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SYNTHESIS, ANTIMICROBIAL ACTIVITY, AND STRUCTURE–ACTIVITY RELATIONSHIPS OF EUGENOL, MENTHOL, AND GENISTEIN ESTERS

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This study concentrates on the extraction of eugenol, menthol, and genistein, and the synthesis, antimicrobial activity, and structure-activity relationships of their ester derivatives. The esters were tested for antimicrobial activity against several bacteria and yeast, and the correlation between biological properties and various physicochemical properties was examined. The ester derivatives gave intense emissions upon irradiation by UV light and have photoluminescence quantum yields of 39, 35, and 36% and long excited-state lifetimes of 3.62, 3.23, and 3.40 ns, respectively. These esters may be used in herbal medicinal therapy, and additionally as a base for the development of new drugs for phytomedicine.

Keywords: natural products, esterification, antimicrobial activity, structure–activity relationships.

Basil (*Ocimum basilicum* L.), mint (*Mentha piperita*), and parsley (*Petroselinum crispum*) have long been a part of various folk medicine traditions around the world. The essential oil eugenol (**1**) derived from basil and menthol (**2**) from mint oil has been used in Chinese medicine as early as 600 A.D. for many bacterial infections [1]. The use of eugenol as an antimicrobial agent has been explored [2]. Menthol and its derivatives are important industrial compounds due to their cooling and refreshing properties [3]. Menthol, like eugenol, has analgesic properties that are mediated through selective activation of opioid receptors [4]. The isoflavone genistein (**3**) is among the oldest and richest sources of Chinese herbal medicine, and traditional use of genistein has shown its inhibitory effects on both estrogen- and peptide-growth-factor-stimulated growth of breast cancer cells [5].

The composition of the extracts from basil (*Ocimum basilicum* L.), mint (*Mentha piperita*), and parsley (*Petroselinum crispum*) were analyzed by GC-MS, where identification of the components was made by a typical library search. The major components were found to be eugenol, menthol, and genistein, respectively. Purification of the extracts involves the use of large amounts of environmentally unfriendly solvents and expensive analysis procedures. Thus the crude extracts were used for the next step of esterification without further purification.

The synthesis of esters via acylation of alcohols is a fundamental and well-known transformation and is generally achieved by the reaction of alcohols with acid anhydrides or acid chlorides [6, 7]. Acylation using acid anhydrides works well, but is a rather wasteful reaction as only one acyl group is used for acylation. Acyl chlorides are not particularly efficient acylating agents [8]. Further, their use is limited due to them being extremely moisture sensitive and corrosive, and them having disturbing lacrymating properties. Generally, esterification by the direct condensation of carboxylic acids with alcohols is generally avoided as equilibrium is prevalent between the substrates and the products, thus necessitating the rapid removal of water from reaction mixtures. However, the use of a dehydrant or azeotrope shifts the equilibria in favor of products. Traditionally, this is achieved by condensing carboxylic acid and alcohol, with one being in large excess to drive the reaction in the forward direction. The studies herein present the synthesis of ester derivatives of eugenol, menthol, and genistein by reacting with a carboxylic acid via Fischer esterification.

Eugenol, menthol, and genistein were extracted from basil, mint, and parsley and their ester derivatives were tested for antimicrobial activity against several bacteria and yeast. The results for eugenol, menthol, and genistein were compared with those of their esters. The structures of these esters were characterized using spectroscopic techniques including GC-MS, IR, UV-Vis, and photoluminescence.

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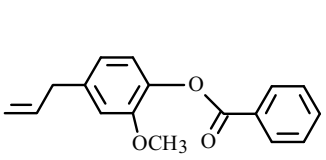
TABLE 1. Antimicrobial Activity Data and Selected Physicochemical Properties for Natural Compounds and Their Derivatives

