KSÜ Tarım ve Doğa Derg 22(6):922-927, 2019
KSU J. AgricNat 22(6):922-927, 2019
DOI:10.18016/ksutarimdoga.vi.546844

Purification of Capsaicin and Molecular Biological Activity Evaluation

Sibel BAYIL OĞUZKAN [1], Halil İbrahim UĞRAŞ[2]
[1]Department of Medical Services and Techniques, Vocational School of Health Services, University of Gaziantep, Gaziantep, Turkey, [2]Department of Chemistry, Arts and ScienceFaculty, Düzce University, Düzce, Turkey
[1]https://orcid.org/0000-0003-0254-6915, [2]https://orcid.org/0000-0002-1633-967X
✉: bayil@gantep.edu.tr

ABSTRACT
Red peppers, belonging to *Dicotyledonae* class, are produced and consumed all over the world including the southern regions of Turkey. Capsaicin is the most important active ingredient in red peppers. For this purpose, in this study, some molecular biological activities such as oxidant status, antiradical activity and DNA preservation of purified capsaicin from red peppers were investigated. Pure capsaicin was used into dichloromethane and methanol solution. Antiradical activity was determined by DPPH method. DNA protective activities was analyzed by using pBR322 plasmid DNA. The highest antioxidant activity was determined in methanol solution from the purified capsaicin extracts. Also the highest antiradical activity was found in red sweet pepper. In addition, DNA protective activity of the extraction in dichloromethane was found to be higher than that of methanol.

Research Article

Article History
Received : 01.04.2019
Accepted : 17.05.2019

Keywords
Red peppers
Capsaicin
Ultra violet
Oxidative

Kapsaisin Saflaştırılması ve Moleküler Biyolojik Aktivite Değerlendirmesi

ÖZET
Kırmızı biberler Dicotiledon sınıfına ait bitki türleridir. Bu biberler Türkiye'nin güney bölgesinde yaygın olmakla beraber tüm dünyada hem üretilmekte hem de tüketilmektedir. Kapsaisin, kırmızı biberlerdeki en önemli etken maddedir. Bu amaçla, bu çalışmada hem tatlı kırmızı biberlerden saflaştırdığımız kapsaisinin oksidan durumu, antiradikalik aktvitesive DNA koruyuculuğu gibi bazı moleküler biyolojik aktiviteleri araştırılmıştır. Bu çalışmada saflaştırılan kapsaisinin diklormetan ve metonal çözücüsündeki ekstrakları kullanıldı. Antiradikalik aktivite için DPPH metodu kullanıldı. DNA koruyucu aktivite için pBR322 plazmid DNA yöntemi kullanılmıştır. En yüksek antioksidan aktivite, saflaştırılmış kapsaisin ekstraklarından methanol çözeltisinde belirlendi, ayrıca en yüksek antiradikalik aktivite kırmızı tatlı biberde bulundu. Ayrıca, diklormetandaki ekstraksiyonda DNA koruyucu aktivitesinin metanolünkinden daha yüksek olduğu bulundu.

Araştırma Makalesi

Makale Tarihçesi
GelişTarihi : 01.04.2019
Kabul Tarihi : 17.05.2019

Anahtar Kelimeler
Kırmızıbiberler
Kapsaisin
Ultra Viyole
Oksidatif

ToCite : Bayıl Oğuzkan S, Uğraş Hİ 2019. Purification of Capsaicin and Molecular Biological Activity Evaluation. KSU J. AgricNat22(6): 922-927. DOI: 10.18016/ksutarimdoga.vi.546844.

