JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Eugenol derivatives as potential anti-oxidants: is phenolic hydroxyl necessary to obtain an effect?

Marília d' Avila Farias[a], Pathise Souto Oliveira[a], Filipe S. Pereira Dutra[c], Thiely Jacobsen Fernandes[c], Claudio M. P. de Pereira[a], Simone Quintana de Oliveira[d], Francieli Moro Stefanello[a], Claiton Leonetti Lencina[c], Alethéa Gatto Barschak[a,b]

[a]Programa de Pós-Graduação em Bioquímica e Bioprospecção, UFPel, Campus Universitário s/n, [b]Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, [c]Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, RS, and [d]Centro de Ciências da Saúde, Departamento de Ciências Farmacêuticas, UFSC, Campus Universitário, Florianópolis, SC, Brazil

Keywords
anti-oxidant activity; eugenol; eugenol derivatives; phenolic compounds

Correspondence
Alethéa Gatto Barschak, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 254, Porto Alegre, CEP: 90050-170, RS, Brazil.
E-mail: aletheagatto@gmail.com

Received July 13, 2013
Accepted November 16, 2013

doi: 10.1111/jphp.12197

Abstract

Objectives Eugenol, obtained from clove oil (*Eugenia caryophyllata*), possess several biological activities. It is anti-inflammatory, analgesic, anaesthesic, antipyretic, antiplatelet, anti-anaphylactic, anticonvulsant, anti-oxidant, antibacterial, antidepressant, antifungal and antiviral. The anti-oxidant activity of eugenol have already been proven. From this perspective testing, a series of planned structural derivatives of eugenol were screened to perform structural optimization and consequent increase of the potency of these biological activities.

Methods In an attempt to increase structural variability, 16 compounds were synthesized by acylation and alkylation of the phenolic hydroxyl group. Anti-oxidant activity capacity was based on the capture of DPPH radical (2,2-diphenyl-1-picrylhydrazyl), ABTS radical 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), measure of TBARS (thiobarbituric acid-reactive species), total sulfhydryl and carbonyl content (eugenol derivatives final concentrations range from 50 to 200 μM).

Key findings Four derivatives presented an efficient concentration to decrease 50% of the DPPH radical (EC_{50}) < 100 μM, which has a good potential as a free-radical scavenger. Three of these compounds also showed reduction of ABTS radical. Eugenol derivatives presenting alkyl or aryl (alkylic or arylic) groups substituting hydroxyl 1 of eugenol were effective in reducing lipid peroxidation, protein oxidative damage by carbonyl formation and increase total thiol content in cerebral cortex homogenates. In liver, the eugenol derivatives evaluated had no effect.

Conclusions Our results suggest that these molecules are promising anti-oxidants agents.

Introduction

Eugenol (4-allyl-2-metoxy-phenol) is a phenolic compound and the main component of clove oil (*Eugenia caryophyllata*).[1,2] Eugenol is a natural pharmacologically active aromatic substance present in essential oils of several plants[3] and known for its aroma and medicinal values.[4] Currently, the most common way to obtain this product is extraction by vapour drag of flower buttons and floral stems of some plants belonging to the family *Myrtaceae*.[5,6]

Eugenol already has several proven biological activity. They include anti-inflammatory,[1,7–11] analgesic,[1,10,12] anaesthesic,[13] antipyretic,[8,10] antiplatelet,[10] anti-anaphylactic,[10] anticonvulsant,[1] anti-oxidant,[1,5,9,11,12] antibacterial,[1,5,8,11,12] antidepressant,[12] antifungal[1,6] and antiviral[6] activity. In traditional medicine, eugenol has been used as an antispasmodic in gastrointestinal disorders[6,8,9] without mutagenic and carcinogenic effects.[1,9,12] Furthermore, there is evidence of its hepatoprotective effect.[6,9]

Eugenol can prevent lipid peroxidation in the early stages.[14] Several studies showed the anti-oxidant capacity of eugenol and its derivatives, such as isoeugenol to inhibit the lipid peroxidation induced by reactive oxygen species. It likewise inhibits the formation of the superoxide radical in the xanthine-xanthine oxidase system, the generation of hydroxyl radical, preventing the oxidation of Fe^{2+} in the Haber-Weiss and Fenton reaction.[15,16] A study of eugenol

structure activity revealed that in addition to the phenolic ring, the side chain has an important role in anti-oxidant activity.[2,5]

It is known that cellular redox state is a consequence of the balance between the levels of oxidizing and reducing agents, and endogenous anti-oxidants. Reactive species are kept at physiological levels by anti-oxidant defence systems. Anti-oxidants are endogenous or exogenous substances that reduce the formation of reactive species or react promoting their inactivation. To prevent cellular damage, which can be caused by the presence of these species, the organism has enzymatic and non-enzymatic anti-oxidant defences.[17,18]

Anti-oxidant therapies have emerged as alternatives for treating chronic degenerative diseases including cancer, inflammation, cardiovascular diseases and neurodegenerative diseases (such as Parkinson and Alzheimer, multiple sclerosis), and thus, biological research aimed at anti-oxidants and free radicals have produced promising results with regard to new therapeutic approaches.[18] Furthermore, it has been shown that anti-oxidants may be involved in signalling pathways and cellular responses, and that many anti-inflammatory agents also present anti-oxidant activity.[19]

The technological advances that have contributed to the search for new compounds involve the discovery of new molecular tools and evolution of analytical techniques, purification and organic synthesis, resulting in more effective active or less toxic substances, which can be used as prototypes of drugs with pharmacological activity similar to or larger than the originals.[20]

The anti-oxidant activity of eugenol has been already confirmed. Thus, the attempt at optimization and consequent increase in the biological activity of eugenol requires knowledge of its mechanism of action, its therapeutic targets and its pharmacophoric groups. Hence, testing a range of planned structural derivatives of eugenol helped increase this knowledge.

The aim of this study was to perform structural changes in eugenol to obtain compounds and evaluate their anti-oxidant capacity and radical scavenging effect. The radical scavenging activity was evaluated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) scavenging tests; anti-oxidant activity was assessed by thiobarbituric acid-reactive species (TBARS) (measure of lipid peroxidation), and carbonyl and thiol content (parameters of protein oxidation).

Materials and Methods

All the reagents were used as purchased from commercial suppliers without further purification, and solvents were freshly distilled. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Brucker Advance III instrument (400 MHz) and AC200 (200 MHz). Trimethylsilane was used as internal standard for ^{1}H NMR and $CDCl_3$ for ^{13}C NMR.

Chemical reactions to synthesis of compounds was monitored on a thin layer chromatography (TLC) and Shimadzu GC-MS-2010SE Gas Chromatograph equipped with an Rtx-Wax polyethylene glycol capillary column (0.25 mm × 30 m) and a mass spectrometry detector (Shimadzu, Kyoto, Japan).

Chemistry

Chemical data on synthesized compounds

The structures of eugenol derivatives were confirmed by ^{1}H NMR, ^{13}C NMR, GC-MS spectral data (see Appendix).

Synthesis of eugenol derivatives 11–19

Eugenol (10 mmol) was dissolved in the solution of NaOH (12 mmol). The solution was placed in a flat-bottomed flask, and the chloride derivative (10 mmol) was added. The mixture was stirred at room temperature on agitation for 30 min. After consumption of the starting material, the reaction medium was extracted with dichloromethane (3 × 10 ml) and washed with Na_2CO_3 5%. The solvent was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure; the pure products were obtained by recrystallization from ethanol.

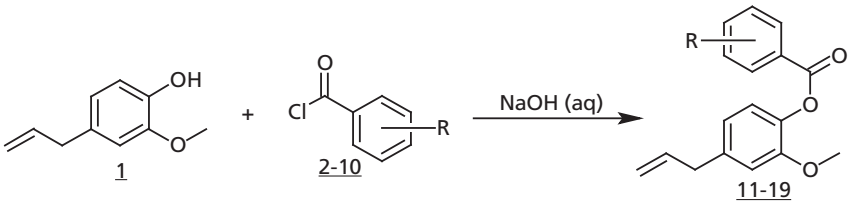
Synthesis of eugenol derivatives 27–33

In one flask, K_2CO_3 (20 mmol) was added, dissolved dried acetone (40 ml) and eugenol (10 mmol), followed by the respective addition of alkyl chloride (10 mmol) and stirred at reflux for 2 h. After complete consumption of the starting material (TLC), the reaction was cooled, filtered and washed with acetone (20 ml). The organic solvent was evaporated under vacuum. The final product was resuspended in dichloromethane (50 ml) and washed with H_2O (2 × 25 ml). The organic extract was dried under anhydrous Na_2SO_4, and the residual solvent was evaporated in vacuum. Pure product was obtained by column (*n*-Hexane: AcOEt gradient) (Schemes 1 and 2).

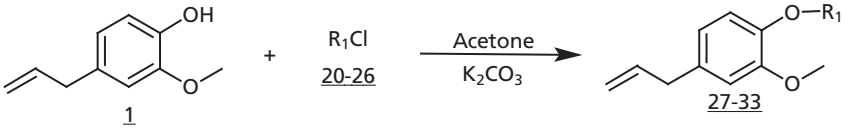
Anti-oxidant activity

2,2-diphenyl-1-picryl-hydrazyl and 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid radical scavenging activity

The DPPH free radical scavenging assay was performed in accordance with the procedure reported by Brand-Williams *et al.*[21] with some modifications. The antiradical activity



Scheme 1 Reaction of obtaining derivatives 11–19.



