Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

MODULATIONOF PUNGENCY AND MAJOR BIOACTIVE COMPOUNDS IN PEPPER DUE TO AGRO-CLIMATIC DISCREPANCY: A CASE STUDY WITH *CAPSICUM CHINENSE* BHUT JOLOKIA FRUIT

SUBRATA KUNDU, ANUPAM DAS, BISWAJIT GHOSH*

Plant Biotechnology Laboratory, Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata 700118, India.
Email: ghosh_b2000@yahoo.co.in

Received: 24 Nov 2014 Revised and Accepted: 20 Dec 2014

ABSTRACT

Objectives: The use of pepper fruit is renowned for its capsaicin content that has numerous beneficial effects in food and pharmaceutical industry. However pepper fruits exhibit wide variations in the accumulation of capsaicin depending on agro-climatic conditions. Therefore, we have performed experiments to evaluate the variation of major bioactive compounds including capsaicin of world second hottest chili pepper Bhut Jolokia fruit across two different agro-climatic conditions.

Methods: All the analysis was done according to standard protocols.

Results: Biochemical combined with High Performance Thin layer Chromatography (HPTLC) analyses confirmed significant differences in major bioactive compounds including capsaicin due to the variation in agro-climatic conditions as well as elevation. It was observed that total phenolic and flavonoid content of Bhut Jolokia fruit (BJ-N) cultivated in Nagaland were more than 2-fold higher compared to the fruits (BJ-W) cultivated in West Bengal. The amount of capsaicin was found to be 4.345–folds higher in BJ-N compared to BJ-W. The Gas chromatography–mass spectrometry (GC-MS) analysis revealed that the chemical composition of the Bhut Jolokia fruits growing in two different geographical location also exhibit chemodiversity.

Conclusion: An overabundance of the agro-climatic conditions likely to differ across the two different elevations. There may be a complex interaction among these different features that play a key role of this significant variation of fruit phenotype as well as major bioactive compounds. The decrease in elevation may be the key reason of the trade-offs of pungency and important secondary metabolites in pepper fruit cultivated in West Bengal.

Keywords: Bhut Jolokia, HPTLC, Antioxidant, Pungency, GC-MS.

INTRODUCTION

Chili pepper (*Capsicum* sp.), a non-climacteric fruit is an important source of nutrients and vitamins in the human diet. The fruits are also enriched with phenolic compounds, carotenoids, compounds with antioxidant and anti-carcinogenic capacity [1-3]. The consumption of peppers may prevent various ailments including cardiovascular disease, cataract, diabetes and neurological disorders [4,5]. Among the chili pepper *Capsicum chinense*' Bhut Jolokia' is an interspecific hybrid chili pepper cultivated in the Northeastern Indian states. It has been reported that Bhut Jolokia is the second world's hottest chili [6].

A series of alkyl vanillylamides including capsaicin (8-methyl-Nvanillyl-6-nonenamide) is predominantly responsible for the pungency in pepper fruit. Biosynthesis of capsaicin is taxonomically restricted to the genus *Capsicum* and is confined to the pepper fruit, specifically in the seeds and pericarp [7]. The capsaicin content is modulated by differences in the growing environment. The degree of pungency and amount of major bioactive compounds in pepper fruits is also regulated by environment and by genotype–environment interaction [8-10].

In the pharmaceutical industry the application of capsaicin is increasing enormously in recent years. The detailed information is desirable on the environmental effects on this important trait as well as for important bioactive compounds. The explicit investigation of difference in pungency and bioactive compounds of mature pepper fruits will corroborate our understanding of plant–environment interactions. The study will also be helpful in manipulating the environment to maintain the pungency and other important compound according to market demand. Chili peppers are grown worldwide, but information is limited on the differential response of chili at different elevations on phytochemical and capsaicin contents. Therefore, the objective of our study was to evaluate the effects of environment on major bioactive compounds including capsaicin in Bhut Jolokia at two different elevations as well as agro-climatic conditions.

