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SYNTHESIS AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF THYMOL AND CARVACROL DERIVATIVES

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Abstract: Fourteen esters of thymol and carvacrol were synthesized and characterized on the basis of spectral data. The NMR data for some of these are being given for the first time. The antibacterial activity screening of thymol, carvacrol and their esters were carried out against four Gram-positive (*Streptococcus mutans* MTCC 890, *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Staphylococcus epidermidis* MTCC 435) and one Gram-negative (*Escherichia coli* MTCC 723) bacteria. The enhancement in activity was noticed in the thymyl ester derivatives **4a**–**c** (against *S. mutans, B. subtilis* and *S. epidermidis*) in comparison to thymol, whereas the carvacrol derivatives were found to be much less active than carvacrol.

Keywords: thymol; carvacrol; thymyl ester; carvacryl ester

Natural products as such or as their chemical modifications have been developed as antibacterial agents (1). Among diverse chemical structures, lower terpenoids have provided effective and less toxic antibacterial compounds (2–5). Thymol and carvacrol, phenolic monoterpenes, isolated from *Thymus vulgaris*, *Origanun vulgare*, *Satureja thymbra* and *Thymbra capitata* (6–9) have been shown to possess antimicrobial and antioxidant activities (10–14). Several hydroxyl derivatives of thymol have shown activity against *S. aureus* and *S. flexneri* (15). 10-Isobutyryloxy-8,9-epoxythymol isobutyrate was found to be active against *S. aureus*, *P. aeruginosa* and *C. albicans* (16). Chemical modification of natural monoterpenoids to various ether and ester derivatives has been reported to result in change in biological activity (17–20).

Keeping the diverse therapeutic activities of phenolic monoterpenes in view, it was attempted to synthesize a novel ester series of thymol and carvacrol to improve the antibacterial activities of title compounds. We report herein the synthesis and antibacterial evaluation of fourteen esters, analogs of thymol (**1**) and carvacrol (**2**).

EXPERIMENTAL

Chemistry

Chemicals and solvents used were from Fluka and Merck. All the reagents were of analytical grade. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker Avance NMR 500 MHz and 125 MHz spectrometer in $CDCl_3$. Chemical shifts are expressed as δ (ppm). Mass spectra were recorded on ThermoQuest Trace GC 2000 interfaced with Finnigan MAT PolarisQ Ion Trap Mass Spectrometer fitted with an Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 μm film coating) in EI mode (70 eV) with mass scan range of 40–450 amu. IR spectra were recorded with FT-IR, Perkin Elmer spectrum BX-II apparatus. The reactions were monitored by TLC on aluminium sheets with silica gel (60 $F_{254,}$ Merck) and GC (FID). All the reactions were carried out under nitrogen atmosphere.

General procedure for the synthesis of ester derivatives 4a–g and 5a–g

To a solution of **1** or **2** (1.0 equiv) and triethylamine (1.1 equiv) in anhydrous dichloromethane (DCM) acid chlorides **3a**–**g** (1.1 equiv) were added at 0°C. The reaction mixture was stirred at 0°C for about 1 h and the stirring was continued at room temperature for about 10 h (progress of reaction was monitored by TLC and GC). After completion, the reaction mixture was quenched with distilled water and extracted with dichloromethane (3 × 25 mL). Finally, the combined organic layer was washed with distilled water, brine and dried over anhydrous

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Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by CC using silica gel (60–120 mesh) with hexane/diethyl ether (19 : 1, v/v) as eluent to afford pure ester derivatives **4a**–**g** and **5a**–**g** in 75–85% yields.

2-Isopropyl-5-methylphenyl acetate (**4a**)

Yield 80%; GC-MS (EI, 70 eV): m/z (%): 192 [M^+], (5), 150 (36), 135 (100), 115 (6), 107 (10), 91 (8); IR (KBr, cm^{-1}): 3028, 2962, 2872, 1760, 1622, 1368, 1207, 1149, 1089, 899, 671. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.18 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.01 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 2.93–3.00 (m, 1H, CH), 2.30 (s, 6H, CH_3), 1.18 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz,: δ ppm): 169.7 (C=O), 147.8 (C_{Ar}), 136.9 (C_{Ar}), 136.3 (C_{Ar}), 127.0 (CH_{Ar}), 126.4 (CH_{Ar}), 122.6 (CH_{Ar}), 27.0 (CH), 25.4 ($COCH_3$), 22.9 (2 × CH_3), 20.8 (CH_3).

