JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. XXXX, XXX, XXX−XXX

pubs.acs.org/JAFC

Semisynthetic Phenol Derivatives Obtained from Natural Phenols: Antimicrobial Activity and Molecular Properties

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Supporting Information

ABSTRACT: Semisynthetic phenol derivatives were obtained from the natural phenols: thymol, carvacrol, eugenol, and guaiacol through catalytic oxychlorination, Williamson synthesis, and aromatic Claisen rearrangement. The compounds characterization was carried out by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The natural phenols and their semisynthetic derivatives were tested for their antimicrobial activity against the bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Listeria innocua*, *Pseudomonas aeruginosa*, *Salmonella enterica* Typhimurium, *Salmonella enterica ssp. enterica*, and *Bacillus cereus*. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined using concentrations from 220 to 3.44 μg mL^{-1}. Most of the tested compounds presented MIC values ≤220 μg mL^{-1} for all the bacteria used in the assays. The molecular properties of the compounds were computed with the PM6 method. Through principle components analysis, the natural phenols and their semisynthetic derivatives with higher antimicrobial potential were grouped.

KEYWORDS: *natural phenols, semisynthetic phenols, chlorophenol, antimicrobial activity*

INTRODUCTION

After A. Fleming accidentally discovered penicillin, in 1928, the first effective antibiotic diffused worldwide, researchers have been investigating alternatives for the discovery of other potential antimicrobials produced from either natural resources, fungi,[1] bacteria,[2] seaweed,[3] or obtained synthetically.[4,5]

Despite considerable innovation, expansion, and evolution in the production of efficient drugs in the combat against bacterial infections, the excessive use of antibiotics has enabled bacteria to develop defenses, known as bacterial resistance, leading to threatens for public health, and causing a series of limitations in the conventional treatments.[6]

With the appearance of several pathogenic microorganisms, which are resistant to classical antibiotics, new antimicrobial agents are necessary.[7] Therefore, compounds with natural origin, the so-called secondary metabolites, isolated from plants, algae, and microorganisms, may be useful as model templates for the production of new drugs.[8] In addition, semisynthetic compounds, natural compounds that possess modifications in their structure, may be prospective candidates as potential antimicrobial agents.[9]

Phenols are examples of a class of compounds that present high antimicrobial potential and are part or the main constituent of a large variety of natural products.[10] Several phenolic compounds with low molecular weight are volatile constituents of plants and are easy to be obtained, for example, we may cite: thymol, carvacrol, guaiacol, and eugenol (Figure 1). There are various applications for these compounds in the drug and food areas, where thymol and carvacrol are generally recognized as safe additives, widely used as food preservatives.[11]

Thymol **1a** (2-isopropyl-5-methylphenol) and carvacrol **1b** (5-isopropyl-2-methylphenol) are constitutional isomers found in thyme (*Thymus vulgaris*),[12] oregano (*Origanum vulgare*),[13] peppermint (*Plectrantus amboinicus*),[14] and other plant essential oils. These compounds present antibacterial,[15] antifungal,[16] antioxidant,[17] phytotoxic, and cytotoxic activities.[18]

Received: September 23, 2017
Revised: November 30, 2017
Accepted: December 9, 2017

DOI: 10.1021/acs.jafc.7b04418
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

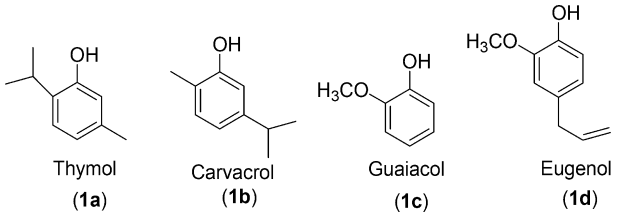

Figure 1. Structure of phenolic compounds with low molecular weight.

Guaiacol **1c** (2-methoxyphenol) is a phenol of natural origin, derived from lignin, which may be obtained from wood creosotes from eucalyptus tar distillation.[19] Guaiacol presents sedative activity in odontological treatments and has properties in the induction of cellular proliferation.[20] This phenol may be found in several nutritive products, as wines, teas, cocoa, and tomatoes.[21]

Eugenol **1d** is the major compound identified in *Syzygium aromaticum* (clove)[22] essential oil and is also present in *Cinnamomum zeylanicum* (cinnamon),[23] *Ocimum gratissimum* L. (clove basil),[24] *Pimenta dioica* (jamaican pepper),[25] and in other plants and condiments. Eugenol antimicrobial activity has been reported as important pathogenic microorganisms such as *Helicobacter pylori*[25] and *Salmonella* Typhi.[26]

Phenolic compounds generally present antimicrobial activity and may be used to reduce or to restrain the multiplication of pathogenic or deteriorating microorganisms.[27] Aiming to potentiate and amplify the application of natural phenols against the microorganisms development, we had as objective to obtain semisynthetic derivatives from the natural phenols thymol, carvacrol, guaiacol, and eugenol, in catalytic oxychlorination reactions, Williamson synthesis, and aromatic Claisen rearrangement and to evaluate the products according to their antimicrobial activities against the pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Listeria innocua*, *Pseudomonas aeruginosa*, *Salmonella enterica* Typhimurium, *Salmonella enterica ssp. enterica*, and *Bacillus cereus*.

MATERIALS AND METHODS

General Experimental Procedures. The phenols used in the reactions (**1a**−**1d**) were purchased from Sigma-Aldrich. Mass spectra for all the synthesized compounds were obtained with a GC-MS QP Plus 2010 from Shimadzu using the electron ionization mode of 70 eV. The spectra of hydrogen and carbon nuclear magnetic resonance (^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz, respectively) were obtained from a Varian Mercury 300 spectrometer. Deuterated chloroform ($CDCl_3$) was used as solvent and tetramethylsilane (TMS) as an internal standard (δ_H = 0). The scalar coupling constants (J) are expressed by Hertz (Hz).