MATERIALS AND METHODS

Plant material

The Bhut Jolokia (*Capsicum chinense*) fruits were used for the study. The field experiments were conducted at Dimapur, Nagaland (lat. 25°906' N, long. 93°727' E, 195 m asl) and Rahara, West Bengal (lat. 22°726' N, long. 88°380' E, 15 m asl) during their main growing period. The Bhut Jolokia sample cultivated at Nagaland was designated as BJ-N whereas the West Bengal sample was termed as BJ-W (Fig. 1). In order to maintain soil moisture and key plant nutrients irrigation and fertigation was applied at two locations. The morphological characters of the pepper fruits were analyzed. The fruits from multiple plants at the two geographic locations were separately bulk-sampled to create a single homogenous sample for subsequent analysis. All fruit samples were harvested at fully mature stage, sun dried and ground to a uniform particle size using a mixture grinder.

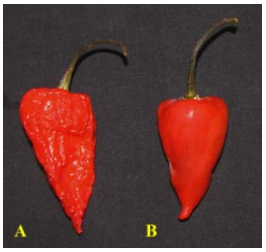

Fig. 1: The Bhut Jolokia fruits cultivated at two agro-climatic conditions. (A) Cultivated at Nagaland (BJ-N) (B) cultivated at West Bengal (BJ-W)

Determination of phenolic and total flavonoid content

Total phenolic content was measured following Folin–Ciocalteau method [11]. Total flavonoid and phenolic content was expressed as mg g^{-1}fresh weight (fw). The total flavonoid content was determined following the aluminum chloride colorimetric assay [12].

Estimation of ascorbic acid

The ascorbic acid was estimated by using Folin phenol reagent using different concentrations of standard ascorbic acid [13]. The amount of ascorbic acid was expressed as mg g^{-1}fw.

Estimation of carbohydrate and protein

Total carbohydrate content was determined in the aqueous solution with anthrone sulfuric acid reagent [14]. The amount of reducing sugar was estimated by dinitrosalicylic acid method [15]. The total protein content was determined following the method reported by Bradford [16]. The total carbohydrate, reducing sugar and protein content was expressed as mg g^{-1}fw.

Determination of antioxidant capacity

Free radical scavenging by the use of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity of the ethanolic extracts was determined according to the DPPH method [17]. Briefly, 50 µL of different concentrations of ethanolic extract was mixed with 1950 mL of 6.34 × 10^{-5} M DPPH radical solution in ethanol. The mixture was allowed to stand for 30 min in the dark. The absorbance of the solution was measured at 517 nm. The ascorbic acid was used as standard. Free radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_B - A_E)/A_B] \times 100 (1)$$

Where, A_B and A_E are the absorbance at 30 min of the blank and the sample, respectively. The antioxidant activity was calculated as IC_{50} (µg mL^{-1}), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lower IC_{50} value corresponds to a higher antioxidant activity. The antiradical activity was expressed as 1/ IC_{50}.

Free radical scavenging by the use of the ABTS radical

The free radical scavenging activity was studied using the ABTS radical cation decolorization assay [18]. ABTS radical cation was produced by reacting 7.0 mM ABTS solution with 2.45 mM potassium persulfate and the mixture was kept in dark at room temperature for 16 h. For the analysis, the solution was diluted in double distilled water to an absorbance of 0.7 (±0.02) at 734 nm. Fifty microliter of the ethanolic extract of different concentrations was added to 1950 µL of ABTS solution and the absorbance was recorded after incubation of 30 min at 30 °C. The different amount of ascorbic acid was used as standard. The percentage of inhibition of ABTS+• was calculated using above formula (eq 1). The antiradical activity was expressed as $1/IC_{50}$.

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity was also determined by ferric reducing power using a spectrophotometer at 700 nm [19]. Briefly, 1 mL of extract was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 20 min. Then 1 mL supernatant was added to the mixture of 0.5 mL of 0.1% $FeCl_3$ and 2.5 mL of double distilled water. The absorbance was measured at 700 nm after incubation for 10 min. Different concentrations of ascorbic acid was used as control. Increase in absorbance was interpreted as increased ferric reducing activity. The IC_{50} value (µg/mL) is the concentration giving an absorbance of 0.5. The antiradical activity was expressed as $1/IC_{50}$.