Scheme 2 Reaction of obtaining derivatives 27–33.

was defined as the amount of anti-oxidant necessary to decrease the initial DPPH concentration by 50% (EC50).

The ABTS radical scavenging activity was evaluated as described by Re *et al.*[22] with some modifications. Trolox was used as standard at concentrations 200, 150, 100, 50 and 10 μM. The percentage of radical scavenging was calculated as trolox equivalent anti-oxidant capacity (TEAC).

Eugenol derivatives were dissolved in dimethyl sulfoxide (DMSO), and final concentrations were 120, 60, 30 and 15 μM for DPPH assay and for ABTS radical scavenging activity.

Subjects and reagents

Male Wistar rats were obtained from the Central Animal House of Universidade Federal de Pelotas, Pelotas, RS. The animals were maintained on a 12/12 h light/dark cycle in an air-conditioned constant temperature (22 ± 1°C) colony room. Rats had free access to a 20% (w/w) protein commercial chow and water. The Guide for the Care and Use of Laboratory Animals[23] was followed in all experiments. The study was approved by the Ethics Committee of Universidade Federal de Pelotas, Brazil (CEEA 10263). All chemicals were purchased from Sigma (St. Louis, MO, USA).

Tissue and homogenate preparation

Thirty-day-old Wistar rats were sacrificed by decapitation without anaesthesia. The cerebral cortex and liver were immediately dissected out and homogenized in 10 volumes (1 : 10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 750*g* for 10 min at 4°C, the pellet was discarded and the supernatant was immediately separated and used for the biochemical measurements.

Thirty microlitres of hydrogen peroxide (5 mM) and ferrous sulfate (20 μM), which are responsible for inducing oxidative damage (TBARS assay, total sulfhydryl content and carbonyl assay), were added to 440 μl of cerebral cortex and liver homogenates. Different concentrations of eugenol derivatives (final concentrations were 50, 100 and 200 μM dissolved in DMSO and diluted in distilled water) were added to the mixture and incubated for 1 h at 37°C with inducing damage. After that, an aliquot of tissue homogenates were used in the different assays.

Butylated hydroxytoluene (BHT) 25 μM was used as standard anti-oxidant.[4]

Thiobarbituric acid-reactive species assay

TBARS, an index of lipid peroxidation, were determined according to the method described by Buege and Aust[24] with some modifications. Trichloroacetic acid and thiobarbituric acid were added to samples and incubated for 25 min at 100°C. A calibration curve was performed using 1,1,3,3-tetramethoxypropane following the same treatment as that of the samples. The absorbance was read at 535 mm, the results were reported as nmol TBARS/mg protein.

Total sulfhydryl content

Total thiol content was determined using the 5,5′-dithiobis-2-nitrobenzoic acid method, as described by Aksenov and Markesbery[25] with some modifications. The amount of 5-thio-2-nitrobenzoic acid (TNB) formed was determined at 412 nm. The results were reported as nmol of TNB/mg protein.

Carbonyl assay

Oxidatively modified proteins present an enhancement of carbonyl content.[26] In this study, protein carbonyl was

assayed by the method of Reznick and Packer,[27] which is based on the reaction of protein carbonyls with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm. Results were reported as nmol carbonyl/mg protein.

Protein determination

Protein was determined by the Lowry *et al*. method[28] using bovine serum albumin as standard.

Statistical analysis

Data were expressed as mean ± standard deviation. The comparisons of means were analysed by one-way analysis of variance followed by the Duncan test when the F value was significant. A value of $P < 0.05$ was considered to be significant.

Results

Good yields of the products were obtained after short reaction times using simple synthetic routes. At first, the anti-oxidant activity of 16 eugenol derivatives was evaluated by DPPH and ABTS assay. The eugenol derivatives 28, 30, 31 and 32 showed an efficient concentration to decrease DPPH radical with values of EC50 of 60, 43, 19 and 59 μM, respectively. Regarding the ABTS radical test, only compounds 28, 30 and 31 showed a TEAC higher than 0.50 mM (Table 1).

These four compounds, 28, 30, 31 and 32, were tested on lipid peroxidation in the brain and liver of rats. As shown in Figure 1, these eugenol derivatives were effective in protecting against lipid peroxidation induced by hydrogen peroxide and ferrous sulfate in cerebral cortex homogenate. All compounds reduce TBARS levels at concentrations from 50 to 200 μM 28 ($F(5,26) = 103.39$; $P < 0.01$)); 30 ($F(5,30) = 89.99$; $P < 0.01$); 31 ($F(5,42) = 263.32$; $P < 0.01$) and 32 ($F(5,36) = 46.09$; $P < 0.01$) compared with induced control. In liver (Table 2), 31 ($F(5,36) = 32.11$; $P < 0.001$) and 32 ($F(5,36) = 34.26$; $P < 0.001$) reduce lipid peroxidation at concentrations from 50 to 200 μM, while 28 reduced TBARS at 100 and 200 μM ($F(5,30) = 20.74$; $P < 0.001$) compared with induced control.

We tested the effect of eugenol derivatives on total thiol content in the cerebral cortex and liver of rats. Figure 2 shows that the compounds 30 and 32 significantly modify sulfhydryl levels in the cerebral cortex of rats. Compound 30 ($F(5,27) = 9.86$; $P < 0.01$) increased at concentrations from 50 to 200 μM, while 32 ($F(5,26) = 10.80$; $P < 0.01$) increased thiol content at concentrations of 100 and 200 μM compared with induced control. In the liver of rats, tested compounds did not modify the total thiol content compared with induced control (Table 2).

We also evaluated the effect of eugenol derivatives on protein oxidative damage by carbonyl formation. The compounds reduced carbonyl content at concentrations from 50 to 200 μM compared with induced control (Figure 3) 28 ($F(5,36) = 8.55$; $P < 0.01$); 30 ($F(5,19) = 5.98$; $P < 0.01$) and 31 ($F(5,34) = 5.14$; $P < 0.01$). However, compound 32 reduced carbonyl content only at 200 μM ($F(5,33) = 12.96$; $P < 0.01$). In liver (Table 2), compound 32 ($F(5,23) = 10.37$; $P < 0.001$) significantly reduced carbonyl formation at concentrations from 50 to 200 μM compared with induced control.

Anti-oxidant and radical scavenging activity of the compounds were compared with BHT (standard anti-oxidant) as positive control.

Discussion

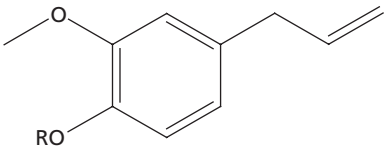
The main structural characteristic responsible for the anti-oxidant activity of phenolic derivatives, such as eugenol, is the presence of hydroxyl that can donate hydrogen atoms, interrupting chain propagation of the oxidative process.[4,7,29] Thus, these compounds play an important role donating hydrogen atoms, suppressing ROS formation, acting as metal chelants (which catalyse the Fenton reaction) or also as enzyme inhibitors/activators.[1,30,31]

In this study, we synthesized eugenol derivatives attempting to find compounds with anti-oxidant action. Some of these compounds have already been tested for other activity such as 15-lipoxygenase inhibitors and herbicidal agents.[32] Sixteen compounds were synthetized with different modifications in the hydroxyl group at 1-position of eugenol. The synthesis was divided into two blocks. These conferred different physical-chemical characteristics on the lateral chain of the compounds obtained.

There are important alterations in position 1 of the eugenol. Differences in size and in the nature of the carbon chain (aliphatic or aromatic) cause stereo-electronic variations that help identify characteristics needed to increment the activity of these molecules. It is known that the pharmacological activity of eugenol are related to its chemical and structural characteristics because different substitutions of the ring can influence the biological activity.[2] As to anti-oxidant activity, eugenol has the property of neutralizing the alkyl radicals, peroxy and superoxide because of the difference in the reduction potential between the radical and the phenol.[33] Insaturations are very valuable for the eugenol activity towards free radicals.

