

ORIGINAL RESEARCH ARTICLE

Estimation of Capsaicin Content of Two Varieties of Green Chilies by TLC Method

Bhuvaneshwari S^{1*}, Prasanna Karthik S², Alice Kuruvilla³

¹Professor, Department of Pharmacology, ²Former MBBS Student, ³Former Professor & HOD, Department of Pharmacology, PSG IMS &R, Peelamedu, Coimbatore-641004, Tamilnadu, India

Received 14 Sep 2013; Revised 08 Dec 2013; Accepted 17 Dec 2013

ABSTRACT

This study was aimed at finding out capsaicin content from two commonly used pungent varieties of green chilies in Tamilnadu, belonging to *Capsicum frutescens* namely, small variety (*Capsicum annuum annuum*) used for cooking and an another variety used for making bajji (*Capsicum annuum grossum*) were estimated by TLC (Thin Layer Chromatography) method. Using varying doses of standard capsaicin calibration curve was obtained using regression analysis. The unknown concentration of capsaicin content in two varieties of chilies was found out from the standard curve by extrapolation method. The results were analysed by student t test. The capsaicin content in whole fruit and seeds of small chili and bajji are not statistically significant ($p > 0.05$). This suggests that capsaicin content is almost equal in whole fruit and seeds of both the chilies. Whereas there is significant difference between the two chilies in capsaicin content of pericarp ($p < 0.05$) suggesting that *Capsicum.annuum annuum* is having more capsaicin concentration than *Capsicum.annuum grossum*.

Key words: *Capsicum annuum annuum*, *Capsicum.annuum grossum*, capsaicin, TLC Method.

INTRODUCTION

Chilies are widely consumed by people of Tamilnadu. Capsaicin is the active compound in chilies, which gives pungency^[1]. Pungency varies with different varieties of chilies. The capsaicin content in chili extracts or oleoresins (crude extract of *Capsicum* fruits) can be determined colorimetrically.

The common names of chili are *Capsicum*, chili, pepper, hot pepper, cayenne, red pepper, Tabasco paprika pepper, sweet pepper, bell pepper, green pepper^[2]. The scientific name of pungent varieties of chilies (Chile peppers or cayenne peppers) is called *Capsicum frutescens* and non pungent varieties varieties (bell peppers, sweet peppers, and green peppers) are called as *Capsicum annuum*^[3]. It is belonging to the family Solanaceae.

The various contents of chili are nitrogenous compounds like capsaicin (0.14%)^[4], dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, terpinoid compounds like steroidal glycosides, other compounds like carotenoid like carotenoid pigments, fat, protein, vitamin C, vitamin A, volatile oils, scopoletin.

Capsaicin is soluble in ethanol, chloroform,

methanol, ether, benzene and petroleum ether. Also soluble in vegetable oils, mineral oil, acetone, methylene chloride and alkali solution.

In earlier days pungency of chily was determined subjectively by organoleptic, method. But there are various reliable methods for extraction of capsaicin from chili by chemical method. They are Thin layer chromatography (TLC), Gas liquid chromatography and High pressure liquid chromatography.

Dyah Juliana *et al* and his colleagues studied on capsaicin content of various varieties of Indonesian chilies, they have studied on 16 varieties. And extraction was done by TLC method and estimated by using spectrophotometer. They have done a comparison between chemical method and organoleptic method. They have found that there was a positive correlation between the capsaicin content of the chilies and the organoleptic heat unit testing using Scoville method^[5].

The same variety of chili from different place may have variable pungency. The irritant property of chili is genetically determined and also influenced

by environmental condition and quality soil where it is grown [6]. Knowing the amount of capsaicin in different chilies is of important, with regard to nutrient value, toxicology and therapeutic applications.

In this study capsaicin content from two commonly used pungent varieties of green chilies in Tamilnadu, belonging to *Capsicum frutescens* namely, small variety (*C.annuum annuum*) used for cooking and an another variety used for making bajji (*Capsicum annuum grossum*) were estimated by TLC method.