High Performance Thin layer Chromatography

Five microliters of the ethanolic extract (500 mg/mL) was applied (band length –5.0 mm; distance between bands – 14.5 mm; distance from left edge - 20.0 mm; distance from lower edge - 10.0 mm) on a precoated TLC aluminum sheets of silica gel G60 F_{254} of 200 µm thickness plate- 20x10 cm (Merck, Germany) using Linomat 5 automated TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-µL syringe (Hamilton, Nevada, USA). The standard capsaicin at concentration of 0.1mg/ml was spotted as a reference on the TLC plate. Prior application, the plate was pre-washed with methanol and dried at 60°C. TLC plates were developed using the mobile phase Chloroform: Methanol: Acetic acid: Hexane (2.85: 0.15: 0.15: 1, v/v/v) in a Camag HPTLC twin-trough chamber (20x10 cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed upto 85.0 mm and dried under a stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode at 282 nm operated by Win CATS software (version 1.4.8). Quantitative analysis of the extracts was done by comparative densitometric analysis via height and area with the standards.

Scoville heat unit conversions

The capsaicin contents obtained from different fruits were converted to Scoville heat units (SHU) in order to classify them according to their various pungency levels. This conversion to SHU was done by multiplying the capsaicin content by the coefficient corresponding to the heat value for pure capsaicin that is 1.6 × 10^7[20].

GC-MS analysis

The BJ-N and BJ-W fruit samples were homogenized with 1 mL of ethanol and centrifuged at 5000 rpm for 10 mim. The 1 µL aliquot of supernatant was injected splitless by an Agilent 7683B Series autosampler (Agilent, Atlanta, GA) into an Agilent 6890 GC equipped with a 30 m x 0.25 mm fused-silica capillary column chemically bonded with 0.25 µm DB5-MS stationary phase (Agilent, Atlanta, GA). The injector temperature was set at 275 °C.

Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1} through the column. The analysis was carried out in Split mode and the Split Ratio was 50. The column temperature was initially kept at 40 °C for 2 min and then increased from 40 to 150 °C at 25 °C min^{-1}, where it was held for 3 min. The column effluent was introduced into the ion source of Mass spectrometer (Waters).

The transfer line temperature was set at 250 °C and ion source temperature at 200 °C. Ions were generated by a 70-eV electron beam at a current of 2.0 mA. Masses were acquired from m/z 40 to 640 at a rate of 30 spectra s^{-1}, and the acceleration voltage was turned on after a solvent delay of 120 s. The identification of metabolites was performed based on NIST 11 (National Institute of Standards and Technology) mass spectra database. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular weight and retention time (R_t) of the components were ascertained.

RESULTS AND DISCUSSION

Morphological characters of the fruits

The morphological characters of the Bhut Jolokia fruits cultivated at two different agro-climatic conditions were analyzed (Table 1). It was observed that the different parameters including fruit length, fruit diameter and fruit weight significantly varied between the samples (BJ-N and BJ-W). The fruit length and diameter of the BJ-N were found to be more than 1.3-fold higher compared to BJ-W sample.

Table 1: Morphological comparison of the Bhut Jolokia fruits cultivated at two agro-climatic conditions

Parameters	BJ-N	BJ-W	Fold change
Fruit length (cm)	11.5 ± 0.058	8.3 ± 0.1	1.39
Fruit diameter (cm)	9.0 ± 0.153	6.8 ± 0.2	1.32
Pedicle length (cm)	5.2 ± 0.208	3.2 ± 0.265	1.63
Fruit weight (g)	4.2 ± 0.379	3.5 ± 0.252	1.20
Number of seeds	70 ± 3.606	68 ± 4.933	1.02

Comparative analysis of bioactive compounds

The variation of major bioactive compounds at two different agro-climatic conditions of Bhut Jolokia were analyzed (table 2). It was found that total phenolic and flavonoid content of BJ-N was more than 2-fold higher compared to the fruits of BJ-W. Although the total carbohydrate content was comparable in both samples but reducing sugar of BJ-N fruit was 1.3-fold higher compared to BJ-W. The ascorbic acid content of the fruits cultivated in Nagaland was 1.6-fold higher compared to BJ-W.

The higher levels of the plethora of selective pressures at high elevation may have contributed to these significance variations of chemical components of Bhut Jolokia fruit.

