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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of halogenated derivatives of thymol and their antimicrobial activities

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Abstract In order to test the antibacterial and antifungal activities of different halogenated thymol derivatives, thymol has been converted into chlorothymol, dichlorothymol with *N*-chlorosuccinimide; monobromothymol, dibromothymol with *N*-bromosuccinimide; *O*-methylated iodothymol with ceric ammonium nitrate and iodine from methylated thymol. Among the different derivatives tested, 4-chlorothymol was found to be most active against *Staphylococcus aureus* and *Staphylococcus epidermis* at a concentration of 12.5 and 25 ppm, respectively. Also it was tested to be active against C*andida albicans* (AI).

Keywords Thymol derivatives · *N*-Halosuccinimide · Antibacterial activity · Antifungal activity

Introduction

The genus of *Thymus* comprises 300–400 species, some of which are used in folk medicine. The main medicinal *Thymus* is *Thymus vulgaris* (common thyme) which is used for dry coughs, bronchitis, laryngitis, indigestion, and gastritis (Rustaiyan *et al*., 1997; Blumenthal, 2000). There are also some reports about antimicrobial effects of *Thymus* essential oil (Rahimifard *et al.,* 2008). It has been demonstrated that the biological effects of *T. vulgaris* are mainly due to the presence of phenolic compounds, especially thymol and carvacrol (Blumenthal, 2000; Cherallier, 1996).Thymol content in thyme essential oil is much higher than carvacrol content (Fig. 1). This compound shows 30 times higher antiseptic effect and four times lower toxicity than phenol (Zekovic *et al*., 2000; Hajimehdipoor *et al*., 2009). In India major source of thymol is an essential oil from Ajowain, botanically known as *Trachyspermum ammi* (Linn.) sprague (Family; Umbelliferae). The plant is a native of Mediterranean region and is cultivated and found growing wild in South West Asia. In India it is grown in north-western states of Gujarat, Maharashtra, Rajasthan, Madhya Pradesh, Uttar Pradesh, and Bihar (Husain *et al.,* 1988; Anonymous, 1976). The oil of Ajowain contains about 53.8 % thymol. Thymol has been known and isolated since 1853. Expensive synthetic thymol has been in the market since 1960 and is commonly used for its antiseptic effect in toothpaste and flavour in cough drops, mouth washes, gargles, and chewing gum. It is often used partly in such products (Arctander, 1969). Recent medical application of thymol extract on rats established that it had relaxing effects on organs possessing β_2-receptors of uterus and trachea (Weinkotter *et al.,* 2007). Recently, two new natural thymol derivatives together with five known thymol derivatives were isolated from *Centipeda minima* and their antimicrobial activities have been tested (Liang *et al.,* 2007). Various derivatives of thymol were also reported in literature (Ahmad *et al.,* 2002). In this paper, we are going to report thymol derivatives i.e. the reaction of thymol and *N*-halosuccinimide under different reaction conditions as outlined in Scheme 1. All the products formed from the above reactions were evaluated for their antimicrobial activities.

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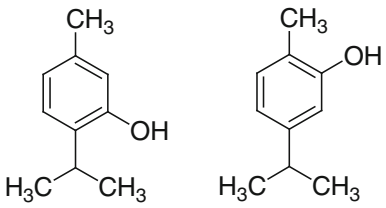


