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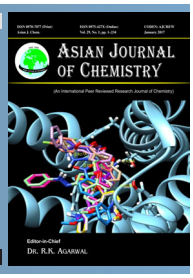

Synthesis and Antibacterial Study of Eugenol Derivatives

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A series of eugenol derivatives (**2-14**) were synthesized and evaluated for their antibacterial activity against five bacterial test strains; three Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) using well-diffusion method. Among the compounds tested, compounds **2-4** displayed susceptible activity toward *S. epidermidis* with 16-18 mm whereas compounds **12** exhibited susceptible inhibition towards *S. aureus* only with inhibition diameter of 16 mm, respectively. Other compounds possessed varied antibacterial activities classified as intermediate or resistance indicating that eugenol derivatives have narrow spectrum activity and specifically to Gram-positive bacteria.

Keywords: Eugenol derivatives, Well-diffusion method, Antibacterial activities.

INTRODUCTION

Eugenol (4-allyl-2-methoxyphenol) is a major component of the essential oil extracted from the dried flower buds of the clove tree (*Eugenia caryophyllata*). Recently, much attention has been paid to eugenol derivatives due to their diverse biological activities [1-6]. Eugenol has been shown to possess many medicinal properties such as antiseptic and analgesic [7,8], anesthetic [9], antispasmodic [10], antipyretic [11] and antioxidant [9,11-13]. Despites, eugenol analogues have also been synthesized due to their variety of applications. Among the other derivatives, eugenol ethers and eugenol esters are two types of eugenol derivatives commonly studied due to their simple synthesis, stability and broad spectrum of biological activity [14-17]. In previous report, the synthetic pathway for the preparation of eugenol esters has been described [18]. These derivatives were synthesized from the reaction between eugenol with long chain acyl chloride such as palmitoyl, myristoyl and lauroyl chloride in comparison to many other aromatic eugenol ethers and eugenol esters commonly reported [19-21]. Besides, several derivatives of eugenol esters and eugenol ethers have been evaluated for antispasmodic activity and have promising antispasmodic activity of eugenol esters derivatives [10]. Derivatives like eugenol-related biphenyl were also believed to enhance the activity of antiproliferative and pro-apoptotic activity against malignant melanoma activity [22].

Eugenol itself is believed to be toxic in high dose [23,24], which was subsidized to central nervous system depression and hepatic failure [25-28] Apart from that, the free radicals formations by this phenolic compound was act as prooxidant and lead to cytotoxic that causes tissue damages [29-31]. Therefore, the modification of eugenol structure were done as reported in literatures to evaluate the biological activities such as antioxidant, antimicrobial, antiproliferative and pro-apoptotic activity.

Hence, as part of our ongoing effort to search for promising antibacterial agent from natural product, in this present study, we report a series of eugenol ethers and eugenol esters (**2-14**) and their antibacterial activity.

EXPERIMENTAL

All reagents used were obtained from Sigma-Aldrich Co., Merck Chemical Co., Acros Organics Co. and R&M Chemical without further additional purification. All reactions were performed under nitrogen atmosphere. The reactions were monitored by thin layer chromatography (TLC) and were visualized under UV 254 nm without treatment. Column chromatography was performed by using silica gel 60 (230-400 mesh, Merck). Infrared spectra were recorded in KBr disc on Perkin Elmer 100 FT-IR spectrometer. UV-visible spectra were recorded on Shimadzu UV-1601 PC spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR were recorded by

using Bruker Spectrospin-400 Spectrometer. Elemental analyses were performed on CHNS Analyzer FlashEA 1112 series. All compounds obtained were evaluated against three Gram-positive bacterial strains (*B. subtilis* ATCC 11774, *S. aureus* ATCC 25983 and *S. epidermidis* ATCC 13528) and two Gram-negative bacteria (*E. coli* ATCC11775 and *S. typhimurium* ATCC 14128) respectively for antibacterial activity by using standard well-diffusion method [32].