2-Isopropyl-5-methylphenyl propionate (**4b**)

Yield 80%; GC-MS (EI, 70 eV): m/z (%): 206 [M^+], (6), 150 (36), 135 (100), 115 (4), 107 (6), 91 (8); IR (cm^{-1}): 3028, 2964, 2872, 1759, 1621, 1461, 1344, 1194, 1151, 897. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.17 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.99 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 2.95–2.97 (m, 1H, CH), 2.58 (q, *J* = 7.5 Hz, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.27 (t, *J* = 7.5 Hz, 3H, CH_3), 1.18 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR: ($CDCl_3$, 125 MHz, δ, ppm): 172.5 (C=O), 147.8 (C_{Ar}), 136.8 (C_{Ar}), 136.3 (C_{Ar}), 126.9 (CH_{Ar}), 124.0 (CH_{Ar}), 122.6 (CH_{Ar}), 34.9 (CH_2), 27.0 (CH), 22.8 (2 × CH_3), 20.6 (CH_3), 12.0 (CH_3).

2-Isopropyl-5-methylphenyl isobutyrate (**4c**)

Yield 76%; GC-MS (EI, 70 eV): m/z (%): 220 [M^+], (8), 150 (46), 135 (100), 107 (6), 91 (8). IR (cm^{-1}): 3029, 2964, 2871, 1756, 1618, 1461, 1344, 1224, 1149, 878. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm):7.20 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 3.24 (hept, *J* = 7.0 Hz, 1H, CH), 2.83 (hept, *J* = 7.0 Hz, 1H, CH), 2.30 (s, 3H, CH_3), 1.32 (d, *J* = 6.5 Hz, 6H, CH_3), 1.18 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 175.3 (C=O), 148.1 (C_{Ar}), 137.1 (C_{Ar}), 136.1 (C_{Ar}), 126.5 (CH_{Ar}), 126.2 (CH_{Ar}), 122.5 (CH_{Ar}), 33.4 (CH), 26.9 (CH), 22.6 (2 × CH_3), 20.5 (CH_3), 18.7 (2 × CH_3).

2-Isopropyl-5-methylphenyl 3-methylbutanoate (**4d**)

Yield 80%; GC-MS (EI, 70 eV): m/z (%): 234 [M^+], (4), 150 (48), 135 (100), 107 (4), 91 (8). IR (cm^{-1}): 3028, 2931, 2872, 1757, 1621, 1463, 1364, 1238, 1150, 816. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.19 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.01 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.79 (s, 1H, Ar-H), 2.96–2.99 (m, 1H, CH), 2.46 (d, *J* = 7.0 Hz, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.25–2.29 (m, 1H, CH), 1.18 (d, *J* = 7.0 Hz, 6H, CH_3), 1.07 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 171.6 (C=O), 147.7 (C_{Ar}), 136.8 (C_{Ar}), 136.3 (C_{Ar}), 126.8 (CH_{Ar}), 126.2 (CH_{Ar}), 122.5 (CH_{Ar}), 43.2 (CH_2), 26.8 (CH), 25.6 (CH), 22.9 (2 × CH_3), 22.3 (2 × CH_3), 20.7 (CH_3).

(*E*)-2-Isopropyl-5-methylphenyl but-2-enoate (**4e**)

Yield 84%; GC-MS (EI, 70 eV): m/z (%): 218 [M^+], (4), 150 (46), 135 (100), 107 (6), 91 (8); IR (cm^{-1}): 3025, 2927, 2871, 1761, 1657, 1621, 1458, 1363, 1231, 1154, 816. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.16 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 6.00–6.08 (m, 1H, CH), 5.25 (d, *J* = 11.0 Hz, 1H, CH-), 2.88–3.01 (m, 1H, CH), 2.29 (s, 3H, CH_3), 1.94 (d, *J* = 6.0 Hz, 3H, CH_3), 1.17 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR: ($CDCl_3$, 125 MHz, δ, ppm): 169.0 (C=O), 146.8 (C_{Ar}), 145.5 (CH), 135.8 (C_{Ar}), 135.4 (C_{Ar}), 126.0 (CH_{Ar}), 125.2 (CH_{Ar}), 121.7 (CH_{Ar}), 118.0 (CH), 25.9 (CH), 21.8 (2 × CH_3), 19.7 (CH_3), 17.0 (CH_3).

