

Spice plants: Chemical composition and antioxidant properties of *Pimenta* Lindl. essential oils, part 1: *Pimenta dioica* (L.) Merr. leaf oil from Jamaica*

Gewürzpflanzen: Chemische Zusammensetzung und antioxidative Aktivitäten von ätherischen Ölen von *Pimenta* Lindl., Teil 1: *Pimenta dioica* (L.) Merr. Blattöl aus Jamaika

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Summary

The chemical composition of an essential pimento leaf oil from Jamaica was analyzed by GC and GC-MS. As major compounds of the sample eugenol (76.02 %), methyl eugenol (7.14 %) and β -caryophyllene (6.47 %) were identified. The antioxidant properties of this *Pimenta dioica* essential leaf oil were assayed by study of the capacity to counteract DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxyl (OH•) and superoxide radicals. The scavenging capacity of the pimento leaf oil was strongest in the case of OH• – its IC₅₀ value was determined to be 0.29 μ g/mL compared to an IC₅₀ value for DPPH of 1.79 μ g/mL. Xanthine oxidase activity was inhibited 74.83 % by the essential *P. dioica* oil, while superoxide scavenging was 95.93 %, at 50 μ g/mL concentration. The essential pimento leaf oil demonstrated antioxidant activity in a linoleic acid emulsion model system, where at a concentration of 0.005 % the sample inhibited conjugated dienes formation by 65.47 % and the generation of secondary linoleic acid oxidation products by 72.98 %.

Keywords:

Pimenta dioica (L.) Merr., essential leaf oil, chemical composition, GC-MS, antioxidant properties

Zusammenfassung

Die chemische Zusammensetzung eines ätherischen Blattöles von Piment aus Jamaika wurde mittels GC und GC-MS analysiert. Als Hauptkomponenten der Probe wurden Eugenol (76,02 %), Methyleugenol (7,14 %) und β -Caryophyllen (6,47 %) identifiziert. Die antioxidativen Eigenschaften dieses ätherischen *Pimenta dioica* Blattöles wurden auf die Fähigkeit, DPPH- (2,2-Diphenyl-1-picrylhydrazyl), Hydroxyl-(OH•) und Superoxid-Radikale entgegenzuwirken, erprobt. Die Radikalfangkapazität des Pimentblattöles war im Falle von OH• am stärksten – der IC₅₀-Wert wurde mit 0,29 μ g/mL bestimmt, verglichen mit einem IC₅₀-Wert von 1,79 μ g/mL für DPPH. Die Aktivität von Xanthinoxidase wurde durch das ätherische Öl von *P. dioica* zu 74,83 % gehemmt, während die Radikalfangkapazität von Superoxid bei einer Konzentration von 50 μ g/mL 95,93 % betrug. Das ätherische Pimentblattöl zeigte antioxidative Aktivitäten in einem Modellsystem einer Linolensäure-Emulsion, in welcher die Probe bei einer Konzentration von 0,005 % die konjugierte Dien-Bildung zu 65,47 % und die Bildung von sekundären Oxidationsprodukten aus Linolensäure zu 72,98 % hemmte.

Kennwörter:

Pimenta dioica (L.) Merr., ätherisches Blattöl, chemische Zusammensetzung, GC-MS, antioxidative Eigenschaften

1. Introduction

Pimenta dioica (L.) Merr. syn. *Eugenia pimenta* DC., *Myrtus pimenta* L., *Pimenta officinalis* Lindl., *Pimenta vulgaris* Lindl. belongs to the botanical spice-group of *Pimenta* Lindl. ("Allspice"), Myrtaceae family [1]. The tropical, evergreen dried fruits of the *P. dioica* tree are used worldwide as valuable spice [1]. Many common names of this spice are known, such as allspice, clove pepper, English spice, Jamaican pepper, pimen-

to (English); bahar (Arabic); pimenta, Jamaica pepper (Dutch); piment, piment des anglais, toute épice, piment de la Jamaïque, quatre-épice, également appelé (French); Allerleigewürz, Englischgewürz, Jamaikapfeffer, Nelkenpfeffer, Neugewürz, Piment (German); kabab cheene (India); pimento, pimento inglese, pepe della Giamaica, pepe garofanato (Italian); pimenta da Jamaica (Portuguese); Jamajskij perez, wosditschnij perez, ormusch, piment (Russian); nové korenje (Slovenian); malequeta, pimienta de Jamaica, pimienta

* Part of an international project for a systematical investigation of the antioxidant properties of various aroma compounds, essential oils and plant extracts

inglesa (Spanish) [1]. The pimento-tree grows or is cultivated especially in Central America, West Indies, Venezuela, Mexico, Honduras, Guatemala, Grenada, Jamaica, Cuba, Haiti and Brazil.