There are studies proving that the phenolic compounds are more potent anti-oxidants than vitamins E, C and carotenoids. For a compound to be classified as an anti-oxidant, several methods have been used to evaluate the anti-oxidant activity *in vitro*.[34]

Table 1 Radical scavenging of eugenol derivatives



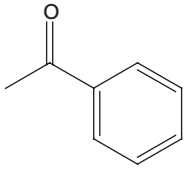
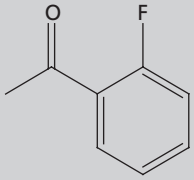
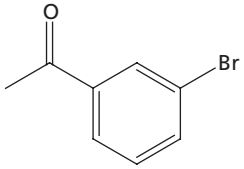
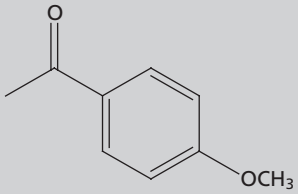
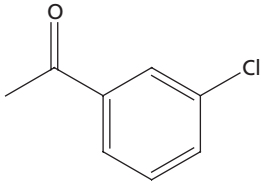
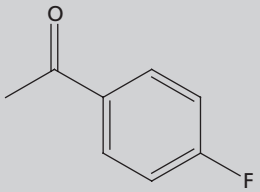
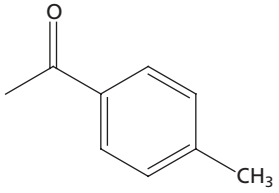
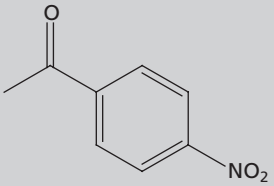
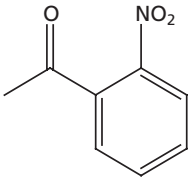
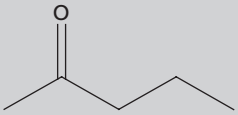
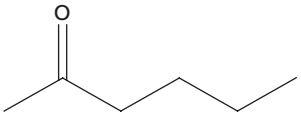
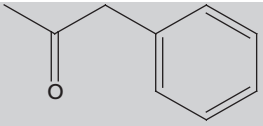
Compound	R	DPPH EC50 (μM)	$ABTS^+$ (TEAC)
Eugenol	H	72	6.8
11		>100	<0.50
12		>100	<0.50
13		>100	<0.50
14		>100	<0.50
15		>100	<0.50
16		>100	<0.50
17		>100	<0.50

Table 1 Continued

Compound	R	DPPH EC50 (μM)	ABTS^{+} (TEAC)
18		>100	<0.50
19		>100	<0.50
27		>100	<0.50
28		60	0.77
29		>100	<0 .50
30	-CH_2CH_2Ph	43	2.10
31	-$CH_2CH_2CH_2Ph$	19	12.44
32	-$CH_2CH_2CH_3$	59	<0.50
33	-$CH_2CH_2CH_2CH_3$	>100	<0.50

ABTS, 2,2′-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picryl-hydrazyl; EC50, efficient concentration to decrease 50% of the DPPH radical; TEAC, trolox equivalent anti-oxidant capacity.

The anti-oxidant capacity of the different eugenol derivates was screened based on the capture of radical DPPH and of radical ABTS. The latter methods are widely used because they are simple, quick, sensitive methodologies.[35]

Four compounds of the 16 eugenol derivatives tested presented EC50 < 100 μM. Derivatives 28, 30, 31 and 32 were promising as they presented a smaller EC50 than eugenol, and with an EC50 lower than standard anti-oxidants such as BHT (EC50 = 60.25 μM).

The DPPH test is an indirect method to determine anti-oxidant activity. This method is based on the measure of the reducing ability of anti-oxidants when dealing with the DPPH radical, usually expressed as EC50. The lower the EC50, the greater the radical scavenging efficiency. One of the limitations of this methodology is that there is no DPPH and similar compounds in biological systems.[36] To complete the screening of the anti-oxidant activity, the TEAC was determined. This method is based on the compound capacity to neutralize the ABTS radical.[22,37] In this test, 28, 30, 31, presented TEAC > 0.50, but only 31 presented TEAC higher than eugenol.

Lipid peroxidation and protein oxidation are two important indicators of oxidative damage of macromolecules induced by ROS.[38] Hence, the compounds that presented activity in the screening tests (28, 30, 31, 32) were evaluated for their anti-oxidant capacity on TBARS, and carbonyl and total thiol content in rat brain and liver. The eugenol derivatives obtained presented significant results in the TBARS test, proving similar to eugenol at the different concentrations tested (50–200 μM) in cerebral cortex of 30-day-old rats, reducing the damaged provoked by the reactive species. But in the liver of these same animals, only a few compounds presented significant results in lipoperoxidation.

These results are due to the fact that the brain is a lipid-rich organ with a low level of anti-oxidant defences. Moreover, it has auto-oxidable neurotransmitters,

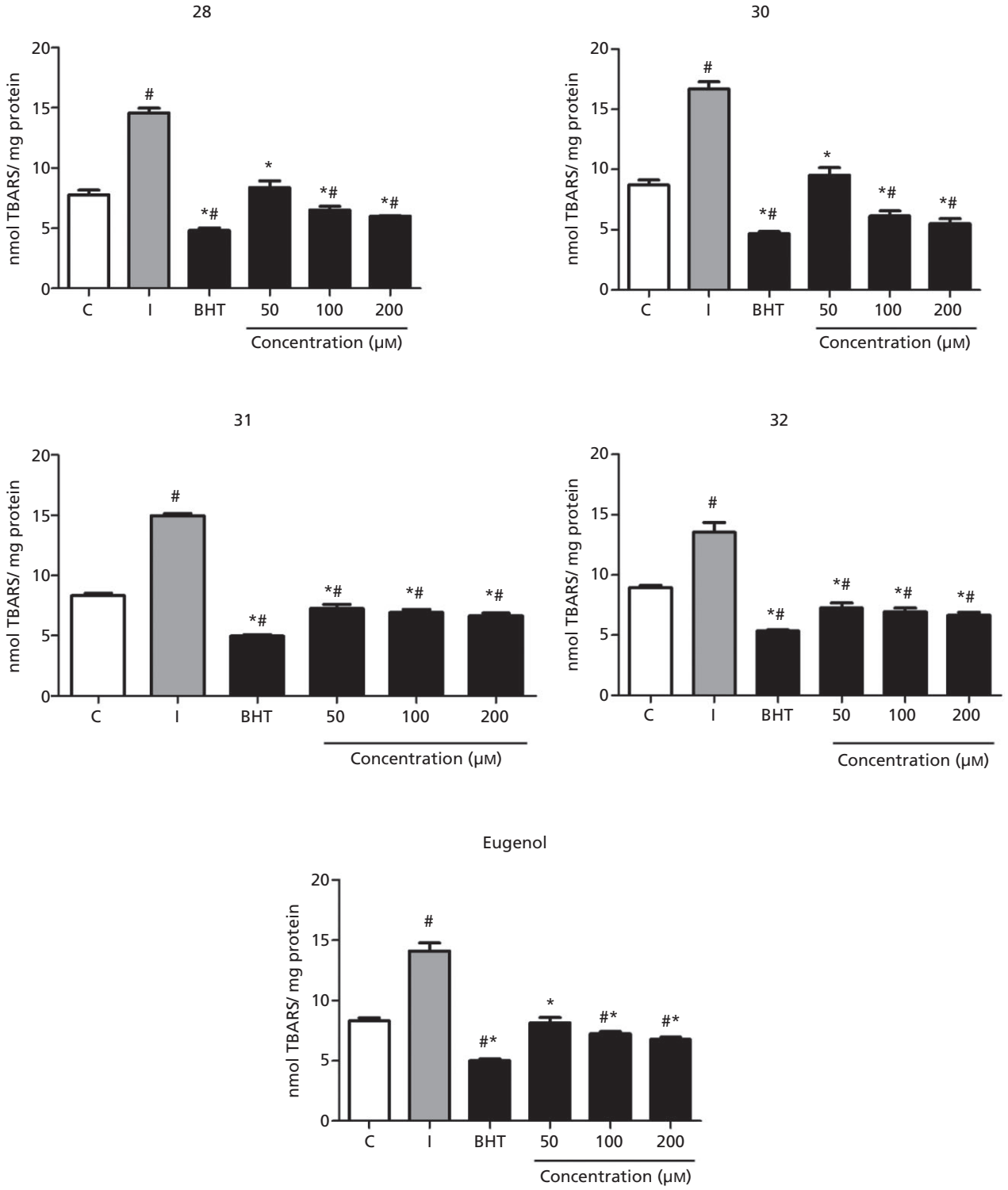


Figure 1 Effect of eugenol derivatives on thiobarbituric acid reactive species in the cerebral cortex of rats. Data are expressed as mean ± standard deviation ($n = 5–15$). The levels of thiobarbituric acid reactive species were reported as nmol thiobarbituric acid reactive species per mg protein. #Significantly different from control ($P < 0.05$). *Significantly different from induced control ($P < 0.05$). Differences were determined by one-way analysis of variance followed by Duncan's *post hoc*. C, control; I, induced control; BHT, butylated hydroxytoluene.

