EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 9
75-80
Year: 2019

PHYTOCHEMICAL CONSTITUENT AND ANTIOXIDANT ACTIVITY OF EXTRACT FROM THE LEAVES OF *PIMENTA OFFICINALIS*

[1]Dr. M. Rama and [2]*Prof. B. Syama Sundar

[1]Dept. of Chemistry, Ch.S.D.St Theresa's College for Women(A), Eluru, WG.

***Corresponding Author: Prof. B. Syama Sundar**
Dept. of Chemistry, Ch.S.D.St Theresa's College for Women(A), Eluru, WG.

Article Received on 08/06/2019 **Article Revised on 28/06/2019** **Article Accepted on 18/07/2019**

ABSTRACT
Pimenta officinalis or Pimenta dioica, commonly known as Allspice plant. As a medicine, Allspice has much the same use as Cloves and their oils. The % composition of essential oil in different seasons in 2010 calendar year were identified and reported that in the month of November the composition of essential oil is more and in the month of May it is very low. Extracts from the leaves of *Pimenta dioica* was investigated for phytochemical constituents and antioxidant activity. Tests for phenols, flavonoids and antioxidants of leaf extracts of *Pimenta dioica* was conducted and the value of phenols found to be 133.6±3.1TAE/gm. The value is compared with tannic acid. The flavonoids contents of *Pimenta dioica* is 0.322 ±0.04 mg/gm. This value is compared with quercetin. The antioxidant content is evaluated by using ascorbic acid as standard and the value is 1.241±0.03 mg/gm. The percentage inhibition of DPPH (scavenging activity) of *Pimenta dioica* is 49.24%. In the present study the phenols, flavonoids and antioxidants of *Pimenta dioica* was rich, it showed that *Pimenta dioica* could be the source of natural antioxidants and phenolic compounds. These findings shows that the rich content of phenols and antioxidants of *Pimenta dioica* may responsible for a large number of pharmacological activities.

KEYWORDS: *Pimento dioica, essential oil, percentage Eugenol, total phenols, flavonoids, antioxidants, DPPH assay.*

1. INTRODUCTION
Pimenta dioica (L.), ***Pimenta*** *officinalis* Lindl. belongs to the botanical spice-group of ***Pimenta*** Lindl. ("Allspice"), Myrtaceae family.[1] Many common names of this spice are known, such as allspice, clove pepper, English spice, Jamaican pepper.[2]

Pimenta dioica is indigenous to West Indies and tropical America and grown in gardens in India. As a medicine, Allspice has much the same use as Cloves and their oils. It works well as a digestive and has an antiseptic and slightly anaesthetic action.[3] The tropical, evergreen dried fruits of the *P.* ***dioica*** tree are used worldwide as valuable spice.[1] The dried unripe berries are used mainly as spice and condiment.[4] They are also used for flatulent indigestion, as a febrifuge and are considered to have tonic and antihelmintic properties.[5]

The essential oil is obtained from fruits or leaves with yields of 1.5–4.5%. The dried leaves on steam distillation yield 0.7-2.9% of an essential oil, which contains eugenol as its main component.[6] The compounds of the *Pimenta* ***dioica*** oil is eugenol (70–80%), eugenol methyl ether [9.6%], phellandrene, 1,8-cineole, α-humulene, β-caryophyllene, a terpene alcohol and cadinene-derivatives were found as further important constituents in higher concentrations.[7-10]

The therapeutic properties of the essential oil of allspice are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient, stimulant and tonic. The essential oil of *P. dioica* leaf and fruit is also used in perfumes, aftershaves and commercial food flavoring.[1,7,11] The leaves of this tree known popularly as "jamaica" have been used in Costa Rican folk medicine as an antihypertensive.[12]

Analgesic and hypothermic effects have also been documented.

The objective of this research was to examine the % composition of essential oil, % composition of eugenol, total phenols, flavonoids, antioxidants and DPPH assay of *P. dioica*. % composition of essential oil and % composition of eugenol of *P. dioica* was identified and compared in four seasons of 2010 calendar year.

2. MATERIALS AND METHODS
2.1 Collection and Identification of Plant material
The selected plant was in the spacious domestic home garden at Gavaravaram village, Eluru mandal in West

Godavari district of Andhra Pradesh is situated between 16.7 ^{0} North 81.1^{0} East, elevation 22mts 72 feet. Plant material was collected and vouchers of specimens were deposited at the Botany department. Each specimen was labeled, numbered, annotated with the date of collection. In the present study the specimen numbers are F6, M6, A6, and N6. Each specimen was subjected for identification at plant systematic laboratory, Kakatiya University, Warangal, Andhra Pradesh, India.