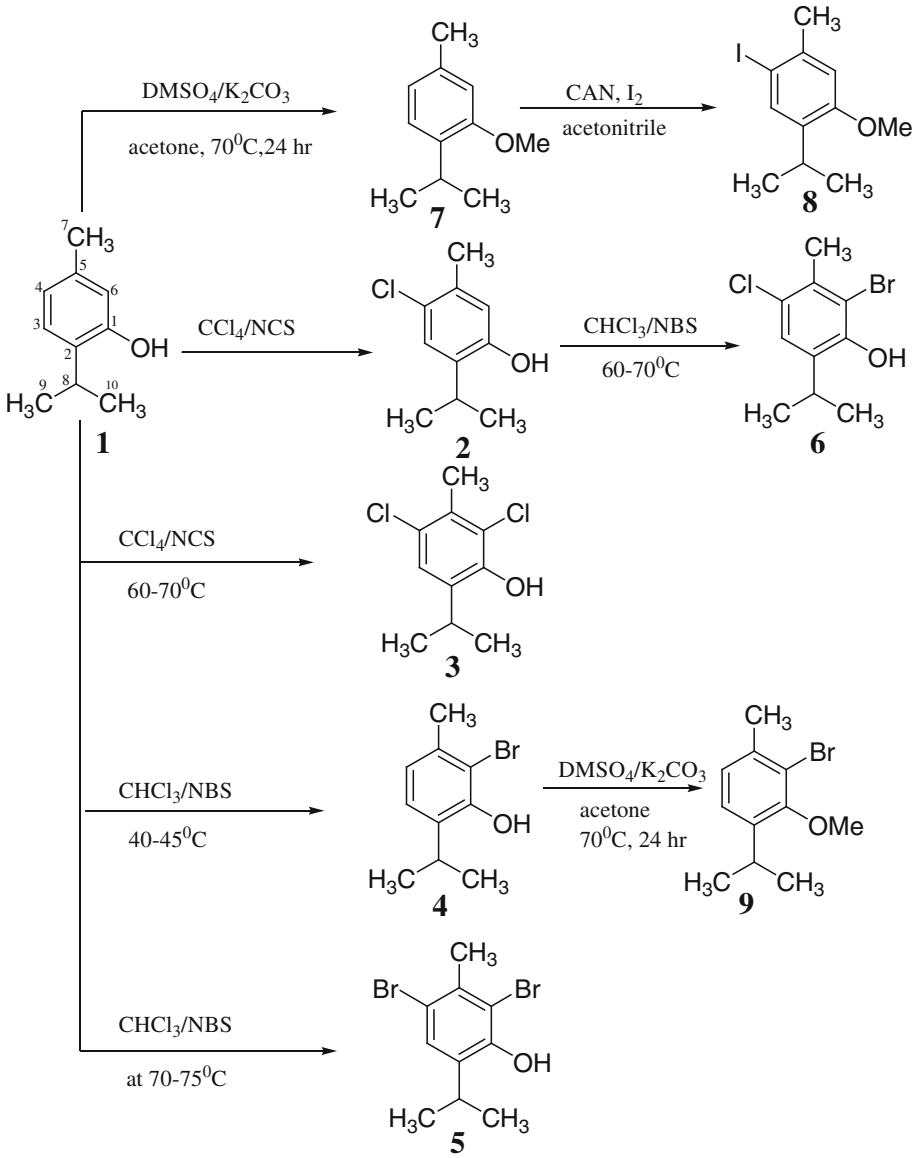
Fig. 1 Structure of thymol and carvacrol

Materials and methods

Experimental

Melting points were determined in open capillary tube on a JSGW melting point apparatus and are uncorrected. Chromatographic purifications were performed using silica gel (60–120-mesh size, Merck chemicals). FT-IR spectra were recorded in KBr or neat on Perkin Elmer AC-1 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Brucker Avance DRX-300 spectrometer. Chemical shift are given in δ values, downfield from TMS as internal standard. Coupling constant (J) values are given in Hz. ESI mass spectra were recorded on a API-3000, LC–MS/MS (Applied Biosystem/MDS SCIEX, Toranto, Canada) mass spectrometer using a standard ESI source coupled with LC separation system. All the solvents and reagents were of LR/AR grade. Dry solvents were prepared as per standard methods. Elemental analysis (C, H, N) was performed on a Elementar Vario EL III (Carlo Erbe 1108). The ^{1}H and ^{13}C NMR data of known compounds are in agreement with those reported in literature (Ahmad *et al.,* 2002).

