Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 3100-3104
Received: 11-03-2018
Accepted: 15-04-2018

Santanu Malakar
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Sudipto Sarkar
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Nitin Kumar
Department of Food Engineering, National Institute of Food Technology Entrepreneurship and Management, Sonepat, Haryana, India

R Jaganmohan
Department of Food Product Development, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India

Correspondence
Santanu Malakar
Department of Agricultural Engineering, Assam University, Silchar, Assam, India

Studies of biochemical characteristics and identification of active phyto-compounds of king chili (*Capsicum chinense* Jacq.) using GC-MS

Santanu Malakar, Sudipto Sarkar, Nitin Kumar and R Jaganmohan

Abstract
This study investigates the possible nutritional composition at two different stage and identification of different active compounds of freshly harvested king chili grown in Northeast, India. The proximate composition were found to be (g/100) moisture 86.75%, ash 1.39%, protein 1.75%, carbohydrate 7.81%, fiber 1.75%, fat 0.1%, energy 39.14 (kcal)and vitamin C 109.36 mg/100g, TSS 3.09 % ^{O}Brix at green stage also nearly same results were found in red stage. Also color indices changed of chroma (C) value 25.11 to 45.87 and hue angle (H) 121.32 to 23.10 at green and red stage respectively. Sample of king chili was extracted with ethanol and the ethanolic extract was analyzed through Perkin-Elmer GC-MS for the identification of different compounds. A total of 11 active compounds were identified with ethanolic extracts. Capsaicin(14.99%), α-D-Glucopyranose, 4-O-α-D-galactopyranosyl-(24.92%),4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (19.07%) and Squalene which were major compounds identified in the fruit and considered to have pungency, antioxidant, and anti-cancerous properties. The results of this study are very encouraging for designing new drugs with the help of these active compounds for the treatment of many infectious diseases and to improve the health status of the consumers.

Keywords: bioactive compounds, capsaicin, GC-MS, king chili, squalene

Introduction
Chillies are used as food additives or spices in many national cuisines due to their sensory attributes of colour, heat, pungency flavour, and aroma and are a good nutritional value [1]. King chili (*Capsicum chinense* Jacq.) is a highly potential crop in North-east, India also known variously by other names in its native region like bhut jolokia, ghost chili, ghost pepper or naga morich is a chili pepper generally recognized as the hottest chili in the world [2] as shown in Fig.1. Previous researched in Indian chili peppers showed results of high nutritional value of fresh green chili on average 85.5 % moisture, 1.2 % ash, 1.1 % fat, 5.4 % protein, 110 mg per % vitamin C and carbohydrate content average of 56.25% respectively [3-5]. Sweet pepper was found to have the highest crude protein value while the least value was found in bell pepper [6-8]. Nutritional composition varies in chilies from variety to variety and also location to location [9-12].

King chili is a spice-cum-vegetable with high potential value like capsaicin content has been found to be very high in comparison to the fruits of the other chili species [13, 14]. The aromas of chili pepper are the consequence of the characteristic components of their presence of bioactive compound [15]. Hot taste is due to the presence of non-volatile capsaicinoids, particularly capsaicin and dihydrocapsaicin which are responsible for the spiciness [16, 17].

Fig 1: Sample of king chili (a) green stage (b) red stage

The GC-MS analysis revealed the presence of 43 compounds in acetone extract of 'Adorno' cultivar and 33 compounds in acetone extract of 'Etna' cultivar [13, 18]. Capsicum annum 'antillais' has the numbers of polar and non-polar compounds was identified [19, 20]. The bioactive compounds have revealed some important pharmacological and medicinal properties such as antimicrobial, antioxidant, anti-carcinogenic, analgesic properties [19, 21, 22]. But there is no information on the origin of the types and cultivars studied for the proximate composition and identification of phyto-chemical compound of king chili. Therefore, present work is aimed to determine biochemical characteristics and identification of bioactive compound of king chili to quantify its contribution to the nutritional value and it's potentially to use in pharmaceutical and food industry.

Material and Methods

Sample collection

Fresh matured materials king chili are procured from the local farmer's field located at Guwahati (Northeast, India)and transported within one hour under ambient conditions to the laboratory and washed, allowed to dry at room temperature (≈27 °C) for 1 hour and fruits of good visual quality were sampled for the experiment.