1 Kg of leaves of the sample was collected in four different seasons of 2010 calendar year i.e., in February, May, August, and in November. These samples were dried under shade, grinded to fine powder in an electric blender (80 mesh) and stored in air tight containers at room temperature in the dark until used.

2.2 Preparation of plant extract

One gram of dried leaf powder was grinded with 20ml of 50% methanol and filtered. The filtrate was made up to the volume 50ml with 50% methanol. This extract was used to analyze the total phenol content, the flavonoids content and the antioxidant capacity.

2.2.1 Soxhlet extraction

Extraction of total essential oil content of plant materials was carried out by soxhlet extraction[13] method. 5gm of dry powder was subjected to soxhulet extraction with 250ml methanol, extraction was carried out for 3hrs, 10 cycles and temperature was maintained at 65°C.This extract was used to analyze DPPH assay.

2.2.2 Steam distillation

Extraction of volatile oils from the plant materials was carried out by steam distillation using Clevenger type apparatus.[14] 100g powdered sample was water distilled by using a Clevenger oil arm fitted with condensers through which cooled water was circulated to prevent low volatiles from escaping. The temperature was maintained at 60°C. The volatile oil was collected and dried over anhydrous Sodium Sulphate and stored at -4 °C. 1mg of volatile extract was dissolved in 1ml of methanol, from that solution 10µl was taken and made up to 100 µl with methanol. This solution was used for GC analysis. (Same procedure followed for the preparation of standard eugenol).

2.3 Gas chromatography analysis

The essential oils were analyzed using a Shimadzu gas chromatograph model 17 A Japan(2014), equipped with flame ionization detector (FID) and DB-Wax capillary column (30mx0.32mm , film thickness 0. 5 µm). Injector and detector temperatures were set at 240 and 250°C, respectively. Column oven temperature was programmed from 40°C to 220°C at the rate of 8°C min-1; initial and final temperatures were held for 3 and 10 minutes, respectively. Helium was used as a carrier gas with a flow of 1.5 mL min-1. A sample of 0.1 µL was injected using slit mode (split ratio, 1:20). Quantification was completed by built-in data-handling software supplied by the manufacturer (spin chrome CFR) of the gas chromatograph. The results (composition) were reported as a relative percentage of the total peak area.

2.4 Estimation of total phenolics

Total phenolic content was determined using Folin-Ciocalteau reagent as previously described.[15] The plant extract solution (250 µl) was mixed with 5ml of Folin-Ciocalteu reagentand 4 ml of (20%) sodium carbonate, and they were vortexed for 50sec and they were let to stand for 30mins in water bath at 40°C. The optical density was measured by using systronics (C1117) colorimeter using at 680nm.

The total phenol content of the extracts was obtained by using the standard curve. The total phenol content was expressed as tannic acid (0.1mg/ml) equivalent in % w/w of the extracts.

$$Total\ phenolic\ content = \frac{\text{Optical density of sample} \times Concentration\ of\ tannic\ acid}{\text{Optical density of standard}}$$

2.5 Total flavonoids content

The total flavonoid content was determined using the Dowd method.[16] The plant extract solution (250 µl) was mixed with 0.1ml of 2% aluminium chloride and 1ml of 0.1M potassium acetate, mixed well and allowed to stand for 30min. at room temperature. The colour developed in each test tube was measured by using systronics (C1117) colorimeter at 420nm. Total flavonoid contents were calculated as quercetin(0.1mg/ml) equivalent from a calibration curve.

$$Total\ flavonoid\ content = \frac{\text{Optical density of sample} \times Concentration\ of\ quercetin}{\text{Optical density of standard}}$$

2.6 Reducing Power Assay

The reducing power of the extracts was measured by using ascorbic acid.[17] The plant extract solution (250 µl) was mixed 2.5 ml of phosphate buffer (PH 6.6) and 2.5ml of(1%) potassium ferricyanide and were incubated in water bath at 50°C for 20min. Then the test tube was centrifuged for 10min. at 10,000 rpm. 2.5ml of supernatant was taken in a test tube 2.5ml of distilled water and 0.5ml freshly prepared (0.1%)ferric chloride were added and observed the colour change. The optical density was measured by using UV–VIS spectrophotometer 2.2. (Double-beam) (SL191 series) at 680nm. Total antioxidants contents were calculated as ascorbic acid (0.1mg/ml) equivalent from a calibration curve.