Scheme 1 Synthesis and structures of thymol derivatives



Isolation of thymol (**1**)

Ajowain oil was kept in a freezer and crystals were obtained by filtering the crystallized portion through sintered funnel and obtained as colourless pure crystals of thymol, 98 % purity (on the basis of GC analysis); m.p. 51–52 °C; IR (KBr) ν_{max}: 3464, 3361, 2963, 2872, 2367, 1719, 1582, 1513, 1420, 1299, 1225, 1154, 1099, 1060, 730 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.07 (d, 1H, $J = 7.8$ Hz, C_3–H), 6.71 (d, 1H, $J = 7.8$ Hz, C_4–H), 6.50 (br s, 1H, C_6–H), 5.09 (s, 1H, Ar–OH), 3.16 (m, 1H, C_8–H), 2.30 (br s, 3H, C_7–H), 1.22 (d, 6H, $J = 6.9$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 152.5 (C-1), 136.6 (C-5), 131.7 (C-2), 126.3 (C-3), 121.9 (C-4), 116.3 (C-6), 26.7 (C-8), 22.7 (C-9), 22.7 (C-10), 20.8 (C-7); EI-MS: *m/z* 150 $[M]^+$. Elemental analysis (% found) C 80.00 (80.40); H 9.33 (10.08).

4-Chlorothymol (**2**)

To a solution of thymol 2 g (13.33 mmol) in carbon tetrachloride (20 ml), *N*-chlorosuccinimide (2.5 g, 18.605 mmol) was added and refluxed over water bath 60–70 °C temperature. The reaction mixture was cooled to room temperature, diluted with water and extracted with chloroform (3 × 25 ml). Organic layer was washed with water, dried over sodium sulphate and evaporated to dryness. The crude reaction product was purified by silica gel column and eluted with hexane/ethyl acetate. It crystallized from hexane as colourless needles (purity 97–98 %). Yield: 65 %; m.p. 57–61 °C; IR (KBr) ν_{max}: 3320, 2966, 1504, 1452, 1337, 1254, 1164, 880, 757 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.12 (s, 1H, C_3–H), 6.60 (s, 1H, C_6–H), 4.76 (s, 1H, Ar–OH) 3.12 (m, 1H, C_8–H), 2.26 (s, 3H, C_7–H), 1.22 (d, 6H, $J = 6.9$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 151.4 (C-1), 134.4 (C-5), 134.3 (C-6), 127.3 (C-2), 126.4 (C-3), 118.2 (C-4), 19.9 (C-7), 27.2 (C-8), 22.1 (C-9, 10); ESI–MS (Negative mode) *m/z* 183 $[M-H]^+$; MS *m/z* 184 $[M]^+$ (on the basis of GC–MS). Elemental analysis (% found) C 65.04 (65.52), H 7.10 (7.58).

4,6-Dichlorothymol (**3**)

To a solution of thymol 2 g (13.33 mmol) in carbon tetrachloride (20 ml), *N*-chlorosuccinimide (5 g, 37.31 mmol) was added and refluxed over water bath 60–70 °C temperature. The reaction mixture was cooled to room temperature, diluted with water, and extracted with chloroform (3 × 25 ml). Organic layer was washed with water, dried over sodium sulphate, and evaporated to dryness. The reaction product was purified by silica gel column and eluted with hexane/ethyl acetate as colourless oil (purity, 97.5 %). Yield: 55 %; IR (neat) ν_{max}: 3527, 2964, 1470, 1402, 1313, 1213, 1172, 1035, 879, 812, 766 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.07 (s, 1H, C_3–H), 5.65 (s, 1H, Ar–OH), 3.22 (m, 1H, C_8–H), 2.22 (s, 3H, C_7–H), 1.12 (d, 6H, $J = 6.3$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 147.9 (C-1), 134.7 (C-5), 131.5 (C-6), 126.2 (C-2), 125.7 (C-3), 121.5 (C-4), 28.2 (C-8), 22.1 (C-9, 10) 17.9 (C-7); ESI–MS (positive mode) *m/z* 219 $[M + H]^+$, MS *m/z* 218 $[M]^+$ (on the basis of GC–MS). Elemental analysis (% found) C 54.82 (54.98), H 5.52 (5.68).