2-Isopropyl-5-methylphenyl benzoate (**4f**)

Yield 85%; GC-MS (EI, 70 eV): m/z (%): 254 [M^+], (10), 150 (10), 149 (82), 105 (100), 91 (4), 77 (48), 51 (8). IR (cm^{-1}): 3032, 2926, 2870, 1736, 1621, 1451, 1363, 1236, 1150, 816. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 8.27 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.69 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.57 (t, *J* = 8.0 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.12 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.99 (s, 1H, Ar-H), 3.07–3.15 (m, 1H, CH), 2.39 (s, 3H, CH_3), 1.26 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 165.4 (C=O), 148.1 (C_{Ar}), 137.2 (C_{Ar}), 136.7 (C_{Ar}), 133.5 (C_{Ar}), 130.1 (2 × CH_{Ar}), 129.6 (CH_{Ar}), 128.6 (2 × CH_{Ar}), 127.2 (CH_{Ar}), 126.5 (CH_{Ar}), 122.9 (CH_{Ar}), 27.3 (CH), 23.1 (2 × CH_3), 20.9 (CH_3).

2-Isopropyl-5-methylphenyl 2-phenylacetate (**4g**)

Yield 85%; GC-MS (EI, 70 eV): m/z (%): 268 [M^+], (2), 150 (68), 135 (100), 118 (26), 91 (34), 65 (6). IR cm^{-1}): 3031, 2927, 2870, 1753, 1621, 1454, 1363, 1230, 1149, 817. ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.35 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.30 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.10 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.94 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.74 (s, 1H, Ar-H), 3.80 (s, 2H, CH_2), 2.69–2.75 (m, 1H, CH), 2.23 (s, 3H, CH_3), 1.02 (d,

J = 8.0 Hz, 6H, CH_3). ^{13}C NMR: ($CDCl_3$, 125 MHz, δ, ppm): 170.2 (C=O), 148.0 (C_{Ar}), 137.1 (C_{Ar}), 136.5 (C_{Ar}), 133.7 (C_{Ar}), 129.4 (2 × CH_{Ar}), 128.8 (2 × CH_{Ar}), 127.4 (CH_{Ar}), 127.2 (CH_{Ar}), 126.4 (CH_{Ar}), 122.7 (CH_{Ar}), 41.7 (CH_2), 27.0 (CH), 23.0 (2 × CH_3), 19.7 (CH_3).

5-Isopropyl-2-methylphenyl acetate (**5a**)

Yield 78%; GC-MS (EI, 70 eV): m/z (%): 192 [M^+], (4), 150 (66), 135 (100), 107 (12), 91 (10). IR (cm^{-1}): 3025, 2961, 2928, 2871, 1766, 1623, 1460, 1369, 1215, 1169, 819. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.13 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, Ar-H), 6.85 (d, *J* = 1.5 Hz, 1H, Ar-H), 2.85–2.87 (m, 1H, CH), 2.29 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 1.21 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$,: δ, ppm): 167.8 (C=O), 147.8 (C_{Ar}), 146.6 (C_{Ar}), 129.4 (C_{Ar}), 125.7 (CH_{Ar}), 122.7 (CH_{Ar}), 118.3 (CH_{Ar}), 32.1 (CH), 22.4 (2 × CH_3), 19.3 (CH_3),14.3 (CH_3).