$$Total\ flavonoid\ content = \frac{\text{Optical density of sample} \times Concentration\ of\ ascorbic\ acid}{\text{Optical density of standard}}$$

2.7 DPPH radical scavenging assay (Antioxidant assay)

The following assay procedure was modified from those described by Blois[18] and Govindaragan.[19] Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/ml in methanol. A portion of sample solution (500 µl) was

mixed with an equal volume of 6x10-5 M DPPH (1,1-diphenyl-2-picrylhydrazyl; in methanol) and allowed to stand at room temperature for 30 min.

In each experiment methanol (1ml) plus plant extract solution was used as blank while the DPPH solution alone in methanol was used as control. The absorbance (A) of sample solution was measured in spectrophotometer at 520 nm, compared with that of control solution (maximum absorbance). The scavenging activity of samples corresponded to the intensity of quenching DPPH. The results were expressed as percentage inhibition.

$$\% \, inhibition = \frac{[\,(\text{A Control} - \text{A sample})}{\text{A Control}} \times 100$$

3. RESULTS AND DISCUSSION

In the present study several phytochemical constituents present in *Pimenta dioica* such as total phenols, flavonoids and antioxidants were evaluated. The free radical scavenging activity was also evaluated. Table 1 shows the yield of essential oil and percentage composition of eugenol in *Pimenta dioica* in four different seasons of 2010 calendar year. It shows that the yield was affected by seasonal changes. The highest amount of the oil in these plants was found in winter i.e in November and very low in summer i.e in May.

Table 1: Total essential content and Percentage composition of eugenol in *Pimenta dioica* in four different seasons.

Month and Year	Total essential oil content (%)	Percentage composition of eugenol
February 2010	2.2%	64.262%
May 2010	1.73%	38.951%
August 2010	1.9%	51.884%
November 2010	2.8%	74.331%

Values are mean ± standard deviation of three samples

In *Pimenta officinalis* the percentage composition of essential oil is high in November (2.8%) and low in May (1.73%). The dried leaves of *Pimenta* on steam distillation yield 0.7-2.9% of an essential oil.[20] The percentage composition of Eugenol in *Pimenta officinalis* in the month of November is 74.331%.The dried leaves of *Pimenta* on steam distillation yield an essential oil, which contains Eugenol as its main componen[20] t. Purseglove and Brown[21] reported that *P.dioica* leaf oil of Jamaican origin contains more Eugenol [95-98.5%], than the other Caribbean leaf oils. Two oils produced by steam distillation of the leaves of *P. dioica* of Jamaican origin were examined by Tucker and Maciarello[22], the oils were found to be rich in Eugenol [66.38-79.24%]. The leaf oil of *P. dioica* of Cuban origin, the main constituent was found to be Eugenol- 54.26%.[23] GC–MS analysis of *P. dioica* essential oils revealed high abundance of Eugenol (64.29%) and methyl-eugenol (20.55%) as main components.[24]

Table 2: Total phenols, flavonoids, antioxidants and DPPH scavenging assay in *Pimenta officinalis.*

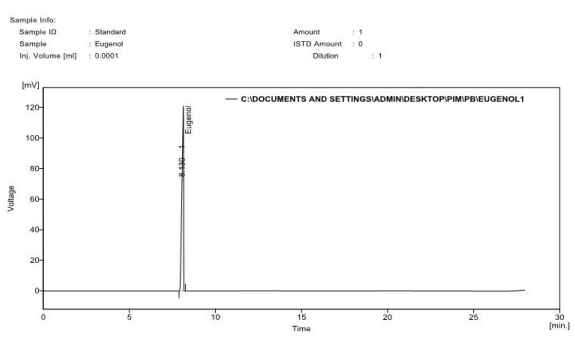
Month and year	Totalphenols mg TA equivalent/gm dw	Total flavonoids mg of Quercetin /gmdw	Antioxidants mg of ascorbic acid equivalent /gm dw	DPPH scavenging % inhibition
November 2010	133.6±3.1	0.322±0.04	1.241±0.03	49.24%

Values are mean ± standard deviation of three samples

Pimenta officinalis also show high value of total phenol content 133.6±3.1TAE/g. The total phenol content of *Pimenta dioica* in the present study was higher (133.6±3.1TAE/g) than the value (122 ±7.0 mg GA/g dry)reported by Rebecca et al.[25]

It is possible that the Eugenol-rich plant materials contain low levels of flavonoids because Eugenol, also a phenylpropenoid, may compete more successfully than the flavonoids for the amino acid precursor phenylalanine.[26] It was true when we take the values of *Pimenta officinalis*, 0.322±0.04 mg/gm.