Table 2: Comparative analysis of the major bioactive compounds of Bhut Jolokia fruits

Parameters	BJ-N mg g^{-1}fw	BJ-W mg g^{-1}fw	Fold change
Total phenolics	13.987± 0.664	6.085± 0.643	2.299
Flavonoid	0.431± 0.059	0.194± 0.058	2.221
Ascorbic acid	2.651± 0.088	1.627± 0.093	1.629
Total protein	8.924± 0.135	6.904 ± 0.247	1.293
Total carbohydrate	39.753 ± 2.774	34.953± 2.901	1.137
Reducing Sugar	258.365 ± 5.527	193.065± 4.066	1.338

Comparative analysis of antioxidant potential

The ethanolic extract of the Bhut Jolokia samples cultivated at two agro-climatic conditions showed enhanced scavenging activity over DPPH free radicals. A dose dependent increase in scavenging activity was recorded with both samples. The antiradical activity was expressed as 1/ IC_{50}. A higher value corresponds to a higher antioxidant activity. The antiradical activity of BJ-N sample was found to be 2.063 ± 0.037 whereas it was 0.382±0.006 in BJ-W sample (fig. 2). The ethanolic extract showed maximum ABTS radical scavenging activity (0.775 ± 0.044) in BJ-N compared to BJ-W sample (0.141 ± 0.004). The reducing power of ethanolic extract was significantly higher in fruit cultivated in Nagaland compared to West Begal (fig. 2). It has been reported by several researchers that there is a positive correlation between the total phenolic content and antioxidant activity [21,22]. Our results clearly revealed that the higher phenolic content in the BJ-N sample coincided with higher antioxidant activity compared to BJ-W sample.

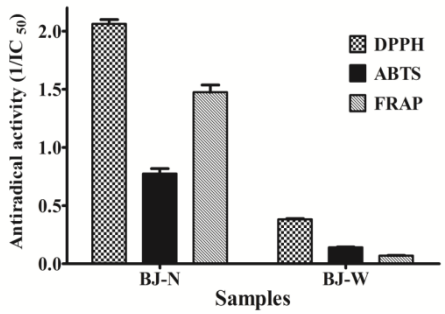

Fig. 2: Comparative analysis of antioxidant activity of the Bhut Jolokia fruits

Method optimization for HPTLC and comparative analysis of capsaicin content

Various solvent compositions were used to determine the suitable mobile phase to obtain sharp, well resolved peaks for precise HPTLC analysis of capsaicin. The mobile phase composed of Chloroform: Methanol: Acetic acid: Hexane (2.85: 0.15: 0.15: 1, v/v/v) provided sharp peak for capsaicin at R_f value of 0.78 (Fig. 3A). The UV spectra measured for the peak of capsaicin showed maximum absorbance at approximate 282 nm. The ethanolic extract of both BJ-N and BJ-W samples were subjected to TLC analysis. The samples resolved on TLC plate were analyzed under the UV light at 282 nm (Fig. 3A). The comparative densitometric analysis of height and peak area of the capsaicin from the two samples was performed using WinCATs software. It was found that height and area of BJ-N sample was found to be 147.953 ± 4.529 and 7389.767 ± 103.590, respectively, whereas it was 20.133 ± 2.061 and 2097.810 ± 95.48 for BJ-W (Fig. 3B).

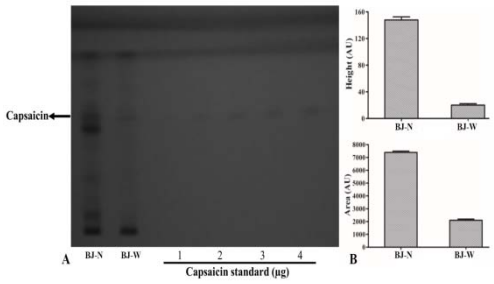

Fig. 3: (A) HPTLC fingerprints of standard capsaicin and the Bhut Jolokia fruits cultivated at two agro-climatic conditions(B)HPTLC-densitometric analysis of capsaicin extracted with respect to mean height and area

The amount of capsaicin was found to be 4.345 –folds higher in BJ-N compared to BJ-W (Table 3). At higher temperature, higher respiration used most of the photosynthetic apparatus for growth of the plant and the rest of the photosynthates were shared among other biosynthetic pathways [23]. The capsaicin is a product of the secondary metabolic pathway, therefore competition between capsaicin and other compounds might have resulted in less capsaicin at relatively higher temperatures of West Bengal. Our results were also agreed with previous reports [24]. They have reported that total capsaicinoid content increased significantly with increasing elevation in wild *Capsicum chacoense* fruits. In addition, the lower partial pressures of atmospheric gases such as oxygen and carbon dioxide at the higher elevated region of Nagaland could also have influenced the higher capsaicin production in Bhut Jolokia fruits.