MATERIALS AND METHODS

- Two varieties of green chilies belonging to *Capsicum frutescens* were selected. Green chilies were procured locally and authenticated by Pharmacognosy department of the Institution. Among this one was a small (*Capsicum.annuum annuum*) variety which is used for cooking and another variety commonly used for making bajji (*Capsicum.annuum grossum*).
- The green chilies were dried and powdered. Two grams of chili powder of whole fruit, two grams of chili powder of pericarp and two grams of chili powder of seeds were extracted with chloroform at 60-62°C for 20 minutes.
- The residue was washed several times until the filtrate was colourless. The final volume was made upto 10ml.
- 50µl of extracted volume containing test was applied to silica gel 254 plate. The chromatogram was developed with diethyl ether [7].
- After development the plate was dried in the air & blue spot located by 0.01% solution of Gibb's reagent in acetone [8].
- A blue spot was appeared at Rf 0.47 within a few minutes (**Figure 1**). The spot was scraped into a centrifuge tube. In each of tube three ml of borate buffer solution with PH 9.4 was added. The tubes were shaken on an electric shaker for five minutes.
- The supernatant was transferred into a graduated tube and to each of these tubes were added 0.5 ml saturated solution of Gibb's reagent.
- The residue was washed three times with borate buffer solution and final volume was made upto five ml.
- The tubes were kept in dark for 30 minutes for colour development and the intensity of

blue colour was measured at 600 nm with UV visible calorimeter.

- Using varying doses of standard capsaicin calibration curve was obtained using regression analysis with same procedure as above (**Figure 2**).
- The unknown concentration of capsaicin content in two varieties of chilies was found out from the standard curve by extrapolation method.
- The results were analysed by student t test.

RESULTS

The absorbency for different concentrations of standard capsaicin was tabulated (**Table 1**).

The calibration curve for the standard had a linear function as $Y=0.0084x+0.0285$, $r=0.9$

The mean absorbency for the two test chilies was found out (**Figure 3**).

Mean concentration of capsaicin present in different parts of two chilies was found out using the standard curve (**Table 2**). The mean concentration was higher in pericarp of both chilies compared to seeds. *Capsicum.annuum annuum* was having higher capsaicin concentration than *Capsicum.annuum grossum*.

Table 1: Mean Absorbency of standard Capsaicin

Concentration (µg)	Absorbency	Predicted absorbency
1.25	0.04	0.04
2.5	0.08	0.05
5	0.08	0.07
10	0.10	0.11

Table 2: Mean concentration of capsaicin in green chilies

Parts of chili	Small chili (<i>C. annuum annuum</i>)	Bajji chili (<i>C. annuum grossum</i>)	P value
	Mean concentration in µg/50µl ± SD (n=4)		
Whole fruit	7.02 ± 2025	6.43 ± 3.55	> 0.05
Pericarp	5.8 ± 1.14	3.2 ± 1.19	< 0.05
Seeds	4.9 ± 1.68	2.6 ± 0.97	> 0.05