Syntheses of Compounds 2a−2d. Syntheses of **2a**−**2d** were exemplified by synthesis of 4-chlorothymol (**2a**). Reactions were carried out in a three neck round-bottomed flask (25.0 mL), under heating and with magnetic agitation, connected to a buret for the volumetric monitoring of oxygen consumption. The reactions were followed by gas chromatography (GC) coupled to a *Shimadzu* GC2010-Plus equipment using a Carbowax 20 m column. As in a typical oxychlorination experiment, a solution of the total volume of 5 mL containing the substrate (0.3011 g; 0.4 mol/L), dodecane (internal pattern, 0.114 mL; 0.1 mol/L), chlorinating agent (LiCl, 0.1696 g; 0.8 mol/L) and copper catalyst ($CuCl_2$, 0.0336 g; 0.05 mol/L) in pure acetic acid as solvent was agitated for approximately 6 h at a temperature of 80 °C in dioxygen atmosphere with periodic sampling.[28] Compounds **2b**, **2c**, and **2d** were synthesized with a methodology similar to the one described for compound **2a**. However, the synthesis of compound **2c** was conducted at 60 °C. After the syntheses, the solvent was neutralized, and the products were characterized by mass spectrometry and NMR analysis of ^{1}H and ^{13}C.

*4-Chloro-2-isopropyl-5-methylphenol (**2a**).* The compound is a reddish brown oil. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.23 (d, 6H, J = 6.9 Hz, $CH(C\underline{H}_3)_2$); 2.27 (s, 3H, $C\underline{H}_3$); 3.13 (sept. 1H, J = 6.9 Hz, $C\underline{H}(CH_3)_2$); 4.82 (s, 1H, OH); 6.62 (s, 1H, Ar−H_6); 7.13 (s, 1H, Ar−H_3). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 19.54 ($\underline{C}H_3$); 22.46 ($CH(\underline{C}H_3)_2$); 26.84 ($\underline{C}H(CH_3)_2$); 117.60 (C_6); 125.77 (C_2); 126.80 (C_3); 133.67 (C_2 or C_5)*; 133.92 (C_2 or C_5)*; 151.16 (C_1). *, attributions may be inverted. MS (EI) m/z (%): 186 ($C_{10}H_{13}ClO$ [M + 2]$^+$·12); 184 ([M]$^+$·4); 171 (33); 169 (100); 134 (12); 105 (16); 91 (6); 77 (9); 51 (6).

*4-Chloro-5-isopropyl-2-methylphenol (**2b**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.20 (d, 6H, J = 6.9 Hz, $CH(C\underline{H}_3)_2$); 2.18 (s, 3H, $C\underline{H}_3$); 3.30 (sept. 1H, J = 6.9 Hz, $C\underline{H}(CH_3)_2$); 4.79 (s, 1H, OH); 6.70 (s, 1H, Ar−H_6); 7.08 (s, 1H, Ar−H_3). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 15.14 ($\underline{C}H_3$); 22.64 ($CH(\underline{C}H_3)_2$); 29.89 ($\underline{C}H(CH_3)_2$); 113.03 (C_6); 122.62 (C_2); 124.19 (C_4); 131.19 (C_3); 144.37 (C_5); 152.65 (C_1). MS (EI) m/z (%): 186 ($C_{10}H_{13}ClO$ [M + 2]$^+$·12); 184 ([M]$^+$·36); 171 (32); 169 (100);149 (6); 134 (14); 115 (5); 105 (17); 91 (6); 77 (12); 65 (4); 51 (7).

*4-Chloro-2-methoxyphenol (**2c**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 3.79 (s, 3H, $OC\underline{H}_3$); 5.53 (s, 1H,OH); 6.83 (m, 2H, Ar−$H_{5;\ and\ 6}$); 7.26 (s, 1H, Ar−H_3). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 56.10 ($O\underline{C}H_3$); 121.09 (C_4); 115.11 (C_3); 111.37 (C_4 and C_5); 144.32 (C_1 or C_2)*; 146.91 (C_1 or C_2)*. *, attributions may be inverted. MS (EI) m/z (%): 160 ($C_7H_7ClO_2$ [M + 2]$^+$·30); 158 ([M]$^+$·93); 145 (33); 143 (100); 117 (15); 115 (46); 99 (3); 87 (7); 79 (8); 73 (4); 63 (8); 51 (24).

*4-Allyl-2-chloro-6-methoxy-phenol (**2d**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 3.27−3.30 (m, 2H, $C\underline{H}_2$); 3.88 (s, 3H, $OC\underline{H}_3$); 5.05−5.10 (m, 2H, = $C\underline{H}_2$); 5.73 (s, 1H, OH); 5.84−5.99 (m, 1H, $C\underline{H}$=); 6.59 (d, 1H, J = 1.8 Hz; Ar−H_5), 6.77 (d, 1H, J = 1.8 Hz; Ar−H_3). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 39.58 ($\underline{C}H_2$); 56.28 ($O\underline{C}H_3$); 109.81 (C_5); 116.17 (=CH_2); 121.59 (C_2); 133.03 (C_3); 136.92 (CH=); 140.20 (C_4); 147.30 (C_1 or C_6)*; 147.96 (C_1 or C_6)*. *, attributions may be inverted. MS (EI) m/z (%): 200 ($C_{10}H_{11}ClO_2$ [M + 2]$^+$·33); 198 ([M]$^+$·100); 185 (7); 171 (14); 163 (21); 155 (9); 131 (89); 119 (14); 103 (39); 91 (37); 77 (14); 65 (16); 51 (11); 39(9).