Table 2 Effect of eugenol derivatives on thiobarbituric acid reactive species (TBARS), carbonyl and total thiol content in liver of rats

	Eugenol Derivatives	Control	Induced control	BHT	Concentration (μM) 50	100	200
TBARS	Eugenol	1.86 ± 0.11	2.33 ± 0.19†	2.13 ± 0.17*†	2.25 ± 0.13†	2.21 ± 0.10†	2.19 ± 0.13*†
	28	1.93 ± 0.06	2.80 ± 0.26†	2.51 ± 0.16*†	2.62 ± 0.17†	2.45 ± 0.09*†	2.50 ± 0.12*†
	30	1.85 ± 0.73	2.25 ± 0.14†	2.07 ± 0.09*†	2.50 ± 0.17*†	2.48 ± 0.09*†	2.46 ± 0.15*†
	31	1.80 ± 0.013	2.69 ± 0.16†	2.33 ± 0.20*†	2.49 ± 0.12*†	2.43 ± 0.11*†	2.51 ± 0.14*†
	32	1.66 ± 0.08	2.42 ± 0.11†	2.10 ± 0.15*†	2.12 ± 0.09*	1.84 ± 0.14*†	1.80 ± 0.18*
Total thiol	Eugenol	69.58 ± 16.0	44.60 ± 8.23†	44.93 ± 9.04†	49.00 ± 6.74†	48.27 ± 8.98†	44.68 ± 12.36†
	28	58.69 ± 9.63	44.50 ± 9.63†	50.91 ± 3.73†	42.87 ± 6.16†	43.24 ± 3.74†	41.42 ± 3.96†
	30	69.58 ± 16.0	44.40 ± 8.23†	44.93 ± 9.04†	40.90 ± 17.35†	53.50 ± 15.33†	50.92 ± 9.18†
	31	58.69 ± 9.63	44.50 ± 3.79†	50.91 ± 3.73	43.28 ± 9.28†	41.75 ± 6.32†	51.54 ± 4.84†
	32	69.58 ± 16.0	44.40 ± 8.23†	44.93 ± 9.04†	42.31 ± 11.11†	57.81 ± 12.08†	51.95 ± 16.71†
Carbonyl	Eugenol	5.27 ± 0.42	4.95 ± 0.94	4.92 ± 0.66	5.13 ± 0.65	5.10 ± 0.43	4.53 ± 0.50
	28	2.30 ± 0.10	2.52 ± 0.24	2.56 ± 0.23	2.44 ± 0.15	2.58 ± 0.22	2.67 ± 0.16†
	30	3.16 ± 0.23	3.39 ± 0.19	3.33 ± 0.22	3.16 ± 0.36	3.19 ± 0.44	3.48 ± 0.48
	31	2.30 ± 0.10	2.52 ± 0.24	2.56 ± 0.23	2.65 ± 0.12†	2.46 ± 0.10	2.72 ± 0.14†
	32	5.27 ± 0.42	4.95 ± 0.94	4.92 ± 0.66	3.55 ± 0.54*†	3.43 ± 0.21*†	3.49 ± 0.39*†

BHT, butylated hydroxytoluene. Data are expressed as mean ± standard deviation (n = 6–12). The levels of TBARS were reported as nmol TBARS per mg protein, thiol content as nmol TNB per mg protein and carbonyl content as nmol of carbonyl per mg protein. Differences were determined by one-way analysis of variance followed by Duncan's *post hoc*. *Significantly different from induced control ($P < 0.05$). †Significantly different from control ($P < 0.05$).

a polyunsaturated fatty acid-rich neuronal membrane, and a high level of iron.[39] On the other hand, the liver resists damage caused by free radicals because besides having high anti-oxidant levels, it adapts easily to metabolic changes.[40]

The sulfhydryl content is inversely correlated with the oxidative damage to the proteins. Therefore, the reduction of protein thiol groups seen in the *in vitro* experiment supplies evidence of reduction of oxidative damage to the proteins. Thiol groups (SH) are recognized as being essential anti-oxidants that have the role of protecting the cellular and extracellular functions against oxidative stress. The mammal cells from different tissues, including the brain, have a system that regulates the redox state of thiol protecting the proteins from excessive oxidation.[41]

The carbonyl content is the main marker of oxidative damage to proteins.[42] The carbonyls can present higher levels because of protein glycation by sugars, the linkage of aldehydes to proteins, or also because of direct oxidation of the lateral chains of amino acids caused by free radicals.[25,43] The great majority of compounds tested, reduced, in the cerebral cortex, the carbonyl content at the concentrations tested, but in the liver, only two compounds had satisfactory results.

Conclusion

Sixteen compounds were synthetized based on structural modifications in the eugenol. Among these, compounds 28, 30, 31 and 32 presented radical scavenging activity in the DPPH and ABTS assays. These compounds also showed anti-oxidant activity, reducing the lipid peroxidation and protein oxidation in biological systems. Although interesting, these results should be treated with caution because it is a first study involving these eugenol derivatives. Further research is necessary to better understand their anti-oxidant activity before establishing beneficial effects of eugenol derivatives. The derivatives that presented promising activity have alkyl or aryl groups substituting the hydroxyl group of eugenol. They vary mainly the size of this lateral chain. Alkyl and linear characteristics appear to favour the activity. These results suggest the existence of other anti-oxidant mechanisms in addition to those involving phenolic hydroxyl. The latter, widely cited as responsible for the anti-oxidant activity of eugenol, is replaced in the derivatives that presented more marked anti-oxidant activity than the aforementioned compound, both in *in vitro* and *ex vivo*. Our results show signs that the presence of phenolic hydroxyl in position 1 of the eugenol is not essential for anti-oxidant activity. This creates a possibility for the occurrence of mechanisms different from those that go through the donation of a proton or metal chelation.

Acknowledgements

This work was supported in part by grants from 'Fundação de Amparo à Pesquisa do Rio Grande do Sul' (FAPERGS), 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior' (CAPES) and 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq), Brazil. The authors also thank Hedy Hoffmann for the corrections of the English version.

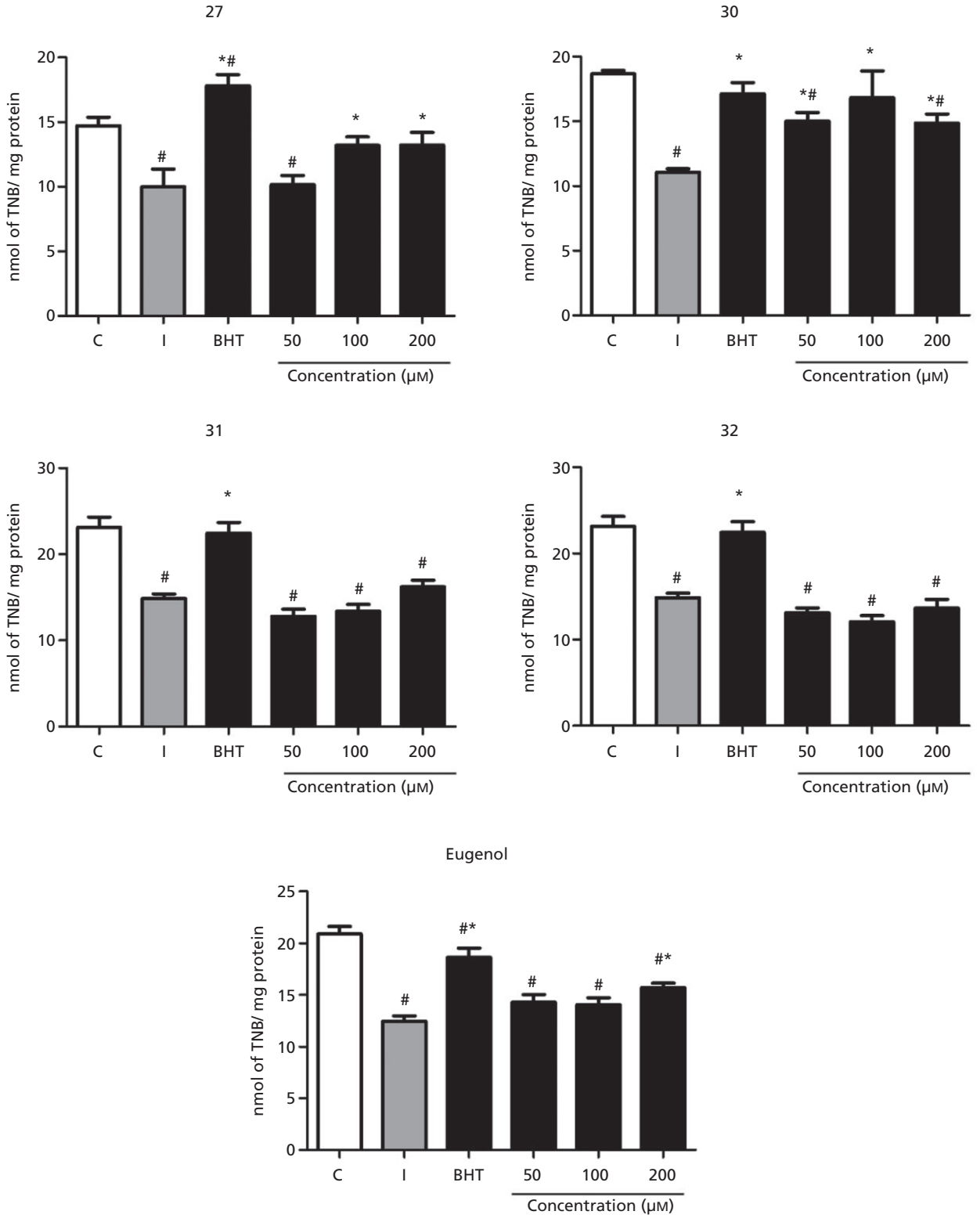


Figure 2 Effect of eugenol derivatives on total thiol content in the cerebral cortex of rats. Data are expressed as mean ± standard deviation (n = 5–14). The levels of thiol content were report as nmol TNB per mg protein. #Significantly different from control ($P < 0.05$). *Significantly different from induced control ($P < 0.05$). Differences were determined by one-way analysis of variance followed by Duncan's *post hoc*. C, control; I, induced control; BHT, butylated hydroxytoluene.