General procedure: The preparation of compounds **2-5** was done by known method [33]. Potassium carbonate (1.5 equiv) was added to a solution of eugenol (**1**) (1.0 equiv) and benzyl halide (1.5 equiv) in anhydrous acetone (20 mL) under an inert atmosphere. The mixture was refluxed upon completion (TLC monitoring). The reaction crude was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed by rotary evaporator. The residue was purified by column chromatography to give compounds **2** to **5** in various yields (57 to 72 %).

4-Allyl-1-benzyloxy-2-methoxybenzene (2) [34]: Yield: 72 %; IR (KBr, ν_{max}, cm^{-1}) 2934, 2907, 1637, 1590, 1512, 1454, 1261; UV (MeOH) λ_{max} (log ε) 205.0 (4.65), 234.5 (4.00), 276.0 (4.18) nm; ^{1}H NMR (400 MHz) δ 3.20 (d, *J* = 5.4 Hz, 2H, H-3), 3.77 (s, 3H, OCH_3), 4.94-5.02 (m, 2H, CH_2, H-1), 5.02 (s, 2H, H-8), 5.80-5.88 (m, 1H, H-2), 6.54 (d, *J* = 5.5 Hz, 1H, CH_{ar}), 6.62 (s, 1H, CH_{ar}), 6.70 (d, *J* = 6.5 Hz, 1H, CH_{ar}), 7.24 (d, *J* = 6.1 Hz, 3H, CH_{ar}), 7.33 (d, *J* = 6.3 Hz, 2H CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 39.7, 40.0, 55.9, 71.1, 76.8, 77.2, 112.4, 114.3, 115.7, 120.5, 127.5, 127.9, 128.3, 128.5, 133.3, 137.7, 146.5 ppm; EIMS *m/z* $[M + Na]^+$ 277.11 (m.f. $C_{17}H_{18}O_2$, m.w. 254.10).

4-Allyl-1-(4-isopropyl-benzyloxy)-2-methoxybenzene (3): Yield: 61 %; IR (KBr, ν_{max}, cm^{-1}) 2960, 2935, 1638, 1588, 1513, 1426, 1232; UV (MeOH) λ_{max} (log ε) 205.2 (4.69), 233.8 (4.05), 277.8 (3.99) nm; ^{1}H NMR (400 MHz) δ 1.30 (s, 6H, H-12), 2.92-2.95 (m, 1H, H-11), 3.36 (d, *J* = 5.6 Hz, 2H, H-3), 3.90 (s, 3H, OCH_3), 5.08-5.13 (m, 2H, CH_2, H-1), 5.12 (s, 2H, H-8), 5.96-6.01 (m, 1H, H-2), 6.70 (d, *J* = 6.5 Hz, 1H, CH_{ar}), 6.76 (s, 1H, CH_{ar}), 6.87 (d, *J* = 6.5 Hz, 1H, CH_{ar}), 7.35 (d, *J* = 5.7 Hz, 2H, CH_{ar}), 7.40 (d, J 6.7 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 24.0, 33.9, 39.8, 46.2, 55.9, 71.1, 76.8, 112.4, 114.2, 115.6, 120.4, 126.9, 127.5, 128.7, 133.2, 134.8, 137.8, 146.7, 148.5 ppm; EIMS *m/z* $[M + Na]^+$ 319.16 (m.f. $C_{20}H_{24}O_2$, m.w. 296.10).