The essential oil is obtained from fruits or leaves with yields of 1.5–4.5 %. The major compound of the *Pimenta dioica* oils is eugenol (70–80 %). In the pimento leaf oils 1,8-cineole, α -humulene, β -caryophyllene and cadinene-derivatives were found as further important constituents in higher concentrations (up to 10 % for single compounds) [2–4].

The essential oils of *P. dioica* leaves and fruits are utilized in food industry – mainly meat and canning industries – as well as in perfumery compositions and cosmetic products. An oleoresin from the pimento berries is also produced in small quantities. The therapeutic properties of the essential allspice oils are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient, stimulant and tonic. Pimento oil can be helpful for the digestive system, for cramp, flatulence, indigestion and nausea. Further, the essential oils can help in cases of depression, nervous exhaustion, tension, neuralgia and stress and is used as natural repellent. The essential *P. dioica* leaf and fruit oil is also used in perfumes, aftershaves and commercial food flavoring [1, 2, 5].

It has been long known that oxygen consumption incorporates – via cell metabolism – an increasing generation of free radicals and reactive oxygen species (ROS). The role of free radical reactions in disease pathology is well established. These reactions are necessary for the normal metabolism, but can be detrimental to health as well. Free radicals cause diseases like arteriosclerosis, arthritis, ischaemic heart attack, diabetes, neuro-degenerative diseases and others [6]. Thus, antioxidant defense systems have co-evolved with aerobic metabolism to counteract oxidative damage from ROS. Antioxidant enzymes as well as some other natural antioxidants such as reduced glutathione, phenolics, flavonoids, pigments, constitute a system that keeps ROS at a low steady-state concentration in cells and tissues and prevents oxidative situations. Experimental data have been filed, concerning some of polyphenols' potential biological activities: 1) inhibition or reduction of various enzymes like cyclooxygenases [7, 8], lipoxygenases [9], xanthine oxidase [10], which promote the production of superoxide radicals; 2) initiation of detoxifying enzymes [11]; 3) intensification of the generation of vasodilating agents such as nitric oxide [12]. These are the properties that furnish polyphenols execute protective effect and evoke an ever-growing attention as therapeutic agents fighting cancer and cardiovascular diseases [13].

Currently there exists a great worldwide interest in finding new and safe antioxidants from natural sources to prevent oxidative deterioration of foods and to minimize oxidative damage to living cells. Our study has focused on spice constituents or isolates, which are functional antioxidants yet do not have the overpowering or undesirable organoleptic properties of the whole spice. Essential oils and extracts of *Pimenta sp.* are very interesting samples to investigate in this field, but only a few data are available until now [14–16].

2. Materials and methods

2.1. Essential oil

The essential leaf oil of *Pimenta dioica* (L.) Merr. from Jamaica is a product from Kurt Kitzing Co., Wallerstein, Germany, with number 800675 (charge 11864).

2.2. GC analysis

GC/FID analyses were carried out using a GC-14A with split/splitless-injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany) and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen; injector temperature 250 °C; detector temperature 320 °C. The temperature programme was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. The columns were 30 m x 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 μ m (Biorad, Germany) and 30 m x 0.32 mm bonded Stabilwax, with a film thickness of 0.50 μ m (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was carried out partly using correlations between retention times [17–21].

2.3. GC-MS analysis

For GC/MS measurements a GC-17A with QP5000 (Shimadzu), split/splitless injector and Compaq-ProLinea data system (class5k-software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and ChemStation software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan-Spectronex, Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ-software) were used. The carrier gas was helium; injector temperature 250 °C; interface-heating at 300 °C, ion-source-heating at 200 °C, EI-mode was 70 eV electron energy, and the scan-range was 41–450 amu. For other parameters, see description of GC/FID above. Mass spectra correlations were done using Wiley, NBS, NIST and our own library as well as published data [17, 19, 20].

2.4. Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)

The radical scavenging capacity was determined according to the method described by [22]. 1.0 mL

from 0.3 mM alcohol solution of DPPH was added to 2.5 mL from the samples with different concentration of pimento oil and of eugenol. The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 518 nm. The optic density of the samples, the control and the empty samples were measured in comparison with ethanol. Ascorbic acid, rutin, butylhydroxy toluene (BHT) and butylated hydroxyl anisole (BHA) were used as positive control.