5-Isopropyl-2-methylphenyl propionate (**5b**)

Yield 75%; GC-MS (EI, 70 eV): m/z (%): 206 [M^+], (4), 150 (66), 135 (100), 107 (8), 91 (6). IR (cm^{-1}): 3028, 2961, 2871, 1761, 1622, 1460, 1351, 1236, 1148, 898. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.13 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.01 (d, *J* = 7.5 Hz, 1H, Ar-H), 6.86 (s, 1H, Ar-H), 2.84–2.88 (m, 1H, CH), 2.60 (q, *J* = 7.5 Hz, 2H, CH_2), 2.12 (s, 3H, CH_3), 1.28 (t, *J* = 7.5 Hz, 3H, CH_3), 1.22 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 172.5 (C=O), 149.1 (C_{Ar}), 147.9 (C_{Ar}), 130.7 (C_{Ar}), 127.0 (CH_{Ar}), 123.9 (CH_{Ar}), 119.6 (CH_{Ar}), 33.4 (CH), 27.5 (CH_2), 23.8 (2 × CH_3), 15.6 (CH_3), 9.1 (CH_3).

5-Isopropyl-2-methylphenyl isobutyrate (**5c**)

Yield 85%; GC-MS (EI, 70 eV): m/z (%): 220 [M^+], (10), 150 (92), 135 (100), 107 (8), 91 (8). IR (cm^{-1}): 3024, 2963, 2931, 2874, 1757, 1622, 1469, 1343, 1232, 1132, 818. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.13 (d, *J* = 7.0 Hz, 1H, Ar-H), 7.01 (d, *J* = 5.0 Hz, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 2.81–2.88 (m, 2H, CH), 2.13 (s, 3H, CH_3), 1.35 (d, *J* = 6.5Hz, 6H, CH_3), 1.23 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 175.0 (C=O), 149.1 (C_{Ar}), 147.8 (C_{Ar}), 130.6 (C_{Ar}), 126.9 (CH_{Ar}), 123.7 (CH_{Ar}), 119.5 (CH_{Ar}), 34.0 (CH), 33.0 (CH), 23.7 (2 × CH_3), 18.9 (2 × CH_3), 15.5 (CH_3).

5-Isopropyl-2-methylphenyl 3-methylbutanoate (**5d**)

Yield 75%; GC-MS (EI, 70 eV): m/z (%): 234 [M^+], (6), 150 (88), 135 (100), 107 (4), 91 (8). IR (cm^{-1}): 3024, 2961, 2930, 2872, 1759, 1622, 1462, 1364, 1233, 1115, 818. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (dd, *J* = 1.5 Hz, 6.5 Hz, 1H, Ar-H), 6.84 (d, *J* = 1.5 Hz, 1H, Ar-H), 2.84–2.91 (m, 1H, CH), 2.46 (d, *J* = 7.00 Hz, 2H, CH_2), 2.22–2.30 (m, 1H, CH), 2.13 (s, 3H, CH_3), 1.22 (d, *J* = 7.0 Hz, 6H, CH_3), 1.07 (d, *J* = 6.5 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 171.1 (C=O), 149.0 (C_{Ar}), 147.8 (C_{Ar}), 130.6 (C_{Ar}), 126.9 (CH_{Ar}), 123.7 (CH_{Ar}), 119.6 (CH_{Ar}), 43.0 (CH_2), 33.3 (CH), 25.6 (CH), 23.7 (2 × CH_3), 22.3 (2 × CH_3), 15.6 (CH_3).

(*E*)-5-Isopropyl-2-methylphenyl but-2-enoate (**5e**)

Yield 78%; GC-MS (EI, 70 eV): m/z (%): 218 [M^+], (20), 150 (100), 135 (52), 107 (5), 91 (10), 69 (25). IR (cm^{-1}): 3024, 2961, 2928, 2871, 1738, 1657, 1622, 1459, 1363, 1232, 1157, 819. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.16–7.19 (m, 1H, CH), 7.13 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (dd, *J* = 1.5 Hz, 6.5 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 6.06 (dd, *J* = 1.5Hz, 14 Hz, 1H, CH), 2.86 (m, 1H, CH), 2.12 (s, 3H, CH_3), 1.94 (dd, *J* = 1.5 Hz, 7.0Hz, 3H, CH_3), 1.22 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 164.5 (C=O), 149.1 (C_{Ar}), 147.8 (CH), 146.5 (C_{Ar}), 130.7 (C_{Ar}), 127.2 (CH_{Ar}), 123.8 (CH_{Ar}), 121.9 (CH), 119.7 (CH_{Ar}), 33.4 (CH), 23.7 (2 × CH_3), 18.0 (CH_3), 14.0 (CH_3).