Biochemical composition of king chili

The proximate chemical composition such as moisture, ash, protein, fat, fiber carbohydrate, TSS energy and ash content of green as well as red king chili were determined. The standard official AOAC methods 19th Ed, 2012 are used for the determination of the above mentioned parameters also as described by [7]

Moisture and ash were determined gravimetrically by drying at 105 °C and by incineration at 550 °C, respectively, following AOAC method 930.15 for moisture and fiber, AOAC method 923.03 for ash and Carbohydrates. Crude protein was obtained by Kjeldahl method using AOAC method 941.12. Fat and total energy determined using AOAC method 941.12. The ascorbic acid was determined by volumetric method [23] with the help of (2, 6-dichlor-ophenol indophenol) and expressed as mg/100 g fresh weight. Soluble solids content was measured with a portable refractometer.

Color indices

Color parameters changes at two different stage was determined by Hunter Lab Colorimeter (Model Color Quest II, Reston, USA), on CIE L*a*b* chromatic space, L (degree of lightness to darkness), a (degree of redness to greenness), b (degree of yellowness to blueness) values by [24]. Also, the chroma (C) (Eq-1) and hue angle (H) (Eq-2) were calculated from the values of L, a, b.

$$C = \sqrt{a^2 + b^2} \quad \text{…. (1)}$$

$$H = \tan^{-1}(\frac{b}{a}) \quad \text{…. (2)}$$

Gas Chromatography and Mass Spectrometry (GC–MS)

Sample preparation

The fruits were cut open, the seeds removed, and a sample 50 g and pulverized to powder using a mechanical grinder. The required quantity of the fruit powder of chili was weighed, transferred to a flask, and treated with ethanol until the powder was fully immersed, incubated overnight and filtered through a filter paper along with sodium sulfate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulfate was wetted with absolute alcohol. The filtrate is then concentrated to 1 ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the plant material, and 2 µl of the sample of the solutions was employed in GC-MS for analysis of different compounds [25, 26].

GC-MS analysis of the ethanol extract of King chili was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µmdf). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

Biochemical compositions of king chili

The King chilies was analyzed for moisture, protein, fat, ash, carbohydrate, fiber, total soluble solid, vitamin C, energy and color parameters at green and red stage and these results are presented in Table 1. The fresh green chili contained 86.75 % moisture, 1.75 % protein, 0.1 % fat, ash 1.39%, fiber 1.75%, carbohydrate 7.81% and energy 39.14 kcal and vitamin C 109.36 mg/100g, TSS 3.09 % ^{O}Brix at green stage but in red stage the values found to be 83.26 % moisture, 1.86 % protein, 0.12 % fat, ash 1.18%, fiber 1.95%, carbohydrate 8.19% and energy 46.21 kcal and vitamin C 110.19 mg/100g, TSS 3.48 % ^{O}Brix. The results were similar with that of [12] which were based on the proximate chemical composition of fresh green chilies (*Capsicum frutescens* L.) per 100 gm as 85.7 % moisture content, 5.4 % protein, 0.6 % fat, 1.0 % mineral and 110 mg per % of vitamin C.

Table 1: Biochemical composition of king chili

Parameter Analyzed	Green stage	Red stage
Moisture (g/100g)	86.75 ±0.82	83.26 ±0.56
Ash (g/100g)	1.39 ±0.04	1.18 ±0.07
Protein (g/100g)	1.75 ±0.06	1.86 ±0.05
Carbohydrate (g/100g)	7.81 ±0.09	8.19 ±0.08
Fiber (g/100g)	1.75 ±0.02	1.94 ±0.05
Fat (g/100g)	0.1 ±0.01	0.12 ±0.01
Energy (K.Cal)	39.14 ±0.45	46.21 ±0.93
Vitamin C (mg/100g)	109.36 ±0.90	110.19 ±0.45
TSS % ^{O}Brix	3.09±0.05	3.48±0.05
L	31.58 ±0.87	27.85±0.63
a	-13.05±0.28	42.13±0.39
b	21.46±0.76	18.15±0.84
chroma (C)	25.11±0.75	45.87±0.69
hue angle (H) °C	121.32±1.45	23.10±1.07

Means ±SD (n=3)

Some researchers reported that for different varieties of pepper the proximate composition varied in moisture 82.54%-85.19%, crude protein 2.64-3.52%, ash 1.21-3.03%, fat 1.52-2.87% and crude fiber 2.37-4.71% [3, 4, 7, 8]. The small variations may be due to varietal difference, soil property, and growing condition, harvesting period, maturity stage, agro-ecological condition and methods of analysis [11, 12]. Chilies contain 27 to 28 g per cent of carbohydrate, 19.68 to 20.78 g per cent of fiber [5]

