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Research Article

EFFECT OF VEGETABLE OIL IN THE SOLUBILITY OF CAPSAICINOIDS EXTRACTED FROM CAPSICUM CHINENSE BHUT JOLOKIA

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ABSTRACT

Capsicum chinense Bhut Jolokia is in fact the world's hottest known chilli pepper owing to the presence of alkaloids collectively known as capsaicinoids. It has become important to establish efficient technique for extraction of capsaicin and its analogues. The present research was conducted to analyze the effect of vegetable oils in the solubility of capsaicinoids. The capsaicinoids extracted in the oils were quantified by UV spectrophotometer and Phosphomolybdic acid reduction methods. Total phenol content in the extracts was estimated by Folin- Ciocalteu method. Among the tested oils gingelly oil, Olive oil and Sunflower oil showed high capsaicin content. The result of this investigation clearly concludes that the different oils used for the extraction showed diverse solubility of capsaicinoid. Sunflower oil extract can be formulated as topical applications. Neem and castor oil extract can be used as pesticide. Oils would be better solvent for the efficient extraction of capsaicinoids for its biological applications.

Keywords: Vegetable oils, Capsicum, Bhut Jolokia, capsaicin, capsaicinoids, phosphomolybdic acid,

INTRODUCTION

The genus Capsicum belongs to the Solanaceae family which includes more than 30 species [1]. ***Capsicum chinense*** Naga Jolokia (also called Bhut Jolokia, Naga Morich, Bih Jolokia etc.) is in fact the world's hottest known chili pepper with a pungent level of 879,953 to 1,001,304 SHU [2]. The Naga chilli has massive scope in domestic as well as international market due to its major high capsaicin content. Capsicum fruits are known for its flavor, color and to add tang and taste to the insipid food. It is cooked as whole or ground and alone or in combination with other flavoring agents, primarily in the curry powder and pickles. Despites of its indispensable spice capability, this genus has recognizable pharmacological properties due to the presence of alkaloids collectively known as capsaicinoids [3]. The economic, nutritious and pharmacological significance of it is responsible for its high demand. 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 forms (ointments, high-dose dermal patches, creams, large bandages) for a number of diverse clinical conditions, such as the pain relief of peripheral neuropathy, symptomatic treatment of arthritis, muscle and joint pains and other types of neurogenic pains such as Herpes zoster. It is worn in the treatment of obesity and ulcer in the forms of oral herbal supplements. Its chemical agent used in riot control and personal defense pepper spray. Furthermore, it was demonstrated that capsaicin induces apoptosis in various cancer cells [3,4]. 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. The use of essential oil in herbal medicine would be base for the development of novel potent drugs. [5]. Hence the present study was conducted to find the solubility of capsaicinoids in different vegetable oils extracted from *Capsicum chinense* Bhut Jolokia fruits.

MATERIALS AND METHODS

Sample Collection

Capsicum chinense fruits were obtained from Manipur, North India. The morphology of the fruit shape, color, seed color and size of the *C. chinense* were examined according to the description of [6]. The 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. Chemicals used for the analysis were from Merck.

Extraction of capsaicinoids by cooking oils and medicinal oils

500mg of sieved chilli powder was boiled at 65°C for an hour with 5ml of following oils: *Brassica juncea* (mustard oil), *Helianthus annuus* (sunflower oil), *Olea europaea* (olive oil), *Cocos nucifera* (coconut oil), *Elaeis guineensis* (palm oil), *Ricinus communis* (castor oil), *Azadirachta indica* (neem oil), *Arachis hypogaea* (ground nut oil) and *Sesamum indicum* (gingelly oil). The extract was centrifuged for 10,000 rpm for 10 min. The supernatant was subjected to quantification of capsaicinoids.

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 280nm. The linear regression equation was generated using the online Statistics and forecasting software (www. wessa.net). The capsaicinoid extracted from the different oils were estimated by UV visible spectrophotometer (Hitachi- U1800). The optical density was recorded at 280nm. The capsaicin concentrations in samples were calculated using capsaicin linear regression equation and it was expressed as mg/g of the chili powder and finally converted to Scoville Heat Unit.