In *Pimenta officinalis* the antioxidants value was 1.241±0.03. The antioxidant activity of allspice may be associated with eugenol.[27,28] In general essential oils exhibited weak to strong free DPPH scavenging activity rang can be shown as (weak: <25%; moderate: 25-50%; strong: >50%) at the tested concentration of 0.1% (v/v). The extracts of *Pimenta officinali* (49.24%), showed the strongest DPPH radical scavenging activity. *Pimenta officinalis* showed good free radical scavenging activity by the DPPH Method.[29]

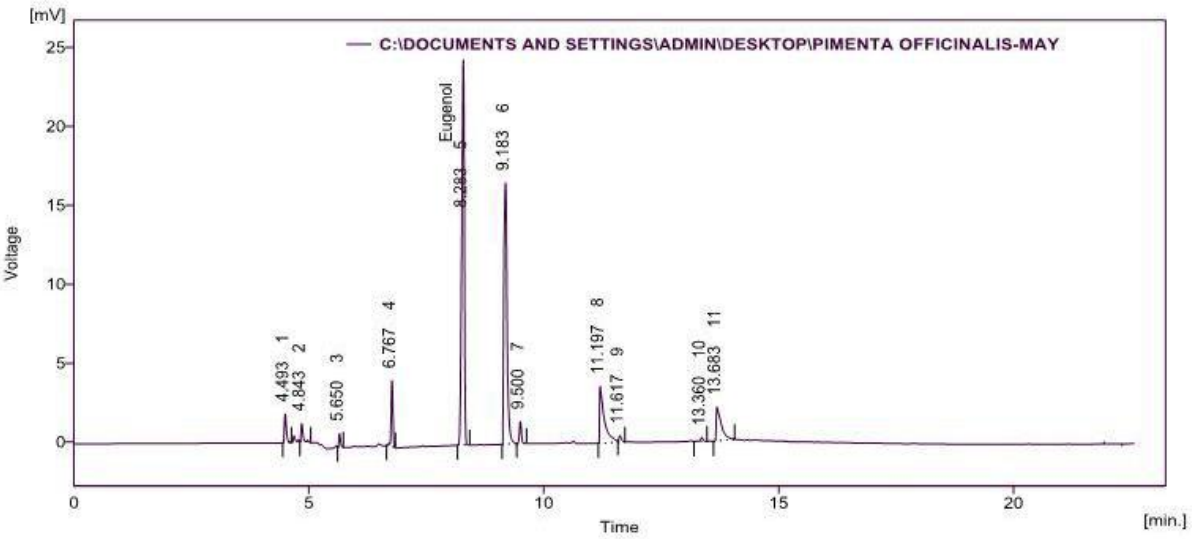


Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\PIM\PB\EUGENOL1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	8.130	798.431	120.739	100.000
	Total	798.431	120.739	100.000