Table 3: Comparative analysis of capsaicin content and SHU of BhutJalokia fruits

Sample	Amount of capsaicin (mg g^{-1}) Mean ± SD	SHU
BJ-N	5.040± 0.076	80633.346
BJ-W	1.160± 0.057	18562.158

Comparative analysis of GC-MS

The various chemical constituents identified in the Bhut Jolokia fruits cultivated in Nagaland (BJ-N) and West Bengal (BJ-W) are represented in table 4 with their corresponding chromatograms in Fig. 4. A total of 13 constituents were identified in the fruits of Nagaland. The chemical composition is dominated by the presence of capsaicin constituting 32.82% of the total composition followed by n-Hexadecanoic acid (13.03%) and Nonivamide (7.93%). The principal components were found to be Linoleic acid ethyl ester (69.90%), n-Hexadecanoic acid (8.62%) and capsaicin (2.98%) in BJ-

W. The results of our study reveal that the chemical composition of the Bhut Jolokia fruits growing in two different geographical location exhibit chemodiversity. Our HPTLC analysis of capsaicin was also corroborated by the GC-MS analysis.

Table 4: Comparative analysis of chemical compounds of BhutJalakia fruits identified by GC-MS analysis

S. No.	Compounds	Retention time (RT)	Molecular formula	Molecular weight	Percentage of peak area	
					BJ-N	BJ-W
1	Pentadecanoic acid	35.484	$C_{15}H_{30}O_2$	242	2.76	0.29
2	Hexadecenoic acid	38.457	$C_{16}H_{30}O_2$	254	6.89	-
3	l-(+)-Ascorbic acid 2,6-dihexadecanoate	40.026	$C_{38}H_{68}O_8$	653	2.12	-
4	n-Hexadecanoic acid	40.804	$C_{16}H_{30}O_2$	256	13.03	8.62
5	Heptadecanoic acid	42.906	$C_{17}H_{34}O_2$	270	1.86	0.96
6	Oleic Acid	43.143	$C_{18}H_{34}O_2$	282	-	0.23
7	9,12-Octadecadienoic acid	45.160	$C_{18}H_{32}O_2$	280	5.08	2.83
8	Linoleic acid ethyl ester	46.170	$C_{20}H_{36}O_2$	308	-	69.90
9	9-Octadecenamide	47.663	$C_{18}H_{35}NO$	281	1.18	-
10	Nonadecylamine	48.077	$C_{19}H_{41}N$	284	0.47	-
11	Heptacosane	54.107	$C_{27}H_{56}$	381	0.48	-
12	Capsaicin	56.437	$C_{18}H_{27}NO_3$	305	32.82	2.98
13	Nonivamide	56.759	$C_{17}H_{27}NO_3$	293	7.93	2.10

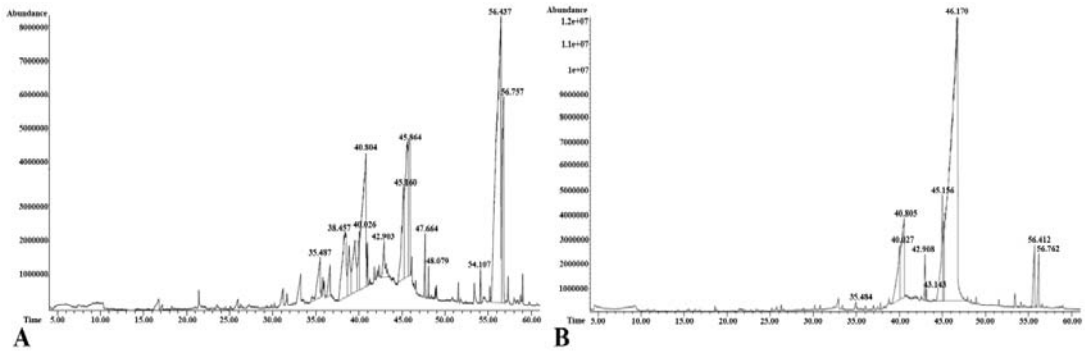

Fig. 4: GC-MS chromatogram of Bhut Jolokia fruits. (A) BJ-N (B) BJ-W

CONCLUSION

A plethora of the agro-climatic conditions differ across the 180 meter elevation gradient. There may be a complex interaction among these different aspects that favor the variation of fruit morphology as well as major bioactive compounds. The decrease in elevation may be the major cause of the trade-offs of pungency as well as major secondary metabolites in pepper fruit cultivated in West Bengal. Thus, there are potentially many selective pressures that could influence the chemical composition of pepper fruits across this elevation. Therefore, by manipulating growing environment we will be able to produce pepper fruits with the pungency according to requirements of the food and pharmaceutical industries.