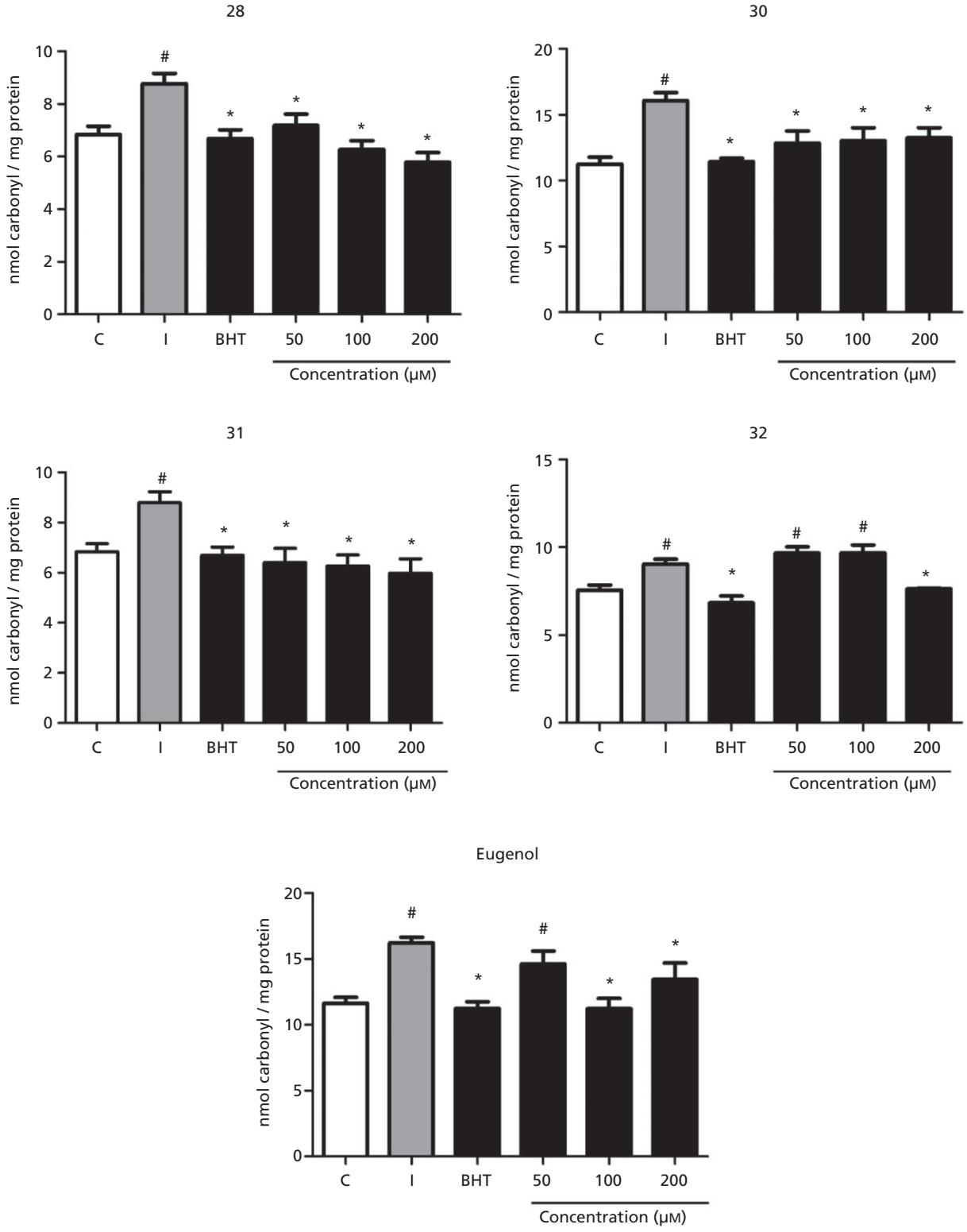


Figure 3 Effect of eugenol derivatives on carbonyl content in the cerebral cortex of rats. Data are expressed as mean ± standard deviation ($n = 4$–8). The levels of carbonyl content were report as nmol of carbonyl per mg protein. #Significantly different from control ($P < 0.05$). *Significantly different from induced control ($P < 0.05$). Differences were determined by one-way analysis of variance followed by Duncan's *post hoc*. C, control; I, induced control; BHT, butylated hydroxytoluene.

References

1. Gülçin I. Antioxidant activity of eugenol: a structure–activity relationship study. *J Med Food* 2011; 14: 975–985.
2. Ito M *et al.* Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. *Food Chem Toxicol* 2005; 43: 461–466.
3. Escobar RG. Eugenol: [Propriedades farmacológicas y toxicológicas, Ventajas y desvantajas de su uso.]. *Rev Cubana Estomatol* 2002; 39: 139–156. in Spanish.
4. Nagababu E *et al.* Assessment of antioxidant activities of eugenol by in vitro and in vivo methods. *Methods Mol Biol* 2010; 610: 165–180.
5. Tangerino LMB. Estudo das propriedades antimicrobianas de copolímeros derivados do eugenol. Universidade Federal de Itajubá, 2006 (dissertation).
6. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* linn (tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian J Physiol Pharmacol* 2005; 49: 125–131.
7. Moreira AVB, Mancini-Filho J. Influence of spices phenolic compounds on lipoperoxidation and lipid profile of rats tissues. *Rev Nutr* 2004; 17: 411–424. in Portuguese.
8. Sadeghian H *et al.* Design and synthesis of eugenol derivatives, as potent 15-lipoxygenase inhibitors. *Bioorg Med Chem* 2008; 16: 890–901.
9. Baskaran Y *et al.* Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. *Toxicology* 2010; 268: 204–212.
10. Rojo L *et al.* Eugenol functionalized poly(acrylic acid) derivatives in the formation of glass-ionomer cements. *Dent Mater* 2008; 24: 1709–1716.
11. Carrasco HA *et al.* Eugenol and its synthetic analogues inhibit cell growth of human cancer cells (part I). *J Braz Chem Soc* 2008; 19: 543–548.
12. Awasthi P *et al.* Eugenol derivatives as future potential drugs. *J Pharm Res* 2008; 1: 215–220.
13. Magalhaes DV Atividade antifúngica de derivados sintéticos do eugenol e timol frente a cepas de *Candida spp*. e *Microsporum canis*. Universidade Estadual do Ceará, 2009 (dissertation).
14. Okada N *et al.* Radical modulating activity and citotoxic activity of synthesized eugenol related compounds. *Anticancer Res* 2000; 20: 2955–2960.
15. Hidalgo ME *et al.* Antioxidant capacity of eugenol derivatives. *Quim Nova* 2009; 32: 1467–1470.
16. Amber K *et al.* Induction of oxidative stress as a possible mechanism of the antifungal action of three phenylpropanoids. *FEMS Yeast Res* 2011; 11: 114–122.
17. Halliwell B. Role of free radicals in the neurodegenerative diseases. *Drugs Aging* 2001; 18: 685–716.
18. Halliwell B, Gutteridge JM. *Free Radicals in Biology and Medicine*, 4th edn. New York, NY: Oxford University Press, 2007.
19. Gacche R *et al.* Antioxidant and anti-inflammatory related activities of selected synthetic chalcones: structure-activity relationship studies using computational tools. *Chem Pharm Bull* 2008; 56: 897–901.
20. Rishton GM. Natural products as a robust source of new drugs and drug leads: past successes and presents day issues. *Am J Cardiol* 2008; 101: S43–S49.
21. Brand-Williams W *et al.* Use of free radical method to evaluate antioxidant activity. *Lebensm-Wiss Technol* 1995; 28: 25–30.
22. Re R *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26: 1231–1237.
23. National Institute of Health. *Guide for the Care and Use of Laboratory Animals*, 8th edn. Washington, DC: NIH, 1996.
24. Buege JA, Aust SD. Microssomal lipid peroxidation. *Methods Enzymol* 1978; 52: 302–309.
25. Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes on the hippocampus and cerebellum in Alzheimer's disease. *Neurosci Lett* 2001; 302: 141–145.
26. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 2003; 25: 207–218.
27. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol* 1994; 233: 357–363.
28. Lowry OH *et al.* Protein measurement with the folin phenol reagente. *J Biol Chem* 1951; 193: 265–275.
29. Fındık E *et al.* Isoeugenol-based novel potent antioxidants: synthesis and reactivity. *Eur J Med Chem* 2011; 46: 4618–4624.
30. Leopoldini M *et al.* The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* 2011; 125: 288–306.
31. Degáspari CH, Waszczynskyj N. Antioxidants properties of phenolic compounds. *Visão Acadêmica – Curitiba* 2004; 5: 33–40. in Portuguese.
32. Cutler SJ *et al.* The synthesis and biological evaluation of eugenol derivatives as potencial herbicidal agents. *PGRSA* 2002; 29: 93–98.
33. Fujisawa S *et al.* Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. *Toxicology* 2002; 177: 39–54.
34. Villãno D *et al.* Comparison of antioxidant activity of wine phenolic compounds and metabolites in vitro. *Anal Chim Acta* 2005; 538: 391–398.
35. Huang W *et al.* The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 2005; 53: 1841–1856.
36. Gülçin I. Antioxidant activity of food constituents: an overview. *Arch Toxicol* 2012; 86: 345–391.
37. Bortolomeazzi R *et al.* Formation of dehydrodiisoeugenol and dehydrodieugenol from the reaction of isoeugenol and eugenol with DPPH radical and their role in the radical scavenging activity. *Food Chem* 2010; 118: 256–265.
38. Nemmiche S *et al.* Cadmium chloride-induced oxidative stress and DNA damage in the human Jurkat T cell line is not linked to intracellular trace elements depletion. *Toxicol in Vitro* 2011; 25: 191–198.