4-Allyl-2-methoxy-1-(4-trifluoromethyl-benzyloxy)-benzene (4): Yield: 57 %; IR (KBr, ν_{max}, cm^{-1}) 2961, 2935, 1641, 1588, 1515, 1420, 1232; UV (MeOH) λ_{max} (log ε) 205.6 (4.90), 232.6 (4.28), 275.6 (4.22) nm; ^{1}H NMR (400 MHz) δ, 3.36 (d, *J* = 5.4 Hz, 2H, H-3), 3.91 (s, 3H, OCH_3), 5.08-5.13 (m, 2H, CH_2, H-1), 5.21 (s, 2H, H-8), 5.94-6.02 (m, 1H, H-2), 6.70 (d, *J* = 6.5 Hz, 1H, CH_{ar}), 6.79 (d, *J* = 5.7 Hz, 1H, CH_{ar}), 6.81 (s, 1H, CH_{ar}), 7.58 (d, *J* = 6.4 Hz, 2H, CH_{ar}), 7.65 (d, *J* = 6.5 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 39.8, 55.9, 70.4, 77.9, 112.5, 114.3, 115.8, 120.4, 123.0, 125.2, 125.5, 127.3, 129.7, 133.9, 137.5, 141.5, 146.1, 149.7 ppm. EIMS *m/z* $[M + He]^+$ 327.07 (m.f. $C_{18}H_{17}F_3O_2$, m.w. 322.32); Anal. Calcd. for $C_{18}H_{17}O_2F_3$: C, 67.07; H, 5.32; found: C, 68.14; H, 6.30 %.

4-Allyl-2-methoxy-1-(4-nitrobenzyloxy)benzene (5) [35]: Yield: 57 %; IR (KBr, ν_{max}, cm^{-1}) 2934, 2907, 1637, 1590, 1512, 1454, 1261; UV (MeOH) λ_{max} (log ε) 205.0 (4.75), 233.5 (3.03), 278.0 (4.12) nm; ^{1}H NMR (400 MHz) δ, 3.33 (d, *J* = 6.8 Hz, 2H, H-3), 3.83 (s, 3H, OCH_3), 4.98-5.09 (m, 2H, CH_2, H-1), 5.24 (s, 2H, H-8), 5.90-6.00 (m, 1H, H-2), 6.68 (d, *J* = 2.0 Hz, 1H, CH_{ar}), 6.76 (s, 1H, CH_{ar}), 6.95 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.75 (d, *J* = 7.8 Hz, 2H, CH_{ar}), 8.25 (d, *J* = 9.0 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.3, 56.1, 70.6, 113.7, 115.6, 115.9, 121.2, 124.2, 128.8, 134.9, 138.7, 146.6, 147.2, 148.3, 150.9 ppm. EIMS *m/z* $[M + 2H]^+$ 300.14 (m.f. $C_{17}H_{17}NO_4$, m.w. 322.10); Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68; found: C, 69.22; H, 6.42; N, 4.70 %.

General procedure for the preparation of compounds 6-14 [36]: Eugenol (**1**) (1.0 equiv) was dissolved in dichloromethane (30 mL) and was added with triethylamine (9.3 mL). The solution was stirred for 0.5 h in 0 °C. Then, an excess of acyl chloride was added dropwise in 15 min periods. The solution was stirred for another 0.5 h at 0 °C before removed to room temperature and continued stirring for another 24 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After the completion of the reaction, the residue was purified with column chromatography using hexane and ethyl acetate (3:1 ethyl acetate in hexane) and yielded products of various colours of compounds **6** to **14** with yields varied from 49 to 99 %.

4-Allyl-2-methoxyphenyl propanoate (6) [37]: Yield: 75 %; IR (KBr, ν_{max}, cm^{-1}) 3079, 2980, 2940, 1761, 1637, 1422, 1269; UV (MeOH) λ_{max} (log ε) 203.5 (3.45), 221.0 (3.81) nm; ^{1}H NMR (400 MHz) δ 1.18 (t, *J* = 7.4 Hz, 3H, CH_3), 2.52-2.58 (q, *J* = 7.5 Hz, 2H, CH_2), 3.37 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.77 (s, 3H, OCH_3), 5.01-5.12 (m, 2H, CH_2, H-1), 5.09 (d, *J* = 3.4 Hz, 2H, CH_2, H-1), 5.92-6.02 (m, 1H, CH, H-2), 6.76 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 6.92 (d, *J* = 3.2 Hz, 1H, CH_{ar}), 6.95 (s, 1H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 9.4, 27.4, 40.4, 56.0, 112.8, 113.7, 115.5, 121.2, 123.3, 137.9, 145.7, 152.0, 172.4 ppm; EIMS *m/z* $[M + N]^+$ 243.09 (m.f. $C_{13}H_{16}O_3$, m.w. 220.10).