Entity**	Microorganism*				Physicochemical properties			
	Bacillus megaterium	*Staphylococcus aureus*	*Escherichia coli*	*Rhodotorula rubra*	MW	MV (E^3)	MR (E^3)	logP
1[a]	12.8	16.0	7.2	7.2	164.20	571.64	52.46	0.31
4[a]	51.4	77.3	34.6	24.0	156.27	580.56	47.44	2.78
2[b]	16.7	16.7	11.8	8.8	270.24	729.04	78.68	2.05
5[b]	–	1.71	0.96	0.9	268.31	849.78	86.21	1.24
3[b]	17.8	27.6	15.8	13.8	260.38	849.99	80.91	4.06
6[b]	11.8	19.4	8.8	8.2	582.57	1474.62	179.93	0.74
Control[c]	–	–	–	–	NA	NA	NA	NA

*Microorganism inhibition zone, mm; – denotes no activity; *Klebsiella pneumoniae* (not shown here) was also used; however all entities showed no bacterial activity against *Klebsiella pneumoniae.*

**Entity refers to compounds. [a]3200 μg/well; [b]500 μg/well; [c]methanol.



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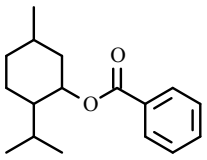

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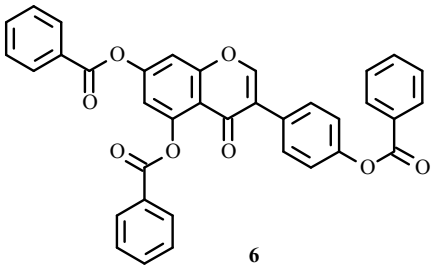

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Evaluation of Antimicrobial Studies. The test solutions were prepared in methanol. Inhibition zones on the medium were measured and recorded in mm. The results of the antimicrobial activities are summarized in Table 1.

Interestingly, the synthesized compounds were found to have no bacterial activity against *Klebsiella pneumoniae*. As shown in Table 1, eugenol ester **4** displayed the greatest antimicrobial activity compared to its unsubstituted analogue (**1**) for all microorganisms. Menthol ester **5** showed a significant decrease in activity relative to its unsubstituted analogue **2**, showing that esterification of menthol effectively destroys any prevalent antimicrobial activity. In general, genistein ester **6** showed the greatest antimicrobial activity among all esters. Esterification of menthol and genistein resulted in losses in activity against all microorganisms.

Structure–Activity Relationships. The lipophilicity of compounds has an important effect on their biological activity [9, 10]. It is expressed as logP, the octanol–water partition coefficient, and high values indicate good permeation of compounds through lipid layers of cell membranes. The prediction of lipophilicity and other physicochemical properties such as molar weight (MW), molecular volume (MV), and molecular refractivity (MR) for compounds **1–6** was calculated using HyperChem Software in an attempt to correlate physicochemical properties of the compounds with their antimicrobial activity [11]. The physicochemical properties MW, MV, MR, and logP of the compounds studied are presented in Table 1.

Interestingly, eugenol ester **4** has 4 times greater lipophilicity compared to its unsubstituted analogue **1**, and as a result this may be expressed as a 4-fold increase in biological activity for **4** compared to its unsubstituted analogue **1**. The lipophilic character of menthol ester **5** was the highest among all compounds; however, its antimicrobial activity showed the opposite tendency, with a substantial decrease in activity upon esterification of its unsubstituted analogue **2**. The steric factors of genistein **3** and its ester **6** played no adverse role in antimicrobial activity. Possible hydrogen bonding via carbonyl groups with the active cell components may be more influential, resulting in the interference and disruption of normal cell processes. In general, the results show there was a dependence between biological activity and lipophilicity, where antimicrobial activity increased with increasing lipophilicity. MR, as with lipophilicity, is also a molecular descriptor used to relate chemical structure to observed behavior. It is the combined effect of a molecule/substituent′s size and polarizability. It can be seen from Table 1 that, in general, MR increased with increasing lipophilicity, and thus MR too may be related to biological activity, where antimicrobial activity increased with increasing MR.

TABLE 2. Photoluminescence Data for Eugenol Ester, Menthol Ester, and Genistein Ester Derivatives **4–6**

Entity	λ_{max} Ex, nm	In Ex	λ_{max} Em, nm	In Em	ϕ_f, %	τ_f, ns
4	278 (258; 307; 324)	659	443 (376; 571)	651	39	3.62
5	287 (243; 258; 307)	577	406 (379; 432; 473)	568	35	3.23
6	279 (243; 259; 328)	617	381 (335; 434; 554)	609	36	3.40

λ_{max} Ex: maximum excitation wavelength; in Ex: maximum excitation intensity; λ_{max} Em: maximum emission wavelength; in Em: maximum emission intensity; ϕ_f : quantum yield; τ_f : excited-state lifetime.

