CHAPTER 5

SYNTHESIS OF COQ$_{10}$, NONAPRENYL SULPHATES AND STUDIES OF ITS BIOLOGICAL ACTIVITIES
5.1. Introduction:

Prenylated aromatics constitute an interesting group of natural products characterized by the presence of a prenylated side chain. The prenyl group is a long chain unsaturated functional moiety generally containing 5 to 50 carbons, which is composed of a fundamental and ubiquitous building block in plants and animals called isoprene (3-methyl-but-2-en-1-yl). The prenyl groups varies with respect to the chain length and structural modifications such as cyclization and hydroxylation of the prenyl chain.1-2 Prenylated flavonoids in higher plants protect the host by exhibiting strong antibacterial and antifungal activities.3 Many prenylated flavonoids have been identified as active components in medicinal plants with biological activities, such as anti-cancer, anti-androgen, anti-leishmania and anti-nitric oxide production.4-6 Prenyl groups seem to play a role in anchoring their host proteins and secondary metabolites to cell membranes.

Owing to their beneficial effects in human health, prenylated aromatics are of particular interest as lead compounds for producing new drugs and functional foods. The prenylation of the flavonoid or aromatic core increases the lipophilicity and the membrane permeability, which is one of the proposed reasons for the enhanced biological activities of prenylated compounds.7-9

A broad range of biological activities have been reported for polyphenolic compounds having prenyl residues and these plant products have been rich resources for many natural medicines.10 Thus far, more than 1000 prenylated polyphenols have been isolated from plants, and some of which have drawn substantial attention in the field of health sciences, such as food industries, breweries and cosmetic companies.

Plant polyphenols are classified into several groups according to the basic ring system, e.g., phenylpropanoids, flavonoids, coumarins, phloroglucinols and xanthones. There seems to be a chemotaxonomical tendency in the occurrence of some polyphenols, e.g., xanthone derivatives mostly occur in Guttiferae (Clusiaceae)11 and isoflavonoids are derived from Leguminosae (Fabaceae)12 and other groups of polyphenols are widely distributed in the plant kingdom. The occurrence of prenylated polyphenols is rather limited to a few plant families.
Representatives families include Leguminosae (Fabaceae), Moraceae, Cannabaceae, Guttiferae (Clusiaceae), Umbelliferae and Rutaceae whereas some other plant families like Euphorbiaceae and Compositae (Asteraceae) also comprise plant species that contain prenylated polyphenols. Plants containing these compounds have often been utilized as medicinal plants in many countries, for example, *Glycyrrhiza glabra* (Leguminosae) is used as an anti-inflammatory in Chinese traditional medicine, *Calophyllum inophyllum* (Guttiferae) is used against bronchitis and diarrhea in Latin America and *Maclura pomifera* (Moraceae) is used for cancer treatment.

The biological activities exhibited by prenylated polyphenols include anti-tumor, anti-bacterial, anti-virus, anti-oxidant, anti-tyrosinase, estrogenic, inhibition of sulfotransferase, anti-nitric oxide production and inhibition of phospholipase activity. A representative of the prenylated flavonoids, for example 8-dimethylallyl naringenin, has been identified in some leguminosaeous plants and is recognized as a strong phytoestrogen. It showed potential for the prevention of osteoporosis and for the enhancement of collagen synthesis in the skin. Xanthohumol is another example of important prenylated flavonoid, which is well known for its divergent biological activities, such as estrogenic, anti-oxidant and anti-tumor activity. This is the main component of hops (*Humulus lupulus* L., Cannabaceae), constituting nearly 80 - 90% to its total flavonoids, which added desired bitterness and flavor to beer. Other prenylated compounds humulone, lupulone, and phloroglucinol found in hops exhibit anti-tumor activity via the inhibition of cyclooxygenase-2 expression, which is mediated by a signal transduction pathway with transcriptional regulators NF-kB and AP-1. Another prenylated xanthone (rubraxanthone) identified in *Garcinia dioica* (Guttiferae) shows antithrombotic, anti-allergic and anti-inflammatory activities via suppression of the binding of platelet activation factor to its receptor. The enhancement of drug effects has been reported for prenylated furanocoumarin of grapefruit, which is caused by the inhibition of enzymes of drug metabolism in the human intestine.