INTRODUCTION
Red peppers (*Capsicum annuum L.*) are the species of plant that belong to Dicotyledonae class, Tubiflorae of Solanaceae family. The species that grows in Eastern Mediterranean and South Eastern Anatolia regions known as *Capsicum annuum L.* (Aırakı et al., 2012), which is a valuable plant frequentlyused in the pharmaceutical, chemical industry and by public (Hayman and Kam, 2008). Capsaicin is animportant secondary metabolite phenolic component synthesized from peppers (Materska and Perucka, 2005). The antioxidant and nutritional properties of capsaicin are known to be dependent on bioactive phenolic phytochemical ratios (Kavindra and Zhihong, 2014; Kempaiah and Srinivasan 2004). Red peppers contain different amounts of capsaicin. Epidemiological studies reported that there was a positive correlation between the consumption of pepper and the prevention of gastric, pancreatic and lung cancers (Filomena et al., 2007). In living organisms, the oxygen activity consumed during catabolism can be transformed to different radical molecules. These reactive oxygen radicals take DNA as heredity material as a target (Ai et al. 2008). Typically, oxidative damage from free radicals can be responsible for many chronic diseases,

such as cancer, inflammation and some of other chronic diseases. In recent years, antioxidant properties of natural compounds were investigated by examining potential antioxidant components (Repetto and Llesuy, 2002, Camilo and Ana, 2014). For this reason, it is very important to understand the antioxidant capacity of the capsaicin. The sweet peppers, generally regarded as bell peppers, are the most commonly known chili peppers in the Capsicum annuum family. Typically, sweet peppers contain much lower amounts of capsaicin compared to hot peppers (Reinbach et al., 2009).For this purpose, this study searches the radical scavenging and antioxidant activities of the capsaicin by isolating them from *C. annuum L.*, a red-colored species, which are both sweet and hot, and widely served as diet in the Middle East. Free radical is one or more single electron containing high reactivity molecules or groups. The effect of these free radicals on DNA is defined as indirect effect (Halliwell, 2007). Breaks in the linear DNA molecule due to the effect of UV rays. The high energy of ultraviolet rays especially UV-C can cause chemical effects on DNA molecules. It can alter the chemical structure and lead to the breakdown of some bonds. This may cause skin cells to become weaker or die, making the skin appear older than it is (Ertaş et al., 2015). Thus, this study determines the antioxidant properties of capsaicin with its effect on DNA protective activity. Studies of the antioxidant effect of capsaicin have been carried out revealing its antioxidant properties (Mateos et al., 2013).In this study, the antioxidant capacity of capsaicin was determined throughtests using the DPPH radical scavenging, total antioxidant capacity and total oxidant levels. While the literature on the red hot pepper's capsaicin composition is available, however, their antioxidant properties of capsaicin have not been evaluated because there is a lesser amount of red sweet pepper capsaicin available. In our previous published article, some biological activities were determined in green hot peppers (Bayil Oğuzkan et al., 2018). Firstly, this study determines and compares antioxidant capacity of the capsaicin in the sweet red peppers with the hot peppers. Several studies have investigated capsaicin's antioxidant capacity, however, no studies on DNA preservation have been found both of hot and sweet peppers. Thus, we have shown that the protective mechanism of capsaicin extracts purified from the red pepper species against DNA, as well as demonstrating the mutagenic effects of UV and H_2O_2.

MATERIAL and METHODS

Plant Material

The samples were twored pepper varieties, one was sweet bell pepper and the second one was the hot type of *C.annuum L.,* obtained from Gaziantep area in June and September 2017 and were identified by the Botanic Department of Biology Faculty at Gaziantep University.