39. Halliwell B. Oxidative stress and neurodegeneration: where are we now? *J Neurochem* 2006; 97: 1634–1658.
40. Genet S *et al*. Alterations in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues: effect of vanadate and fenugreek (*Trigonella foenun* graecum). *Mol Cell Biochem* 2002; 236: 7–12.
41. Penz J *et al*. Effect of 3-butyl-1-phenyl-2-(phenyltelluro)oct-en-1-one on oxidative stress in cerebral cortex of rats. *Food Chem Toxicol* 2009; 47: 745–751.
42. Levine RL. Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 2002; 32: 790–796.
43. Dalle-Donne I *et al*. Protein carbonylation in human diseases. *Trends Mol Med* 2003; 9: 169–176.

Appendix

Spectroscopic data

4′-allyl-2′-methoxyphenyl-benzoate (11): Chemical Formula: $C_{17}H_{16}O_3$; MS *m/z*: 268.11; NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.19 (2H, $C_6\mathbf{H}_5$); 7.55 (1H, $C_6\mathbf{H}_5$); 7.46 (2H, $C_6\mathbf{H}_5$); 7.06 (1H, $C_6\mathbf{H}_3$-O); 6.79 (2H, $C_6\mathbf{H}_3$); 5.97 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.10 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.87 (3H, OC$\mathbf{H}_3$); 3.38 (2H, CH_2=CH_2-C$\mathbf{H}_2$),^{13}C NMR (100 MHz): 40.1 (CH_2-**C**H_2-C_6H_5); 56.0 (O-**C**H_3); 113.1 (**C**m-**C**$_6H_3$); 116.0 (**C**H_2=CH_2-CH_2); 120.7 (**C**o-**C**$_6H_3$); 122.6 (**C**m-**C**$_6H_3$-O); 128.6 (O=C-**C**i-**C**$_6H_5$); 133.2 (**C**p-**C**$_6H_5$); 137.0 (CH_2-**C**p-**C**$_6H_5$); 138.4 (O-**C**i-**C**$_6H_5$); 138.9 (CH_2=**C**H_2-CH_2); 151.4 (**C**o-**C**$_6H_3$-OCH_3); 164.8 (**C**=O); Yield = 86%; MP = 57–58°C.

4′-allyl-2′-methoxyphenyl-2-fluorobenzoate (12): Chemical Formula: $C_{17}H_{15}FO_3$; MS *m/z*: 286.10; NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.22 (2H, $C_6\mathbf{H}_4$-F); 7.22 (2H, $C_6\mathbf{H}_4$-F); 7.16 (1H, $C_6\mathbf{H}_3$); 6.83 (2H, $C_6\mathbf{H}_3$); 5.98 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.11 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.78 (3H, OC$\mathbf{H}_3$); 3.39 (2H, CH_2=CH_2-C$\mathbf{H}_2$) ^{13}C NMR (100 MHz): 39.9 (CH_2-**C**H_2-C_6H_3); 55.9 (O-**C**H_3); 113.2 (**C**m-**C**$_6H_3$-O); 115.5 (**C**m-**C**$_6H_4$(2-F)-C=O); 115.7 (**C**H_2=CH_2-CH_2); 116.1 (**C**i-**C**$_6H_3$-C=O); 120.8 (**C**o-**C**$_6H_3$-O); 122.6 (**C**m-**C**$_6H_3$-O); 125.9 (**C**m-**C**$_6H_4$-C=O); 132.8 (**C**o-**C**$_6H_4$-C=O); 137.1 (CH_2-**C**p-**C**$_6H_3$); 138.3 (**C**p-**C**$_6H_4$-C=O); 139.2 (**C**i-**C**$_6H_3$-O); 151.2 (CH_2=**C**H_2-CH_2); 163.8 (CH_3O-**C**o-**C**$_6H_3$-O); 164.8 (F-**C**o-**C**$_6H_4$); 167.4 (**C**=O); Yield = 67%; MP = 59–60°C.

4′allyl-2′-methoxyphenyl-3-bromobenzoate (13): Chemical Formula: $C_{17}H_{15}BrO_3$; MS *m/z*: 346.02, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.33 (1H, $C_6\mathbf{H}_4$-Br); 8.11 (1H, $C_6\mathbf{H}_4$-Br); 7.71 (1H, $C_6\mathbf{H}_4$-Br); 7.34(1H, $C_6\mathbf{H}_4$-Br); 7.05 (1H, $C_6\mathbf{H}_3$); 6.80 (2H, $C_6\mathbf{H}_3$); 5.95 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.11 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.77 (3H, OC$\mathbf{H}_3$); 3.38 (2H, CH_2=CH_2-C$\mathbf{H}_2$),^{13}C NMR (100 MHz): 40.1 (CH_2-**C**H_2-C_6H_3); 55.9 (O-**C**H_3); 113.2 (**C**m-**C**$_6H_3$-O); 116.1 (**C**H_2=CH_2-CH_2); 120.8 (**C**m-**C**$_6H_3$-O); 122.6 (Br-**C**m-**C**$_6H_4$); 122.5 (**C**o-**C**$_6H_3$-O); 128.7 (**C**o-**C**$_6H_4$(3-Br)C=O); 130.0 (**C**m-**C**$_6H_4$(3-Br)C=O); 131.6 (**C**i-**C**$_6H_4$(3-Br)C=O); 133.1 (**C**p-**C**$_6H_3$-O); 136.2 (**C**i-**C**$_6H_3$-O); 137.0 (CH_2=**C**H_2-CH_2); 138.2 (**C**p-**C**$_6H_4$ (3-Br)-C=O); 139.2 (**C**o-C_6H_4-C(Br); 151.2 (OCH_3-**C**o-C_6H_3-O); 163.4 (**C**=O); Yield = 90%; MP = 54–56°C.

4′-allyl-2′-methoxyphenyl-4-methoxybenzoate (14): Chemical Formula: $C_{18}H_{18}O_4$; MS *m/z*: 298.12, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.10 (2H, $C_6\mathbf{H}_4$-OCH_3); 7.28 (2H, $C_6\mathbf{H}_4$-OCH_3); 7.05 (1H, $C_6\mathbf{H}_3$); 6.80(2H,$C_6\mathbf{H}_3$); 5.98 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.12 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.78 (3H, OC$\mathbf{H}_3$); 3.38 (2H, CH_2=CH_2-C$\mathbf{H}_2$), 2.42 (3H, OC$\mathbf{H}_3$); ^{13}C NMR (100 MHz): 21.8 (CH_2-**C**H_2-C_6H_3); 40.3 (C_6H_3-O-**C**H_3); 56.1 (C_6H_4-O-**C**H_3); 113.4 (**C**m-**C**$_6H_3$-O); 116.1 (**C**H_2=CH_2-CH_2); 121.0 (**C**o-**C**$_6H_3$-O); 123.0 (**C**m-**C**$_6H_3$-O); 127.1 (**C**i-**C**$_6H_4$(4OCH_3)-C=O); 129.1 (**C**m-**C**$_6H_4$(4OCH_3)-C=O); 130.3 (**C**o-C_6H_4-(4OCH_3)-C=O); 137.1 (**C**p **C**$_6H_3$-O); 138.6 (**C**i-**C**$_6H_3$-O); 139.0 (CH_2=**C**H_2-CH_2); 144.3 (OCH_3-**C**o-**C**$_6H_3$-O); 151.5 (**C**p-**C**$_6H_4$ (4OCH_3)-C=O); 165.1 (**C**=O); Yield = 43%; MP = 170–171°C.