2.5. Detection of hydroxyl radicals by deoxyribose assay

The assay was performed as described elsewhere [23] with minor changes. All solutions were freshly prepared. 1.0 mL of the reaction mixture contained 28 mM 2-deoxy-D-ribose (dissolved in $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer 10 mM pH 7.4), 500 μL solution of various concentrations of the pimento oil or eugenol, 200 μM FeCl_3 and 1.04 mM EDTA (1:1 v/v), 10 mM H_2O_2 and 1.0 mM ascorbic acid. After an incubation period of 1 h at 37 °C the extent of deoxyribose degradation was measured by the thiobarbituric acid (TBA) reaction. 1.0 mL of TBA (1 % in 50 mM NaOH) and 1.0 mL of trichloroacetic acid (TCA) were added to the reaction mixture and the tubes were heated at 100 °C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank (containing only buffer and deoxyribose). The percentage inhibition was calculated by the formula:

$$I(\%) = 100 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

The IC_{50} value represented the concentration of the compounds that caused 50 % inhibition of radical formation. Quercetin was used as a positive control.

2.6. Assay of superoxide radical anion

Superoxide anions were generated in an enzymatic system (xanthine-xanthine oxidase) and assayed by the reduction of nitroblue tetrazolium. The former comprised a solution of 100 μM xanthine, 60 μM nitroblue tetrazolium in 0.1 M phosphate buffer at pH 7.4 and 0.07 U mL^{-1} xanthine oxidase in a total volume of 1 mL. This mixture was incubated at 25 °C for 10 min and the optical density was recorded at 560 nm against a blank, which did not contain the enzyme [24]. In order to check the inhibitory effect of pimento oil on xanthine oxidase activity, the enzyme was assayed by measuring the formation of uric acid from xanthine [24, 25]. Concentrations of 50 $\mu\text{g}/\text{mL}$ of pimento oil, eugenol, BHT were added to the samples before the enzyme was added. The percentage inhibition of xanthine oxidase was calculated by the formula:

$$I(\%) = 100 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

2.7. Evaluation of antioxidant activity in linoleic acid model system

Linoleic acid emulsions were prepared by mixing 0.285 g of linoleic acid, 0.289 g of Tween 20 as emulsifier and 50 mL phosphate buffer (pH 7.2). The mixture was homogenized for 5 min according to [26]. The antioxidant was added at the final concentrations of 0, 0.002 and 0.005 % wt/vol of pimento oil, BHT 0.01 % was used as control. The mixture was incubated in an oven at 37 °C for 10 d. The course of oxidation was monitored by measuring the conjugated dienes formation (CD) and thiobarbituric acid reactive substances (TBARS). The antioxidant activity at the end of the assay time was expressed for each indicator as reduction percent of peroxidation (RP %) with the control containing no antioxidant (= 0 %).

$$\text{RP \%} = \left[\frac{(\text{peroxidation indicator value w/o antioxidant}) - (\text{peroxidation indicator value with antioxidant})}{\text{peroxidation indicator value w/o antioxidant}} \right] \times 100$$

A higher percentage indicates a higher antioxidative activity.

2.8. Determination of conjugated diene (CD) formation

Aliquots of 0.02 mL were taken at different intervals during incubation. After incubation, 2 mL of methanol in de-ionised water (60 %) were added, and the absorbance of the mixture was measured at 233 nm. The conjugated diene concentration was expressed in mL/mg in each sample. The results were calculated as $\text{CD} = B \times \text{vol}/\text{wt}$; where B is the absorbance reading, vol denotes the volume (mL) of the sample and wt is the mass (mg) of emulsion measured [27].

2.9. Determination of thiobarbituric acid reactive substances

A modified thiobarbituric acid reactive substances (TBARS) method was used to measure the antioxidant activity of the essential oil in terms of inhibition on lipid peroxidation. 0.1 mL of sample was taken every day, from the emulsion, the following were sequentially added: the TBA-TCA solution (20 mm TBA in 15 % TCA). The mixture was heated in a 100 °C water bath for 15 min and cooled at room temperature. After 2 mL of chloroform were added, the mixture was mixed and centrifuged at 2000 rpm for 15 min. The chloroform layer was separated and the absorbance of the supernatant was measured at 532 nm against a blank containing TBA-TCA solution. Malonic aldehyde standard curves were prepared by 1,1,3,3-tetramethoxypropane and TBARS were expressed as mg of malonic aldehyde/kg dry matter [28].