4-Allyl-2-methoxyphenyl butyrate (7) [38]: Yield: 69 %; IR (KBr, ν_{max}, cm^{-1}) 3077, 2966, 2937, 1726, 1638, 1418, 1268; UV (MeOH) λ_{max} (log ε) 270 (5.5), 322.5 (4.0) nm; ^{1}H NMR (400 MHz) δ 1.00 (t, *J* = 7.4 Hz, 3H, CH_3), 1.67-1.76 (m, 2H, CH_2), 2.49 (t, *J* = 7.2 Hz, 2H, CH_2), 3.36 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.75 (s, 3H, OCH_3), 5.02-5.12 (m, 2H, CH_2, H-1), 5.91-6.01 (m, 1H, CH, H-2), 6.75 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 6.91 (d, *J* = 4.0 Hz, 1H, CH_{ar}), 6.93 (s, 1H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 13.7, 19.0, 36.1, 56.0, 113.5, 116.0, 121.0, 123.3, 138.3, 139.1, 139.6, 152.0, 171.6 ppm; EIMS *m/z* $[M + Na]^+$ 257.12 (m.f. $C_{14}H_{18}O_3$, m.w. 234.10).

4-Allyl-2-methoxyphenyl 4-methylbenzoate (8) [19,38]: Yield: 74 %; IR (KBr, ν_{max}, cm^{-1}) 3067, 2974, 2936, 1730, 1608, 1420, 1268; UV (MeOH) λ_{max} (log ε) 203.5 (4.8), 240.50 (4.3), 273.5 (3.8) nm; ^{1}H NMR (400 MHz) δ 2.44 (s, 3H,CH_3), 3.41 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.78 (s, 3H, OCH_3), 5.04-5.15 (m, 2H, CH_2, H-1), 5.96-6.06 (m, 1H, CH, H-2), 6.83 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 6.99 (s, 1H, CH_{ar}), 7.10 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.39 (d, *J* = 8.0 Hz, 2H, CH_{ar}), 8.05 (d, *J* = 8.4 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 21.6, 40.5, 56.1,

113.7, 116.1, 121.2, 123.6, 127.7, 130.2, 130.6, 131.2, 138.4, 139.2, 139.9, 145.2, 152.2, 165.0 ppm; EIMS *m/z* $[M + H]^+$ 283.13, EIMS *m/z* $[M + Na]^+$ 305.11, (m.f. $C_{18}H_{18}O_3$, m.w. 282.10); Anal. Calcd. for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43; found: C, 72.09; H, 6.02 %.

4-Allyl-2-methoxyphenyl 4-ethylbenzoate (9): Yield: 63 %; IR (KBr, ν_{max}, cm^{-1}) 3067, 2971, 2937, 1732, 1608, 1418, 1268; UV (MeOH) λ_{max} (log ε) 203.0 (4.8), 242.5 (4.4), nm; ^{1}H NMR (400 MHz) δ 1.26 (t, *J* = 7.6 Hz, 3H, CH_3), 2.73-2.79 (q, *J* = 7.6 Hz, 2H, CH_2), 3.42 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.78 (s, 3H, OCH_3), 5.04-5.15 (m, 2H, CH_2, H-1), 5.96-6.06 (m, 1H, CH, H-2), 6.83 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.00 (s, 1H, CH_{ar}), 7.10 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.43 (d, *J* = 8.4 Hz, 2H, CH_{ar}), 8.08 (d, *J* = 8.0 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 51.9, 56.0, 56.1, 113.8, 114.8, 116.1, 121.2, 122.7, 123.7, 132.1, 132.8, 138.4, 139.8, 152.3, 164.7, 164.9 ppm; EIMS *m/z* $[M + Na]^+$ 319.13 (m.f. $C_{19}H_{20}O_3$, m.w. 296.10); Anal. Calcd. for $C_{19}H_{20}O_3$: C, 73.60; H, 6.79; found: C, 76.45; H, 7.61 %.