Synthesis of Compounds 3a−3c and 4a−4c. *Exemplified by Synthesis of 2-(Allyloxy)-1-isopropyl-4-methylbenzene (**4a**).* In a round-bottomed flask adapted to a condenser in reflux position, thymol **1a** (10 g; 67 mmol) and acetone (40 mL) were added for the preparation of a NaOH (5.08 g; 0.127 mol) solution in 50 mL of water. The reaction was heated between 50 and 60 °C for 20 min, and allyl bromide (6.0 mL; 69 mmol) was added through the most elevated part of the condenser. More allyl bromide (2 mL; 23 mmol) was slowly added when the solution reached ebullition temperature. The reaction remained in reflux and agitation for 2 h. Thereafter, the reaction was cooled at room temperature and extracted with hexane (3 × 50 mL). Extracts from the organic phase were dried upon anhydrous sodium sulfate and concentrated in rotary evaporator. The product purification was carried out by column chromatography using the mixture hexane/ethyl acetate (40:1) as eluent.[29]

Compounds **3b** and **3c** were synthesized with a methodology similar to the one described for compound **3a**. For the obtention of compounds **4a**−**4c**, the methodology was similar; nevertheless, the compounds were obtained as unique products in the reactions.

*1-(Allyloxy)-4-chloro-2-isopropyl-5-methylbenzene (**3a**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.20 (d, 6H, J = 6.9 Hz, $CH(C\underline{H}_3)_2$); 2.32 (s, 3H, CH_3); 3.29 (sept. 1H, J = 6.9 Hz, $C\underline{H}(CH_3)_2$); 4.52 (dt, 2H, J = 5.1; 1.5 Hz, $C\underline{H}_2$); 5.25−5.46 (m, = $C\underline{H}_2$); 5.99−6.10 (m, 1H, $C\underline{H}$=); 6.68 (s, 1H, Ar−H_3); 7.14 (s, 1H, Ar−H_6). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 20.00 ($\underline{C}H_3$); 22.55 ($CH(\underline{C}H_3)_2$); 26.65 ($\underline{C}H(CH_3)_2$); 69.05 ($\underline{C}H_2$); 114.28 (=$\underline{C}H_2$); 116.92 (C_6); 125.70 (C_4); 126.59 (C_3); 127.26 (C_2); 133.33 (C_5); 136.61($\underline{C}H_2$=); 154.19 (C_1). MS (EI) m/z (%):

DOI: 10.1021/acs.jafc.7b04418
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226 ($C_{13}H_{17}ClO$ [M + 2]$^{+}$·33); 224 ([M]$^{+}$·100); 211(19); 209 (57); 189 (13); 185 (20); 183 (66); 167 (19); 155 (73); 143 (88); 139 (14); 119 (23); 105 (23); 91 (2); 77 (26); 65 (7); 41 (69).

*1-(Allyloxy)-4-chloro-5-isopropyl-2-methylbenzene (**3b**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.22 (d, 6H, J = 6.9 Hz,CH($C\underline{H}_3$)$_2$); 2.18 (s, 3H,$C\underline{H}_3$); 3.33 (sept. 1H, J = 6.9 Hz, $C\underline{H}(CH_3)_2$); 4.54 (dt, 2H, J = 5.1; 1.5 Hz, $C\underline{H}_2$); 5.22−5.44 (m, =$C\underline{H}_2$); 5.26−5.46 (m, 2H; =$C\underline{H}_2$); 6.10 (m, 1H, $C\underline{H}$=); 6.72 (s, 1H, Ar−H6); 7.10 (s, 1H, Ar−H_3). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 15.58 (CH_3); 22.71 (CH($\underline{C}H_3$)$_2$); 30.16 ($\underline{C}H(CH_3)_2$); 69.82 (CH_2); 109.67 (C_6); 117.15 (=CH_2); 124.09 (C_2); 126.01 (C_4); 130.97 (C_3); 133.44 ($\underline{C}H$=); 143.60 (C_5); 155.63 (C_1). MS (EI) m/z (%): 226 ($C_{13}H_{17}ClO$ [M + 2]$^{+}$·26); 224 ([M]$^{+}$· 77); 211(32); 209 (100); 181 (9); 174 (48); 158 (31); 145 (19); 141 (6); 128 (10); 115 (14); 105 (4); 91 (10); 77 (8); 51 (6); 43 (7).

*1-(Allyloxy)-4-chloro-2-methoxybenzene (**3c**).* The compound is a reddish brown liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 3.86 (s, 3H, $OC\underline{H}_3$); 4.57 (dt, 2H, J = 5.4 Hz; 1.5 Hz; $C\underline{H}_2$); 5.26−5.42 (m, 2H, =$C\underline{H}_2$); 5.99−6.12 (m, 1H, $C\underline{H}$=); 6.77−7.26 (m, 3H,Ar−H). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 56.05 ($O\underline{C}H_3$); 70.10 ($\underline{C}H_2$); 112.34 (C_3); 114.24 (C_6); 118.19 (=$\underline{C}H_2$); 120.19 (C_5); 126.00 (C_4); 132.97 ($\underline{C}H$=); 146.67 (C_1); 150.04 (C_2).MS (EI) m/z (%): 200 ($C_{10}H_{11}ClO_2$ [M + 2]$^{+}$·2); 198 ([M]$^{+}$·6); 157 (100); 143 (5); 129 (24); 111 (14); 99 (8); 93 (50); 79 (16); 65 (30); 51 (13); 41 (16).

*2-(Allyloxy)-1-isopropyl-4-methylbenzene (**4a**).* The compound is an uncolored liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.22 (d, 6H, J = 6.9 Hz, CH($C\underline{H}_3$)$_2$); 2.23 (s, 3H, $C\underline{H}_3$); 3.28−3.51 (m, 1H, $C\underline{H}(CH_3)_2$); 4.52−4.56 (m, 2H, $C\underline{H}_2$−CH=CH_2); 5.25−5.49 (m, 2H, CH=$C\underline{H}_2$); 5.88−6.16 (m, 1H, $C\underline{H}$=CH_2); 6.76 (d, 1H, J = 7.5 Hz, Ar−H_5); 6.67 (s, 1H,Ar−H_3),7.11 (d, 1H, J = 7.5 Hz, Ar−H_6). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 21.40 ($C\underline{H}_3$); 22.75 (CH($\underline{C}H_3$)$_2$); 26.59 ($\underline{C}H(CH_3)_2$); 68.73 ($\underline{C}H_2$); 113.86 (C_3); 112.65 (=$\underline{C}H_2$);116.61 (C_5); 125.88 (C_1); 127.10 (C_6); 133.74 ($\underline{C}H$=); 136.20 (C_4); 155.64 (C_2). MS (EI) m/z (%): 190 ($C_{13}H_{18}O$ [M]$^{+}$· 82); 175 (100); 147 (57); 133 (47); 121 (85); 105 (47); 91 (53); 77 (25); 65 (11); 41 (52).