The data obtained at each point for all experiments were the average of three measurements.

2.10. Statistical analysis

The experimental data (in triple repetition) were included in an approximation model through polynomial dependences from fourth order. For all cases the plural correlation coefficient R^2 was determined. The level of the concentration, which corresponds to 50 % of inhibition, was calculated according to this approximated dependence for which R^2 is maximum. The statistical processing of the data was carried out with the special software MATLAB (5.3/6.0).

3. Results and discussions

Using GC and GC-MS 36 constituents of the essential leaf oil of *Pimenta dioica* from Jamaica were found with eugenol (76.02 %), methyl eugenol (7.14 %) and β -caryophyllene (6.47 %) as the main compounds (see *Table 1*). Further components in concentrations higher than 1.00 % (calculated as relative %-peak area of GC with FID and apolar column, mean-value of three measurements) of the pimento leaf oil were identified as α -humulene (1.41 %) and α -selinene (1.04 %).

Results of the investigations of the DPPH radical-scavenging activity are as follows: DPPH is a stable free radical and accepts an electron or a hydrogen radical to become a stable diamagnetic molecule. It is typically used as a substrate to evaluate the antioxidant activity of various antioxidants [29]. *Table 2* shows the concentrations of the essential pimento leaf oil, and of eugenol, rutin, ascorbic acid, BHT and BHA that resulted in a 50 % inhibition of the free radical DPPH (IC_{50}). Following the decrease in antioxidant activities, the components under study were arranged in the order: BHA > eugenol > pimento > ascorbic acid > BHT > rutin. IC_{50} values were approximated with statistical significance $p \leq 0.01$ and with high regression coefficients.

Hydroxyl radical-scavenging activity investigations showed following data: Hydroxyl radicals were generated in a reaction mixture containing ascorbate, hydrogen peroxide and iron III-EDTA at pH 7.4 and measured by their ability to degrade the sugar deoxyribose [23, 30]. The presence of the essential pimento leaf oil in the reaction mixture protected deoxyribose against degradation by eliminating the highly reactive hydroxyl radicals ($OH\cdot$) (*Figure 1*). Results from this study characterized pimento oil as a strong $OH\cdot$ -scavenger in rivalry with 2-deoxy-D-ribose, the effect intensifying with the increase of concentration and reaching as high as 87.27 %, while pimento oil's major component – eugenol, performed 91.20 % in-

Compound*	% ⁺	RI*
cis-3-Hexenol	0.01	860
α -Thujene	0.08	930
α -Pinene	0.15	939
β -Pinene	0.01	979
Myrcene	0.11	991
α -Phellandrene	0.42	1003
p-Cymene	0.51	1025
Limonene	0.12	1027
β -Phellandrene	0.28	1030
1,8-Cineole	0.31	1032
trans- β -Ocimene	0.21	1048
γ -Terpinene	0.15	1059
Terpinolene	0.47	1088
Linalool	0.06	1093
Terpinen-4-ol	0.19	1177
α -Terpineol	0.09	1186
Chavicol	0.12	1248
Eugenol	76.02	1357
β -Elemene	0.24	1390
Methyl eugenol	7.14	1402
Isoeugenol	0.01	1405
α -Gurjunene	0.05	1411
β -Caryophyllene	6.47	1418
γ -Elemene	0.02	1436
Aromadendrene	0.26	1439
α -Humulene	1.41	1454
γ -Gurjunene	0.07	1477
Alloaromadendrene	0.26	1481
β -Selinene	0.58	1490
α -Selinene	1.04	1497
α -(E,E)-Farnesene	0.14	1508
γ -Cadinene	0.18	1513
δ -Cadinene	0.37	1522
α -Cadinene	0.08	1538
trans-Nerolidol	0.11	1561
Viridiflorol	0.04	1582

Tab. 1: Chemical composition of the essential oil of *Pimenta dioica* leaves from Jamaica.

in order of their retention-times

+ relative %-peak area of GC-FID analyses using an apolar column

* retention indices using an apolar OV-5-type column

Test compound	IC ₅₀ *	R ² **
Pimento oil	1.79	0.998
Eugenol	1.26	0.999
Rutin	14.65	0.991
Ascorbic acid	4.20	0.994
Butylated hydroxytoluene (BHT)	4.47	0.998
Butylated hydroxyanisole (BHA)	1.12	0.996