4′-allyl-2′-methoxyphenyl-3-chlorobenzoate (15): Chemical Formula: $C_{17}H_{15}ClO_3$; MS *m/z*: 302.07, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.18 (1H, $C_6\mathbf{H}_4$-Cl); 8.17 (1H, $C_6\mathbf{H}_4$-Cl); 7.56 (1H, $C_6\mathbf{H}_4$-Cl); 7.05 (1H,$C_6\mathbf{H}_3$); 6.0(2H, $C_6\mathbf{H}_3$); 5.97 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.12 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.78 (3H, OC$\mathbf{H}_3$); 3.37 (2H, CH_2=CH_2-C$\mathbf{H}_2$),^{13}C NMR (100 MHz): 40.1 (CH_2-**C**H_2-C_6H_3); 56.0 (C_6H_3-O-**C**H_3); 113.2 (**C**m-**C**$_6H_3$-O); 116.1 (**C**H_2=CH_2-CH_2); 120.8 (**C**m-**C**$_6H_3$-O); 122.6 (**C**o-**C**$_6H_3$-O); 128.3 (**C**i-**C**$_6H_4$(3Cl)-C=O); 129.7 (**C**o-**C**$_6H_4$(3Cl)-C=O); 130.2 (**C**m-**C**$_6H_4$-(3Cl)-C=O); 131.5 (**C**o-**C**$_6H_4$ (3Cl)-C=O); 133.3 (**C**p-**C**$_6H_4$ (3Cl)-C=O); 134.7 (**C**m-**C**$_6H_4$-Cl-(C=O)); 137.0 (**C**p-**C**$_6H_3$-O); 128.2 (**C**i-**C**$_6H_3$-O); 139.2 (CH_2=**C**H_2-CH_2); 151.2 (OCH_3-**C**o-C_6H_3-O); 163.6 (**C**=O); Yield = 31%; MP = 48–50°C.

4′-allyl-2′-methoxyphenyl-4-fluorobenzoate (16): Chemical Formula: $C_{17}H_{15}FO_3$; MS *m/z*: 286.10, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.21 (2H, $C_6\mathbf{H}_4$-F); 7.12 (2H, $C_6\mathbf{H}_4$-F); 7.05 (1H, $C_6\mathbf{H}_3$); 6.8 (2H, $C_6\mathbf{H}_3$); 5.98 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.11 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.78 (3H, OC$\mathbf{H}_3$); 3.49 (2H, CH_2=CH_2-C$\mathbf{H}_2$), ^{13}C NMR (100 MHz): 40.1 (CH_2-**C**H_2-C_6H_3); 56.0 (C_6H_3-O-**C**H_3); 113.2 (**C**m-**C**$_6H_3$-O); 115.7 (**C**m-**C**$_6H_4$-(4F)-C=O); 120.7 (**C**H_2=CH_2-CH_2); 122.6 (**C**o-**C**$_6H_3$-O); 125.9 (**C**m-**C**$_6H_3$-O); 132.8 (**C**o-**C**$_6H_4$(4F)-C=O); 137.0 (**C**i-**C**$_6H_4$(4F)-C=O); 138.3 (**C**p-**C**$_6H_3$-O); 139.1 (**C**i-**C**$_6H_3$-O); 151.2 (CH_2=**C**H_2-CH_2); 163.7 (OCH_3-**C**o-**C**$_6H_3$-O); 164.7 (**C**=O); 167.3 (**C**p-**C**$_6H_4$(4F)-C=O); Yield = 64%; MP = 59–60°C.

4′-allyl-2′-methoxyphenyl-4-methylbenzoate (17): Chemical Formula: $C_{18}H_{18}O_3$; MS *m/z*: 282.13, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.07 (2H, $C_6\mathbf{H}_4$-CH3); 7.20 (2H, $C_6\mathbf{H}_4$-CH3); 7.03 (1H, $C_6\mathbf{H}_3$); 6.77 (2H, $C_6\mathbf{H}_3$); 5.92 (1H, CH_2=C$\mathbf{H}_2$-CH_2); 5.06 (2H, C$\mathbf{H}_2$=CH_2-CH_2); 3.76 (3H,

OC**H**$_3$); 3.37 (2H, CH_2=CH_2-C**H**$_2$), 2.4 (3H, CH3); ^{13}C NMR (100 MHz): 21.6 (**C**H_3); 40.1 (CH_2-**C**H_2-C_6H_3); 55.9 (C_6H_3-O-**C**H_3); 113.2 (**C**m-**C**$_6$H$_3$-O); 115.9 (**C**H_2=CH_2-CH_2); 120.8 (**C**o-**C**$_6$H$_3$-O); 122.8 (**C**m-**C**$_6$H$_3$-O); 126.7 (**C**i-**C**$_6$H$_4$(4CH3)-C=O); 129.2 (**C**m-**C**$_6$H$_4$(4CH3)-C=O); 130.3 (**C**o-**C**$_6$H$_4$-(4CH_3)-C=O); 137.1 (**C**p-**C**$_6$H$_3$-O); 138.5 (**C**i-**C**$_6$H$_3$-O); 144.4 (CH_2=**C**H_2-CH_2); 151.3 (**C**p-**C**$_6$H$_4$-(4CH_3)-C=O); 164.8 (OCH_3-**C**o-**C**$_6$H$_3$-O); 171.5 (**C**=O); Yield = 64%; MP = 93–95°C.

4′-allyl-2′-methoxyphenyl-4-nitrobenzoate (18): Chemical Formula: $C_{17}H_{15}NO_5$; MS *m/z*: 313.10, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.11 (2H, C_6**H**$_4$-CH3); 7.26 (2H, C_6**H**$_4$-CH3); 7.00 (1H, C_6**H**$_3$); 6.82 (2H, C_6**H**$_3$); 5.97 (1H, CH_2=C**H**$_2$-CH_2); 5.14 (2H, C**H**$_2$=CH_2-CH_2); 3.78 (3H, OC**H**$_3$); 3.39 (2H, CH_2=CH_2-C**H**$_2$), 2.40 (3H, CH3); ^{13}C NMR (100 MHz): 21.6 (**C**H_3); 40.2 (CH_2-**C**H_2-C_6H_3); 55.9 (C_6H_3-O-**C**H_3); 113.1 (**C**m-**C**$_6$H$_3$-O); 115.9 (**C**H_2=CH_2-CH_2); 120.8 (**C**o-**C**$_6$H$_3$-O); 122.8 (**C**m-**C**$_6$H$_3$-O); 124.3 (**C**i-**C**$_6$H$_4$(4NO_2)-C=O); 126.1 (**C**m-**C**$_6$H$_4$(4NO_2)-C=O); 133.2 (**C**o-**C**$_6$H$_4$-(4NO_2)-C=O); 137.1 (**C**p-**C**$_6$H$_3$-O); 138.5 (**C**i-**C**$_6$H$_3$-O); 144.2 (CH_2=**C**H_2-CH_2); 151.3 (**C**p-**C**$_6$H$_4$-(4CH_3)-C=O); 164.8 (OCH_3-**C**o-**C**$_6$H$_3$-O); 171.5 (**C**=O); Yield = 77%; MP = 75–77°C.

4′-allyl-2′-methoxyphenyl-2-nitrobenzoate (19): Chemical Formula: $C_{17}H_{15}NO_5$; MS *m/z*: 313.10, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.10 (2H, C_6**H**$_4$-CH3); 7.26 (2H, C_6**H**$_4$-CH3); 7.02 (1H, C_6**H**$_3$); 6.82 (2H, C_6**H**$_3$); 5.97 (1H, CH_2=C**H**$_2$-CH_2); 5.11 (2H, C**H**$_2$=CH_2-CH_2); 3.78 (3H, OC**H**$_3$); 3.39 (2H, CH_2=CH_2-C**H**$_2$), 2.41 (3H, CH3); ^{13}C NMR (100 MHz): 21.6 (**C**H_3); 40.1 (CH_2-**C**H_2-C_6H_3); 55.9 (C_6H_3-O-**C**H_3); 113.2 (**C**m-**C**$_6$H$_3$-O); 115.9 (**C**H_2=CH_2-CH_2); 120.8 (**C**o-**C**$_6$H$_3$-O); 122.8 (**C**m-**C**$_6$H$_3$-O); 126.9 (**C**i-**C**$_6$H$_4$(4NO_2)-C=O); 129.2 (**C**m-**C**$_6$H$_4$(4 NO_2)-C=O); 130.2 (**C**o-**C**$_6$H$_4$-(4 NO_2)-C=O); 137.1 (**C**p-**C**$_6$H$_3$-O); 138.5 (**C**i-**C**$_6$H$_3$-O); 144.2 (CH_2=**C**H_2-CH_2); 151.3 (**C**p-**C**$_6$H$_4$-(4CH_3)-C = O); 164.8 (OCH_3-**C**o-**C**$_6$H$_3$-O); 171.5 (**C**=O); Yield = 81%; MP = 57–58°C

1-(4′-allyl-2′-methoxyphenyl)-3-methylbutanoate (27): Chemical Formula: $C_{15}H_{20}O_3$; MS *m/z*: 248.14, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 6.89 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10(2H, C**H**$_2$=CH_2-CH_2); 3.8(3H, OC**H**$_3$); 3.4 (2H, CH_2=CH_2-C**H**$_2$); 2.4 (2H,C-C**H**$_2$-CH); 2.3 (1H, CH_3-C**H**-CH_3); 1.10 (3H, C**H**$_3$-CH-C**H**$_3$); ^{13}C NMR (100 MHz): 22.4 (**C**H_3-CH-CH_3); 25.9 (CH_3-CH-**C**H_3); 40.1 (CH_3-**C**H-CH_3); 43.1 (CH_2-**C**H_2-C_6H_5); 55.7 (O-**C**H_3); 112.7 (**C**m-**C**$_6$H$_3$); 116.1 (**C**H_2=CH-CH_2); 120.7 (**C**m-**C**$_6$H$_3$-O); 122.5 (**C**o-**C**$_6$H$_3$); 137.1 (CH_2=**C**H-CH_2); 138.8 (CH_2-**C**p-**C**$_6$H$_5$); 150.9 (O-**C**i-**C**$_6$H$_5$); 171.2 (**C**o-**C**$_6$H$_3$-OCH_3); 191.74 (**C**=O); Yield = 61%; Oil.