Fig 1: Extraction capsaicin from green chilies – chromatography plate

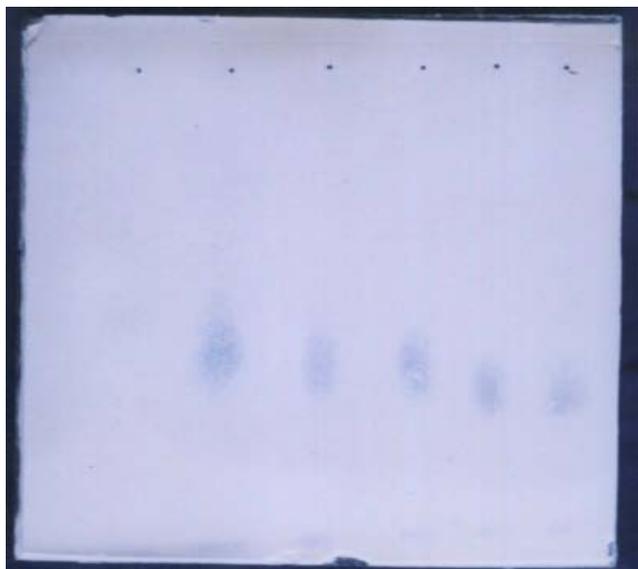


Fig 2: Standard capsaicin – chromatography plate

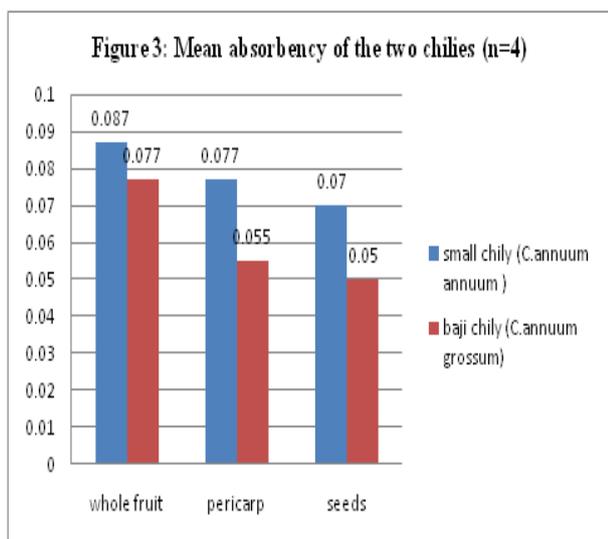


Fig 3: Mean absorbency of the two chilies

DISCUSSION

The capsaicin content in whole fruit and seeds of small chili and bajji are not statistically significant ($p > 0.05$). This suggests that capsaicin content is almost equal in whole fruit and seeds of both the chilies.

Whereas there is significant difference between the two chilies in capsaicin content of pericarp ($p < 0.05$) suggesting that *Capsicum.annuum annuum* is having more capsaicin concentration than *Capsicum.annuum grossum*.

These results correlate with study done by Margen *et al.* He has found that the content was more in white ribs of chili peppers, its placenta that runs down the sides and middle of chili peppers. It is also unevenly distributed throughout flesh of chili. So certain areas of chili can be spicier than others. Less content was found in seeds^[9].

Knowing the concentration of capsaicin is important for the following reasons:

- To have knowledge of their nutrient and non nutrient profile, which is required for health workers, food technologists and regulatory bodies who wish to evaluate for its components.
- To determine the upper permissible limit of particular variety of chili to avoid toxic manifestations like development of esophageal cancer^[10].
- For medical applications in treatment of disorders.

This study concludes that small chili (*Capsicum.annuum annuum*) that is used for cooking is having more capsaicin content than in bajji chili (*Capsicum.annuum grossum*) and pericarp is having more capsaicin content than seeds of both the chilies.

REFERENCES

1. <http://en.wikipedia.org/wiki/Capsicum>.
2. Rosengarten F. The Book of Spices. Wynnewood,PA: Livingston, 1969.
3. Ensiminer EH, Ensminger ME, Konlande JE. Foods and Nutrition Encyclopedia. Second edition. Boca Rator, FL: CRC press 1993.
4. Cordell GA and Araujo OE. Capsaicin: Identification, nomenclature and pharmacotherapy. Annals of Pharmacotherapy 1993; 27:330-36.
5. Dyah Juliana S, Oen LH, Azizawati and Winarno FG. Capsaicin content of various varieties of Indonesian chilies. Asia Pacific J Clin Nutr 1997; 6(2):99-101.
6. Purseglove JW. Spices Vol 1, London. Longman, 1981:331-439.
7. Wagner H. Plant Drug analysis- A Thin Layer Chromatography Atlas, Springer-Verlag, Berlin Heidelberg 1984:1-15.
8. Pankar DS and Margar NG. New method for determination of capsaicin by using Multi Band Thin Layer Chromatography. J Chrom 1977; 144:1949-52.
9. Margern S. The wellness encyclopedia of food and nutrition: How to buy,store and prepare every variety of fresh foods. Random House (1992).
10. Mamta Srivastava, Umesh Kapil, Chattopadhyay *et al.* Nutritional factors in carcinoma oesophagus: a case control study. Asia Pacific J Clin Nutr 1997; 6(2):96-8.