4-Allyl-2-methoxyphenyl 4-fluorobenzoate (10) [19,38]: Yield: 49 %; IR (KBr, ν_{max}, cm^{-1}) 3085, 2981, 2939, 1736, 1603, 1412, 1265; UV (MeOH) λ_{max} (log ε) 202.0 (4.5), 227.0 (4.4), 263.0 (3.2) nm; ^{1}H NMR (400 MHz) δ 3.42 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.79 (s, 3H, OCH_3), 5.05-5.15 (m, 2H, CH_2, H-1), 5.98-6.05 (m, 1H, CH, H-2), 6.84 (d, *J* = 8.4 Hz, 1H, CH_{ar}), 7.01 (s, 1H, CH_{ar}), 7.12 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.35 (t, *J* = 9.0 Hz, 2H, CH_{ar}), 8.23 (d, *J* = 8.8 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 56.1, 113.8, 116.1, 116.5, 116.7, 121.3, 123.5, 127.0, 127.1, 133.5, 138.3, 139.1, 140.2, 152.2, 164.1, 165.6 ppm.

4-Allyl-2-methoxyphenyl 4-chlorobenzoate (11) [19,21]: Yield: 90 %; IR (KBr, ν_{max}, cm^{-1}) 2914, 1739, 1591, 1463, 1265, 1068, 848; UV (MeOH) λ_{max} (log ε) 203.0 (4.7), 248.0 (4.3), 273.5 (3.5) nm; ^{1}H NMR (400 MHz) δ 3.42 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.79 (s, 3H, OCH_3), 5.05-5.15 (m, 2H, CH_2, H-1), 5.96-6.04 (m, 1H, CH, H-2), 6.84 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.01 (s, 1H, CH_{ar}), 7.12 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.63 (d, *J* = 8.8 Hz, 2H, CH_{ar}), 8.16 (d, *J* = 8.8 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.6, 52.5, 56.2, 116.2, 121.3, 121.5, 129.2, 129.6, 129.9, 131.9, 132.7, 133.0, 138.3, 139.1, 152.1, 164.2 ppm.

4-Allyl-2-methoxyphenyl 4-bromobenzoate (12): Yield: 79 %; IR (KBr, ν_{max}, cm^{-1}) 3075, 2968, 2935, 1738, 1588, 1420, 1263; UV (MeOH) λ_{max} (log ε) 203.0 (4.7), 245.5 (4.3) nm; ^{1}H NMR (400 MHz) δ 3.42 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.79 (s, 3H, OCH_3), 5.05-5.15 (m, 2H, CH_2, H-1), 5.96-6.06 (m, 1H, CH, H-2), 6.84 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.01 (s, 1H, CH_{ar}), 7.12 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.79 (d, *J* = 8.8 Hz, 2H, CH_{ar}), 8.09 (d, *J* = 8.8 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 52.5, 56.2, 113.8, 116.1, 121.3,123.5, 128.9, 129.2, 130.3, 132.5, 132.6, 133.9, 138.3, 139.1, 140.2, 152.1, 164.2, 166.5 ppm; EIMS *m/z* $[M + N]^+$ 360.19 (m.f. $C_{17}H_{15}BrO_3$, m.w. 346.10); Anal. Calcd. for $C_{17}H_{15}BrO_3$: C, 58.81; H, 4.35; found: C, 53.10; H, 4.02 %.

4-Allyl-2-methoxyphenyl 4-nitrobenzoate (13) [38]: Yield: 57 %; IR (KBr, ν_{max}, cm^{-1}) 2940, 1746, 1605,1530, 1344, 1264, 1076; UV (MeOH) λ_{max} (log ε) 203.5 (4.7), 258.0 (4.4), 273.5 (4.3) nm; ^{1}H NMR (400 MHz) δ 3.43 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.81 (s, 3H, OCH_3), 5.05-5.16 (m, 2H, CH_2, H-1), 5.96-6.09 (m, 1H, CH, H-2), 6.86 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 7.03 (s, 1H, CH_{ar}), 7.17 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 8.41-8.42 (m, 4H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.3, 56.1, 70.6, 113.7, 115.6, 115.9, 121.2, 124.2, 128.8, 134.8, 146.5, 147.2, 148.3 ppm; Anal. Calcd. for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47; found: C, 64.34; H, 5.257; N, 4.66 %.