5-Isopropyl-2-methylphenyl benzoate (**5f**)

Yield 82%; GC-MS (EI, 70 eV): m/z (%): 254 [M^+], (12), 105 (100), 77 (26), 51 (4). IR (cm^{-1}): 3062, 2960, 2927, 2870, 1737, 1621, 1451, 1341, 1238, 1116, 819. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 8.23 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.65 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.52 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.20 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.06 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 2.89-2.92 (m, 1H, CH), 2.19 (s, 3H, CH_3), 1.25 (d, *J* = 7.0 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 164.7 (C=O), 149.2 (C_{Ar}), 148.0 (C_{Ar}), 133.3 (CH_{Ar}), 130.7 (CH_{Ar}), 130.0 (2 × CH_{Ar}), 129.4 (C_{Ar}), 128.4 (2 × CH_{Ar}), 127.2 (C_{Ar}), 124.0 (CH_{Ar}), 119.7 (CH_{Ar}), 33.4 (CH), 23.8 (2 × CH_3), 15.7 (CH_3).

5-Isopropyl-2-methylphenyl 2-phenylacetate (**5g**)

Yield 78%; GC-MS (EI, 70 eV): m/z (%): 268 [M^+], (2), 150 (100), 135 (96), 118 (35), 91 (42), 65 (8). IR (cm^{-1}): 3088, 3064, 3031, 2960, 2926, 2870, 1747, 1622, 1454, 1234, 1119, 820. ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm): 7.41 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.37 (t, *J* = 7.0 Hz, 1H, Ar-H), 7.30 (t, *J* = 6.0 Hz, 2H, Ar-H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.99 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, Ar-H), 6.83 (d, *J* = 1.5

Hz, 1H, Ar-H), 3.87 (s, 2H, CH_2), 2.83–2.86 (m, 1H, CH), 1.97 (s, 3H, CH_3), 1. 20 (d, J = 6.5 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 169.7 (C=O), 149.24 (C_{Ar}), 148.0 (C_{Ar}), 133.6 (C_{Ar}), 130.9 (CH_{Ar}), 129.4 (2 × CH_{Ar}), 128.7 (2 × CH_{Ar}), 127.3 (C_{Ar}), 127.1 (CH_{Ar}), 124.1 (CH_{Ar}), 119.7 (CH_{Ar}), 41.5 (CH_2), 33.6 (CH), 23.9 (2 × CH_3), 15.6 (CH_3).

Antibacterial activity

The antibacterial activity of the synthesized compounds was screened against the Gram positive bacteria *Streptococcus mutans* (MTCC 890), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus epidermidis* (MTCC 435) and Gram negative bacteria *Escherichia coli* (MTCC 723) using disc diffusion and broth dilution methods (21, 22).

Paper disc diffusion method

The *in vitro* antibacterial activity was tested by the paper disc diffusion method, performed in sterilized (autoclaved at 120°C for 1 h) Petri dish. Compounds with 100 µg/disc concentrations were impregnated on the discs, which were placed on the surface of agar plates already inoculated with pathogenic bacteria. The plates were incubated at 37°C and examined after 24 h for zone of inhibition. Ampicillin was used as a standard. An additional control disc with an equivalent amount of solvent (DMSO) was also used in the assay. The results showed that some of the compounds exhibited significant zones (Table 1).