6-Bromothymol (**4**)

To a solution of thymol (5 g, 33.33 mmol) in chloroform (25 ml), (5 g, 28.08 mmol) *N*-bromosuccinimide was added and refluxed over water bath for 5–6 h at temperature of 40–50 °C. To this distilled water was added and extracted with chloroform (3 × 25 ml). Organic layer was washed with water dried over sodium sulphate and evaporated to dryness. The reaction product was purified by silica gel column and eluted with hexane/EtOAc as colourless oil (97.6 %). Yield: 65 %; light yellow viscous; IR (neat) ν_{max}: 3506, 2962, 2870, 1465, 1373, 1398, 1208, 1169, 1123, 1028, 879, 789, 735, 651 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.02 (d, 1H, $J = 7.8$ Hz, C_3–H), 6.76 (d, 1H, $J = 7.8$ Hz, C_4–H), 5.68 (Ar–OH), 3.22 (m,1H, C_8–H), 2.31 (s, 3H, C_7–H), 1.27 (d, 6H, $J = 6.9$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 149.8 (C-1), 135.8 (C-6), 134.7 (C-5), 125.4 (C-2), 122.5 (C-3), 114.1 (C-4), 28.5 (C-8), 23.3 (C-7), 22.7 (C-9, 10); MS *m/z* 228 $[M]^+$; Elemental analysis (% found) C 52.42 (51.93), H 5.72 (5.78).

4,6-Dibromothymol (**5**)

To a solution of thymol 5 g (33.33 mmol) in chloroform (25 ml), (12 g, 67.41 mmol) *N*-bromosuccinimide was added and refluxed over water bath for 5–6 h at temperature of 60–70 °C. To this distilled water was added and extracted with chloroform (3 × 25 ml). Organic layer was washed with water dried over sodium sulphate and evaporated to dryness. The reaction product was purified by silica gel column and eluted with hexane/ethyl acetate. It was obtained as yellow viscous oil (98 %). Yield: 56 %; IR (neat) ν_{max}: 3506, 2962, 2870, 1596,1465, 1343, 1306, 1208, 1169, 1123, 1028, 879, 607 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.29 (s, 1H, C_3–H), 5.66 (s, 1H, Ar–OH), 3.28 (m, 1H, C_8–H), 2.43 (s, 3H, C_7–H), 1.20 (d, 6H, $J = 6.9$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 149.3 (C-1), 135.2 (C-6), 134.6 (C-4), 129.6 (C-5), 115.50 (C-2), 114.4 (C-3), 28.5 (C-7), 24.3 (C-8), 22.9 (C-9, 10); ESI–MS (negative mode) *m/z* 307 $[M–H]^+$; MS *m/z* 308 $[M]^+$ (on the basis of GC–MS). Elemental analysis (% found) C 38.99 (39.25), H 3.93 (4.07).

6-Bromo, 4-chlorothymol (**6**)

To a solution of chlorothymol (5 g, 27.17 mmol) in chloroform (25 ml), *N*-bromosuccinimide (10 g, 56.17 mmol) was added and the mixture was refluxed over water bath for 5–6 h at temperature 60–70 °C. To this distilled water was added and extracted with chloroform (3 × 25 ml). Organic layer was washed with water dried over sodium sulphate and evaporated to dryness. The reaction product was purified by silica gel column and eluted with hexane/chloroform as light yellow oil (97.6 %). Yield: 50 %; IR (neat) ν_{max}: 3416, 2890, 2780, 1620, 1560, 1430, 1303, 1210, 1110, 1026, 816, 676 cm^{-1}. ^1H NMR ($CDCl_3$; 300 MHz): δ 7.13 (s, 1H, C_3–H), 5.64 (s, 1H, Ar–OH), 3.26 (m, 1H, C_8–H), 2.44 (s, 3H, C_7–H), 1.22 (d, 6H, $J = 6.9$ Hz, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 149.3 (C-1), 135.2 (C-6), 134.6 (C-4), 129.6 (C-5), 115.5 (C-2), 114.4 (C-3), 28.54 (C-7), 24.3 (C-8), 22.9 (C-9, 10); MS *m/z* 264 $[M]^+$ (on the basis of GC–MS). Elemental analysis (% found) C 45.57 (45.33), H 4.59 (4.56).