Capsaicin Purification

Extracts were obtained in dichloromethane and methanol solution from sold commercially Sigma company. Soxhlet device was used to obtain the extracts of the plant, and the dried plant pulverized with a disintegrant. Dichloromethane and methanol extracts were extracted from the red pepper plant through Soxhlet method. Dichloromethane and methanol were removed from the extract through evaporation process using a rotary evaporator. This study dissolved each of the extracts in 30 ml of acetone solvent obtained from the sample petri dish. Moreover, we added 30 ml of 0.1 M $AgNO_3$ solution to the experiment and added 0.1 M NaOH solution drop wise (approximately 3 drops) to pH 7 at 30 ℃, which was followed by extraction on a magnetic stirrer for 3 hours. Then, the solution we rested. The aqueous phase was separated by filtration through ordinary filter paper. The aqueous phase was extracted with liquid-liquid by adding 100 ml of hexane. Moreover, 25 mL of dichloromethane was added to the aqueous phase, separated the aqueous phase, and added 25 mL of dichloromethane to the aqueous phase. Dichloromethane phases were pooled, and approximately 50 ml of the solution was evaporated on a half of rotary evaporator. The liquid containing the capsaicin solution was again subjected to liquid-liquid extraction with 25 ml of saturated NaCl solution, after which the dichloromethane phase was taken and the solvent was removed. The mixture was evaporated with 3 ml of dichloromethane solution and then the solvent was removed. The method was according to Segi and et al. (1999) which was the purification of industrial capsaicin. In this way, purification amount of dichloromethane of sweet and hot red peppers and methanol of hot and sweet red peppers are obtained and compare to commercially pure capcaicin (Sigma Aldrich company, purity 98%). as well as analytical degree. We have used the capsaicin in the extracts obtained from pure capsaicin pepper in two different solvents. The amounts of capsaicin obtained in the two peppers are shown in Table 1.

Table 1: Purification amount of capcaicin from red peppers (mg)

Solvents	Red hot pepper capcaicin	Red sweet pepper capcaicin
Dichlormethane	64.5 mg	18.9 mg
Methanol	6.8 mg	29.4 mg

Determining the Antiradical Activity with the DPPH method

This study also determined the antiradical activity of the extracts by using DPPH (1,1-diphenyl-2-picrilhidrazyl) radical according to Blois method [16].

The resultswith the calculation of the DPPH Radical Scavenging Activity were evaluatedwith the IC_{50}. The DPPH Radical Scavenging Activity was calculated with the following formula:

DPPH Radical Scavenging Activity % =(Control Absorbance – Sample Absorbance) / Control Absorbance x 100.

Determination of the Total Antioxidant Capacity and Total Oxidant Capacity

The Total Antioxidant Capacity (TAC) test principle was used ABTS (2,2 azinobis) (3-ethyl benzo thiazoline-6-sulfonic acid) as radical cation and evaluated with trolox equivalent (Vitamin E) as positive control. The total oxidant test principle was that the presence of oxidants in the sample oxidizes ferrous ions to ferric ion. According to the presence of oxidant in the environment, the color is increased. This color density is measured by spectrophotometry. The absorbance value indicates the oxidant value in the example. The TOC (Total Oxidant Capacity) value is detected by comparing the obtained results with µmol H_2O_2Equiv./L (Mateos et al., 2013). In this study Real Assay Diagnostics TAC and TOC Assay Kit were used (Tarpey et al., 2000).

The Determination of DNA protective activity by Capcaisin

pBR322 plasmid DNA was purchased from Sigma Chemicals which used for detecting to protective activity of DNA. Plasmid is a small, circular, double-stranded DNA molecule that showed clearly band on agorose gel electropresis. pBR 322 DNA was often used DNA protector analysis that was optimized by experimentally. Moreover, the study subjected the DNA of the plasmid to damage in the presence of the extracts by applying UV and H_2O_2 by performing the visualization on 1.25% agarose gel following the method specified by Russo et al. (Vanella et al., 2002,Russo et al., 2000).

RESULTS

In this study, the ratio of the oxidant and the antioxidant capacities measured by the experimentally measured oxidative stress index parameters was calculated mathematically as follows:

OSI= (TOC, µmol H_2O_2 equivalent/L) / (TAC, µmol Trolox equivalent/L) x100

Smaller OSI values indicate less oxidative stress. The capsaicin's OSI values were detected at very low levels. This showed that the mean of the oxidant activity of OSI low levels is quite low, and both different samples are quite good antioxidants. OSI values are at the very low level of all the samples and the best one among them is red pepper methanol extract as being revealed at Table 2.

When the antioxidant values of capsaicin were examined, it was found that the best antioxidant properties were found in each of the samples showing that red hot pepper was the best specimen of methanol extract. Thus, the radical scavenging effects of capcaicin were demonstrated in this study, which was tested on DPPH, and found to be stable radical.