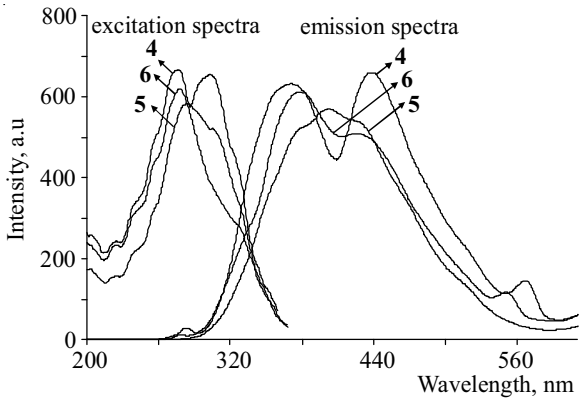

Fig. 1. Photoluminescence spectra of eugenol ester (**4**), menthol ester (**5**), and genistein ester (**6**) derivatives in dichloromethane; samples were excited at 268, 268, and 269 nm, respectively.

Ultraviolet-Visible Spectrophotometry (UV-Vis). The UV-Vis spectrum of each sample indicates the presence of several distinct absorption bands, including shoulder peaks at different wavelengths as given in the experimental section. Almost all samples revealed sharp or broad and intense absorption peaks at about 300 to 340 nm. These signals are attributed to the π–π* transition peaks of compounds with different chemical structures.

Fourier Transform Infrared Spectroscopy (FT-IR). FT-IR spectra of eugenol, menthol, and genistein ester derivatives showed several intense peaks at different wavelengths as given in the experimental section. The peak at about 3050 cm^{-1} belongs to C-H stretching; the region 1600–1450 cm^{-1} can be prescribed to aromatic ring stretching, and the region 1200–500 cm^{-1} belongs to the in-plane and out-of-plane bending of C-H bonds on aromatic rings. The IR bands at 1700–1750 cm^{-1} are due to C=O, and those at 1000–1300 cm^{-1} are due to C-O stretching of carbonyl and ether groups of the esters. The esters revealed peaks at 1735, 1737, and 1743 cm^{-1}, indicating formation of carbonyl bonds, and 1160, 1195, and 1209 cm^{-1}, indicating formation of ether bonds.

Fluorescence Measurements. Absorption and photoluminescence spectra were studied for solutions of eugenol ester derivative (**4**) excited at 268 nm. The most striking feature was that the eugenol ester derivative gave an intense emission upon irradiation by UV light. The photoluminescence spectrum of the eugenol ester derivative in dichloromethane is shown in Fig. 1. Maximum luminescent intensity was observed at 443 nm, and the full width at half maximum was 165 nm. The eugenol ester derivative exhibited a photoluminescence quantum yield of 39% and a long excited-state lifetime of 3.62 ns. The high quantum yield is due to extensive π-electron delocalization in the large molecular structure. Thus, it is evident that the fluorescence emission intensity of the compound increases with more π bonds in the larger compound or with the formation of an electron-rich cyclic molecule. This electron-rich cyclic ester molecule increases electron delocalization and/or energy transfer from the excited state of the eugenol ester, thus increasing the nonradiated transition of the eugenol ester excited state and increasing the fluorescence emission.

The absorption and photoluminescence spectra were studied for solutions of menthol ester derivative (**5**) excited at 268 nm. The most striking feature was that the menthol ester derivative gave an intense emission upon irradiation by UV light. The excitation and emission spectra of the menthol ester derivative in dichloromethane are shown in Fig. 1. Maximum luminescent intensity was observed at 406 nm, and the full width at half maximum was 132 nm. The menthol ester derivative

exhibited a photoluminescence quantum yield of 35% and a long excited-state lifetime of 3.23 ns. The photoluminescence intensities and quantum yield of the derivative increased with respect to that of menthol due to the formation of a larger electron-rich molecule. Thus, it is evident that the fluorescence emission intensity of the compound increases with more π bonds in the larger compound or with the formation of an electron-rich molecule.