Thymol methyl ether (**7**)

To a solution of 150 mg thymol (1 mmol) in acetone (10 ml), 550 mg of K_2CO_3 (3.9 mmol), and 0.5 ml of dimethyl sulphate was added. After stirring at 70 °C under reflux for about for 24 h the reaction was worked up by removal of solvent. The residue was partitioned between chloroform and water. Organic layer was washed with water and dried over Na_2SO_4. The residue obtained was purified by column chromatography on silica gel column with petroleum ether-ethyl acetate as elutant to give 135 mg of methyl ether of thymol as colourless oil (98 %). Yield: 80 %, IR (neat) ν_{max}: 2962, 2928, 1490, 1462, 1369, 1260, 1244, 1194, 1172, 1046, 959 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.00 (d, 1H, $J = 7.8$, C_3–H), 6.65 (d, 1H, $J = 7.8$, C_4–H), 6.58 (s, 1H, C_6–H), 3.71 (s, 3H, OMe), 3.16 (m, 1H, C_8–H), 2.23 (s, 3H, C_7–H) 1.11 (d, 6H, $J = 6.9$, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 157.1 (C-1), 136.6 (C-5), 134.3 (C-2), 126.1 (C-3), 121.4 (C-4), 111.8 (C-6), 26.8 (C-8), 23.1 (C-9,10), 21.6 (C-7), 58.9 (O–Me); ESI–MS (positive mode) *m/z* 165 $[M+H]^+$; Elemental analysis (% found) C 80.44 (80.38), H 9.82 (9.97).

Mono iodo methyl ether of thymol (**8**)

To a solution of 50 mg of methyl ether of thymol in acetonitrile (5 ml) was added 16.4 mg ceric ammonium nitrate and 38 mg of Iodine. After stirring at room temperature for about 8 h the reaction was worked up adding 5 % Na_2SO_4 solution and extracted with EtOAc. Organic layer was washed with water and dried over Na_2SO_4. Residue obtained was purified by column chromatography using hexane/EtOAc as eluant to give 20 mg of product as colourless oil (97.8 %). Yield: 64 %, IR (neat) ν_{max}: 2927, 2343, 1489, 1461, 1242, 1192, 1068, 949, 763 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.44 (s, 1H, C_3–H), 6.64 (s, 1H, C_6–H), 3.60 (s, 3H, OMe), 2.299 (s, 3H, C_7–H), 1.10 (d, 6H, $J = 6.9$, C_9–H and C_{10}–H); ^{13}C NMR ($CDCl_3$; 75 MHz): δ 157.5 (C-1), 139.6 (C-5), 137.4 (C-3), 136.1 (C-2), 112.8 (C-6), 90.4 (C-4), 28.3 (C-8), 26.8 (C-7), 23.6 (C-9, 10), 58.9 (O-Me); ESI–MS (positive mode) *m/z* 313 $[M+Na]^+$; *m/z* 291 $[M+H]^+$. Elemental analysis (% found) C 45.54 (45.21), H 5.21 (5.35).

Methyl ether of mono bromo thymol (**9**)

Hundred mg of mono bromo thymol was taken in 5 ml acetone, to this solution, 500 mg of K_2CO_3 and 0.5 ml of dimethyl sulphate was added. After refluxing for about 24 h the reaction was worked up by removal of solvent. The residue was fractionated between chloroform and water. Organic layer was dried over Na_2SO_4. The residue obtained was purified by column chromatography on silica gel to give 124 mg of product as yellow colourless oil (96.8 %). IR (neat) ν_{max}: 2962, 2920, 1490, 1462, 1369, 1244,1172, 1046, 885, 670 cm^{-1}; ^1H NMR ($CDCl_3$; 300 MHz): δ 7.02 (d, 1H, $J = 7.8$, C_3–H), 6.928 (d, 1H, $J = 7.8$, C_4–H), 3.71 (s, 3H, O-Me), 3.19 (m, 1H, C_8–H), 2.30 (s, 3H, C_7–H), 1.11 (d, 6H, $J = 6.9$, C_9–H and C_{10}–H), ^{13}C NMR ($CDCl_3$; 75 MHz): δ 157.1 (C-1), 135.8 (C-5), 134.3 (C-2), 126.1 (C-3), 125.4 (C-4), 113.8 (C-6), 21.6 (C-7), 26.8 (C-8), 23.1 (C-9, 10), 58.9 (O–Me); ESI–MS (positive mode) *m/z* 242.7 $[M+H]^+$. Elemental analysis (% found) C 54.34 (54.58), H 6.22 (6.08).