Table 2: TAC, TOC, OSI and IC 50 values of capsaicin

	TAC	TOC	OSI	IC50	Reference value
RHPD	2,405618	0,032	1,3	1,309543	TAL>2 verygood
RSPD	2,343446	0,026	1,11	1,20723	TOL<5 verygood
RHPM	2,954307	0,031	1,05	0,408675	
RSPM	2,934082	0,041	1,39	0,055627	

RHPD:Red hot pepper dichlormethane
RSPD:Red sweet pepper dichlormethan
RHPM:Red hot pepper methanol
RSPM:Red sweet pepper dichlormethan

In our study the IC50 values for the radical elimination capacities of the capsaicin in the dichloromethane methanol samples were calculated. As the concentration of red-hot pepper dichloromethane, red sweet pepper dichloromethane, red-hot pepper methanol and red sweet pepper dichloromethane increased in all four samples of capsaicin obtained from red peppers, and its success to eliminate DPPH increased directly. As shown in Table 2, red-hot pepper and red sweet pepper were found to be the best radical elimination effects of the extract of capsaicin purified from the methanol and lower than the other samples of dichloromethane.

3 types of forms are seen in the plasmid DNA agarose gel imaging system. These are nicked DNA, linear DNA, supercoiled DNA according to the separation state. When the DNA structure exposed to any agent begins to form in this three-band form, the agarose gel is observed and the DNA band that is nicked stops at the top and brightly close to the loading well, and then the brightness is reduced and observed in 3 forms of DNA[20]. In the evaluations of DNA protective protocols, the band-luster up to the form of the band is indicative of this protective effect DNA preservation studies were performed on constructions. Displayed on the agarose gel were firstly compared with the control

group, and all samples were shown to show protective activity relative to the control group as figure 1 and figure 2.

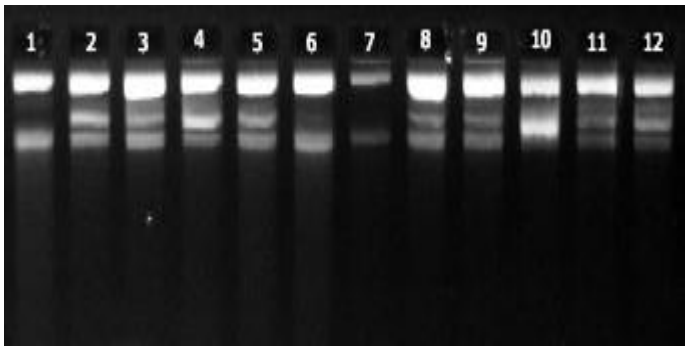

Figure 1:DNA protective activities' bands of samples.

1 [RHPD (40)] : Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
2 [RHPD (20)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
3 [RHPD(10)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
4 [RSPD (40 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
5 [RSPD (20 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
6 [RSPD (10 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
7 [RHPM (40 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
8 [RHPM (20 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
9 [RHPM (10 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
10 [RSPM (40 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
11 [RSPM (20 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)
12 [RSPM (10 mg)]: Plasmid DNA (3 µl) + capcaicin (5 µl)+ UV+ H_2O_2 (1 µl)

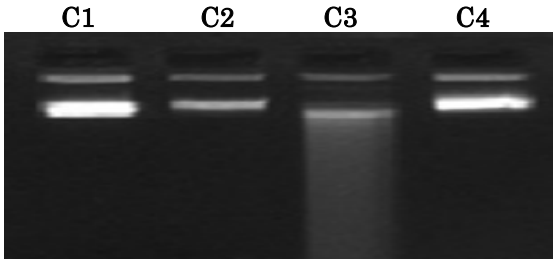

Figure 2: Bands of DNA protective activities (Control Group)
C1: Plasmid DNA (3 µl) + dH_2O (6 µl)
C2: Plasmid DNA (3 µl) + dH_2O (6 µl)+ UV
C3: Plasmid DNA (3 µl) +dH_2O (6 µl)+ H_2O_2 (1 µl)
C4: Plasmid DNA (3 µl) + dH_2O (6 µl)+ UV+ H_2O_2 (1 µl)

Also, DNA protection activities at different concentrations of extracts of both dichloromethane and capsaicin in methanol were evaluated.It has been observed that when the samples are compared between the bands in Fig. 1, the extract of the red pepper in the methanol shows lower activity than the dichlormethane. Although the amounts of capsaicin in red sweet peppers are very low, DNA protective activity was found to be higher than the methanol extract of red hot peppers, as can be seen from the band images. Red hot pepper (40 mg) was lower in the methanol extract than in the low concentration of DNA protective activity (10 mg). According to this result, we can say that the capsaicin in the methanol extract shows better protective activity in low concentrations.