Absorption and photoluminescence spectra were studied for solutions of genistein ester derivative (**6**) excited at 269 nm. The most striking feature was that the genistein ester derivative gave an intense emission upon irradiation by UV light. The excitation and emission spectra of genistein ester derivative (**6**) in dichloromethane are shown in Fig. 1. Maximum photoluminescence intensity was observed at 381 nm, and the full width at half maximum was 142 nm. The genistein ester derivative (**6**) exhibited a photoluminescence quantum yield of 36% and a long excited-state lifetime of 3.40 ns. The high quantum yield is due to extensive π-electron delocalization in the large molecular structure. Thus, it is evident that the fluorescence emission intensity of the compound increased with more π bonds in the larger compound or with the formation of an electron-rich cyclic molecule.

The photoluminescence data for eugenol, menthol, and genistein ester derivatives **4–6** are summarized in Table 2. The photoluminescent properties of these compounds may indicate great potential for numerous optical applications and for medicinal biomarkers.

EXPERIMENTAL

Materials and Instrumentation. Commercially available and/or reagent grade solvents and reagents were purchased from Aldrich Co. or Merck and were used without purification unless otherwise stated. All reactions were carried out under an atmosphere of nitrogen gas. Reaction temperatures were measured either externally or by a thermometer inserted into the reaction mixture. The melting point was recorded on an Electrothermal 9200 apparatus and were determined using sealed capillaries and reported uncorrected.

In this study, basil, mint, and parsley were collected from Mersin and Kahramanmaras in Turkey. Preweighed dried basil, mint, and parsley leaves (10% moisture) were used. Extracts from basil, mint, and parsley were stored in the refrigerator (4°C) and kept in the dark when not in use. Ester derivatives of eugenol (basil), menthol (mint), and genistein (parsley) were synthesized by reacting with a carboxylic acid via Fischer esterification.

The products obtained were investigated by GC-MS (Agilent Technologies System) and identification of compounds was made by a typical library search (NIST, Wiley). UV-Vis spectra were recorded from 190 to 900 nm using a Shimadzu UV-160A scanning spectrophotometer and 0.05 wt/vol sample in dichloromethame in a 1 cm optical path quartz cuvette. FTIR spectra were obtained using a Shimadzu spectrometer, Model 8300 FTIR. FTIR spectra were obtained using samples prepared as KBr pellets; samples were dry-ground using a mortar and pestle and then pressed into transparent pellets.

The single-photon fluorescence spectra were collected on a Perkin–Elmer LS55 luminescence spectrometer. All the samples were prepared in spectrophotometric grade dichloromethane and analyzed in a 1 cm optical path quartz cuvette. The solution concentration of the eugenol ester, menthol ester, and genistein ester derivatives **4–6** in dichloromethane was 1.0×10^{-5} mol L^{-1}, and the samples were excited at 268, 268, and 269 nm wavelengths, respectively. The photoluminescence quantum efficiencies of the eugenol, menthol, and genistein derivatives were calculated using 9,10-diphenylanthracene as the standard [12].

Extraction of 4-Allyl-2-methoxyphenol (eugenol) (1). To a 1000 mL single-neck round-bottomed flask containing 250.0 mL of ethyl acetate and 250.0 mL distilled water, 25 g dry ground basil leaves (10% moisture) was added. The resulting mixture was refluxed for 24 h. After cooling, the reaction mixture was diluted with water, and the residue was filtered out. The filtrate was extracted with ethyl acetate, washed with water and brine, and then dried ($MgSO_4$), filtered, and concentrated by evaporation *in vacuo* to afford 0.47 g (1.9 wt%) of light green oil used in the next step without further purification. IR spectrum (KBr, cm^{-1}) 3110, 3016, 2940, 1784, 1698, 1548, 1502, 1412, 1316, 1204, 1108, 1030, 740. UV spectrum (λ_{max}, nm): 282, 317, 414, 479, 532, 558, 603, 656. The spectral properties were as expected.