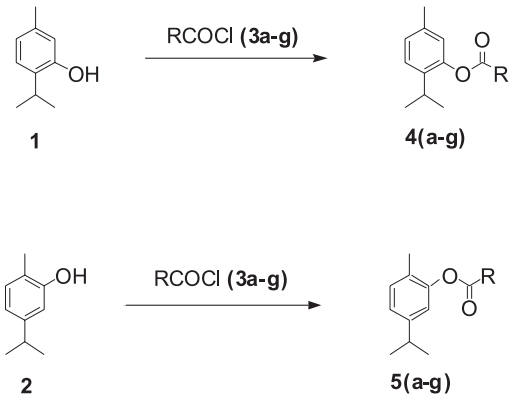
Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of the derivatives, the lowest concentration of compound giving complete inhibition of visible growth, were determined by micro titer plate broth dilution method. Various concentrations of compounds were prepared by two-fold dilution method. The last well of micro titer plate was considered as control, having no test compound. The inoculum was prepared using a 16 h broth culture of each bacterial strain adjusted to a turbidity equivalent to a 0.5 Mc Farland standard (3 × 10^6 cfu mL^{-1} bacteria). The micro titer plates were incubated for 24 h at 37°C.

Table 1. Antimicrobial activity of thymol, carvacrol and their ester analogues **4a-g** and **5a-g**

Compounds	*In-vitro* activity – zone of inhibition (in mm)[a] and MIC (in µg×mL^{-1})									
	S. mutans		*S. aureus*		*B. subtilis*		*S. epidermidis*		*E. coli*	
	zone of inhibition	MIC	zone of inhibition	MIC	zone of inhibition	MIC	zone of inhibition	MIC	zone of inhibition	MIC
Thymol	17 ± 0.79	125	25 ± 0.98	62.5	15 ± 0.68	125	14 ± 0.57	125	13 ± 0.51	250
4a	**30 ± 1.31**	**11.7**	18 ± 0.81	93.7	**30 ± 1.28**	**11.7**	**32 ± 1.37**	**11.7**	12 ± 0.53	375
4b	12 ± 0.53	187.5	10 ± 0.41	187.5	**25 ± 1.07**	**46.8**	**28 ± 1.21**	**46.8**	7 ± 0.27	>1000
4c	**18 ± 0.86**	**93.7**	17 ± 0.78	93.7	**21 ± 0.96**	**46.8**	**20 ± 0.87**	**46.8**	7 ± 0.26	>1000
4d	–	ND	–	ND	8 ± 0.29	ND	7 ± 0.27	ND	–	ND
4e	12 ± 0.53	750	9 ± 0.37	750	10 ± 0.46	750	9 ± 0.35	ND	7 ± 0.28	>1000
4f	–	ND	–	ND	7 ± 0.29	ND	10 ± 0.39	ND	–	ND
4g	8 ± 0.31	187.5	15 ± 0.66	93.7	8 ± 0.33	750	12 ± 0.49	187.5	7 ± 0.28	>1000
Carvacrol	30 ± 1.29	23.4	25 ± 1.07	23.4	35 ± 1.41	11.7	32 ± 1.39	11.7	35 ± 1.49	11.7
5a	17 ± 0.78	93.7	15 ± 0.66	93.7	25 ± 0.98	46.8	20 ± 0.89	93.7	–	ND
5b	12 ± 0.46	187.5	15 ± 0.74	187.5	20 ± 0.87	46.8	18 ± 0.72	93.7	–	ND
5c	7 ± 0.29	>1000	9 ± 0.37	375	9 ± 0.38	375	12 ± 0.56	187.5	–	ND
5d	22 ± 1.03	46.8	21 ± 0.91	46.8	25 ± 1.12	23.4	21 ± 0.96	46.8	10 ± 0.42	375
5e	17 ± 0.82	187.5	20 ± 0.89	93.7	20 ± 0.88	93.7	18 ± 0.86	93.7	10 ± 0.46	375
5f	–	ND	–	ND	–	ND	–	ND	–	ND
5g	–	ND	–	ND	–	ND	–	ND	–	ND
Ampicillin	27 ± 1.21	4	22 ± 0.94	8	25 ± 1.12	4	25 ± 1.07	2	12 ± 0.49	12

[a] Values are the mean of three determinations, the ranges of which are less than 5% of the mean in all cases. Ampicillin (20 µg/disk) was used as a positive reference. Compounds (100 µg/disk) were used for experiments. (–) = no inhibition. ND = not determined.