Quantification of Total capsaicin

Capsaicin is a protoalkaloid which is responsible for the pungency of chilli, the quality of the chilli fruit; extract of oleoresin is determined by the capsaicin content. The phenolic group in capsaicin reduces the phosphomolybdic acid to lower acids of molybdenum; the resulting compound is blue in color and was read at 650 nm the color intensity directly proportional to the concentration of capsaicin [7]. Standard capsaicin solution was diluted to range of 100µg, 80 µg, 60 µg, 40 µg and 20 µg and liner regression curve was generated using the online Statistics and forecasting software (www.wessa.net). To 0.1 ml of extracted oil into a glass stoppered test tube and added 10 ml of dry acetone (added about 25gm

anhydrous sodium sulphate into 500ml acetone of analytical grade at least 1 day before use) and kept it in shaker for an hour. The content was centrifuged at 10000 rpm for 10 min. 1ml of the clear supernatant was pipette out and evaporated to dryness in a hot water bath. The residue was dissolved in 5ml of 0.4% sodium hydroxide solution and 3ml of Phosphomolybdic acid were added and were kept for an hour. The solution was centrifuged to remove the floating debris. The colored solution was directly read at 650 nm. Capsaicin content calculated from the standard curve was expressed as mg/g of the chilli powder and finally converted to Scoville Heat Unit.

Quantification of Total Phenol

Total phenol contents of hot peppers were analyzed using the modified Folin- Ciocalteu reagent method [8]. To 0.1ml of extract 1ml of 1/10 dilution Folin-Ciocalteu reagent (Fisher) and 2ml of 7.5% (w/v) Na_2CO_3 was added. After vortexing for 10 sec, the mixture was incubated at 45ºC in a 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 from without the extract. The standard curve was was generated using the online Statistics and forecasting software (www. wessa.net).

Scoville Heat Unit (SHU) Conversion

According to the commonly accepted Scoville organoleptic test, the spicy strength of the investigated samples 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 [9].

RESULTS

The simple linear regression curve was plotted for standard capsaicin purchased from Sigma Chemical. The linear regression equation was generated using the online Statistics and forecasting software (www. wessa.net). The capsaicin contents obtained in µg/ml were converted to Scoville heat units in order to classify them according to their various pungency levels. The total capsaicin content in gingelly oil, olive oil and sunflower oil showed high pungency level with 1,379,357 SHU, 1,148,175SHU and 879,596.3SHU respectively(Table 1 and Fig 1). The UV estimation showed range of 150 000 to 300 000 SHU in tested oils, but castor oil showed 62,884 SHU which is said to be less pungent (Table1 and Fig 2). The total phenol estimated in the extracted oils showed contemporise the amount of total capsaicin estimated (Fig 3). Hence the current study summaries that the different oils used for the extraction showed diverse solubility of capsaicinoid.

DISCUSSION

The present study was focused on the solubility of capsaicinoids in vegetable oils categorized into cooking and medicinal oils. The pungency or hotness of chilli peppers is determined by the concentration of capsaicinoids produced as a secondary metabolite in the fruit and it is often presented as "Scoville Heat Units SHU [10]. The capsaicinoids content in the extracts were estimated spectrophotometrically (Anan et al., 1996). The UV-VIS spectrophotometric method is one of the most inexpensive and accessible for capsaicin quantification. However analysis is restricted to capsaicin solutions with microgram-level concentrations [11]. The choice of suitable solvent for the extraction of capsaicioind is crucial, to consider the solubility of pigments which can impact subsequent purification steps. Capsaicinoid yields were significantly affected by time and duration. The rate of capsaicin solubility was relatively high for the solvents, temperature and duration. The optimum extraction conditions reported for air dried *C. frutescens was* 120 ◦C for 15 min with acetone (Barbero *et al.,* 2008). Temperature lower than the 70⁰C recommended in the AOAC [13] official method of analysis for fruits and vegetables was followed to minimize loss of capsaicinoids through volatilization. In the present study, for the better yield of capsaicinoids from Bhut Jolakia the extraction was carried out at 65◦C for one hour. The presence of water during extraction impacts the hydrophilic properties of solvents and interactions with the capsaicinoid compounds, resulting in varying solubility of capsaicin [12, 14]. Previous research has proved that cosmetic-grade plant products can be extracted using hexane, ethanol, or vegetable oil. Capsaicin is fairly resistant to acids and alkali solutions at room temperatures [15]. It is only slightly soluble in water [16], but more soluble in ethanol and vegetable oils [17]. The vegetable oils used in this experiment include cooking and medicinal oils. Vegetable oils penetrate deeply into the skin than paraffin oil [18]. The chemical compounds of olive oil contribute to its overall therapeutic characteristics [19]. Rheumatoid arthritis patients receiving olive oil benefits pain reduction and reduced morning stiffness were reported [20]. Topical application of olive oil caused a significant reduction in stratum corneum integrity and induced mild erythemain. Sunflower seed oil preserved stratum corneum integrity, with improved hydration and without erythema, [21]. Capsaicin extracted in the oil would enhanced the penetration of an anti-inflammatory agent through human skin as well as it will reduced reduce skin irritation. Plant oils protects the skin by dissolving the sebum secreted from oil glands, thus support evaporation from the skin [22]. So we suggest that capsaicin extracted in sunflower oil can be used in the topical application and olive oil can be used in cooking food for better tang and hotness. Capsaicin as agrochemicals has been proven as pesticides against cotton crop pest [23]. Extracts of capsicum fruits has been used as an repellent to some species of stored product beetles such as *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* [24]. Moreover plant oils, are safe, low in cost and eco friendly, beside their effectiveness against several pests [25]. Hence the neem oil and castor oil used in the extraction of capsaicinoids can be formulated and tested against the pests.

