Mode of Action of the Compound
5.1 Introduction

A search in the World Wide Web showed that the compound (1E, 4E)-1,5-bis (4-hydroxy-3,5-dipropyl phenyl) penta-1,4-dien-3-one isolated from the fern *Drynaria quercifolia* has not been reported earlier. Hence, it is assumed that this is the first time the compound is studied and its antibacterial properties are reported. To find its mode of action, the structure of the compound has been compared with other medicinally important phytochemicals. This chapter contains a brief description of the medicinal properties of similar compounds and the mode of action of antibacterial phytochemicals.

5.2 Structurally similar compounds and their reported properties

The compound is compared with different classes of antibiotics for structural similarity and found that it is not structurally similar to any if the ‘antibiotics’. But it has been seen that the compound has a distant similarity with one of the curcumin analogues; 1,5-Bis-(2,4-difluorophenyl)penta-1,4-diene-3-one. The compound has been reported to possess anti-tumor and anti-angiogenic properties (Dennis et al., 2003). Another similar compound 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-1,4-dien-3-one and derivatives have been reported to possess antitumoral properties (Suarez et al., 2006). Several appropriately substituted 4-(dialkylamino-alkyl)-substituted-styryl-alkyl ketones or acetophenones inhibited the interaction between recombinant HIV Env and CD4 (Niharika et al., 2002).
5.3 Mode of action of non-antibiotic antibacterial agents

The possible mechanism of action of the compound can be traced out from the action of similar compound with antibacterial action. In this section mechanism of action of terpenoid and similar phytochemicals were investigated to make a possible conclusion about the action of the compound on bacteria.

A number of plant derived antibacterial agents have been reported. Important targets of the antibacterial agents are the cell wall, the cytoplasmic membrane, and the cytoplasm (Hugo and Russel, 1998). Actions on cytoplasmic membrane may be divided into three categories: action on membrane potentials, action on membrane enzymes and action on general membrane permeability. It has been shown that bacteria, in common with chloroplasts and mitochondria, are able to maintain a gradient of electrical potential making use of membrane-bound electron transport chain such that the interior of the cell is negative. Certain chemical substances are known to make membrane permeable to protons and destroying this potential gradient. Examples of antibacterial agents which are having, at least partly, this ability are tetrachlorosalicylanilide, pentachlorophenol, di-(5-chloro-hydroxyphenyl) sulphide and 2-phenoxyethanol. Antimicrobial effect of menthol, thymol, and linalyl acetate against the gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *Escherichia coli* may result, at least partially, from a perturbation of the lipid fraction of microorganism plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials. Furthermore, the drugs might cross the cell membranes, penetrate into the interior of the cell
and interact with intracellular targets for antibacterial activity (Walsh, 2003, Trombetta et al., 2005).

Thymol, a plant-derived antimicrobial agent, caused rapid efflux of intracellular constituents of Porphyromonas gingivalis, Selenomonas artemidis and Streptococcus sobrinus. Studies suggest that membrane perforation is a principal mode of action of this substance. The thymol-induced decline of intracellular ATP in S. sobrinus and it appears to be entirely attributable to leakage, whereas in P. gingivalis thymol may also inhibit ATP-generating pathways. Relative changes in the transmembrane potential of resting cells of S. sobrinus pulsed with glucose are as sensitive to thymol as is leakage from this organism. The effects of thymol on transmembrane potential are probably secondary to those arising from leakage of intracellular substances (Shapiro and Guggenheim, 1995).

Leaf extract of the of Eremophila duttonii, a traditional Australian medicinal plant previously shown to have potent bactericidal activity against gram-positive bacteria. The extract compromised the integrity of the cytoplasmic membrane of Staphylococcus aureus, leading to increased membrane permeability (indicated by uptake of PI) and a decreased ability to exclude NaCl. The bactericidal action of the E. duttonii extract was concluded to be due to its membrane-active properties (Tomlinson and Palombo, 2005).

Antibacterial effects of three terpene alcohols on Staphylococcus aureus, revealed that terpene alcohols, namely, farnesol, nerolidol and plaunotol might act on cell membranes. The antibacterial activity reflected the initial rate of leakage of K+ ions,
suggesting that damage to cell membranes might be one of the major modes of action of these terpene alcohols. The results also demonstrated that the initial rate of leakage and the amount of leaked K+ ions are useful as indices of the antibacterial activities of hydrophobic compounds (Inoue et al., 2004).