ACKNOWLEDGEMENT

The authors are thankful to Swami Kamal asthananda, Principal, Ramakrishna Mission Vivekananda Centenary College, Rahara, Kolkata (India), for the facilities provided as well as his continuous enthusiastic encouragement for the present study. The authors thank the Department of Science and Technology, West Bengal for the financial assistance (616(Sanc.)/ST/P/S&T/1G-6/2011) and research fellowships to AD We are also thankful to DST-FIST for infrastructural support.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Howard LR, Talcott ST, Brenes CH, Villalon B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. J Agric Food Chem 2000;48:1713-20.
2. Materska M, Perucka I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J Agric Food Chem 2005;53:1750-6.
3. Amruthraj NJ, Raj JPP, Saravanan S, Lebel LA. *In vitro* studies on anticancer activity of capsaicinoids from *Capsicum chinense* against human hepatocellular carcinoma cells. Int J Pharm Pharm Sci 2014;6:254-8.
4. Shahidi F. Functional foods: their role in health promotion and disease prevention. J Food Sci 2004;69:R146-R9.
5. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables. The millenniums health. Int J Food Sci Tech 2001;36:703-25.
6. Bosland PW, Coon D, Reeves G. 'Trinidad moruga scorpion' pepper is the world's hottest measured chile pepper at more than two million scoville heat units. Hort Technol 2012;22:534-8.
7. Stewart C, Mazourek M, Stellari GM, O'Connell M, Jahn M. Genetic control of pungency in *C. chinense* via the Pun1 locus. J Exp Bot 2007;58:979-91.
8. Zewdie Y, Bosland PW. Pungency of Chile (*Capsicum annuum* L.) fruit is affected by node position. Hortsci 2000;35:1174.
9. Gurung T, Techawongstien S, Suriharn B, Techawongstien S. Stability analysis of yield and capsaicinoids content in chili (*Capsicum* spp.) grown across six environments. Euphytica 2012;187:11-8.
10. Musfiroh I, Mutakin M, Angelina T, Muchtaridi M. Capsaicin level of various capsicum fruits. Int J Pharm Pharm Sci 2013;5:248-51.
11. Singleton VL, Orthofer R, Lamuela–Raventos RM. Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol 1999;299:152-78.
12. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.

13. Jagota SK, Dani HM. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal Biochem 1982;127:178-82.

14. Radwan DEM, Fayez KF, Mahmoud SY, Hamad A, Lu G. Physiological and metabolic changes of *Cucurbitapepo* leaves in response to Zucchini yellow mosaic virus (ZYMV) infection and salicylic acid treatments. Plant Physiol Biochem 2007;45:480-9.

15. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-8.

16. Bradford MM. A rapid and sensitive method for quantification of proteins utilizing the principle of protein dye binding. Anal Biochem 1976;72:248-54.

17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995;28:25-30.

18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999;26:1231-7.

19. Hinneburg I, Dorman HJD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem 2006;97:122-9.

20. Sanatombi K, Sharma GJ. Capsaicin content and pungency of different Capsicum spp. cultivars. Not Bot Horti Agrobot Cluj 2008;36:89-90.

21. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004;85:231-7.

22. Gulati V, Harding IH, Palombo EA. Enzyme inhibitory and antioxidant activities of traditional medicinal plants: potential application in the management of hyperglycemia. BMC Complement Altern Med 2012;12:77.

23. Gurung T, Techawongstien S, Suriharn B, Techawongstien S. Impact of Environments on the accumulation of capsaicinoids in capsicum spp. Hort Sci 2011;46:1576-81.

24. Tewksbury JJ, Manchego C, Haak DC, LeveyDJ. Where did the chili get its spice? Biogeography of capsaicinoid production in ancestral wild chili species. J Chem Ecol 2006;32:547-64.