No.	R
a	CH_3
b	CH_2CH_3
c	$CH(CH_3)_2$
d	$CH_2CH(CH_3)_2$
e	$CHCHCH_3$
f	Ph
g	CH_2Ph

Scheme 1.Reagents and conditions TEA, anhydrous DCM, 0°C-rt, 10 h

RESULTS AND DISCUSSION

Chemistry

The esterification of **1** or **2** with RCOCl (R= -CH_3, -CH_2CH_3, -$CH(CH_3)_2$, -$CH_2CH(CH_3)_2$, -$CHCHCH_3$, Ph, -CH_2Ph) in the presence of triethylamine produced fourteen ester analogs. The condensation of 1.0 equiv of **1** or **2** with 1.1 equiv of corresponding acid chloride and 1.1 equiv of triethylamine in dry DCM at 25°C for 10 h gave fourteen ester analogs **4a**–**g** and **5a**–**g**, respectively, as colorless oily liquids in 75–85% yields (Scheme 1) (19). Formation of ester analogs was confirmed by the absence of -OH stretching absorption at 3325–3450 cm^{-1} and the presence of C=O group in their IR spectra, which were further confirmed by MS and NMR spectra (^{1}H and ^{13}C NMR). The synthesis of **4a**–**d** and **4f**–**g** has been previously reported (23–25). However, NMR (^{1}H and ^{13}C NMR) and MS data of **4e**, **4f** and **5a**–**g** have not been reported earlier.

Antibacterial activity

The antibacterial activity of thymol, carvacrol and their derivatives were evaluated against pathogenic bacterial strains *viz*. *Streptococcus mutans* (MTCC 890), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Staphylococcus epidermidis* (MTCC 435) and *Escherichia coli* (MTCC 723) using disc diffusion and broth dilution methods (21, 22). Ampicillin was used as positive control against both Gram-positive and Gram-negative bacteria. The zone of inhibition (in mm) and minimum inhibitory concentration (in µg/mL) of tested compounds are shown in Table 1. The antibacterial activity of the analogous **4a**–**g** and **5a**–**g** are lower in comparison to the standard antibiotic, ampicillin, but some of the synthetic analogs show better activity than the parent compound. Table 1 revealed that the majority of the compounds showed significant to moderate activity, with exception of **5f**–**g**, which were found inactive against all the bacterial strains. Among the thymyl ester derivatives (**4a**–**g**) and carvacryl ester derivatives (**5a**–**g**), **4a**–**c**, **5a**, **5b**, **5d** and **5e** showed significant activity against all the tested Gram-positive bacterial strains. Compounds **4a**–**c**, **5d** and **5e** showed lower activity against *E. coli*, whereas **5a** and **5b** were found inactive. The thymyl ester derivatives **4d** and **4f** showed moderate activity against *B. subtilis* and *S. epidermidis*. The most notable enhancement in the activity was noticed for thymyl ester derivatives **4a**–**c** for Gram-positive bacterial strains. Thymyl acetate **4a** and thymyl isobutyrate **4c** were found to be more effective than thymol (IZ = 17 mm; MIC = 125 µg/mL) and all other esters against *S. mutans* (IZ = 30 mm and 18 mm; MIC = 11.7 and 93.7 µg/mL, respectively), *B. subtilis* (IZ = 30 mm and 21 mm; MIC = 11.7 and 46.8 µg/mL, respectively) and *S. epidermidis* (IZ = 32 mm and 20 mm; MIC = 11.7 and 46.8 µg/mL, respectively), whereas **4b** was found to be more active for *B. subtilis* (IZ = 25 mm; MIC = 46.8 µg/mL) and *S. epidermidis* (IZ = 28 mm; MIC = 46.8 µg/mL) as compared to thymol. All other derivatives possessed much lower activity as compared to thymol itself and, generally, showed a decrease in their activity with an increase in the size of substituent (R). Carvacrol was much more active than its isomer thymol against all the test bacteria even at lower concentration. Nikumbh et al. have also synthesized carvacryl esters, namely: carvacryl acetate and carvacryl phenyl acetate and evaluated their antibacterial activity against *B. japonicum*, *B. megaterium*, *B. substilits*, and *B. polymyx* (20).