* Concentration (μ g/mL) for a 50 % inhibition
 ** R^2 – correlation coefficient

Tab. 2: Effect of the test compounds in the DPPH assay

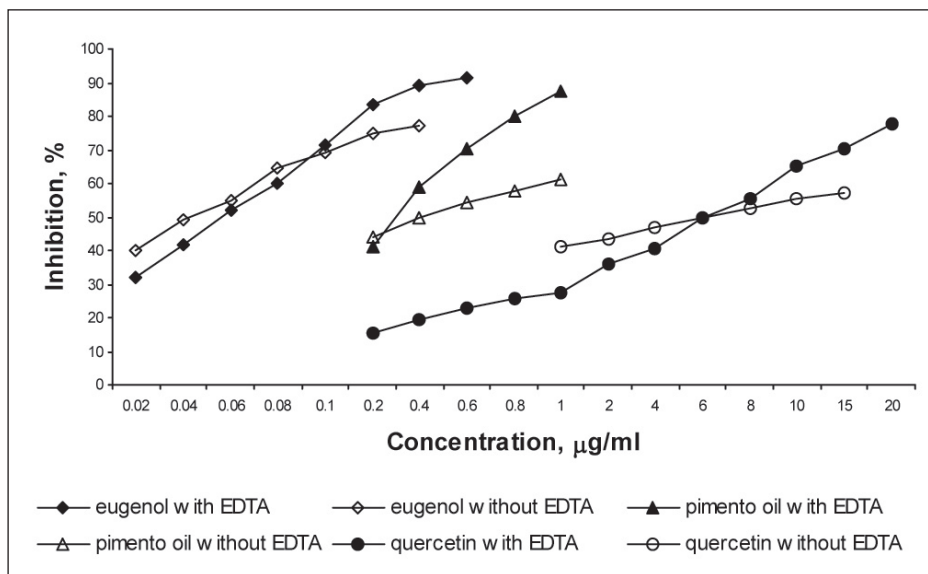


Fig. 1: Metal chelating activity of pimento oil, eugenol and quercetin on deoxyribose degradation by OH•

inhibition of OH• at 0.6 µg/mL concentration (Figure 1). The antioxidant activity of quercetin – 77.8 % at 20 µg/mL was substantially weaker. The three studied antioxidants were arranged by their antioxidant effect (expressed as IC₅₀), in descending order, as follows: eugenol - 0.06 µg/mL (R² = 0.989), pimento oil - 0.29 µg/mL (R² = 0.999), quercetin - 4.61 µg/mL (R² = 0.834). The same analytical method could also be applied for studying the inhibitory power of essential *P. dioica* oil against the metal ion-dependant generation of OH•, and not only for assaying its ability to capture already formed radicals. When Fe³⁺-ions are added to the reaction mixture as FeCl₃ instead of EDTA complex, some of the iron ions form complexes with deoxyribose. The Fe³⁺ may be subsequently reduced by ascorbate to Fe²⁺, which in turn may remain bound to deoxyribose and further react with H₂O₂. The reaction generates the necessary OH•, which immediately triggers the degradation of deoxyribose in a site-specific manner. Only molecules that are able to chelate iron ions and make them inactive may inhibit the degradation of deoxyribose.

Figure 1 (without EDTA) shows pimento oil, eugenol and quercetin as scavengers of OH• and manifests chelative properties, most expressive in the case of eugenol. Like most radicals, OH• can be neutralized by a hydrogen atom. The capture of OH• by pimento oil is attributed to the hydrogen-donating capacity of eugenol, which is found in the essential oil of *Pimenta dioica* leaves at a high concentration (76 %). The evaluation of pimento oil's scavenging activity with respect to the two radicals in concern – DPPH and OH•, showed that it was a more effective scavenger of OH•-radicals – the respective IC₅₀ values were 0.29 µg/ml for OH• against 1.79 µg/ml for DPPH.

The superoxide anion scavenging activity results can be discussed as follows: Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals [31]. Superoxide anions indirectly initiate lipid oxidation serving as precursors of singlet oxygen and hydroxyl radicals [24]. Xanthine-xanthine oxidase is the system, which is often used as a generator of superoxide radicals. The results from this study showed that the essential *P. dioica* leaf oil, eugenol and BHT had inhibitory effect on enzyme activity (Table 3).