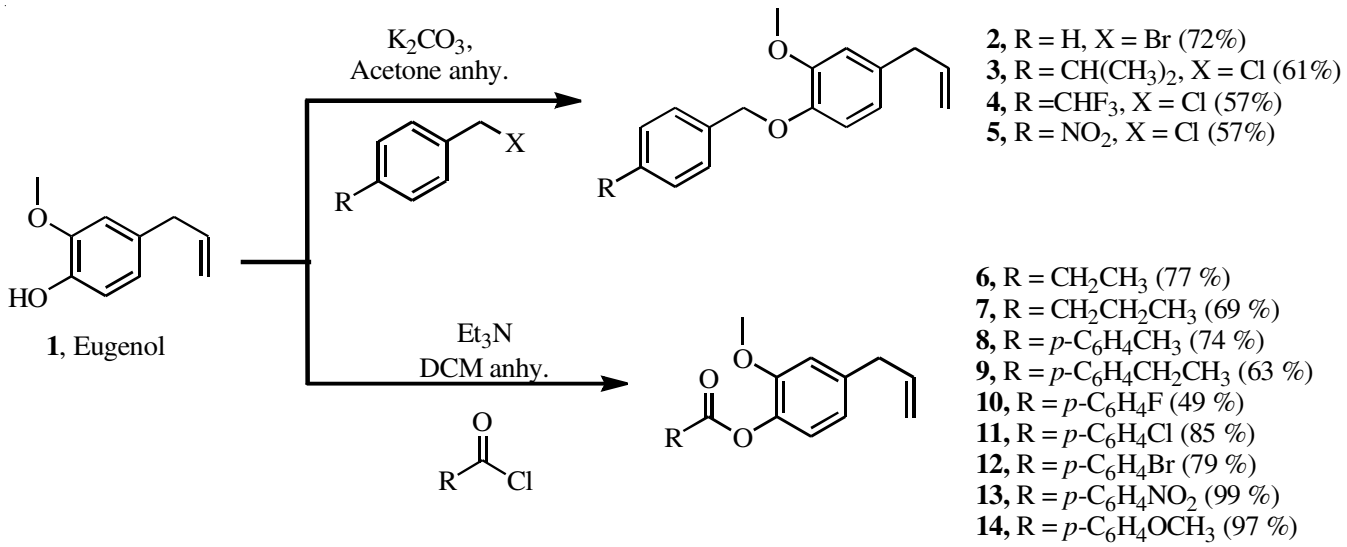
4-Allyl-2-methoxyphenyl 4-methoxybenzoate (14) [18,19,38]: Yield: 97 %; IR (KBr, ν_{max}, cm^{-1}) 3010, 2976, 2941, 1728, 1606, 1421, 1264; UV (MeOH) λ_{max} (log ε) 203.0 (4.7), 259.0 (4.4) nm; ^{1}H NMR (400 MHz) δ 3.42 (d, *J* = 6.8 Hz, 2H, CH_2, H-3), 3.78 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.04-5.15 (m, 2H, CH_2, H-1), 5.96-6.06 (m, 1H, CH, H-2), 6.83 (d, *J* = 8.0 Hz, 1H, CH_{ar}), 6.99 (s, 1H, CH_{ar}), 7.09 (d, *J* = 9.0 Hz, 2H, CH_{ar}), 7.11 (s, 1H, CH_{ar}), 8.11 (d, *J* = 8.8 Hz, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.6, 52.5, 56.2, 116.2, 121.3, 121.5, 129.2, 129.6, 129.9, 131.9, 132.7, 133.0, 138.3, 139.1, 152.1, 164.2 ppm; EIMS *m/z* $[M + H]^+$ 299.12, EIMS *m/z* $[M + Na]^+$ 321.11 (m.f. $C_{18}H_{18}O_4$, m.w. 298.10); Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08; found: C, 70.84; H, 6.67 %.