Extraction of 2-Isopropyl-5-methylcyclohexanol (menthol) (2). This was carried out with 40 g dry ground mint leaves (10% moisture) in a manner similar to that described above to afford 0.90 g (2.3 wt%) of a light green solid as the product, which was used in the next step without further purification: mp 38–40°C (lit. [13] 36–38°C). IR spectrum (KBr, cm^{-1}): 3534, 3020, 2944, 1764, 1660, 1548, 1496, 1414, 1322, 1208, 1060, 1008, 740. UV spectrum (λ_{max}, nm): 282, 319, 411, 502, 535, 561, 605, 666, 720. The melting point behavior and spectral properties were as expected.

Extraction of 5,7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one (genistein) (3). This was carried out with 25 g dry ground parsley leaves (10 % moisture) in a manner similar to that described above to afford 0.49 g (2.0 wt%) of a light brown solid as product, which was used in the next step without further purification: mp 86–88°C. IR spectrum (KBr, cm^{-1}): 3550, 3110, 3022, 2944, 1790, 1708, 1548, 1502, 1416, 1284, 1094, 734. UV spectrum (λ_{max}, nm): 284, 301, 317, 409, 501, 532, 557, 604, 662. The spectral properties coincide with the expected.

General Procedure for Ester Synthesis. Fischer esterification of the natural products eugenol (**1**), menthol (**2**), and genistein (**3**) was carried out as follows. Benzoic acid was added to a DCM solution of the natural product prepared as above. The resulting mixture was placed in an ice/water bath (0°C), and after chilling for several minutes, 3 mL conc. sulfuric acid was added slowly dropwise. The reaction mixture was maintained at this temperature for 5 min, after which the resulting mixture was allowed to warm to room temperature and stirred for 24 h. To quench the reaction, 20.0 mL of water was added dropwise and the mixture extracted with ethyl acetate, washed with water and sodium bicarbonate, and then dried ($MgSO_4$) and concentrated by evaporation *in vacuo* to give a residue. The solid was recrystallized in ethanol, dried *in vacuo,* stored in the refrigerator (4°C), and kept in the dark when not in use.

Synthesis of 4-Allyl-2-methoxyphenyl Benzoate (eugenol ester) (4). The general synthetic procedure described above affords 0.067g (20%) of product as a white solid from eugenol (**1**) (0.200 g, 1.22 mmol) as prepared above and benzoic acid (0.179 g, 1.46 mmol): mp 54–56°C (lit. [14] 55–56°C). IR spectrum (KBr, cm^{-1}): 3116, 2937, 1781, 1735, 1692, 1553, 1506, 1408, 1160, 1204, 745. UV spectrum (λ_{max}, nm): 277, 407, 502, 538, 655, 687. Mass spectrum (EI) *m/z* (I_{rel}, %): 268 (10), 147 (80), 136 (12), 115 (7). The melting point behavior and spectral properties were as expected.

Synthesis of 2-Isopropyl-5-methylcyclohexyl Benzoate (menthol ester) (5). The general synthetic procedure described above affords 0.068 g (20%) as a white solid from menthol (**2**) (0.200 g, 1.28 mmol) as prepared above and benzoic acid (0.188 g, 1.54 mmol): mp 80–82°C. IR spectrum (KBr, ν, cm^{-1}): 3538, 2939, 1756, 1737, 1664, 1541, 1493, 1325, 1195, 1062, 735. UV spectrum (CH_2Cl_2, λ_{max}, nm): 279, 326, 415, 544. Mass spectrum (EI) *m/z* (I_{rel}, %): 260 (20), 138 (68), 109 (15), 85 (10). The spectral properties were as expected.

Synthesis of 5,7-Dibenzoate-3-(4-phenylbenzoate)-chromen-4-one (genistein ester) (6). The general synthetic procedure described above affords 0.095 g (22%) as a white solid from genistein (**3**) (0.200 g, 0.74 mmol) as prepared above and benzoic acid (0.298 g, 2.44 mmol): mp 108–110°C. IR spectrum (KBr, ν, cm^{-1}): 3545, 3115, 2941, 1784, 1743, 1554, 1506, 1411, 1209, 1087, 739. UV spectrum (CH_2Cl_2, λ_{max}, nm): 273, 342, 406, 541, 656, 688. Mass spectrum (EI) *m/z* (I_{rel}, %): 582 (20). The spectral properties were as expected.