Compound tested	A _{295 nm}	% inhibition
Control	0.310	-
Pimento oil (50 µg/mL)	0.078	74.83
Eugenol (50 µg/mL)	0.120	61.29
BHT (50 µg/mL)	0.096	69.03

Tab. 3: Effect of pimento oil, eugenol and BHT on xanthine oxidase activity

At 50 µg/mL concentration, the xanthine oxidase inhibition power of the studied components arranged them in the following order: pimento oil > BHT > eugenol. By inhibiting xanthine oxidase activity pimento oil cut down the generation of superoxide radicals. The superoxide anion scavenging activity of pimento oil, eugenol and BHT is presented in Table 4.

Compound tested	A _{560 nm}	% inhibition
Control	0.295	-
Pimento oil (50 µg/mL)	0.012	95.93
Eugenol (50 µg/mL)	0.051	82.71
BHT (50 µg/mL)	0.082	72.20

Tab. 4: Superoxide anion scavenging activity of pimento oil, eugenol and BHT

The decrease in the absorption at 560 nm suggested superoxide radicals' scavenging, being most significant when pimento oil was added to the reaction mixture – 95.93 %. Superoxide dismutase 100 U/mL with inhibiting effect 77.8 % was used as a standard. The results achieved in the study revealed two ways of influence of pimento oil and eugenol on xanthine-xanthine oxidase function – they acted as xanthine

oxidase inhibitors and superoxide scavengers, thus confirming the interpretation of [32].

The evaluation of antioxidant activity in a linoleic acid model system was as follows: For the task of evaluating the inhibitory effect of pimento oil on lipid peroxidation, a model system of linoleic acid emulsion was applied. The antioxidant capacity was estimated both at the early stages of linoleic acid autoxidation and later, after the emergence of secondary oxidation products, expressed as malonic aldehyde content. Two indicators were referred to, corresponding to a different degree of lipid peroxidation – conjugated diene formation and TBARS. The effect of the essential pimento leaf oil on lipid peroxidation was assayed at body temperature, 37 °C.

For the determination of conjugated diene (CD) formation the following results were found: An increase of pimento oil concentration from 0.002 to 0.005 % caused a corresponding increase of the inhibitory effect of the component on lipid peroxidation, for the entire study period (Figure 2A). Linoleic acid peroxidation leading to CD formation was most intensive on the fifth day of acid incubation. At this stage of incubation, with 0.005 % pimento oil added, significant inhibition of the process was achieved - 53.32 %, compared to the 35.21 % inhibition realized with BHT. The most effective inhibition of the process was observed on the eighth day of the study, when 0.005 % concentration of essential *P. dioica* leaf oil lead to an inhibitory effect greater than that of 0.01 % BHT - 65.47 % and 61.90 %, for the two substances respectively.

Finally, the determination of thiobarbituric acid reactive substances can be discussed as follows: With the second reference used for estimating the presence of sec-

ondary derivatives from linoleic acid oxidation – TBARS – maximum accumulation of malonic aldehyde was found, as the case above, on the fifth day of linoleic acid incubation (Figure 2B), suggesting therefore that the process ran in a manner nearly analogous to the formation of conjugated dienes. The strongest antioxidant action of the compounds under study was exercised on the eighth day of the study, with the inhibitory effect of essential pimento leaf oil being almost equal to that of the synthetic standard BHT; moreover, the effect was executed at a concentration twice as low than that of BHT (0.005 %). The inhibition of lipid peroxidation by pimento oil obtained 72.98 % compared to 76.47 % inhibition realized by BHT.