All the hydrolyzable tannins tested demonstrated dose-dependent membrane-damaging activity. However, it remains to be elucidated whether their membrane-damaging activity directly contributes to their antibacterial action (Funatogawa et al., 2004).

The antimicrobial mechanism of totarol was studied using *Pseudomonas aeruginosa* IFO 3080. This diterpene inhibited oxygen consumption and respiratory-driven proton translocation in whole cells, and oxidation of NADH in membrane preparation. NADH-cytochrome c reductase was inhibited by totarol while cytochrome c oxidase was not. NADH-DPIP reductase and NADH-CoQ reductase were also inhibited. The site of respiratory inhibition of totarol was thought to be near CoQ in the bacterial electron transport chain (Haraguchi et al., 1996).

The antibacterial properties such as growth inhibition, lethal effect and cytological damage of phenolic compounds and aromatic alcohols have been investigated. The role of protein and RNA synthesis in the bactericidal action was also determined. All compounds tested demonstrated lethal properties and the ability to alter membranes, especially in Gram-negative bacteria. Efficacious concentrations, however, varied greatly among the compounds. These data corroborate previous findings, which suggest that the mechanism of action of these compounds is related to their
lipophilia. Moreover, since it was demonstrated that the lethal effect of two aromatic alcohols (phenethyl alcohol and benzyl alcohol) stops when protein synthesis is inhibited, it is likely that both possess specific mechanisms of action (Lucchini et al., 1990)

Carvacrol is a component of several essential oils and has been shown to exert antimicrobial activity. It has been proved that carvacrol interacts with the membranes of bacteria such as \textit{B. cereus} by changing its permeability for cations like H(+) and K(+). The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death (Ultee et al., 1999). The structural requirements for the activity of carvacrol were determined by comparison to structurally related (nonessential oil) compounds. Removal of the aliphatic ring substituents of carvacrol slightly decreased the antimicrobial activity. The effect of the hydroxyl group of carvacrol on activity could not be determined by simply comparing it to p-cymene, because this compound is immiscible with water; therefore, 2-amino-p-cymene, the amino analogue of carvacrol, which has a similar hydrophobicity and structural characteristics, was used. 2-Amino-p-cymene had similar membrane disruption and bacterial killing characteristics as carvacrol showing that, contrary to previous reports, the hydroxyl group of carvacrol itself is not essential for the antimicrobial activity. However, the observed 3-fold lower activity for 2-amino-p-cymene as compared to carvacrol indicates special features in the antimicrobial mode of action of carvacrol due to the hydroxyl group (Veldhuizen et al., 2006)

\textit{(+)-Totarol}, a diterpenoid isolated from \textit{Podocarpus} spp., is a potent antioxidant and antibacterial agent. Although the
mechanism of action of this hydrophobic molecule is poorly understood, recent work has shown that it could be due to membranotropic interactions. The study indicates that (+)-totarol is situated in the upper region of the membrane, with its hydroxyl group located in the vicinity of the C-3/4 carbon atoms of the phospholipid acyl chain, and nearly perpendicular with respect to the phospholipid acyl chain axis. Such a location of (+)-totarol in the membrane would be expected to compromise the functional integrity of the membrane and account, at least in part, for its antibacterial effects (Bernabeu et al., 2002).

Licochalcone A-D and echinatin, retrochalcones isolated from the roots of Glycyrrhiza inflata, showed antimicrobial activity. Among them, licochalcone A and C had potent activity against some gram-positive bacteria. These retrochalcones inhibited oxygen consumption in susceptible bacterial cells. The oxidation of NADH in bacterial membrane preparations was also inhibited by them. NADH-cytochrome c reductase was inhibited by licochalcones, while cytochrome c oxidase was not. NADH-CoQ reductase and NADH-FMN oxidoreductase were not inhibited. The site of respiratory inhibition of licochalcones was thought to be between CoQ and cytochrome c in the bacterial respiratory electron transport chain (Haraguchi et al., 1998).

Farnesol increased beta-lactam susceptibility of methicillin-susceptible Staphylococcus aureus by inhibition of cell wall biosynthesis through reduction of free C55 lipid carrier with subsequent retardation of murein monomer precursor transport across the cell membrane (Kuroda et al., 2007).
The antibacterial compound isolated and characterized in the present study is a terpenoid and might be having properties similar to that of other terpenoids. Though we have not conducted any study that could prove the mechanism of antibacterial action of this compound; from the above findings and available reference, we propose that this compound also might be interacting with cytoplasmic membrane of bacterial cells and destroying it. Hydrophobic nature of the compound may be helping its interaction with the lipid bilayer of the membrane.