Microbial strains and media

The bacterial and fungal cultures were grown on the Mueller–Hinton agar (Hi-Media) and Sabouraud dextrose agar (Hi-Media) media by incubation at the strain wise optimum temperatures. Streptomycin was used as control antibiotic in the bioactivity assays in case of bacteria whereas amphotericin B was used as the standard antifungal compound.

Bacterial strains

Staphylococcus aureus, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Escherichia coli*, *Klebsiella pneumoniae*.

Fungal Strains

Candida albicans (AI), *C. albicans* (MTCC), *C. albicans* (CN).

Disc diffusion assay

Antibacterial activity testing was done as per Bauer *et al.*, (1996). All bacteria were subcultured from −80 °C stock cultures into 5 mL of Mueller–Hinton broth and incubated for 24 h at desired temperatures. For use as an inoculum, the turbidity of the bacterial suspension was adjusted to the McFarland standard 0.5 (ca. 2–4 × 10^6 cfu/ml). About 100 µL amount of bacterial culture was spread plated on solid medium and discs (5-mm diameter) containing test compound were placed on the pre-inoculated agar surface. Observations were recorded after 48 h of incubation of plates at desired temperatures.

Antifungal activity testing was done as per Wannisorn *et al.*, (1966). Fungal cultures were grown on SDA at 28 °C for 7 days except *C. albicans*. Suspension of each fungus was prepared in 0.85 % normal saline containing 0.1 % Tween 80. For use as inoculum, the turbidity of the fungal suspensions was also adjusted to the McFarland standard 0.5. A seeded agar plate was prepared by pouring (a) 20 mL of SDA into a sterile plate and, (b) uniformly overlaying of solid medium with 5 mL of soft agar, pre-inoculated with 0.1 mL of fungal suspension. Each of the essential oil or fractions was diluted/dissolved in DMSO to the required concentration and applied into filter paper discs (diameter 5 mm) @ 5 µL/disc. The discs were transferred into the surface of seeded agar plate (1 disc/plate) and gently pressed down to ensure contact. The plates were incubated at 28 °C for 7–10 days when inhibition zone was measured.

Broth-dilution assay

Two-fold serial dilution technique was employed to assess the minimum inhibitory concentration (MIC) of a given compound using 96-well microplate. The MIC was taken as the lowest concentration of the test compound, which inhibited the appearance of visible growth.

Results and discussion

Previously, the reaction of thymol with sulfuryl chloride was studied and dichloro and trichloro thymol were formed in that reaction (Ahmad *et al.,* 2002; Daniel *et al.*, 1950). Reaction of thymol with *N*-chlorosuccinimide (NCS), *N*-bromosuccinimide (NBS) and iodination with iodine have not been studied thoroughly. Reaction of thymol with *N*-chlorosuccinimide at mild temperature (40–45 °C) in carbon tetra chloride-afforded 4-chlorothymol (**2**) and at higher temperature formed 4,6-dichlorothymol (**3**). Treatment of thymol with *N*-bromosuccinimde at mild temperature (40–45 °C) in chloroform afforded 6-bromothymol (**4**) and at higher temperature (70–75 °C)-afforded 4,6-dibromothymol (**5**). Reaction of 4-chlorothymol (**2**) with *N*-bromosuccinimide at temperature 60–70 °C afforded to 6-bromo, 4-chlorothymol (**6**). Reaction of thymol with *N*-iodo succinimide did not occur. Iodination of thymol with I_2 in the presence of ceric ammonium nitrate also failed. However, when the same reaction was carried out with methyl ether of thymol (**7**) it gave mono iodo derivative (**8**).

The antibacterial activities of different thymol derivatives were studied as per Bauer *et al.*, (1996) and the results are presented in Table 1. Thymol **1** was found to be most active against *S. epidermidis*, *E. faecalis*, and *S. mutans* at a concentration of 32.2, 62.5, and 62.5 ppm, respectively. It was also found to be active against *S. aureus*, *Enterobacter aerogenes* at a concentration of 125 ppm and against *K. pneumoniae* at a concentration of 250 ppm. Among the halogenated derivatives of thymol **1**, 4-chlorothymol **2** was found to be most active against *S. aureus* and *S. epidermidis* at a concentration of 12.5 and 25 ppm and 4,6-dibromothymol **5** was found to be more active against *S. aureus* followed by *S. epidermidis*, respectively. 6-Bromothymol **4** was found to be marginally effective against *S. mutans* at a concentration of 125 ppm and at a high concentration of 1,000 ppm slightly active against *E. aerogenes*. The other thymol derivatives **3**, **6**, **7**, **8**, and **9** did not show any antibacterial activity.