CONCLUSION

To conclude, a series of thymyl and carvacryl esters have been synthesized and evaluated as antibacterial agents. These modifications resulted in change in the antibacterial activity of thymol and carvacrol analogs. The enhancement in activity was noticed in the thymyl ester derivatives **4a–c** against *S. mutans, B. subtilis* and *S. epidermidis*, whereas it diminished in case of carvacryl esters derivatives. Based on these results, compounds **4a, 4b** and **4c** possess a potential for developing as antibacterial agents.

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REFERENCES

1. Newmann D.J., Cragg G.M., Snader K.M.: J. Nat. Prod. 66, 1022 (2003).
2. Aggarwal K.K., Khanuja S.P.S., Ahmad A., Santakumar T.R., Gupta V.K., Kumar S.: Flavour Fragr. J. 17, 59 (2002).
3. Medeiros J.R., Medeiros N., Medeiros H., Davin L.B., Lewis N.G.: J. Essent. Oil Res. 15, 293 (2003).
4. Zgoda-Pols J.R., Freyer A.J., Killmer L.B., Porter J.R.: Fitoterapia 73, 434 (2002).
5. Akbar E., Malik A.: Nat. Prod. Lett. 16, 339 (2002).
6. Puertas-Mejia M., Hillebrand S., Stashenko E., Winterhalter P.: Flavour Fragr. J. 17, 380 (2002).
7. Mirza M., Baher Z.F.: J. Essent. Oil Res. 15, 404 (2003).
8. Goren A.C., Topcu G., Bilsel G., Bilsel M., Wilkinson J.M., Cavanagh H.M.A.: Nat. Prod. Res. 18, 189 (2004).
9. Salgueiro L.R., Pinto E., Gonçalves M.J., Pina-Vaz C., Cavaleiro C., Rodrigues A.G., Palmeira A. et al.: Planta Med. 70, 572 (2004).
10. Yoshida T., Mori K., He G.X.: Heterocycles 41, 1923 (1995).
11. Oke F., Aslim B., Oztirk S., Altundag S.: Food Chem. 112, 874 (2009).
12. Kordali S., Cakir A., Ozer H., Cakmakei R., Kesdek M., Mete E.: Bioresour. Technol. 99, 8788 (2008).
13. Peltoketo A., Dorman H.J.O., Yrjonen T., Summanen J., Laakso I., Vuorela H., Hiltunen R.: Phytomedicine 7, 75 (2000).
14. Liolios C.C., Gortzi O., Lalas S., Tsaknis J., Chinou I.: Food Chem. 77, 112 (2009).
15. Liang H., Bao F., Dong X., Tan R., Zhang C., Lu Q., Cheng Y.: Molecules 12, 1606 (2007).
16. Stojakowska A., Kedzia B., Kisiel W.: Fitoterapia 76, 687 (2005).
17. Kumbhar (alias Mahulikar) P.P., Dewang P.M.: Pestology 23, 27 (1999).
18. Kumbhar (alias Mahulikar) P.P., Dewang P.M.: J. Sci. Ind. Res. 60, 645 (2001).
19. Moszner N., Salz U., Rheinberger V.: Polymer Bull. 33, 7 (1994).
20. Nikumbh V. P., Tare V. S., Mahulikar P. P.: J. Sci. Ind. Res. 62, 1086 (2003).
21. Bauer A.W., Kirby W.M., Snerries J.C., Turck M.: Am. J. Clin. Pathol. 45, 493 (1996).
22. Gupta V.K., Fatima A., Faridi U., Negi A.S., Shankar K., Kumar J.K., Rahuja N. et al.: J. Ethnopharmacol. 116, 377 (2008).
23. Ahmad A., Aggarwal K.K., Kumar S.: Indian Perfumer 46, 145 (2002).
24. Mathela C.S., Tiwari A., Padalia R.C., Chanotiya C.S.: Indian J. Chem. 47B, 1249 (2008).
25. More D.H., Hundiwale D.G., Kapadi U.R., Mahulikar P.P.: J. Sci. Ind. Res. 65, 817 (2006).

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