A few prenylated compounds show multiple biological effects, e.g., Kurarinone (prenylated flavanone) isolated from *Sophora flavescens* (Leguminosae)
for example, exhibited estrogenic, anti-tyrosinase, anti-glycosidase and anti-lipoxygenase activities.\textsuperscript{38-41} It is noteworthy, that the prenyl moiety often plays a crucial role in these divergent biological activities in many of these compounds. This in turn suggests that the addition of a prenyl residue to polyphenol skeletons may contribute to the enhancement of the biological activities of polyphenolic compounds.

Ubiquinone (\textbf{5.01}), also known as Coenzyme Q\textsubscript{10}, (CoQ\textsubscript{10}) is a prenylated 1, 4-benzoquinone, where the number 10 refers to the number of isoprene units the prenyl side chain. CoQ\textsubscript{10} is present in most eukaryotic cells primarily in mitochondria and it is produced naturally in the human body. Ubiquinone is known to be useful in treating congestive heart failure, gum disease and type-2 diabetes. It is a widely used health supplement for replenishing reduced levels of ubiquinone caused by certain cholesterol medications or aging.

Coenzyme Q\textsubscript{10} is an integral part of the mitochondrial respiratory chain, which is essential for ATP synthesis and energy production in the cell. The redox functions of CoQ in cellular energy production and anti-oxidant protection are based on the ability to exchange two electrons in a redox cycle between ubiquinol (reduced CoQ) and oxidized CoQ (ubiquinone). Coenzyme Q\textsubscript{10} has many vital roles in the cells and protects the components of cell from the onslaught of oxygen free radical. It functions as an anti-oxidant in cell membranes and lipoproteins.\textsuperscript{42-43} Coenzyme Q\textsubscript{10} also play an important role in regulating genes, which in turn control energy production and other important functions in the cell. It is found in highest concentration in those organs and tissues with highest oxygen consumption such as the heart, brain and muscle.

As the body ages, the coenzyme Q\textsubscript{10} content decreases. Coenzyme Q\textsubscript{10} is also depleted during disease conditions such as heart and kidney failure. It is estimated that the body synthesizes more than half its own coenzyme Q\textsubscript{10} requirements, and the rest being obtained from the dietary sources. Coenzyme Q\textsubscript{10} is found in red meat, fish and green vegetables, but they contain relatively small amounts of coenzyme Q\textsubscript{10} compared to supplements. Coenzyme Q\textsubscript{10} supplements are virtually without side effects and are used for a variety of conditions including heart failure and brain disease.
CoQ_{10} is ubiquitously present in the mammalian tissues, especially in the heart. CoQ_{10} is not an essential nutrient, because it can be synthesized in the body. Dietary intake of CoQ_{10} is about 2-5 mg per day, which is inadequate for the body under physiological conditions.\textsuperscript{42-43} The natural levels of endogenous CoQ_{10} in the heart decreases during ischemic heart disease including heart failure and oral administration of CoQ_{10} was found to be effective in improving angina episodes, arrhythmias and left ventricular function in patients with acute myocardial infarction.\textsuperscript{44-45}

CoQ_9 differs from CoQ_{10} with respect to the number of isoprenoid units in the tail, CoQ_9 has nine units in contrast to the presence of 10 units in CoQ_{10}. CoQ_9 is found in rodents like mice and rats, while CoQ_6, CoQ_7 and CoQ_8 are found in yeast and bacteria.\textsuperscript{46-47} The majority of CoQ_9 in rat liver is present in its reduced form (ubiquinol), which exerts its anti-oxidative function. Similar to CoQ_{10}, CoQ_9 is not merely a compound responsible for energy transduction in mitochondrial membrane in rat heart.\textsuperscript{48} It also serves as a functional element in the cells and possesses ability for redox cycling.