*2-(Allyloxy)-4-isopropyl-1-methylbenzene (**4b**).* The compound is an uncolored liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 1.24 (d, 6H, J = 6.9 Hz, CH($C\underline{H}_3$)$_2$); 2.22 (s, 3H, $C\underline{H}_3$); 2.86 (sept. 1H, J = 6.9 Hz, $C\underline{H}(CH_3)_2$); 4.84 (dt, 2H, J = 5.1 Hz; 1.5 Hz; $C\underline{H}_2$-CH=); 5.25−5.48 (m, 2H, CH=$C\underline{H}_2$); 6.00 (m, 1H, $C\underline{H}$=CH_2); 6.70 (d, 1H, J = 1.0 Hz, Ar−H_3); 6.75 (dd, 1H,J = 7.5; 1.5 Hz, Ar−H_5), 7.07 (d, 1H, J = 7.5 Hz, Ar- H_6). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 15.85 (CH_3); 24.10 (CH($\underline{C}H_3$)$_2$); 34.09 ($\underline{C}H(CH_3)_2$); 68.73 (CH_2); 109.87 (C_3); 116.80 (=$\underline{C}H_2$); 118.21 (C_5); 124.25 (C_1); 130.44 (C_6); 133.81 ($\underline{C}H$=); 147.79 (C_4); 156.65 (C_2). MS (EI) m/z (%): 190 ($C_{13}H_{18}O$ [M]$^{+}$· 100); 175 (82); 133 (38); 121 (35); 105 (59); 79 (26); 77 (26); 65 (10); 55 (13); 41 (56).

*1-(Allyloxy)-2-methoxybenzene (**4c**).* The compound is an uncolored liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 3.88 (s, 3H, $OC\underline{H}_3$); 4.61 (dt, 2H, J = 5.4; 1.5 Hz, $C\underline{H}_2$); 5.26−5.44 (m, = $C\underline{H}_2$); 6.03−6.16 (m, 1H, $C\underline{H}$=); 6.91 (m, 3H, Ar−H). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 55.85 ($O\underline{C}H_3$); 69.82 ($\underline{C}H_2$); 111.72 (C_3 or C_6); 113.55 (C_3 or C_6); 117.84 (=$\underline{C}H_2$); 120.69 (C_4 or C_5)*; 121.19 (C_4 or C_5)*; 133.39 ($\underline{C}H$=); 147.47 (C_1or C_2)*; 149.47 (C_1 or C_2)*. *, attributions may be inverted. MS (EI) m/z (%): 164 ($C_{10}H_{12}O_2$ [M]$^{+}$·70); 123 (100); 95 (69); 77 (66); 67 (11); 65 (22); 52 (20); 41 (35).

Synthesis of Compound 5c. Synthesis of **5c** was exemplified by the synthesis of the 2-allyl-6-methoxyphenol (**5c**), known as *ortho*-eugenol. The allyl ether obtained, **4c** (**3g**), was introduced in a sealed glass tube, which was heated between 200 and 240° for 6 h in a sand bath. After this period, the tube was submitted to a cooling bath until it reached room temperature, when the tube could be opened and the product purified by column chromatography using the mixture hexane/dichloromethane (5:1) as eluent.[29]

*2-Allyl-6-methoxyphenol (**5c**).* The compound is a yellowish liquid. ^{1}H NMR, δ_H (300 MHz, $CDCl_3$): 3.42−3.44 (m, 2H, $C\underline{H}_2$); 3.89 (s, 3H, $OC\underline{H}_3$); 5.04−5.13 (m, 2H, =$C\underline{H}_2$); 5.72 (s, 1H, OH); 5.96−6.10 (m, 1H, $C\underline{H}$=); 6.74−6.84 (m, 3H, Ar−H). ^{13}C NMR, δ_C (75 MHz, $CDCl_3$): 33.81 ($\underline{C}H_2$); 55.99 ($O\underline{C}H_3$); 108.64 (C_5); 115.38 (=CH_2); 119.36 (C_3); 122.22 (C_4); 125.84 (C_2); 136.64 (CH=); 143.37 (C_1); 146.36 (C_6). MS (EI) m/z (%): 164 ($C_{10}H_{12}O_2$[M]$^{+}$·100); 149 (35); 131 (27); 121 (16); 103 (22); 91 (18); 77 (22); (8); 55 (18); 39 (8).

Antimicrobial Activity. Antimicrobial activity was evaluated by the agar diffusion test using solutions of all components in DMSO at 200 μg mL^{-1} according to the methodology described by the National Committee for Clinical Laboratory Standards.[30] Gram-positive bacterium *Staphylococcus aureus* ATCC 6538 and Gram-negative bacterium *Escherichia coli* ATCC 11229 were used. Suspensions of the microorganisms were activated twice in BHI broth (Himedia, India) and incubated at 35 °C for 18−24 h. To obtain isolated colonies, streaking was done in Petri dishes containing count agar plates (PCA, Himedia, India) and incubated at 35 °C for 18−24 h. Isolated colonies were selected to prepare the bacterial inocula in saline solution at 0.85% (w/v). The optical density of bacterial inocula was spectrophotometrically adjusted at 600 nm (Thermo Scientific Multiskan Go, USA) to give approximately 1.0 × 10^{8} CFU mL^{-1} ($Abs_{600\ nm}$ = 0.1). An aliquot of 0.1 mL of bacterial suspension ($Abs_{600\ nm}$ = 0.1) was spread onto Muller-Hinton agar plate (Sigma-Aldrich, São Paulo, Brazil). Discs (6 mm) impregnated with 5 μL of each tested compound were placed on the surface of the agar. Inoculated plates were incubated at 35 °C for 24 h. At the end of incubation, the diameter of inhibition zones was measured with a ruler and recorded in mm. DMSO was used as negative control and ampicillin (10 μg disc, Bio-Rad, France) as positive control. The experiment was conducted under completely randomized design (CRD) with three repetitions in duplicate. Data from the agar diffusion test were analyzed using the R software[31] and were subjected to analysis of variance (ANOVA) and Duncan test at 5% of probability.