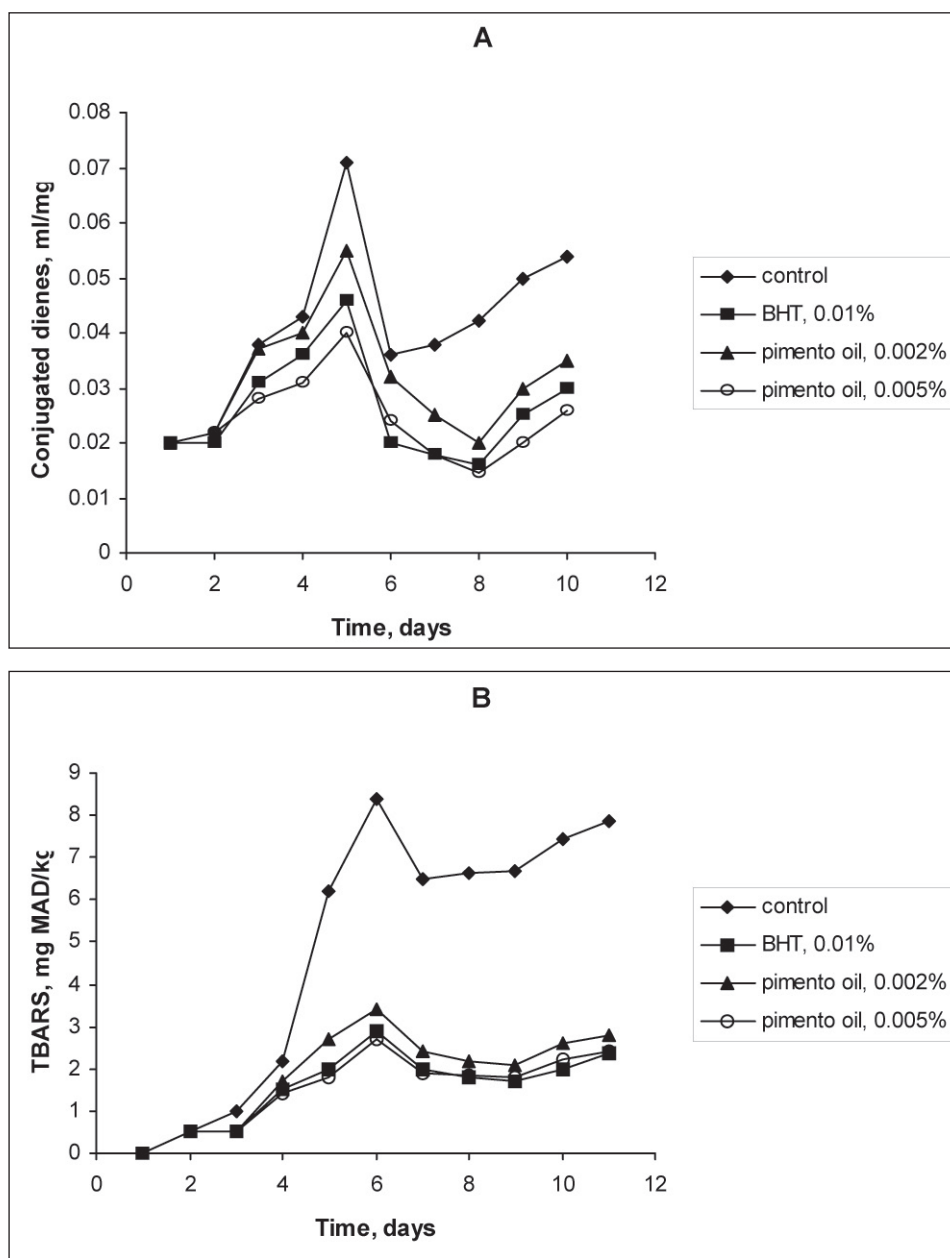


Fig. 2: Effect of pimento oil on (A) conjugated dienes and (B) TBARS in a linoleic acid/water emulsion system.

Therefore, we can conclude for this investigation of chemical composition and antioxidative properties of the essential leaf oil of *Pimenta dioica* from Jamaica that eugenol, methyl eugenol and β -caryophyllene were found as main components with this capacity.

Pimento oil manifested greater antiradical activity with respect to OH• in comparison with DPPH radicals, attested by the respective IC₅₀ values. The inhibitory capacity of pimento oil on OH• exceeded that of quercetin. The oil was a Fe³⁺-chelator, too, thus preventing the initiation of hydroxyl radicals.

Pimento oil inhibited xanthine oxidase activity, which caused a decrease of the generation of superoxide radicals; moreover, there was a second mechanism of action involved – the scavenging of superoxide radicals. Pimento oil was capable of an effective inhibition of both the conjugated diene formation and the generation of secondary products from lipid peroxidation, carried out at a concentration twice lower than that of the reference antioxidant BHT. The use of some methods to determine the antioxidative properties of medicinal plant essential oils confirms the findings that antioxidative capacity detected by only a single method should be interpreted with some caution [33].

The established antioxidant properties of pimento oil thus broaden the scope for its implementation in food industry and medicine.

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Future Trends in Phytochemistry – A Young Scientists Symposium

Unter der Organisation der Phytochemischen Gesellschaft von Europa (PSE) und der Palacký Universität fand das PSE Young Scientists Symposium „Future Trends in Phytochemistry“ in Olmütz (Tschechische Republik) von 28. Juni bis 1. Juli 2006 statt.

Dem Organisationskomitee um *Miroslav Strnad* (PSE) und Labor für Wachstumsregulatoren der Palacký Universität Olmütz) gelang es, ein interessantes Konferenzprogramm, bestehend aus wissenschaftlichen Vorträgen, Kurzpräsentationen und Posterpräsentationen, zusammenzustellen.