Color parameters changes

Color is the major quality attribute considered to have the most impact on consumer selection of the produce. Color change of king chili from green to red in terms of L*, a* and b* values showed that decrease of L* and b* values while a* value rapidly increased when fruit changed color green to red. The initial L, a* and b* of green king chili values are 31.58, -13.05 and 21.26 respectively. These values are changes at red stage of L, a* and b* values were found to be 27.85, 42.13 and 18.15 respectively. The initial chroma (C*) value was 25.11 in fresh green king chili which changes 45.87 at red stage. Similarly green king chili of hue angle (H*) was found to be 121.32 which reduce to 23.10 at red stage. The decrease of hue angle value, which was indicated with the increase in red color in chili, probably due to the color development that occurred more obviously as the maturity increased [27].

Identification of phyto-compounds in king chili

The selected samples of King Chilies were extracted with ethanol and the ethanolic extract was analyzed through GC-MS for the identification of different compounds. The Gas chromatogram is shown in Fig.2. In total, eleven components of phyto-compounds were identified in the chili extract.

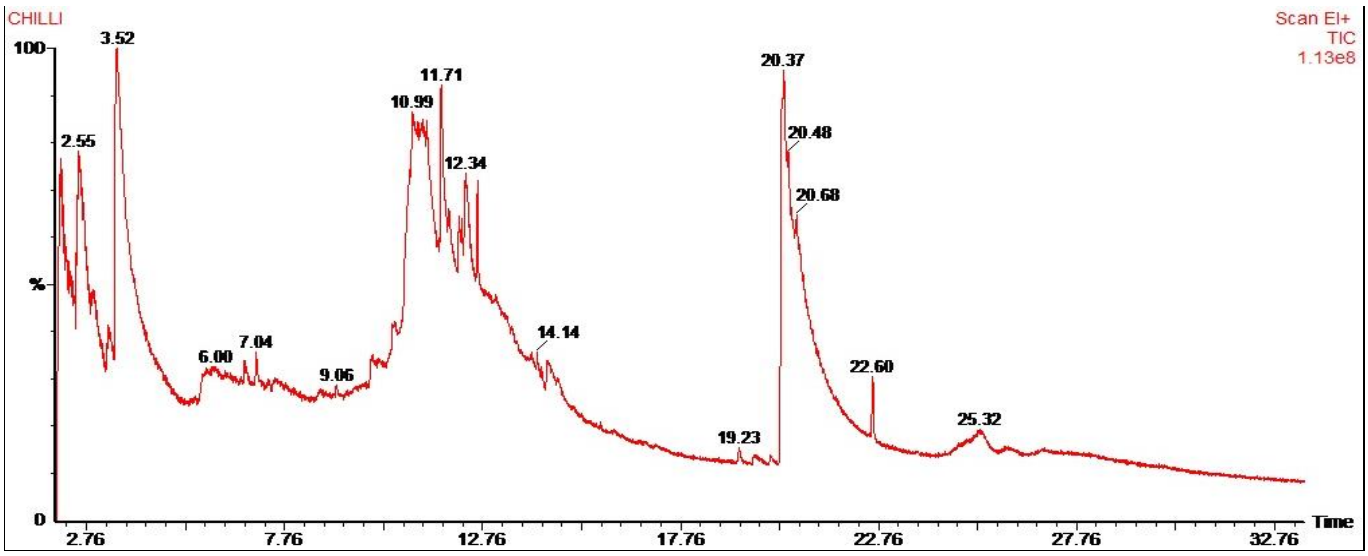

Fig 2: GC-MS chromatogram of king chili

A total of 11 active compounds were identified with ethanolic extracts tabulated in Table 2. Out of these compounds found only Capsaicin (14.99%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (19.07%) α-D-Glucopyranose, 4-O-α-D-galactopyranosyl-(24.92%) and Squalene are the major identified compound in the fruit and considered to have pungency and anti-cancerous properties [16, 17, 28]. Similar compounds identified of different capsicum variety in the GC/MS analysis revealed the presence of 43 compounds in acetone extract of 'Adorno' cultivar and 33 compounds in acetone extract of 'Etna' cultivar [13, 29]. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and percentage composition with their activities in the chili extracted with ethanol and the ethanolic extract is presented in Table 2.