Determination of Minimum Inhibitory Concentration (MIC). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the natural phenol derivatives were tested against strains of: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* Typhimurium (ATCC 13076), *Bacillus cereus* (ATCC 14579), *Listeria innocua* (ATCC 33090), and *Salmonella enterica* ssp. *enterica* isolated from vegetables commercialized in Alegre city, Espirito Santo. The MIC was carried out using the *in vitro* broth microdilution method. Broth BHI (Himedia, India) supplemented with the compounds was added to 96-well titration microplates. A two-fold dilution factor was used ranging from 220 to 3.44 μg mL^{-1}. The concentrations tested were: 220 μg mL^{-1}, 110 μg mL^{-1}, 55 μg mL^{-1}, 27.5 μg mL^{-1}, 13.75 μg mL^{-1}, 6.87 μg mL^{-1}, 3.44 μg mL^{-1}. Microplates were separately, inoculated with 5 × 10^{5} ufc mL^{-1} of each strain of bacteria in exponential growth phase. After the inoculations, the microplates were aerobically incubated at 35 °C for 24 h. MIC was considered the lower concentration of the tested compounds able to inhibit microorganism's visual growth.[32]

Determination of Minimum Bactericidal Concentration (MBC). The minimum bactericidal concentration (MBC) was determined from the inoculation by spreading BHI of 0.1 mL of the microplate wells content that did not present growth in the MIC test in agar. Plates were aerobically incubated at 35 °C for 24 h. MBC was defined as the lower concentration that did not present bacterial multiplication.[32]

Experimental Planning and Statistical Data Analysis. The experiment was conducted under completely randomized design (CRD) with three repetitions. For the diffusion in agar test, the results were analyzed with the R software submitted to analysis of variance (ANOVA). When the different semisynthetic compounds presented significant effect, they were compared by the Duncan test at 5% of probability. For the comparison of the two tested bacteria, results were submitted to t test for the averages comparison ($p > 0.05$)[31].

Molecular Properties and Statistical Analysis. The molecular properties of the natural phenols and their semisynthetic derivatives were computed with the Spartan 6.0[33] software using the semi-empirical PM6 method.[34] Geometries were fully optimized with the

DOI: 10.1021/acs.jafc.7b04418
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PM6 method and properties were obtained for the most stable conformer. The calculated parameters are solvation energy; HOMO energy; LUMO energy; dipole moment; molecular volume, polar surface area, partition coefficient (log *P*), ovality, and polarizability. The obtained parameters were analyzed with principal components analysis (PCA).

RESULTS AND DISCUSSION

Synthesis. The semisynthetic phenols derived from the natural phenols were obtained according to Figure 2. In the

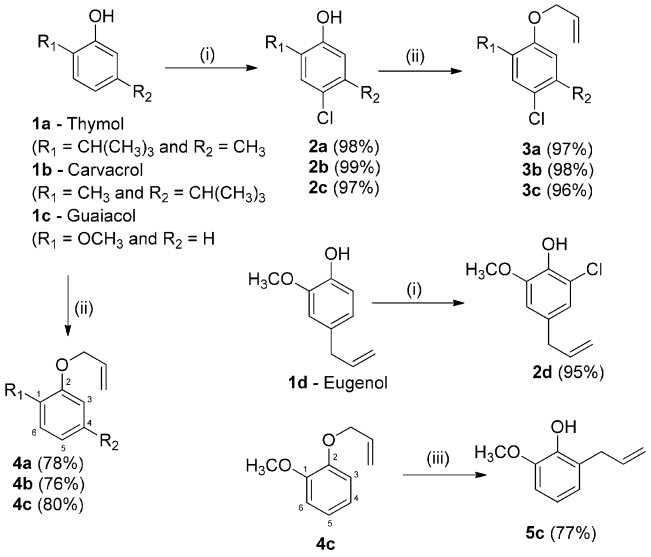

Figure 2. Synthetic route for preparation of semisynthetic derivatives from the natural phenols: thymol, carvacrol, eugenol, and guaiacol.

synthesis of 4-chlorophenol (**2a**–**2d**), the catalytic oxychlorination was used. This has proven to show high regioselectivity.[28] These compounds were obtained as unique products and with excellent yields, with exclusive addition of chlorine at position 4 of the aromatic ring. This high selectivity is important, particularly considering the results of classical phenol chlorination with $Cl_{2(g)}$ in the presence of a Lewis acid ($AlCl_3$ or $FeCl_3$) as catalyst. In addition to the reagent toxicity, the conventional halogenation of the aromatic compounds leads to the formation of an isomer mixture, with chlorophenols substituted in the *ortho* and *para* positions, requiring a posterior purification stage. The compound **2b** (4-chlorocarvacrol) was obtained for the first time with this methodology.

After the Williamson synthesis, the allyl ethers from the chlorinated and nonchlorinated phenols (**3a**–**3c** and **4a**–**4c**) were obtained with good results. The aromatic Claisen rearrangement was carried out for the nonchlorinated allyl ether (**4c**), with formation of the product **5c** (*ortho*-eugenol), an eugenol constitutional isomer. The obtained compounds were characterized by ^1H and ^{13}C NMR and by MS. The structures of all the compounds synthesized were shown in Figure 3. These compounds and their precursors (natural phenols) were used in antimicrobial assays.