R. Ruegg et al. developed a method for synthesis of CoQ_{10} by treatment of 5-methyl-2, 3-dimethoxy hydroquinone with decaprenol.\textsuperscript{49} Toyokichi et al. developed a region selective poly prenyl rearrangement of prenylated 2, 3, 4, 5-tetrasubstituted phenyl ethers promoted by boron trifluoride in synthesis of CoQ_{10}.\textsuperscript{50} Hirozumi et al. also synthesized the CoQ_{10} in two steps by reaction of 2, 3-dimethoxy-5-methyl quinol with isodecaprenol in presence of lewis acid.\textsuperscript{51} Yukio et al. prepared the CoQ_{10} by treatment of solanesyl-p-tolylsulfone with n-butyl lithium and 2, 3, 4, 5-tetramethoxy-6-(1-bromo-2-methyl-but-2-ene-4yl)-toluene in presence of HMPA in THF.\textsuperscript{52} Mutsunobu et al. prepared CoQ_{10} via stereo selective desulphonation of allylic sulphone with LiHBEt_3 in presence of catalytic amount of [PdCl_2(dppp)].\textsuperscript{53} Bruce et al. developed a short and highly efficient synthetic route for CoQ_{10}, it’s a sequence consisting of six operations get crystalline material in over all yield of 64%.\textsuperscript{54} Jae-Hong Min et al. prepared CoQ_{10} by reaction with tetramethoxy toluene and 4-chloro-2-methyl-1-phenyl sulphonyl-2-butene in presence of lewis acid and the intermediate used for total synthesis of CoQ_{10}.\textsuperscript{55} Akira Yanagisawa et al. prepared CoQ_{10} by highly
SN₂ (E)-and anti selective alkylation of allylic phosphates and fícile synthesis. A few number of other methods have been developed for the synthesis of CoQ₁₀.

Nonaprenylhydroquinonesulphates are unique class of secondary metabolites closely related in structure to CoQ compounds. Nonaprenylhydroquinone sulfate (5.11) and octaprenylhydroquinone sulfate (5.11a) are potent α-1, 3-Fucosyltransferase VII inhibitors isolated from an Australian marine sponge Sarcotragus sp. The α-1, 3-Fucosyltransferase VII is a pro-inflammatory protein responsible for P- and E- selection ligand synthesis and play a role in the control of leukocyte recruitment during the inflammation process. It is also known to play a role in the metastasis of colorectal and prostate cancers. Hence, α-1, 3-Fucosyltransferase VII has been considered to be one of the favorite targets for developing anti-inflammatory and anti-cancer drugs.

Presently, there is a great demand for non-steroidal anti-inflammatory agents, especially those derived from natural origin. As part of developing potent anti-inflammatory and anti-cancer agents for doctorial program, the author selected nonaprenyl aromatic sulphates for further investigation. Limited availability of sponge raw material, coupled with tedious extraction and isolation process for nonaprenylsulphates from natural sources prompted us to develop a cost effective synthetic methodology for nonaprenylhydroquinonesulphates to study their in vitro and in vivo efficacy. Due to the commercial importence of CoQ₁₀ and therapeutic potential for nonaprenylsulphates, the author has embarked on the synthesis of CoQ₁₀ and nonaprenylsulphates. In addition, the author also interested to take up their biological evaluation in vivo and in vitro models.

5.2. Chemistry:

Many methodologies were described in literature for the synthesis of CoQ₁₀. Solanesol, a natural compound having nine isoprene units, has been the key intermediate for most of these methodologies. Tobaco is a rich source of solanesol. These methods differ in the process for the development of aromatic moiety,
expansion of the solanesol to decaprenyl intermediate and coupling of prenylgroup to the aromatic intermediate.

As part of developing a new strategy for the synthesis of CoQ₁₀, the author conducted the retro-synthetic analysis as shown in figure 5.1 to identify appropriate precursors. Based on this disconnection, the synthetic process as summarized in scheme 5.1 was designed and executed to obtain CoQ₁₀ in 11.6% overall yield.

Figure 5.1: retro-synthetic analysis of CoQ₁₀
Synthesis of prenylated aromatic compounds

Scheme 5.1: Synthesis of co-enzyme quinone 10
Reagents and conditions: i) CCl₄, isoprenol, BF₃ etherate, rt, 3 h, 96.1%; ii) NaOH, DMS, 90°C, 2 h, 78%; iii) SeO₂, ethanol, rt, 4 h; iv) ethanol, NaBH₄, rt, 2 h; v) THF, pyridine, PBr₃, 0°C, 15 min., 92%; vi) CH₂Cl₂, sodium p-toluenesulfinate, TEA, rt, 3 h, 83.3%; vii) solanesyl bromide, tert-BuOK, THF, rt, 2 h, 74%; viii) THF, ethanol, sodium metal, rt, 9 h, 79%; ix) CH₂Cl₂, acetonitrile, CAN, 10°C, 1 h, 72%.