Conclusions. Eugenol, menthol, and genistein ester derivatives were synthesized and their antimicrobial activity and structure–activity relationships were examined. While genistein had the highest antimicrobial activity, a comparison of the plant precursor compounds with their respective ester derivatives showed that the eugenol ester had the greatest biological activity compared to its unsubstituted analogue. It was observed that, in general, as the lipophilicity and MR of the compounds increased, the antimicrobial activity increased.

Preparation of Microorganism Cultures. Six samples were evaluated for their *in vitro* antibacterial activity against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* 6538, *Bacillus megaterium* DSM 32, and *Klebsiella pneumoniae* FMC 5, and antifungal activity against *Rhodotorula rubra* 116 using the agar-well diffusion method [15]. The microorganisms were provided by the Microbiology Laboratory Culture Collection, Department of Biology, Kahramanmaras, Sutcu Imam University, Turkey. All the bacteria mentioned above were incubated at 37 ± 0.1°C for 24 h by inoculation into nutrient broth (Difco), and the yeast studied was incubated in Sabouraud dextrose broth (SDB) (Difco) for 48 h. The bacteria and yeast (prepared as above) were added to petri dishes (9 cm) in the amount of 0.1 mL (10^8/mL bacteria and 10^8/mL yeast cells); 15 cm^3 of Mueller Hinton agar (MHA, Oxoid) and Sabouraud dextrose agar (SDA) (sterilized in a flask and cooled to 45–50°C) were homogenously distributed onto the sterilized petri dishes.

By using a sterilized cork borer (7 mm diameter), wells were dug in the culture plates, and compounds dissolved in methanol were added to these wells; 3200 μg eugenol (**1**) and its ester **4**; and 500 μg all other compounds **2**, **3**, **5**, and **6**. The petri dishes so obtained were placed at 4°C for 2 h, where the plates inoculated with bacteria were incubated at 37 ± 0.1°C for 18 h, and those with the yeast were incubated at 37 ± 0.1°C for 24 h. At the end of the period, the diameter of the inhibition zones for the growth of the various microorganisms was measured as responses to treatment with the samples. These studies were performed in triplicate, and methanol was used as the control.

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REFERENCES

1. C. Perez and C. Anesini, *Am. J. Chin. Med.*, **22**, 169 (1994).
2. V. Moleyar and P. Narasimham, *Int. J. Food Microbiol.*, **16**, 337 (1992).
3. R. Eccles, *J. Pharm. Pharmacol.*, **46**, 618 (1994).
4. N. Galeottia, L. D. C. Mannellia, G. Mazzantib, A. Bartolinia, and C. Ghelardini, *Neurosci. Lett.*, **322**, 145 (2002).
5. G. Peterson and S. Barnes, *Cell Growth Differ.*, **7**, 1345 (1996).
6. R. C. Larock, *Comprehensive Organic Transformations*, VCH, New York, USA, 1989.
7. J. Otera, *Esterification: Methods, Reactions and Applications*, Wiley-VCH, Weinheim, 2003.
8. G. Zengin and J. W. Huffman, *Synthesis*, **12**, 1932 (2004).
9. T. Fujita, J. Iwassa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
10. A. Leo, C. Hansch, and D. Elkins, *Chem. Rev*., **71**, 525 (1971).
11. HyperChem 7.5 Program, Hypercube Inc.: Toronto, Canada, 2002.
12. G. G. Guilbault (ed.), *Practical Fluorescence*, Marcel Dekker, New York, USA, 1990.
13. R. Eccles, *J. Pharm. Pharmacol.,* **46**, 618 (1994).
14. H. Sadeghian, S. M. Seyedi, M. R. Saberi, Z. Arghiani, and M. Riazi, *Bioorg. Med. Chem.*, **16**, 890 (2008).
15. C. H. Collins, P. M. Lyne, and J. M. Grange, *Microbiological Methods*, Butterworths & Co. Ltd., London, UK, 1989.