Antibacterial assay: All eugenol derivatives synthesized were tested for their antibacterial activity against five bacterial strains of Gram-positive and Gram-negative bacteria; three Gram-positive bacteria (*B. subtilis, S. aureus* and *S. epidermidis*) and two Gram-negative bacteria (*E. coli* and *S. typhimurium*) using standard well-diffusion method [32]. The Mueller-Hinton agar (MHA) plates were seeded with cultured bacterial strains using cotton swab. Wells of 6.0 mm diameter were cut on the media using sterile cork borer and was loaded with 60 µL of diluted compounds. 1 mg/mL of synthesized compounds were prepared by using methanol as solvent. Streptomycin (Abtek Biologicals Ltd) was used as positive control. All plates were incubated at 37 °C for overnight before evaluating the antibacterial activity by measuring the diameter of inhibition zones against bacteria.

RESULTS AND DISCUSSION

A series of eugenol derivatives were synthesized according to the pathways described in **Scheme-I**. The treatment of eugenol (**1**), with various benzyl halides and potassium carbonate by following published method [33] to furnish (**2-5**) in moderate isolated yields. Eugenol esters (**6-14**) have been synthesized following the reported method [36] by the reaction of eugenol (**1**), with various acyl chlorides in the presence of triethylamine.

Antibacterial evaluation of synthesized compounds: All the synthesized compounds **2-14** were subjected to antibacterial study using standard well-diffusion method [32]. The diameters of inhibition zone of all compounds were represented in Table-1. The results showed that most compounds are active against *S. epidermidis*. Notably, compounds **2-4** exhibited susceptible activity against *S. epidermidis* whereas compounds **12** displayed susceptible activity towards *S. aureus*. Compounds **5-6**, **8-10**, **12** and **14** were intermediately active in inhibiting the growth of *S. epidermidis*, respectively. Compounds **7**, **9** and **12** exhibited moderate activity against *S. typhimurium, S. aureus* and *E. coli*.



Scheme-I: Synthesis of compounds **2-14**

TABLE-1
ANTIBACTERIAL ACTIVITY FOR EUGENOL (**1**)
AND ITS DERIVATIVES (**2-14**)

Compounds	Zone of inhibition (mm)				
	Gram-positive bacteria			Gram-negative bacteria	
	BS	SA	SE	EC	ST
1	6	6	12	6	NI
2	7	6	18	7	6
3	6	6	16	6	6
4	7	6	16	6	NI
5	NI	NI	10	8	6
6	9	7	11	9	8
7	7	7	7	NI	10
8	8	NI	11	7	7
9	NI	10	14	7	7
10	NI	7	10	8	8
11	7	7	9	8	6
12	6	16	10	10	7
13	NI	NI	9	8	7
14	NI	9	14	8	9
Streptomycin	15	16	24	17	17

BS = *B. subtilis*; SA = *S. aureus*; SE = *S. epidermidis*; EC = *E. coli*; ST = S. typhimurium; NI = No Inhibition.

Previous study demonstrated that the compounds with the presence of nitro group as substituent showed good antimicrobial activity [39]. However, the result obtained in this study revealed that compound **13** employing nitro substituent displayed intermediate to resistance activity towards tested strains. This might be attributed to substituent position which is *para* directing that deactivate the molecules. This is in accordance with the previous study [40] that showed the nitro substituent at *para* position displayed no activity towards the bacterial strains. Apart from that, the modification of eugenol structure in the presence of bromine as substituent has led to the increase in antibacterial activity as shown by compound **12**. This finding is in agreement with previous work [40,41] that showed potent activity against *S. aureus* in the presence of bromine substituent. Compound **12** emerges as the best antibacterial agent and susceptible to both Gram-positive and negative bacteria strains, indicating broad-spectrum activity.

Conclusion

In conclusion, 13 derivatives of eugenol were successfully synthesized from the reaction of eugenol with benzyl halides and acyl chloride. Antibacterial screening revealed that compounds **12** as the most potential antibacterial agent with broad spectrum of activity against Gram-positive and Gram-negative bacteria.

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