CONCLUSION

Capsicum species are used worldwide not only as a food, but also as medicine for its astounding therapeutic applications such as anticarcinogenis, antioxidants, suppression of fat accumulation, anti-inflammatory, antimicrobial and used in topical creams. Hence this study concludes that the vegetable oils would be better solvent for the efficient extraction of capsaicinoids for its biological applications. In order to get more tang and hot olive oil and giglley oil can be used for cooking in the food. For the ointments and cream preparation Sunflower oil can be used. The capsaicinoids in the oils can be emulsified and it can be used as nano carriers. Future studies are still needed to learn more about oils when used in combination with capsaicin as part of topical cream and pesticides.

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Tabel.1 SHU units of Capsaicnoids content in oils extracts analysed by UV-Visible spectrophotometer and reduction of Phosphomolybdic acid

		Scoville Heat Unit (SHU)			
Oil used		**UV Method**	**Pungency level**	**Phosphomolybdic acid**	**Pungency level**
	Mustard oil	280,464	***	633,116	***
	Sunflower oil	234,047	***	879,596	***
	Olive oil	244,491	***	1,148,175	***
	Coconut oil	298,451	***	865,997	***
Cooking oil	Palm oil	295,549	***	483,527	***

	Groundnut oil	275,242	***	623,766	***
	Gingelly oil	169,949	***	1,379,357	***
	Castor oil	62,884	**	590,619	***
Medicinal oil	Neem oil	69,503	**	135,054	***

***** Very highly pungent (> 80,000SHU), ** Highly pungent (25,000 – 70, 000SHU)**

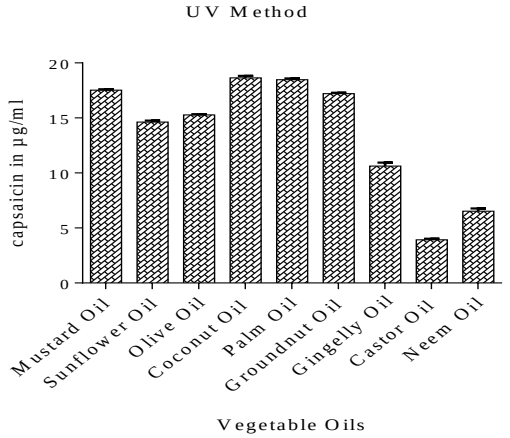

Fig.1: Shows the amount of capsaicin in different vegetable oils used in extraction- quantified by UV spectrophotometer

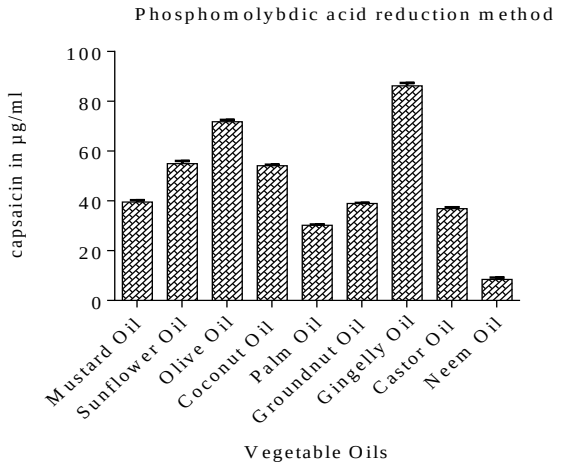

Fig.2: Shows the amount of capsaicin in different vegetable oils used in extraction- quantified by Phosphomolybdic acid reduction method

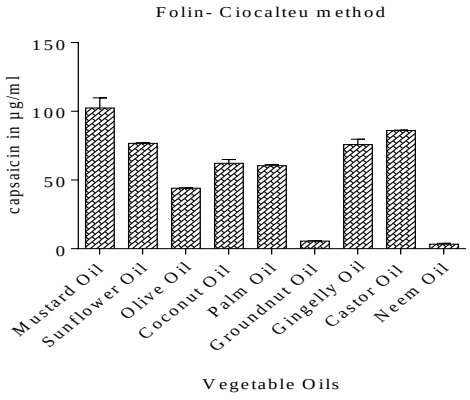

Fig.3: Shows the Total phenol content in different vegetable oils used in extraction- quantified by Folin – Ciocalteu method

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