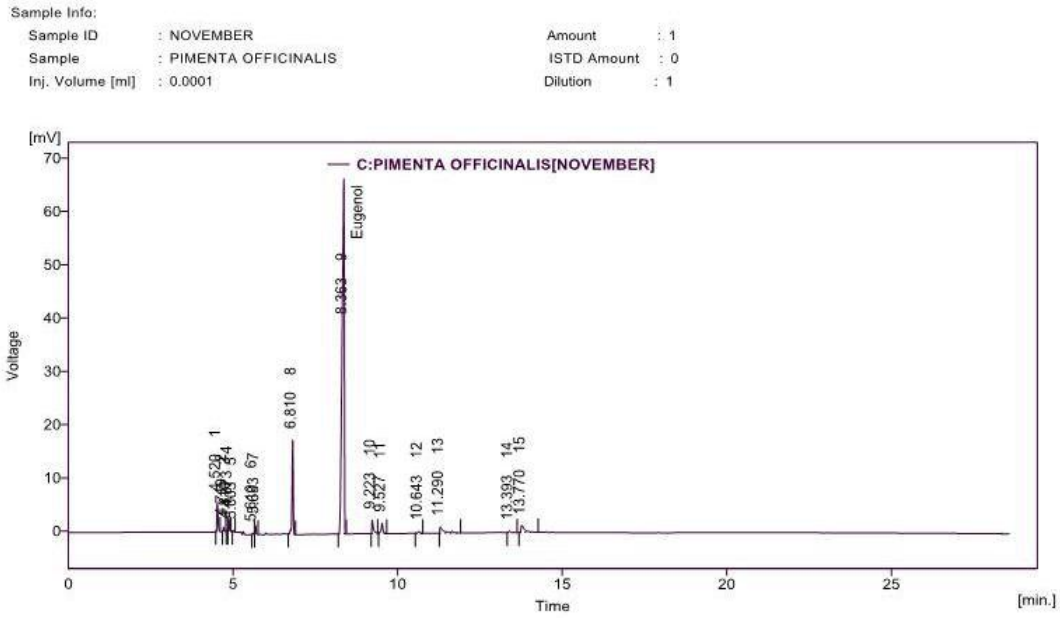
Sample Info:

Sample ID	: MAY	Amount	: 1
Sample	: PIMENTA OFFICINALIS	ISTD Amount	: 0
Inj. Volume [ml]	: 0.0001	Dilution	: 1



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\PIMENTA OFFICINALIS(MAY)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.493	5.299	1.829	2.192
2	4.843	3.561	1.165	1.473
3	5.650	2.153	0.884	0.890
4	6.767	11.204	4.220	4.633
5	8.283	94.185	24.373	38.951
6	9.183	77.942	16.542	32.233
7	9.500	4.222	1.411	1.746
8	11.197	25.033	3.606	10.352
9	11.617	1.657	0.421	0.685
10	13.360	0.774	0.242	0.320
11	13.683	15.774	2.145	6.524
	Total	241.805	56.839	100.000



Result Table (Uncal - C:PIMENTA OFFICINALIS(NOV.)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	4.520	13.114	5.518	3.167
2	4.710	2.135	0.916	0.516
3	4.810	0.535	0.355	0.129
4	4.873	4.864	2.393	1.175
5	5.003	0.577	0.312	0.139
6	5.610	0.624	0.312	0.151
7	5.683	3.549	1.714	0.857
8	6.810	43.431	17.696	10.488
9	8.363	307.789	66.476	74.331
10	9.223	7.749	2.497	1.871
11	9.527	6.495	1.933	1.568
12	10.643	1.348	0.406	0.325
13	11.290	10.598	1.187	2.559
14	13.393	0.953	0.300	0.230
15	13.770	10.319	1.321	2.492
	Total	414.079	103.337	100.000

4. CONCLUSION

Eugenol is an important chemical constituent of the essential oils of many aromatic plants and has great importance in pharmaceutical industry and usually extracted from clove buds (*Eugenia caryophyllata)* belonging to family Mytraceae, and from leaves and barks of *Cinnamomum zeylanicum Breyn* belonging to family Lauraceae. Although these plant sources are rich in eugenol but because of their higher prices the commercial extraction of eugenol from them is costly. In contrast to these sources *Pimenta officinalis* is cheaper sources for commercial extraction of eugenol. The leaves of the plant contain essential oils with good percentage of eugenol. In the present study we have selected the *Pimenta officinalis* plants to get eugenol in cheaper cost. This plant is easily available in our surroundings, cultivation is also very easy. All results showed that *Pimenta officinalis* could be sources of natural antioxidants and phenolic compounds. It would be a blessing in disguise if this plant becomes a medicine for the common man. Still more clinical trials need to be conducted to support its medicinal therapeutic uses.

REFERENCES

1. Seidemann J. Pimenta Lindl. – Allspice – Myrtaceae. World Spice Plants, 286-287.
2. Springer-Ver- lag, Heidelberg, 2005.
3. Rogers, M.C. Flora de Guatemala. Field. Bot., 1963; 24: 382.
4. Ridley H; Spices. London: Mac Milan and Company Ltd, 1983; 197-199.
5. Grieve, M. A Modern Herbal. Dover, New York, 1971; 19-21.