As per the retro synthetic analysis, the precursor 2, 5-dihydroxy-3, 4-dimethoxy tolune (5.02) was prepared from commercially available 2, 3-dimethoxy-5-methylquinone. The other key intermediate solanesyl bromide was prepared from solanesol by a substitution reaction with PBr₃ in THF. Solanesol is produced from tobacco waste using hexane extraction, followed by silica column chromatography and crystallization. The aryl intermediate 2, 5-dihydroxy-3, 4-dimethoxy tolune (5.02) was treated with isoprenol (2-methyl-3-butene-2-ol) in presence of boron tri fluoride etherate to give 2, 3 dimethoxy-5-(3 methyl-2-butene)-6-methyl quinol (5.03). Methylation of quinol 5.03 with sodium hydroxide and dimethyl sulphate gave 2, 3, 4, 5- tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04). The compound 5.04 was subjected to allylic oxidation using selenium dioxide to give a mixture of 2-(2-methyl-2-en-1-al-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.05) and 2-(2-methyl-2-en-1-ol-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.06).
This mixture (5.05 and 5.06) was subjected to hydride reduction using sodium borohydride to give 2-(2-methyl-2-en-1-ol-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.06). The alcohol 5.06 was treated with phosphorus tribromide to obtain the bromide compound 2-(2-methyl-2-en-1-bromo-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.07), which was then treated with sodium-p-toluene sulfinate to give 2-(2-methyl-2-en-1-p-tolenesulphinate-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.08). The sulphone compound 5.08 was treated with solanesyl bromide in presence of potassium tert-butoxide to give compound (5.09). The compound 5.09 was subjected to elimination of p-toluenesulfinate using sodium/ethanol to give 2-decaprenyl-3, 4, 5, 6-tetramethoxy toluene (5.10). Finally the 2-decaprenyl-3, 4, 5, 6-tetramethoxy toluene (5.10) was treated with ceric ammonium nitrate to give CoQ_{10} (5.01) and is shown in scheme 5.1.

The second objective of the current research project was to synthesize the nonaprenyl sulfates and evaluate their biological activities. The synthesis of sodium nonaprenyl hydroquinone sulfate (5.11) and sodium O-nonaprenylphenolic sulphate (5.12) was achieved in a straightforward manner as summarized in the scheme 5.2. Instead of Friedel-Crafts alkylation using solanesyl bromide for prenylation of the aromatic nucleus, solanesol utilized directly in the presence of BF\(_3\)-etherate to prenylate the carbon otho to the phenolic group. Acetylhydroquinone (5.13) or phenol (5.14) was reacted with solanesol in presence of boron trifluoride diethyl etherate\(^{71}\) to obtain nonaprenyl phenols 5.15 and 5.16 respectively. The compounds 5.15 and 5.16 were subjected to O-sulphonation independently using sulfurtrioxide-pyridine\(^{72}\) to yield nonaprenyl phenolic sulfates 5.17 and 5.18 respectively. The compounds 5.17 and 5.18 were converted to their sodium salts using sodium hydroxide\(^{73}\) to give sodium nonaprenylhydroquinone sulfate (5.11) and sodium nonaprenylphenolic sulfate (5.12). The details of the synthetic process were summarized in scheme 5.2. The IR, \(^1\)H NMR, \(^{13}\)C NMR spectral data obtained for synthetic sodium nonaprenylhydroquinone sulfate (5.11) and sodium O-nonaprenylphenolic sulphate (5.12) matched very closely with those reported for their natural counterparts.\(^{70}\)
Scheme 5.2: Synthesis of nonaprenyl aromatic sulphates (5.11 and 5.22)

Reagents and conditions: i) Solanesol, BF₃ etherate, CCl₄, rt, 1 h; ii) sulphurtrioxide-pyridine, pyridine, DMAP, THF, rt, 2 h; iii) NaOH, MeOH, rt, 12 h.