Antimicrobial Activity. The values regarding the diameter of the inhibition zones from the phenol derivatives and their starting compounds against the bacteria *S. aureus* and *E. coli* are presented in Table 1.

The compounds **2a**, **2b**, and **2c** presented higher inhibition halos against *S. aureus* than against *E. coli* ($p < 0.05$). Besides,

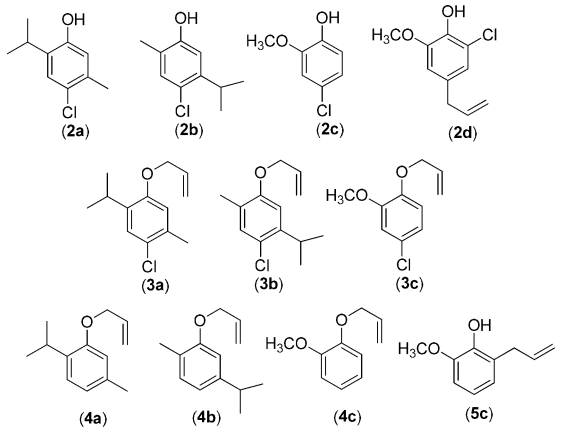

Figure 3. Structures of compounds synthesized from natural phenols: thymol (**2a**–**4a**), carvacrol (**2b**–**4b**), guaiacol (**2c**–**5c**), and eugenol (**2d**).

Table 1. Diameter of Inhibition Zones against *S. aureus* and *E. coli* for Compounds Tested Using Concentration of 200 μg mL^{-1}

	diameter of inhibition zones (mm)[a]	
compounds	*S. aureus*	*E. coli*
thymol (**1a**)	30.1 ± 8.8 cdA	24.0 ± 7.3 aA
2a	75.8 ± 9.0 aA	30.8 ± 4.6 aB
3a	0.0 ± 0.0 fA	0.0 ± 0.0 eA
4a	0.0 ± 0.0 fA	0.0 ± 0.0 eA
carvacrol (**1b**)	31.2 ± 15.3 cA	27.4 ± 3.5 abA
2b	52.7 ± 3.0 bA	22.8 ± 7.9 aB
3b	0.0 ± 0.0 fA	0.0 ± 0.0 eA
4b	0.0 ± 0.0 fA	0.0 ± 0.0 eA
guaiacol (**1c**)	0.0 ± 0.0 fA	0.0 ± 0.0 eA
2c	21.0 ± 5.7 deA	4.30 ± 7.5 deB
3c	0.0 ± 0.0 fA	8.30 ± 14.4 cdA
4c	0.0 ± 0.0 fA	0.0 ± 0.0 eA
5c	11.1 ± 9.6 eA	16.2 ± 14.1 bcA
eugenol (**1d**)	19.4 ± 2.9 eA	24.1 ± 8.2 aA
2d	0.0 ± 0.0 fA	0.0 ± 0.0 eA
control (DMSO)	0.0 ± 0.0 fA	0.0 ± 0.0 eA
ampicillin	20.7 ± 2.5 deA	16.0 ± 1.8 bA

[a]Average and standard deviation of three repetitions. Values followed by the same lower case letter in the column do not differ significantly at 5% by Duncan test. Values followed by the same capital letter in the line do not differ significantly at 5% by *t* test.

these three compounds presented halos against *S. aureus* superior to the phenols that originated them. This may be a consequence of the presence of chlorine atoms in these molecules. The acid character of the phenols may be one of the factors that contributes to the antimicrobial activity presented by the thymol and carvacrol isomers.[35] With the introduction of chlorine in the position 4 (*para*) in relation to the OH group of the phenols, an increase in the acidity of these compounds occurs, what may contribute to potentiate their antimicrobial activity. For the other compounds, there was no significant difference ($p > 0.05$) in relation to the action upon the Gram-positive and Gram-negative bacteria. The chlorophenols present great utility in the agrochemical industries as pesticides as well as in the production of drugs and for obtaining potent disinfectants.[36]

DOI: 10.1021/acs.jafc.7b04418
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Table 2. Values for MIC and MBC Presented by Natural Phenols and Their Semisynthetic Derivatives (220 to 3.44 μg mL^{-1}) against Bacteria: *E. coli*, *S. aureus*, *L. innocua*, *P. aeruginosa*, *S.* Typhimurium, *S. enterica*, and *B. cereus*

	antimicrobial activity (μg mL^{-1})													
compound	*E. coli*		*S. aureus*		*L. innocua*		*P. aeruginosa*		*S.* Thyphimurium		*S. enterica*		*B. cereus*	
	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB
thymol (**1a**)	110	220	220	>220	220	>220	27.5	27.5	110	110	110	110	220	>220
2a	110	110	6.87	6.87	110	110	110	110	110	110	110	110	110	110
3a	110	220	220	220	220	220	110	220	110	220	110	220	220	220
4a	110	220	110	220	110	220	110	220	110	220	110	110	110	220
carvacrol (**1b**)	110	220	220	>220	220	>220	220	220	110	110	110	110	220	>220
2b	110	110	220	>220	220	220	3.44	6.87	110	220	110	110	110	220
3b	110	220	6.87	13.75	>220	>220	55	55	>220	>220	>220	>220	>220	>220
4b	110	220	220	>220	220	220	110	220	110	220	110	110	110	220
guaiacol (**1c**)	110	220	110	220	110	220	220	220	110	220	110	220	110	220
2c	110	220	110	220	110	220	110	220	220	>220	110	220	110	220
3c	110	220	110	220	110	220	110	110	110	220	110	220	110	220
4c	110	220	220	>220	110	220	110	110	220	220	110	220	110	220
5c	110	220	110	220	110	220	110	110	110	220	110	220	110	220
eugenol (**1d**)	110	220	110	220	110	220	220	220	110	220	110	220	110	220
2d	110	220	110	220	110	220	220	220	220	>220	110	220	110	220

In general, chlorinated compounds may present antimicrobial activity. Shi et al.[37] synthesized and determined a relevant antimicrobial activity for a series of Schiff bases by the reaction of 5-chloro-salicylaldehyde with primary amines. The synthesized compounds are active against the bacteria: *Bacillus subtilis*, *E. coli*, *Pseudomonas fluorescence*, and *S. aureus*.