Thymol and its different derivatives were also tested for their antifungal activities as shown in Table 2. Antifungal activity testing was done as per Wannisorn *et al.*, (1966). 4-Chlorothymol **2** was found to be the most active compound. Thymol **1** at a concentration 100 ppm showed marginal activity against *C. albicans* (AI), *C. albicans* (MTCC), and *C. albicans* (CN). 4-Chlorothymol **2** at the

Table 1 Antibacterial activities of thymol and its derivatives (zone of inhibition in mm, MIC in µg/mL given in parenthesis

Compounds	*S. aureus*	*S. epidermis*	*E. faecalis*	*E. aerogenes*	*S. mutans*	*E. coli*	*K. pneumoniae*
Thymol (**1**)	12 (125)	18 (32.25)	10 (62.5)	8 (125)	11 (62.5)	3 (100)	6 (250)
Monobromothymol (**4**)	–	–	–	2 (100)	3 (125)	–	–
Dibromothymol (**5**)	13 (250)	16 (1000)	–	–	–	–	–
Chlorothymol (**2**)	5 (12.5)	6 (250)	–	–	–	–	–
Streptomycin	9 (12.5)	9 (6.35)	10 (0.36)	10 (2.71)	13 (6.35)	9 (1.35)	9 (2.71)

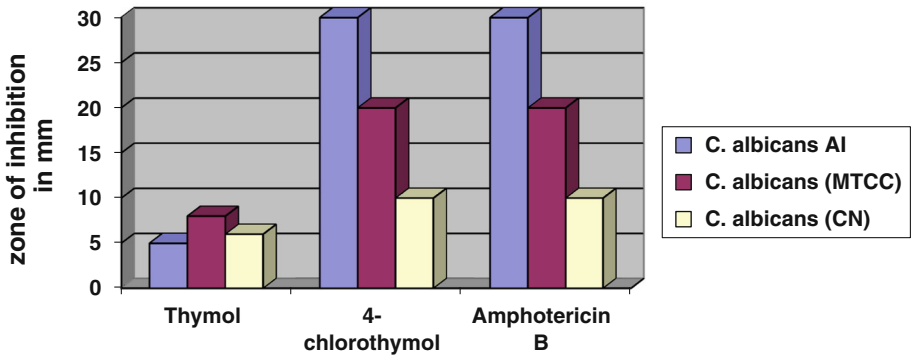


Fig. 2 Antifungal activity of different thymol derivatives

Table 2 Antifungal activities of thymol and its derivatives (zone of inhibition in mm, MIC in μg/ml given in parenthesis

Compounds	*C. albicans* (AI)	*C. albicans* (MTCC)	*C. albicans* (CN)
Thymol (**1**)	5 (100)	8 (100)	6 (100)
Chlorothymol (**2**)	30 (100)	20 (100)	10 (100)
Amphotericin B	30 (100)	20 (100)	10 (100)

same concentration of 100 ppm was found to be six times, 2.5 times and over almost two times more active than thymol against AI, MTCC and CN, respectively. Antifungal activity of 4-Chlorothymol **2** is comparable to that of Amphotericin B used as control (Fig. 2). The other thymol derivatives **3**, **4**, **5**, **6**, **7**, **8**, and **9** did not show any antifungal activity. Halogenation of thymol on position other than four resulted in complete loss of antibacterial activity. Among different halogenated derivatives, chloro substituted derivative were found to be more active as compared to bromo- and iodo-substituted derivatives.

Conclusion

Different halogenated derivatives of thymol have been prepared and they were tested for antimicrobial and antifungal activities. 4-Chlorothymol (**2**) was tested to be most active against *S. aureus*, *S. epidermis* at a concentration of 12.5 and 25 ppm, respectively. Also the same compound 4-chlorothymol (**2**) was found to be six times, 2.5 times and over two times more active than thymol against *C. albicans* (AI), *C. albicans* (MTCC), and *C. albicans* (CN), respectively.

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