5.3. Discussion:

The compounds CoQ₁₀ (5.01), sodium nonaprenylhydroquinone sulfate (5.11) and sodium nonaprenylphenolic sulfate (5.12) were tested for their *in vitro* potential for 5-lipoxygenase enzyme inhibition, brine shrimp lethality and anti-oxidant activity in DPPH and NBT free radical scavenging activity. The compound 5.01 exhibited mild 5-LOX inhibition (IC₅₀: 59.08 μg/mL), where as compounds 5.11 and 5.12 showed potent 5-lipoxygenase inhibition with IC₅₀ values of 1.08 μg/mL and 3.40 μg/mL respectively. During direct comparison, Curcumin as a positive control showed an IC₅₀ value of 3.5 μg/mL as summarized in Table 5.1. Interestingly, compounds 5.11 and 5.12 also showed potent brine shrimp lethality with IC₅₀ values of 2.26 μg/mL and 3.98 μg/mL respectively, which are superior or better to the positive control podophyllotoxin (IC₅₀ 3.80 μg/mL). Compound 5.01 showed
moderate brine shrimp lethality with IC$_{50}$ value of 89.21 µg/mL and the results are summarized in Table 5.1. In addition, compounds 5.11 and 5.12 exhibited potent antioxidant activity in super oxide radical model with IC$_{50}$ values 50.7 µg/mL and 83.98 µg/mL respectively, when compared to vitamin C (IC$_{50}$ 102.4 µg/mL), but CoQ$_{10}$ (5.1) did not show significant activity up to 100 µg/mL and the results are summarized in Table 5.2. The anti arthritic efficacy of 5.11 was evaluated in vivo in FCA induced arthritis model of rats in comparison to prednisolone as a positive control. The sodium nonaprenylhydroquinone sulfate (5.11) showed 26.42% inhibition of paw edema at 50 mg/kg body weight per day, when compared to control group of animals. The positive control prednisolone in comparison showed 37.99% improvement and the results are summarized in Figure 5.2.

5.4. Experimental and Results:

5.4.1. Synthesis of Coenzyme Q$_{10}$:

5.4.1.1. 2, 3-dimethoxy, 5-methyl hydroquinone (5.02): 2, 3-dimethoxy, 5-methyl quinone (6 g, 0.033 mol), acetonitrile (48 mL), water (12 mL) and sodium dithionate (8.6 g, 0.0494 mol) were taken in a round bottomed flask. Reaction mixture was stirred at room temperature for 2 h. After completion of the reaction which was monitored by TLC [Rf: 0.8, hexane:ethyl acetate (8:2)], the reaction mixture was poured into cold water and extracted with ethyl acetate and washed with brine solution. Organic layer was dried over sodium sulfate and concentrated at 40°C temperature to obtain 2, 3-dimethoxy, 5-methyl hydroquinone (5.02, 5.5 g, 90.7 %).

5.4.1.2. 2, 3-dimethoxy-5-(3-methyl-2-butene)-6-methylquinol (5.03): 2, 3-dimethoxy-5-methylquinol (5.02, 5.5 g, 0.029 mol) carbon tetrachloride (28 mL) and 2-methyl-3-buten-2-ol (4.1 g, 0.048 mol) were taken in a round bottomed flask. BF$_3$ (0.85 g, 0.006 mol) was added drop by drop to the reaction mixture at 0-5°C. Then the reaction mixture was allowed to stand at room temperature with continuous stirring for 3 h. After completion of the reaction, which was monitored by TLC [Rf: 0.85, hexane:ethyl acetate (8:2)], the mixture was poured into cold water and extracted with EtOAc and washed with brine solution and the organic layer was dried over sodium sulfate and concentrated at 40°C to yield 8 g of crude mixture. The crude mixture was
chromatographed over silica gel using hexane/ethyl acetate mixtures as eluants, gave 2, 3-dimethoxy-5-(3-methyl-2-butene)-6-methyl quinol (5.03, 15% ethyl acetate in hexane, 8 g, 96.1%).

5.4.1.3. 2, 3, 4, 5-tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04): 2, 3-dimethoxy-5-prenyl-6-methylquinol (5.03, 8 g, 0.032 mol) and sodium hydroxide (5 g, 0.125 mol) were dissolved in water (21 mL) were taken in a round bottomed flask. Dimethylsulphate (19.9 g, 0.159 mol) was slowly added to reaction mixture at room temperature and reaction mixture was stirred at 90°C for 2 h. After completion of the reaction which was monitored by TLC [Rf: 0.89, hexane: ethyl acetate (8:2)], the mixture was poured into cold water and acidified with 5N H2SO4 and extracted with ethyl acetate and washed with brine and the organic layer was dried over sodium sulfate and concentrated to give a crude mixture (9 g). Then the crude mixture was subjected to column chromatography on silica gel using hexane/ethyl acetate mixtures as eluents, obtain 2, 3, 4, 5-tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04, 2% of ethyl acetate in hexane, 6.9 g, 78%).