DISCUSSION

Capsaicin is a valuable bioactive phytochemical used in the pharmaceutical, chemistry and cosmetic industries (William et al., 2014). It is known that phytochemicals are not only used for chemotherapy but also have antioxidant and anti-carcinogen effects (Arct et al.,2008). In contrast to antioxidants, oxidant equilibrium may deteriorate in a direction, resulting in many pathological conditions such as cancer, immune system disorders, cardiovascular diseases. Therefore, preserving this balance is extremely important for health (Bayil Oguzkan et al., 2016).In particular, UV-C (~ 260nm.) Is strongly absorbed by DNA and UV-B rays react with DNA and other biological molecules. Ionizing radiation and many anti-cancer drugs are thought to be the most toxic form of DNA damage induces double chain fractures. Repair of these fractures will continue in the cell's genomic stability and plays a critical role in survival. Many researchers believe that the survival rate of cancer cells inactivation of proteins involved in repair of double chain fractures or uses treatment modalities to reduce their expression (Lien et al., 2008, Hitoshi, 2010). Therefore, the effect of capsaicin, which is the primary active ingredient in peppers, on both oxidant status and genetic material by using some molecular biological methods was investigated. In this study, it was observed that all samples extracted from red hot pepper and red sweet peppers showed DNA protective activity compared to control as shown that figure 1 and figure 2. Low amount of capsaicin obtained from sweet peppers does not mean that it does not show DNA protective activity. Indeed, DNA protective activity evaluations are interpreted by evaluating the band image and brightness. Thus, we can say that all of the samples are associated with DNA protection as compared to control group. As capsaicin is responsible for the bitterness of peppers, sweet peppers are quite low, as shown in our study.

However, it has been shown in this study for the first time that it has a protective effect on DNA even in low amounts. Antioxidants have been reported to be effective in preventing damage to genetic material (André et al., 2017).Although antioxidants have a protective effect on DNA, prolonged exposure to high levels of UV radiation may cause a decrease in cellular

antioxidant levels(Cadet et al., 2005). The radical molecules formed together with the increase in cellular oxidant level can easily be found in nucleotides such as OH and H2O2, which can lead to irreversible DNA damage (Dai and Mumper 2010). Therefore, in this study, we evaluated DNA protective activity and oxidant status together. In fact, these different type pepper's capcaicin extraction suppressed the formation of lin DNA, generated by exposure of PBR 322 plasmid DNA to ·OH generated by H_2O_2 - UV-photo- lysis. The effect of capsaicin on peroxyl radical elimination has been reported to be much better than both caffeine and melatonin (Balasundrama et al., 2006).

In our study, the effect of eliminating the DPPH radical from the capsaicin was also found to be quite high. Many studies have shown that capsaicin has a strong antioxidant capacity (Christopher and Garold 2006, Young 2002), and it is thought that this reaction neutralizes peroxyl radical, which is a very strong radical because of this enzyme(Rosa et al.,2002, Leonardi et al., 2012). In our study, it was determined that the oxidant value of the capsaicin in red peppers was very low. In this study, the results of the antioxidant capacity of the capsaicin are consistent with the literature, and we can say that both the sweet and hot capsaicin have a high level of antioxidant capacity. Capsaicin is an irritant alkaloid that gives chili peppers a sharp scent and soreness, and it is assessed with a measurement unit called Scoville used to measure the hotness of the peppers (Scoville, 1992).Hot peppers, such as chili, tabasco, paprika, cayenne pepper contain these condiments in different quantities. Sweet peppers, also known as bell peppers (so called because of their shape), contain lesser amounts of capsaicin due to their sweetness (Peter, 2012). Although capcaicin is a parameter of hot peppers, in this study, the purifying of capillary from sweet peppers was carried out. As expected, the amount of capcaicin in red chillies was higher than the sweet pepper. The results obtained from *Capsicum annuum L.* was found to be lower capcaicin in the red sweet peppers grown, consumed in the Southeastern Anatolia region than the hot peppers. According to our results, the amounts of capsaicin were found to be different according to the solvent and genotypes in which the peppers were extracted.In terms of bioactivity, this is the first study that focuses on the extract of the capsaicin isolated from sweet peppers.