The natural or semisynthetic phenolic compounds presented inhibition halo against *S. aureus*, or *E. coli*, except guaiacol, the only natural phenol that did not present inhibition halo against the two tested bacteria. The semisynthetic compounds belonging to the class of allyl phenol ethers did not present activity against these bacteria, which suggests that the presence of the OH phenolic group may be important to their antimicrobial action. The action mechanism in bacteria may be associated with their action in the cell membrane. According to Hammer and Heel,[38] carvacrol alters the polarization and permeability of Gram-positive bacteria. This depolarization is possibly associated with the accumulation of molecules inside the membrane in a way that the ionic permeability is increased, provoking a dissipation of the ions transmembrane gradient. Afterward, a higher accumulation of these compounds in the interior of the membrane, possibly associated with a higher period of exposition, leads to the increase of its permeability, interfering with the cell activity.

Values of the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the studied compounds against the analyzed bacteria are given in Table 2.

The compound **2a** presented MIC and MBC values inferior to what was found for thymol, its precursor, against three of the seven tested microorganisms: *S. aureus*, *L. innocua*, and *B. cereus*. These three bacteria have in common the fact that they are *gram*-positive. The compound **2b** presented the lowest

Table 3. Molecular Properties of Natural Phenols and Their Semi-Synthetic Derivatives Obtained with PM6 Method[a]

compound	ΔH_f^o (kJ/mol)	E_{solv} (kJ/mol)	Homo (eV)	Lumo (eV)	μ (debye)	V_{mol} (Å^3)	PSA (Å^2)	Oval (Å)	Log *P*	β (10^{-30} m^3)
1a (thymol)	−208.4	−24.8	−8.74	0.37	1.51	179.29	18.74	1.31	3.37	53.77
2a	−249.8	−25.7	−8.80	−0.02	1.84	192.47	18.72	1.33	3.92	54.91
3a	−166.7	−4.3	−8.62	0.15	2.65	245.11	5.58	1.42	4.88	59.19
4a	−124.2	−5.1	−8.57	0.52	1.54	231.94	5.58	1.40	4.32	58.05
5a	−166.6	−10.4	−8.74	0.38	1.55	228.24	14.03	1.36	2.30	57.74
1b (carvacrol)	−203.3	−25.3	−8.70	0.37	1.38	179.33	19.06	1.31	3.37	53.78
2b	−242.8	−26.4	−8.78	−0.03	2.05	192.33	18.88	1.32	3.92	54.91
3b	−159.8	−6.3	−8.60	0.13	2.84	244.98	5.74	1.41	4.88	59.19
4b	−119.9	−7.1	−8.53	0.50	1.48	231.97	5.77	1.40	4.32	58.06
1c (guaiacol)	−243.1	−32.1	−8.62	0.13	2.99	134.18	25.29	1.21	−0.51	50.19
2c	−285.1	−29.3	−8.78	−0.26	2.84	147.67	25.29	1.25	−0.65	51.34
3c	−195.1	−15.1	−8.58	−0.12	4.31	199.79	10.86	1.34	0.15	55.58
4c	−153.2	−17.1	−8.37	0.26	2.60	186.32	10.91	1.31	0.29	54.45
5c	−205.1	−22.5	−8.61	0.17	2.85	184.80	24.16	1.32	0.23	54.29
1d (eugenol)	−208.1	−27.6	−8.51	0.14	2.92	185.05	25.33	1.33	0.23	54.34
2d	−242.7	−18.6	−8.71	−0.26	0.42	197.77	22.98	1.34	0.09	55.42

[a] ΔH_f^o = Heat of formation (kJ/mol); E_{solv} = solvation energy (kJ/mol); homo = homoenergy (eV); lumo = lumoenergy (eV); μ = dipolemoment (debye); V_{mol} = molecular volume (Å^3); PSA = polar surface area (Å^2); Oval = Ovality (Å); Log *P* = partition coefficient; β = second-order polarizability (10^{-30} m^3).

DOI: 10.1021/acs.jafc.7b04418
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Table 4. Pearson's Correlation Coefficients between Molecular Properties

	E_{solv} (kJ/mol)	homo (eV)	lumo (eV)	μ (debye)	V_{mol} (Å^3)	PSA (Å^2)	Oval (Å)	Log P	β (10^{-30} m^3)
E_{solv} (kJ/mol)	1.0000	0.4328	0.3780	−0.0582	0.9245[a]	−0.9219[a]	0.8992[a]	0.5673[a]	0.9249[a]
homo (eV)		1.0000	0.4446	0.3174	0.2710	−0.4253	0.2607	−0.1705	0.2730
lumo (eV)			1.0000	−0.2486	0.3346	−0.4719	0.3469	0.5046	0.3191
μ (debye)				1.0000	−0.1468	−0.0290	−0.1762	−0.3655	−0.1388
V_{mol} (Å^3)					1.000000	−0.8503[a]	0.9915[a]	0.7275[a]	0.9998[a]
PSA (Å^2)						1.0000	−0.8063[a]	−0.6675[a]	−0.8482[a]
Oval (Å)							1.0000	0.7099[a]	0.9909[a]
Log P								1.0000	0.7179[a]

[a]Significant ($p < 0.05$).

values of MIC (3.44 μg mL^{-1}) and MBC (6.87 μg mL^{-1}) against *P. aeruginosa* (*gram*-negative). Semisynthetic obtained from guaiacol and eugenol presented antimicrobial activity similar to their precursor phenols in relation to the bacteria used in the assays.