Der Schwerpunkt dieses Symposiums lag in der Präsentation neuer Erkenntnisse über Pflanzeninhaltsstoffe sowie deren Funktion, Biosynthese, physiologischen und pathologischen Effekte auf Pflanzen und Tiere und deren Anwendung in Landwirtschaft und Pharmakologie.

Zum Kennenlernen der Symposiumsteilnehmer diente ein Stadtspaziergang zu den historischen Plätzen von Olmütz, die teilweise in die Liste des „UNESCO Weltkulturerbes“ aufgenommen worden sind.

Nach der offiziellen Eröffnung des Symposiums durch *Miroslav Strnad* begann das Symposium mit thematisch sehr verschiedenen Referaten. *Zdeněk Dvořák* (Tschechische Republik) berichtete über „Past, present and future of phytochemistry in Olomouc-Šantavý lecture“, *Maïke Petersen* (Deutschland) über „How to detect enzymes of plant secondary metabolism“, *Iryna Gerasymenko* (Ukraine) über „Metabolic Engineering of *Rauvolfia serpentina*: Approaches to Increase Biodiversity of Indole Alkaloids“, *Ürün Bayindir* (Deutschland) über „Hinokinin Biosynthesis“, *Adinpunya Mitra* (Indien) über „Elucidating the metabolic route to p-hydroxybenzoic acid formation in hairy roots of *Daucus carota* L.“, *Soad Bayoumi* (GB) über „Secondary Metabolite Production and Genetic Analysis in Cassava“, *Jordan Zjawiony* (USA) über „From Phytochemistry to Neuropharmacology: The Story of Salvinorin A“, *Min Xu* (China) über „Chemical composition of *Gentiana rigescens*: Isolation, Identification and HPLC Analysis“ und *Sheila Maragesi* (Belgien) über „Phytochemical Investigation of *Baleria eranthemoides*“.

Am darauffolgenden Kongresstag standen folgende Vorträge auf dem Programm: *Anne Osbourne* (GB) beleuchtete „Plant-derived natural products – Synthesis, function and the basis of metabolic diversity“, *Ying-Jun Zhang* (China) erörterte „New antifungal steroidal saponines from *Smilacina atropurpurea*“, *Pattarawadee Sumthong* (Niederlande) sprach über „Antifungal quinones from Teak (*Tectona grandis* L.f.)“ und *Martina Macková* (Tschechische Republik) über „Consequences of plant-microbe metabolic interactions influencing fate of xenobiotics in the environment“.

Die weiteren Vorträge dieses Vormittags standen unter dem Titel „Hops, Beer and Phytochemicals“. *Pavel Dostálek* (Tschechische Republik) berichtete in seiner Präsentation „Hop-brewing raw material and source of bioactive compounds for beer“ über die antioxidativen Effekte von polyphenolischen Substanzen aus Hopfen-Wasser-Extrakten. In klinischen Studien wurde sowohl die antioxidative Kapazität (TAC, total antioxidant capacity) als auch der Gesamtphenolgehalt im Blutplasma nach dem Genuss von Lager-Bier und alkoholfreiem Bier ermittelt. Es wurde festgestellt, dass die antioxidative Kapazität des Blutplasmas eine Stunde nach dem Biergenuss um 10 % angestiegen war und diese mit dem Gesamtphenolgehalt korreliert.

Die Arbeit von *Jelle De Keukeleire* (Belgien) „Identification of genes involved in the biosynthesis of pharmacologically active prenylchalcones present in hops and beer“ beschäftigt sich mit den Prenylchalconen wie Desmethylxanthohumol, einem Pro-Östrogen, das während des Bierbrauprozesses in das Phytoöstrogen 8-Prenylarginin (8-PN) umgewandelt wird, sowie Xanthohumol und deren Biosynthese in Hopfen. Diese Polyphenole sind bekannt für ihre biologischen Aktivitäten: Sie mindern Wechselbeschwerden, hemmen die Umwandlung von Diacylglycerol in Triacylglycerol und reduzieren somit kardiovaskuläre Erkrankungen. Die geringe Verfügbarkeit dieser Substanzen im Hopfen limitiert die Möglichkeit, diese für medizinische Zwecke zu verwerten. Die Charakterisierung regulatorischer Elemente und von Genen im Hopfen, welche für die Entstehung von Prenylchalconen (Desmethylxanthohumol und Xanthohumol)