However, an extensive amount of capsaicin and bioactivity studies should determine not only on capsaicin isolated from capsicum annum L. peppers, but also on samples that were taken from different types of peppers. Also, this experimentally results can be compared to analytical degree of capcaicin in further studies.

CONCLUSIONS

This study is a preliminary study for further studies, and we are convinced that testing of different bioactivity's properties, and carrying out more extensive research on the antioxidant properties of capsaicin and its effect on DNA protective activity would open the way for its assessment in both health and other areas.

ACKNOWLEDGEMENT and NOTES

Authors would like to thank to Gaziantep University, Biology Department.

REFERENCES

Aırakı M, Leterrıer M, Mateos R, Valderrama R, Chakı M, Juan B, Luıs A, Palma D.R.J, Francısco J.C 2012. Metabolism of reactive oxygen species and reactive nitrogen species in pepper (Capsicum annuum L.) plants under low temperature stress. Plant Cell and Environment, 35: 281–295.

Adebayo AH, Tan NH, Akindahunsi AA, Zeng GZ, Zhang YM 2010. Anticancer and antiradical scavenging activity of Ageratumconyzoides L. (Asteraceae). Pharmacogn Mag, 6 (21) :62-66.

Ai L,Huy P, He H, Huy C.P 2008. FreeRadicals, Antioxidants in Disease and Health. Int J Biomed Sci, 4 : 89-96.

André PS, Natália CM, Natielen JS, Carlos F, Martins M, Camila C, Machado G 2017. Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. Free Rad. Biol. Med, 107:110-124.

Arct J, Pytkowska K 2008. Flavonoids as components of biologically active cosmeceuticals. Clin. Dermatol, 26 : 347–357.

Balasundrama N, Sundramb K, Sammana S 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem, 99:191–203.

Bayil Oguzkan S, Uğraş S, Aksoy ES, Ülger S, Üzmez Ş, Karagül B, Uğraş Hİ 2016. Biological Activity Analysis of Hazelnut Nutshell Extracts. International J. Chem. Natural Sci. 4 :481-485.

Bayıl Oğuzkan S, Can M, Kılıç Hİ, Uğraş Hİ, Özaslan M 2018. Güneydoğu Anadolu Bölgesinde Yetişen Yeşil Acı Biberlerdeki Kapsaisinin DNA Koruyuculuğu ÜzerineEtkisi. KSÜ Tarım ve Doğa Derg, 21(1):26-31.

Cadet J, Sage E, Douki T 2005. Ultraviolet radiation-mediated damage to cellular DNA. Mutat Res, 571 : 3-17.

Camilo LA, Ana D 2014. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Analytica chimica acta, 763:1-10.

Christopher AR, Garold SY 2006. Metabolism of Capsaicinoids by P450 Enzymes: A Review of Recent Findings on Reaction Mechanisms, Bio-

Activation, and Detoxification Processes. J. Drug Metabol. Reviews, 4: 38.

Dai J, Mumper RJ 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15 : 7313–7352.

Ertaş A, Gören AC, Haşimi T, Kolak U 2015. Evaluation of antioxidant, cholinesterase inhibitory and antimicrobial properties of Menthalongifolia subsp. noeana and its secondary metabolites, Records of Natural Products, 9(1): 105-115.