In capsicum fruits the phyto-compound were able to identify 9 components among major and minor capsaicinoids using GC/MS. Capsaicin and dihydrocapsaicin were the dominant

capsaicinoids detected [18, 30]. Similarly 2H-Thiopyran, tetrahydro- antibacterial properties [31], 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one antioxidant properties [32, 33]. Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl,ester had anticancer properties [34] The results of this study are very encouraging for designing new drugs with the help of these active compounds for the treatment of many infectious diseases and to improve the health status of the consumers.

Table 2: Components identified in king chili sample

Name of the compound	PubChem CID/CAS	RT	Molecular Formula	MW	Peak Area (%)	Activity properties
2H-Thiopyran, tetrahydro-	1613-51-0	2.55	C5H10S	102	5.82	Antibacterial
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	3.52	C6H8O4	144	19.07	Antimicrobial, Anti-inflammatory
Lactose	63-42-3	7.04	C12H22O11	342	9.02	Sources of energy on cells
α-D-Glucopyranose, 4-O-α-D-galactopyranosyl-	14641-93-1	10.99	C12H22O11	342	24.92	Preservative
α-Hydroxydodecanoic acid	79034	11.71	C12H24O3	216	6.42	Insectifuge, Antihistaminic Antieczemic,Nematicide
α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-α-D-fructofuranosyl	470-55-3	12.34	C18H32O16	504	4.01	---
Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	7149-36-2	12.63	C16H26O2	250	12.96	Antimicrobial
10-Undecenoic acid, octyl ester	28080-85-5	19.23	C19H36O2	296	0.06	Anti-inflammatory
Capsaicin	404-86-4	20.37	C18H27NO3	305	14.99	Anticancer
Squalene	111-02-4	22.60	C30H50	410	0.91	Antibacterial, Antitumor
9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	55320-01-9	25.32	C25H40O6	436	1.83	Antiageing, Analgesic

Conclusion

The study revealed that there is a great potentially to use the fruit in food and pharmaceutical industry. King chili has an important nutritional quality to improve the benefit for health and therapeutic potential. High fiber content and energy with very less fat content makes the fruit a functional ingredient for food processing industries. Total eleven components were identified in the chili through GC-MS for their different potential activities. Capsaicin, squalene and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- were major compound identified which has great potential in the field of pharmaceutical industries. It seemed important to designing new drugs for the treatment of many infectious diseases and antimicrobial drugs.

Acknowledgments

We, the authors are thankful to our HOD, Coordinator (TEQIP-II) of the Department of Agricultural Engineering, Assam University, Silchar for providing facilities and encouragement.

References

1. Bosland PW, Votava EJ. Peppers: Vegetable and Spice Capsicums. Cambridge, USA. CAB International Publishing, 2000.
2. Verma PK. *et al*. Botanical Enigma of India's Hottest Chilli 'Bhoot Jolokia' (*Capsicum Chinense* Jacq). N Y Sci J. 2013, 11(6).
3. Howard LR, *et al*. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapenos. Journal of Food Science. 1994; 59(2):362-365.
4. Esayas K. *et al*. Proximate composition, mineral content and antinutritional factors of some capsicum (*Capsicum annum*) varieties grown in Ethiopia. Bull. Chem. Soc. Ethiop. 2011; 25(3):451-454.
5. Krithika V, Radhai S. Physicochemical and Nutritional Characteristics of Chilli Cultivars. International Journal of Research in Science. 2014; 1(2):117-123.
6. Simonne AH, *et al*. Ascorbic Acid and Provitamin A Contents in Unusually Colored Bell Peppers (*Capsicum annuum* L.). Journal of Food Composition and Analysis. 1997; 10:299-311
7. Orellana-Escobedo L, *et al*. Capsaicinoids content and proximate composition of Mexican chili peppers (*Capsicum* spp.) cultivated in the State of Chihuahua. CyTA - Journal of Food. 2012; 11(2):179-184.
8. Ogunlade I, Alebiosu AA, Osasona AI. Proximate, mineral composition, antioxidant activity, and total phenolic content of some pepper varieties (*Capsicum species*) Int. J Biol. Chem. Sci. 2012; 6(5):2221-2227.
9. Kaur G, Bajaj KL, Jaiswal SP. Studies on variation in protein and mineral contents in dried red fruits of chilli varieties. Indian Food Packer. 1980; 34(1):21-23.
10. Teotia MS, Raina BL. Evaluation of chillies grown in Himachal Pradesh. Indian Food Packer. 1986; 42:11-14.
11. Naik PJ. *et al*. Packaging and storage studies on commercial varieties of Indian chillies (*Capsicum annuum* L.). J. Food Sci. Technol. 2001; 38(3):227-230.
12. Khatun HA. A Study on the Processing of Green chilies (*Capsicum frutescens* L.) Powder, in Food Engineering. Bangladesh Agricultural University: Mymensingh, 2012.
13. Sanatombi, K. and G.J. Sharma, Capsaicin content and pungency of different Capsicum spp. cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2008; 36:89-90.
14. Baruah S, *et al*. A Review on Recent Researches on Bhut jolokia and Pharmacological Activity of Capsaicin. Int. J Pharm. Sci. Rev. Res. 2014, 24(2).
15. Forero CE, Quijano, Pino JA. Volatile compounds of chile pepper (*Capsicum annuum* L. var. glabriusculum) at two ripening stages. J Flavor Fragrance. 2009; 24:25-30.