1-(4′-allyl-2′-methoxyphenyl)-pentanoate (28): Chemical Formula: $C_{15}H_{20}O_3$; MS *m/z*: 248.14, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 6.89 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10(2H, C**H**$_2$=CH_2-CH_2); 3.8(3H, OC**H**$_3$); 3.3(2H, CH_2=CH_2-C**H**$_2$); 2.6(2H, O-C**H**$_2$-CH_2); 1.8 (2H, O-CH_2-C**H**$_2$); 1.5 (2H, CH_2-C**H**$_2$-CH_3); 1.0 (3H, CH_2-CH_2-C**H**$_3$); ^{13}C NMR (100 MHz): 13.7 (CH_2-**C**H_3); 22.2 (**C**H_2-CH_3); 27.1 (CH_2-**C**H_2-CH_2); 33.8 (O-**C**H_2); 40.1 (CH_2-**C**H_2-C_6H_5); 55.8 (O-**C**H_3); 112.8 (CH_2=**C**H-CH_2); 116.1 (**C**m-**C**$_6$H$_3$); 120.7 (**C**o-**C**$_6$H$_3$); 122.5 (**C**m-**C**$_6$H$_3$-O); 137.1 (**C**H_2=CH-CH_2); 138.8 (CH_2-**C**p-**C**$_6$H$_5$); 146.5 (O-**C**i-**C**$_6$H$_5$); 150.9 (**C**o-**C**$_6$H$_3$-OCH_3); 172.0 (**C**=O); Yield = 43%; Oil.

4′-allyl-2′-methoxyphenyl-2-phenylacetate (29): Chemical Formula: $C_{17}H_{16}O_3$; MS *m/z*: 268.11, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 8.2-7.5 (5H, C_6**H**$_5$); 7.10 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10 (2H, C**H**$_2$=CH_2-CH_2); 3.8 (3H, OC**H**$_3$); 3.4(2H, CH_2=CH_2-C**H**$_2$); ^{13}C NMR (100 MHz): 40.1 (CH_2-**C**H_2-C_6H_5); 55.9 (O-**C**H_3); 112.9 (**C**m-**C**$_6$H$_3$); 116.1 (**C**H_2=CH_2-CH_2); 120.8 (**C**m-**C**$_6$H$_3$-O); 122.7 (**C**o-**C**$_6$H$_3$); 128.5–133.4 (**C**$_6$H$_5$); 137.1 (CH_2-**C**p-**C**$_6$H$_5$); 138.3 (O-**C**i-**C**$_6$H$_5$); 139.1 (CH_2=**C**H_2-CH_2); 151.2 (**C**o-**C**$_6$H$_3$-OCH_3); 164.9 (O=**C**-C_6H_5); Yield = 98%; Oil.

4-allyl-2-methoxy-1-phenethoxybenzene (30): Chemical Formula: $C_{16}H_{20}O_2$; MS *m/z*: 268.15, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 7.3 (5H, C_6**H**$_5$); 6.89 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10 (2H, C**H**$_2$=CH_2-CH_2); 3.89 (3H, OC**H**$_3$); 3.7(2H, O-C**H**$_2$-CH_2); 3.3(2H, CH_2=CH_2-C**H**$_2$); 3.10 (2H, O-CH_2-C**H**$_2$); ^{13}C NMR (100 MHz): 38.2 (CH_2-**C**H_2-C_6H_5); 39.9 (CH_2-**C**H_2-C_6H_5); 46.0 (**C**H_2-CH_2-C_6H_5); 55.9 (O-**C**H_3); 111.2 (**C**m-**C**$_6$H$_3$); 114.3 (**C**o-**C**$_6$H$_3$); 115.5 (**C**m-**C**$_6$H$_3$-O); 121.2 (**C**H_2=CH_2-CH_2); 126.9 (CH_2-**C**p-**C**$_6$H$_5$); 128.6–137.9 (**C**$_6$H$_5$); 131.9 (CH_2=**C**H_2-CH_2); 138.1 (CH_2=**C**H_2-CH_2); 143.9 (O-**C**i-**C**$_6$H$_5$); 146.5 (**C**o-**C**$_6$H$_3$-OCH_3); Yield = 70%; Oil.

4-allyl-2-methoxy-1-phenpropoxybenzene (31): Chemical Formula: $C_{19}H_{22}O_2$; MS *m/z*: 282.16, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 7.3 (5H, C_6**H**$_5$); 6.89 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10 (2H, C**H**$_2$=CH_2-CH_2); 3.89 (3H, OC**H**$_3$); 3.5(2H, O-C**H**$_2$-CH_2); 3.3(2H, CH_2=CH_2-C**H**$_2$); 2.8(2H, CH_2-C**H**$_2$-CH_2); 2.1(2H, CH_2-C**H**$_2$-C_6H_5); ^{13}C NMR (100 MHz): 32.8 (CH_2-**C**H_2-CH_2); 34.0 (CH_2-**C**H_2-C_6H_5); 39.9 (CH_2-**C**H_2-C_6H_5); 44.2 (**C**H_2-CH_2-C_6H_5); 55.9 (O-**C**H_3); 111.1 (**C**m-**C**$_6$H$_3$); 114.3 (**C**o-**C**$_6$H$_3$); 115.5 (**C**m-**C**$_6$H$_3$-O); 121.2 (**C**H_2=CH_2-CH_2); 126.1–126.5 (**C**$_6$H$_5$); 131.9 (CH_2-**C**p-**C**$_6$H$_5$); 137.8 (CH_2=**C**H_2-CH_2); 143.9 (O-**C**i-**C**$_6$H$_5$); 146.5 (**C**o-**C**$_6$H$_3$-OCH_3); Yield = 66%; Oil.

4-allyl-2-methoxy-1-propoxybenzene (32): Chemical Formula: $C_{13}H_{18}O_2$; MS *m/z*: 206.13, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 6.8 (3H, C_6**H**$_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10 (2H, C**H**$_2$=CH_2-CH_2); 3.88 (2H, O-C**H**$_2$-CH_2); 3.82 (3H, OC**H**$_3$); 3.4 (2H, CH_2=CH_2-C**H**$_2$), 2.6 (2H, CH_2-C**H**$_2$-CH_3); 1.3(3H, CH_2-CH_2-C**H**$_3$); ^{13}C NMR (100 MHz): 9.2 (CH_2-**C**H_3); 27.4 (**C**H_2-CH_3); 40.1 (CH_2-**C**H_2-C_6H_5); 55.8 (O-**C**H_3); 112.8 (**C**m-**C**$_6$H$_3$); 116.1 (**C**o-**C**$_6$H$_3$); 120.7 (**C**H_2=CH_2-CH_2); 122.5 (**C**m-**C**$_6$H$_3$-O); 137.1 (CH_2-**C**p-**C**$_6$H$_5$); 138.8 (CH_2=**C**H_2-CH_2); 150.9 (O-**C**i-**C**$_6$H$_5$); 172.7 (**C**o-**C**$_6$H$_3$-OCH_3); Yield = 54 %; Oil.

4-allyl-2-methoxy-1-butoxybenzene (33): Chemical Formula: $C_{14}H_{20}O_2$; MS *m/z*: 220.15, NMR Data ^{1}H NMR (400 MHz): δ (ppm) 7.27 (2H, Ar-O-CH_2); 6.8 (3H, $C_6\mathbf{H}_3$); 5.9 (1H, CH_2=C**H**$_2$-CH_2); 5.10 (2H, C**H**$_2$=CH_2-CH_2); 3.86 (2H, O-C**H**$_2$-CH_2); 3.82 (3H, OC**H**$_3$); 3.4 (2H, CH_2=CH_2-C**H**$_2$); 2.55(2H, C**H**$_2$-CH_2-CH_3); 1.8 (2H, CH_2-C**H**$_2$-CH_3);1.1(3H, CH_2-CH_2-C**H**$_3$); ^{13}C NMR (100 MHz): 13.6 (CH_2-**C**H_3); 18.6 (**C**H_2-CH_3); 35.9 (O-CH_2-**C**H_2); 40.1 (CH_2-**C**H_2-C_6H_5); 55.8 (O-**C**H_3); 112.8 (O-**C**H_2-CH_2); 116.1 (**C**m-$\mathbf{C}_6H_3$); 120.7 (**C**o-$\mathbf{C}_6H_3$); 122.5 (**C**m-$\mathbf{C}_6H_3$-O); 137.1 (CH_2-**C**p-$\mathbf{C}_6H_5$); 138.1 (**C**H_2=CH_2-CH_2); 138.8 (CH_2=**C**H_2-CH_2); 150.9 (O-**C**i-$\mathbf{C}_6H_5$); 171.9 (**C**o-$\mathbf{C}_6H_3$-OCH_3; Yield = 93%; Oil.