6. Germosén-Robineau, L. Hacia una Farmacopea Caribeña. Enda-Caribe, Santo Domingo, República Dominicana, 1995; 481- 483.
7. Anonymous; The Wealth of India [Raw Materials]. New Delhi: Publications Information Division, CSIR Publications, 1969.
8. BACIS-Boelens Aroma Chemical Information Service: Pimento. VCH Volatile Compounds in Food Database, TNO Nutrition and Food Research, Zeist, 2000.
9. Maringiu B., Piras A., Porcedda S., Casu R., Oierucci P. J Essent Oil Res, 2005; 17: 530.
10. Tucker AO and Maciarello MJ. Journal of Essential Oil Research, 1991; 3: 195.
11. Rosilene Aparecida de Oliveira, Tâmara Vieira Reis, Célio Kersul do Sacramento, Lucienir Pains Duarte, Fernando Faustino de Oliveira; Brazilian Journal of Pharmacognosy, 2009; 19(3): 771.
12. Sharma R.: Pimenta. Medicinal plants of India –An encyclopaedia. Data Publishing House Delhi, New Delhi, 2003.
13. Vargas, S.; Plantas Medicinales: la naturaleza como guardián de su salud. Mundo Gráfico, San José costa Rica., 1990; 55.
14. Mustafa Z. Ozel, Hilal Kaymaz,. Superheated water extraction, steam distillation and Soxhlet extraction of essential oils of *Origanum onites.* Analytical and Bioanalytical Chemistry, 2004; 379: 7-8, 1127-1133.
15. Clevenger, J. F. Apparatus for the determination of volatile oil. Journal of the American Pharmacists Association, 1928; 17: 346.
16. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 2001; 73: 73-84.
17. Meda, A., Lamien, C. E., Romito, M., Millogo, J. & Nacoulma, O. G. Determination of the total phenolic, flavonoid and proline contents in Burkina fasan honey, as well as their radical scavenging activity. Food Chemistry, 2005; 91: 571–577.
18. Oyaizu, M. Studies on products of browning reaction–antioxidant activities of products of browning reaction prepared from glucoamine. *Jap. J. Nutr.*, 1986; 44: 307–315.
19. Blois MS. Antioxidant determinations by the use of a stable free radical. Nat., 1958; 26: 1199-1200.
20. Govindarajan R., M. Vijaya Kumar, A.K.S. Rawat, S. Mehrotra. Free radical scavenging potential of *Picrrorhiza kurroa* Royle ex Benth., *Indian J. Exptl. Biol.*, 2003; 41: 875.
21. Anonymous; The Wealth of India [Raw Materials]. New Delhi: Publications Information Division, CSIR Publications, 1969.
22. Purseglove JW, Brown EG, Green CL and Robbins SRJ: Spices. Longman, New York, 198: 287.
23. Tucker AO and Maciarello MJ. Journal of Essential Oil Research, 1991; 3: 195.
24. Pino J and Rosado A; Nahrung, 1989; 33[8]: 717.
25. Martin Zabka, Roman Pavela, Ludmila Slezakova. Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi, Industrial Crops and Products, 2009; 30: 250–253.
26. Rebecca P. Dearlove, Phillip Greenspan, Diane K. Hartle, Ruthann B. Swanson, and James L. Hargrove. Inhibition of Protein Glycation by Extracts of Culinary Herbs and Spices *J Med Food*, 2008; 11(2): 275–281.
27. Roberto F. Vieiraa,1, ReneÂe J. Grayerb, Alan Patonb, James E. Simona,. Genetic diversity of Ocimum gratissimum L. based on volatile oil constituents, favonoids and RAPD markers Biochemical Systematics and Ecology, 2001; 29: 287±304.
28. Rompelberg C.J, Vogels J.T, de Vogel N, Bruijnttjes-Rozier G.C, Stenhuis W.H, Bogaards J.J, Verhagen H. Effect of short-term dietary administration of eugenol in humans. Hum Exp Toxicol, 1996; 15: 129–35.
29. Kluth D, Banning A, Paur I, Blomhoff R, Brigelius-Flohe R. Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic Biol Med., 2007; 42: 315–25.
30. Kikiuzaki, H., Sato, A., Mayahara, Y and Nakatani, N. Galloylglucosides from Berries of Pimenta dioica. Journal of Natural Products, 2000; 63: 749-752.