were observed in all extracts Figure 3. The capsaicin spot was not observed in DMSO and water but observed in other solvent extracts. The standard capsaicin Rf value 0.078 was corresponded to the spot observed in the all extracts. Among 12 extracts the TLC profile of acetonitrile has capsaicin spot with less of other impurities. In our study we have confirmed acetonitrile was the best solvent for the extraction of capsaicinoids from *C. chinense.* In white light the capsaicin was not visible on the TLC plate. The capsaicin band was visible under UV illuminator at 302 nm and in the iodine vapor.

The linear regression equation was generated for standard capsaicin using the online Statistics and forecasting software (www. wessa.net). The amount of capsaicin in different solvent extract was calculated using the following equation ($Y = 0.00919X - 0.0084$), ($Y= 0.082+0.0077X$) and ($Y= 0.0367 + 0.006275$) for the UV spectrophotometer estimation, total phenolic and the total capsaicin estimation

respectively. The capsaicinoid contents obtained in µg/g were converted to Scoville heat units in order to classify them according to their various pungency levels. The UV-VIS spectrophotometric method is one of the most inexpensive and accessible for capsaicin quantification; indeed, most laboratories have a UV-VIS spectrophotometer. However, analysis is restricted to capsaicin solutions with microgram-level concentrations (27). High-performance liquid chromatography (HPLC) has been widely used for characterization of capsaicin and it analogues (28 – 32). Among the 12 tested solvents, acetone and acetonitrile showed high pungency level with 1,347,439 SHU and 1,266,250 SHU respectively, followed by ethanol 1,246,523 SHU. The non polar solvent hexane, benzene, chloroform and polar protic solvents methanol and water have less 1,000,000 SHU level with less capsaicinoid Figure1 and Table 4

According to Menichini, (33) the phenols content of *C. chinese* Habanero extracts level decreased with the increase in the maturity of the fruits. The concentration of carotenoids and capsaicinoids increased as the peppers reached maturity, whereas the concentration of phenols declined (34). Hence our results agree with the above said concept, the total phenol content in all the solvent extracts from the matured dry fruits of *C. chinese* Bhuta Jollakia showed high amount of capsaicin with low amount of total phenols Figure1 and Table 4 The aqueous extract of bell pepper reported high total phenolic content with zero pungency level. Hence the total phenol does not contribute to the pungency of the chilli (Rohanizah, 2012).

The data presented in the Table 1 showed the concentrations of capsaicin, as well as the pungency expressed in Scoville heat units (SHU). The acetonitrile extracts have high pungency level with 4,694,074 SHU (Fig.22). In the comparative study on the UV, total phenol and total capsaicin estimation revealed that acetone and acetonitrile solvents were efficient to extract the high amount capsaicinoids. The efficient extraction of capsaicinoids from *C. chinense* fruits depends on the type of solvents used. Our study designed on capsaicinoid extraction from *C. chinense* fruits using solvents ranges from non polar solvents (hexane, benzene, chloroform and diethyl ether), polar aprotic solvents (ethyl acetate, acetone acetonitrile and dimethyl sulfoxide) and polar protic solvents (n-Propanol, ethanol, methanol and water). The present investigation supports the findings of Peusch et al 1997, reported that the ethanol and acetonitrile are the ideal solvents for the extraction of capsaicin from the fresh sample. It may be inferred that the presence of water during extraction impacts the hydrophilic properties of solvents and interactions with the capsaicinoid compounds, resulting in varying solubility of capsaicin (36). Our results with the solvents used for the extraction showed diverse solubility of capsaicinoid.

CONCLUSION

The hotness or pungency of *Capsicum* is due to synthesis of alkaloids in the fruit collectively called as capsaicinoids in which capsaicin and dihydrocapsaicin were major analogues. The present study clearly reported the efficient phytochemical extraction, separation, identification and quantification of capsaicinoids from *C. chinensis* in different organic solvents by TLC, UV spectrophotometric, colorimetric for the purification of capsaicin. The acetone and acetonitrile extract for the phytochemical extraction showed higher content of capsaicinoids, among the non polar solvents, polar aprotic solvents and polar protic solvents. Further research has to be conducted for the purification of commercially important analogue capsaicin from the acetonitrile extract for pharmacological and its biological applications.

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