1H NMR (CDCl3, 400 MHz): δ 4.99 - 4.95 (1H, m), 3.83 (3H, s), 3.82 (3H, s), 3.72 (3H, s), 3.71 (3H, s), 3.24 (2H, d, J = 6.80 Hz), 2.07 (3H, s), 1.70 (3H, d, J = 0.8 Hz), 1.62 (3H, d, J = 0.8 Hz); 13C NMR (CDCl3, 100 MHz): δ 147.9, 147.7, 144.9, 144.7, 131.3, 129.2, 125.3, 122.9, 61.0, 60.9, 60.6, 25.9, 25.6, 17.9, 11.7; IR (CHCl3, νmax): 2933, 1467, 1407, 1351, 1258, 1196, 1100, 1070, 1042, 1015, 997 cm⁻¹; LC-MS: m/z 303.2 (M+Na)+, +ve ion mode.

5.4.1.4. 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-en-1-al-4yl)-toluene (5.05): Tetramethoxyprenyltoluene (5.04, 7.3 g, 0.066 mol) was slowly added to selenium dioxide (32.5 g, 0.296 mol) in ethanol (100 mL) at room temperature and stirred for 4 h. After completion of the reaction, which was monitored by TLC [Rf: 0.87, hexane: ethyl acetate (8:2)], the mixture was poured into cold water and extracted with ethyl acetate and washed with brine and the organic layer was dried over Sodium sulfate and concentrated. The residue was subjected to silica column using hexane/ethyl acetate as eluents. The fractions eluted in 10-15% ethyl acetate/hexane were monitored by TLC, equal spots are combined and concentrated under reduced
pressure to yield 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-en-1-ol-4yl)-toluene (5.05, 3.5 g, 51%) and 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-en-1-ol-4yl)-toluene (5.06, 2.1 g, 30.5%)

\[ \text{\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz): } \delta 9.30 (1H, s), 6.32 (1H, td, } J = 1.2, 6.8 \text{ Hz), 3.85 (3H, s), 3.83 (3H, s), 3.74 (3H, s), 3.72 (3H, s), 3.59 (2H, dd, } J = 6.8, 0.8 \text{ Hz), } 2.07 (3H, s), 1.83 (3H, d, } J = 1.2 \text{ Hz), } \text{\textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz): } \delta 195.1, 152.6, 148.1, 147.9, 145.9, 144.9, 138.9, 125.5, 125.3, 61.1, 61.0, 60.9, 60.7, 27.0, 11.9, 9.3; IR (CHCl\textsubscript{3}, \nu_{\text{max}}): 2932, 1687, 1467, 1407, 1352, 1219, 1104, 1039 \text{ cm}^{-1}; \text{ LC-MS: } m/z 295.3 (M+H)^{+}, 317.3 (M+Na)^{+}, 333.2 (M+K)^{+}, \text{+ve ion mode.} \]

5.4.1.5. 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-en-1-ol-4yl)-toluene (5.06): But-2-ene-2-methyl-1-one-tetramethoxytoluene (5.05, 5.5 g, 0.019 mol) was dissolved in ethanol (55 mL) and sodium borohydride (413 mg, 0.011 mol) was slowly added to reaction mixture under stirring at room temperature for 2 h. After completion of the reaction, which was monitored by TLC [Rf: 0.67, hexane:ethyl acetate (8:2)], the mixture was poured into cold water and acidified with 5N HCL and extracted with ethyl acetate. The organic layer was washed with brine and the organic layer was dried over Sodium sulfate and concentrated to get crude mixture. The crude mixture was subjected to silica gel column chromatography on silica gel using hexane/ethyl acetate mixtures, to yield 2, 3, 4, 5-tetramethoxy-6-(2-methylbut-2-en-1-ol-4yl)-toluene (5.06, 15%-20% of ethyl acetate in hexane, 3.5 g, 65%).

\[ \text{\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz): } \delta 5.29 - 5.23 (1H, m), 3.93 (2H, brs), 3.83 (3H, s), 3.82 (3H, s), 3.73 (3H, s), 3.71 (3H, s), 3.29 (2H, dd, } J = 6.8, 0.8 \text{ Hz), } 2.07 (3H, s), 1.76 (3H, s); \text{\textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz): } \delta 147.9, 147.7, 145.2, 144.8, 134.7, 128.3, 125.2, 124.5, 68.8, 61.0, 60.9, 60.6, 25.5, 13.8, 11.8; IR (CHCl\textsubscript{3}, \nu_{\text{max}}): 3432, 2935, 2862, 1466, 1407, 1351, 1219, 1105, 1067, 1039, 1013 \text{ cm}^{-1}; \text{ LC-MS: } m/z 319.3 (M+Na)^{+}, 335.3 (M+K)^{+}, \text{+ve ion mode.} \]