One of the antimicrobial action mechanisms of the phenolic compounds, as the isomers thymol and carvacrol, may be associated with the acid character of these molecules and to the fact that they are hydrophobic.[35,37] Bassanetti et al.[32] confirmed the importance of the phenol group in the antimicrobial activity of thymol and carvacrol derivatives. Regarding guaiacol and eugenol, the presence of the methoxy group in the *ortho* position may restrain the hydroxyl groups to easily release the H^+ ion, interfering in the antimicrobial activity. Arfa et al.[39] studied the relation between the chemical structure and the antimicrobial activity of carvacrol, eugenol, and two compounds derived from synthesized carvacrol: carvacrol methyl ether and carvacryl acetate against the bacteria: *E. coli*, *Pseudomonas fluorescens*, *S. aureus*, *Lactobacillus plantarum*, and *Bacillus subtilis*. Eugenol exhibited antimicrobial activity inferior to that presented by carvacrol. The derivatives from carvacrol, carvacryl acetate, and carvacrol methyl ether did not present antimicrobial activity, indicating that the presence of a free hydroxyl group in the phenols is important for the antimicrobial activity.

In the present work, the most part of the natural phenols and their semisynthetic derivatives presented MIC $\leq$ 220 μg mL^{-1} for all the tested bacteria. Therefore, these compounds presented great potential to be used as antimicrobial agent because they possess wide spectrum of action.[40] The natural phenolic compounds are food flavoring and possess evidenced antioxidant activity. Consequently, enhancing their antimicrobial activities may be of great relevance for the food industry, increasing the options of additives that can be used industrially.[35]

Computational Calculations of Molecular Properties. The molecular properties of the natural phenols and their semisynthetic derivatives were obtained with the Spartan 6.0 software using the PM6 semiempirical method. The property values for each compound are given in Table 3. The calculated parameters are heat of formation (ΔH_f°); solvation energy (E_{solv}); homo energy (homo); lumo energy (lumo); dipole moment (μ); molecular volume (V_{mol}), polar surface area (PSA), ovality (oval), partition coefficient (Log P) and polarizability (β). The Pearson's correlation coefficients between molecular properties are given in Table 4. The compounds were grouped by PCA, where the natural phenols thymol (**1a**), and carvacrol (**1b**), and their respective chlorinated semisynthetic derivatives **2a** and **2b** remained in the same group, as shown in Figure 4. Isomers **1d** and **5c** also remained in the same group since they presented differences only in atom connectivities (constitutional isomers).

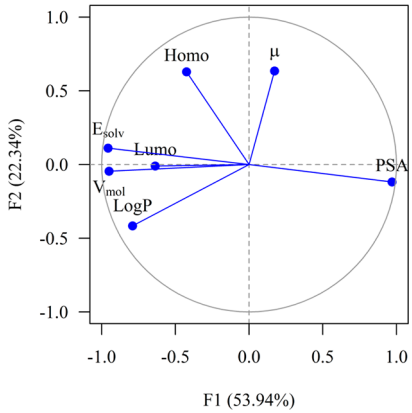

Figure 4. Projection of molecular properties in the factors F1 and F2.

Molecular volume (V_{mol}), ovality (Oval), and polarizability (β) are highly correlated ($r > 0.99$). Thus, ovality and polarizability were excluded from the principal component analysis. The first two factors account for 76.28% of the total variation, with 53.94% of the first component and 22.34% of the second component. Figure 4 shows the projection of the variables in the first two main components.

The molecular properties E_{solv}, PSA, and V_{mol} were the ones that contributed the most to the first factor, with contributions of 22.88%, 23.54%, and 22.58%, respectively. The variables homo and μ were the ones that contributed the most to the second factor, with contributions of 39.5% and 40.29%, respectively. The variable Log P contributed with 15.62% for the first factor and 17.35% for the second factor.

Figure 5 shows the projection of compounds in the first two factors. Two groups are noted, one formed by compounds **1a**, **1b**, **2a**, **2b**, and **2d** and the other formed by compounds **3a**, **3b**, **4a**, and **4b**.

The natural phenols thymol (**1a**), carvacrol (**1b**), guaiacol (**1c**), and eugenol (**1d**), as well as the semisynthetic chlorinated phenols (**2a**−**2d** and **5c**) and their respective allyl phenyl ethers (**3a**−**3c** and **4a**−**4c**) presented great potential to be used as antimicrobials and they may be useful in the food and drug industries, as well as sanitizers and disinfectants. Most of the compounds presented a wide spectrum of antimicrobial action because MIC values equal or inferior to 220 μg mL^{-1} were found for all the tested bacteria: *E. coli*, *S. aureus*, *L. innocua*, *P. aeruginosa*, *S. enterica* Typhimurium, *S. enterica* ssp. *enterica*, and *B. cereus*.

DOI: 10.1021/acs.jafc.7b04418
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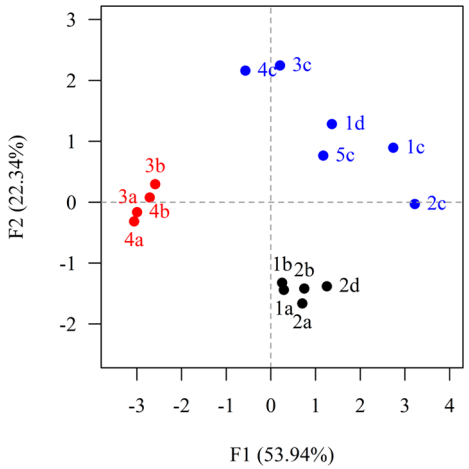

Figure 5. Projection of compounds in the first two factors.

Thymol and carvracol derivatives, **2a** and **2b**, added with chlorine to their molecules, stood out for the antimicrobial potential, with emphasis on *S. aureus* and *P. aeruginosa*. In general, they had antimicrobial activity equal to or greater than their precursor compounds. In future studies, synthetic phenols could be tested directly in food or in food models.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b04418.

Mass spectra of synthesized compounds (PDF)

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Funding

We thank the National Council for Scientific and Technological Development (CNPq) for financial support (484183/2013-3).

Notes

The authors declare no competing financial interest.

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