16. Higashiguchi H, *et al.* Purification and structure determination of glucosides of capsaicin anddihydrocapsaicin from various Capsicum fruits. J Agric. Food Chem. 2006; 54:5948-5953.
17. Davis CB, *et al.* Determination of capsaicinoids in Habanero peppers by Chemometric analysis of UV spectral data. J Agric. Food Chem. 2007; 55:5925-5933.
18. Wesołowska A, Jadczak D, Grzeszczuk M. Chemical Composition of the Pepper Fruit Extracts of Hot Cultivars (*Capsicum annuum* L). Acta Sci. Pol., Hortorum Cultus 2011; 10(1):171-184.
19. Kouassi CK. *et al*. Profiles of Bioactive Compounds of Some Pepper Fruit (*Capsicum* L.) Varieties Grown in Côte D'ivoire. Innovative Romanian Food Biotechnology. 2012; 11:49-55.
20. Koffi-Nevry R, *et al.* Antibacterial activity to two bell pepper extracts: *Capsicum annuum* L and Capsicum frutescens. International Journal of food Properties. 2012; 5:35-43.
21. Hayman M, Kam PCA. Capsaicin: a review of its pharmacology and clinical applications. Current Anaesthesia and Clinical Care. 2008; 19:338-343.
22. Mueller M, Hobiger S, Jungbauer A. Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chemistry. 2010; 122:987-996.
23. Ranganna S. Handbook of Analysis and quality control for Fruits and Vegetables Products, ed. S. Edn. Tata McGraw-Hill Publishing Company Limited, New Delhi, 1999.
24. Singh R, Giri SK, Kotwaliwale N. Shelf-life enhancement of green bell pepper (*Capsicum annuum* L.) under modified atmosphere storage. Food Packaging and Shelf-life 2014a; 1(2):101-112.
25. Kumaravel S, Kumar PP, Vasuki P. GC-MS Study on Microbial degradation of Lindane. International Journal of Applied Chemistry. 2010; 6(3):363-366.
26. Bagavathi PE, Ramasamy N. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacognosy Res. 2012; 4(1):11-14
27. Mercado JA, *et al*. Storage of bell peppers in controlled atmospheres at chilling and non-chilling temperatures. Acta Hort. 1995; 412:134-142.
28. Menichini F. *et al.* The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. *cv Habanero.* Food Chemistry 2008; 114:553-560.
29. Wesołowska A, Jadczak D, Grzeszczuk M. Chemical composition of the pepper fruit extracts of hot cultivars *Capsicum annuum* L. Acta Sci. Pol., Hortorum Cultus. 2011; 10(1):171-184.
30. Rohanizah AR, Ishak M. Phytochemical Contents of *Capsicum frutescens* (Chili Padi), Capsicum Annum (Chili Pepper) and Capsicum Annum (Bell Peper) Aqueous Extracts., in International Conference on Biological and Life Sciences, *IPCBEE*, IACSIT Press, Singapore, 2012.
31. Singh U, *et al*., New Antibacterial Tetrahydro-4(2H)-thiopyran and Thiomorpholine S-Oxide and S, S-Dioxide Phenyloxazolidinones. Bioorganic & Medicinal Chemistry Letters. 2003; 13:4209-4212.
32. Lucie CE, *et al.* On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. Eur Food Res Technol. 2011; 233:367-376.
33. Yu X, *et al*. Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose-histidine Maillard reaction products. Food Research International 2013; 51:397-403.
34. Chen Y, *et al.* Composition and potential anticancer activities of essential oils obtained from myrrh and frankincense. Oncology Letters. 2013; 6:1140-1146.