5.4.1.6. 2, 3, 4, 5-tetramethoxy-6-(1-bromo-2-methylbut-2-en-4yl)-toluene (5.07): But-2-ene-2-methyl-1-ol-tetramethoxytoluene (5.06, 2 g, 0.007 mol) was dissolved in tetrahydrofuran (10 mL). Pyridine (0.1 g, 0.0017 mol) was added to above reaction mixture. PBr\textsubscript{3} (0.66 g, 0.0025 mol) was slowly added to the reaction mixture at 0°C.
for 15 minutes. After completion of the reaction which was monitored by TLC [Rf: 0.9, hexane:ethyl acetate (9:1)], the mixture was poured into cold water and sodium bicarbonate solution. The mixture was extracted with ethyl acetate. The organic layer was washed with brine solution and the organic layer was dried over sodium sulfate and concentrated under vacuum. The residue was subjected to column chromatography on silica gel, hexane/ethyl acetate mixtures used as eluents to give 2, 3, 4, 5-tetramethoxy-6-(1-bromo-2-methylbut-2-en-4yl)-toluene (5.07, 2% ethyl acetate in hexane, 2.2 g, 92%).

\[ ^1 \text{H NMR (CDCl}_3, 400 \text{ MHz):} \delta 5.45 (1H, t, J = 6.8 \text{ Hz}), 3.89 (2H, s), 3.83 (3H, s), 3.82 (3H, s), 3.72 (3H, s), 3.71 (3H, s), 3.28 (2H, d, J = 6.8 \text{ Hz}), 2.06 (3H, s), 1.85 (3H, d, J = 0.8 \text{ Hz}); ^{13} \text{C NMR (CDCl}_3, 100 \text{ MHz):} \delta 147.9, 147.8, 145.4, 144.8, 131.8, 129.7, 127.4, 125.3, 61.1, 61.0, 60.9, 60.6, 41.5, 26.2, 14.8, 11.8; \text{IR (CHCl}_3, v_{\text{max}}): 2934, 1466, 1408, 1351, 1200, 1105, 1073, 1039, 1012, 974 \text{ cm}^{-1}; \text{LC-MS: m/z 361.2 (M+H)\text{+}, 383.2 (M+Na)\text{+}, +ve ion mode.} \]

5.4.1.7. 2, 3, 4, 5-tetramethoxy-6-(1-p-toluenesulphinyl-2-methylbut-2-ene-4yl)-toluene (5.08): But-2-ene-2-methyl-1-bromo-4-yl-tetramethoxytoluene (5.07, 1 g, 2.77 mmol) was dissolved in dried dichloromethane (10 mL). Sodium-p-toluenesulphinate (493 mg, 2.78 mmol) was added portion wise to the reaction mixture. Then triethylamine (0.28 g, 2.77 mmol) was added to the reaction mixture and stirred at room temperature for 3 h. After completion of the reaction which was monitored by TLC [Rf: 0.76, hexane:ethyl acetate (8:2)], the reaction mixture was poured on to cold water and acidified to pH 4 with 5N HCL and extracted with ethyl acetate and washed with brine solution and concentrated. The residue was subjected to column chromatography on silica column, using hexane/ethyl acetate mixtures as eluents to obtain 2, 3, 4, 5-tetramethoxy-6-(1-p-toluenesulphinyl-2-methylbut-2-ene-4yl)-toluene (5.08, 15% of ethyl acetate in hexane, 1 g, 83.3%).