Filomena C, Giancarlo A, Menichini F 2007. Chemical and biological variability of hot pepper fruits (Capsicum annuum var. acuminatum L.) in relation to maturity stage. Food Chem, 102 : 1096–1104.

Hayman M, Kam PC 2008. Capsaicin: A review of its pharmacology and clinical applications. Current Anaesthesia and Critical Care, 19 (5) :338-343.

Hitoshi M 2010. Role of antioxidants in the skin: Anti-agingeffects. J. DermatologSci, 58 : 85–90.

Halliwell,B 2007. Biochemistry of oxidative stress BiochemSoc Trans. 35 (5): 1147-1150.

Kavindra R, Zhihong N 2014. From nature to synthetic systems: shape transformation in soft materials. J. Mater. Chem. B,2 :2357-2368.

Kempaiah RK, Srinivasan K 2004. Influence of dietary curcumin, capsaicin and garlic on the antioxidant status of red blood cells and the liver in high-fat-fed rats. Ann Nutr. Metab, 48: 314–320.

Kumar SS, Devasagayam TPA, Jayashree P, Kesavan CB 2001. Mechanism of protection against radiation induced DNA damage in plasmid pBR322 by Caffeine. Int. J. Radiat. Biol, 77: 617- 623.

Leonardi B, Miron D, Schapoval E, Rodrigues J,Grace O, Gosmann O 2012. Antioxidant and anti-inflammatory properties of Capsicum baccatum: From traditional use to scientific approach. Journal of Ethnopharmacology, 6 : 228-233.

Lien A, Pham H, Hua H, Chuong PH 2008. Free Radicals, Antioxidants in Disease and Health. Int J Biomed Sci, 4: 89-96.

Mateos R, Jiménez A, Román P, Romojaro F, Bacarizo S, Leterrier M, Manuel G,Francisca S, Luis A, Corpas, M. Palma J 2013. Antioxidant Systems from Pepper (Capsicum annuum L.): Involvement in the Response to Temperature Changes in Ripe Fruits.Int J Mol. Sci, 14: 9556–9580.

Materska M, Perucka I 2005. Antioxidant Activity of the Main Phenolic Compounds Isolated from Hot Pepper Fruit (Capsicum annuum L.) J. Agric. Food Chem, 53: 1750–1756.

Peter KV 2012. Handbook of Herbs and Spices. 1 st ed. United states of America: Elsevier Science.

Repetto MG, Llesuy SF 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers.Braz J MedBiol. Res, 35 (5) : 523-534.

Reinbach H.C, Smeets A, Martinussen T, Møller P, Westerterp-Plantenga M.S 2009. Effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance.ClinNutr, 28(3):260-5.

Rosa A, Deiana M, Casu V, Paccagnini S, Appendino G, Ballero M, Dessí MA 2002. Antioxidant activity of capsinoids.J. Agric. Food. Chem, 50 : 7396-401.

Russo A, Acquaviva R, Campisi A, Sorrenti V, DiGiacomo C, Virgata G, Barcellona ML, Vanella A 2000. Bioflavonoids as antiradicals, antioxidantsand DNA cleavage protectors. Cell. Biol. Toxicol, 16: 91-98.

Segi H, Yamada S, Kato S, Murasigo S 1999. Method of industrial purification of capsaicin US. Patent Application no : 5, 955, 631.

Scoville W 1992. Note on capsicums. Journal of the American Pharmaceutical Association. 1: 453–4.

Tarpey MM, Wink DA, Grisham MB 2000. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J. Physiol, 286 : 431–44, 2004.

Vanella A, Russo A, Acquaviva R, Campisi A, Giacomo C.D, Sorrenti V, Barcellona M.L (2002). l - Propionyl-carnitine as super oxidescavenger, antioxidant, and DNA cleavageprotector. Cell Biol. Toxicol, 16: 99-104.

Young JS 2002. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem. Toxicol, 40: 1091-1097.

William DR, Stover CA, Kathleen CB, Haley EP, Cathryn DS, Christopher AM, John G.B, Monica AV, Piyali D 2014. Bioavailability of capsaicin and its implications for drug delivery. J Control Release,28:96–105.