\[ ^1 \text{H NMR (CDCl}_3, 400 \text{ MHz):} \delta 7.58 (2H, d, J = 8.0 \text{ Hz}), 7.15 (2H, d, J = 8.0 \text{ Hz}), 4.86 (1H, t, J = 6.4 \text{ Hz}), 3.84 (3H, s), 3.80 (3H, s), 3.70 (3H, s), 3.63 (3H, s), 3.62 (2H, s), 3.17 (2H, d, J = 6.8 \text{ Hz}), 2.33 (3H, s), 1.91 (3H, s), 1.86 (3H, d, J = 1.2 \text{ Hz}); ^{13} \text{C NMR (CDCl}_3, 100 \text{ MHz):} \delta 147.8, 147.6, 145.3, 144.7, 144.3, 135.6, 134.3, 134.3, 133.6, 132.6, 132.5, 131.6, 130.6, 129.6, 128.6, 127.6, 126.6, 125.6, 124.6, 123.6, 122.6, 121.6. \]
129.4, 128.5, 127.2, 125.1, 123.5, 66.1, 61.0, 60.9, 60.8, 60.6, 26.2, 21.5, 16.9, 11.6; IR (CHCl$_3$) $\nu$$_{max}$: 2933, 1594, 1463, 1410, 1352, 1309, 1212, 1108, 1040, 970 cm$^{-1}$; LC-MS: $m/z$ 435.3 (M+H)$^+$, +ve ion mode.

5.4.1.8. 6-Decaprenyl-2, 3, 4, 5-tetramethoxytoluene-4′-(p methyl phenyl)-sulphinate (5.09): Potassium tert-butoxide (520 mg, 4.63 mmol) was suspended in tetrahydrofuran (10 mL). 2-But-2-en-2-methyl-p-toluene-sulphinate-tetramethoxytoluene (5.08, 1 g, 2.30 mmol) and solanesyl bromide (2.4 g, 3.45 mmol) were dissolved in tetrahydrofuran (10 mL). 2-but-2-en-2-methyl-p-toluene sulphinate-tetramethoxytoluene and solanesyl bromide mixture were slowly added to potassium tert-butoxide solution at -20°C. Then the reaction mixture was allowed to stand at room temperature with constant stirring for 2 h. After completion of the reaction which was monitored by TLC [Rf: 0.87, hexane : ethyl acetate (8:2)], the reaction mixture was poured into cold water and acidified with 5N HCL to pH 4 and reaction mixture was extracted with ethyl acetate, organic layer was washed with brine, dried over sodium sulfate and concentrated. The residue was subjected to column chromatography on silica gel using hexane/ethyl acetate mixtures as eluents to yield 6-decaprenyl-2, 3, 4, 5-tetramethoxytoluene-4′-(p methyl phenyl)-sulphinate (5.09, 5% of ethyl acetate in hexane, 1.78 g, 74%).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.57 (2H, d, $J$ = 8.0 Hz), 7.15 (2H, d, $J$ = 8.0 Hz), 5.06 - 5.00 (9H, m), 4.85 (1H, t, $J$ = 7.2 Hz), 3.83 (3H, s), 3.80 (3H, s), 3.69 (3H, s), 3.58 (3H, s), 3.18 (2H, d, $J$ = 6.4 Hz), 2.33 (3H, s), 2.03 - 1.96 (16H, m), 1.93 - 1.88 (18H, m), 1.86 (3H, s), 1.84 (3H, s), 1.76 (3H, s), 1.61 (6H, s), 1.52 (18H, s), 1.49 (3H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 145.2, 144.7, 138.4, 135.3, 135.0, 134.9, 134.8, 134.5, 134.2, 131.2, 129.5, 129.2, 129.1, 128.9, 126.9, 124.4, 124.3, 124.2, 124.1, 123.7, 118.8, 73.9, 66.3, 60.9, 60.8, 60.6, 39.7, 26.7, 26.6, 25.9, 25.6, 24.3, 21.5, 17.6, 16.3, 15.9, 11.5; IR (CHCl$_3$, $\nu$$_{max}$): 2925, 2854, 1465, 1406, 1383, 1314, 1219, 1145, 1105, 1086, 1041 cm$^{-1}$; LC-MS: $m/z$ 1046.8 (M+H)$^+$, +ve ion mode.

5.4.1.9. 6-Decaprenyl-2, 3, 4, 5-tetramethoxytoluene (5.10): 6-Decaprenyl-2, 3, 4, 5-tetramethoxytoluene-4′-(p methyl phenyl)-sulphinate (5.09, 800 mg, 0.76 mmol) was dissolved in tetrahydrofuran (8 mL) and ethanol (1.23 g, 26.76 mmol) was also
added to reaction mixture. Sodium (146 mg, 10.71 mmol) was slowly added to the reaction mixture at room temperature, and stirred for 9 h. After completion of the reaction which was monitored by TLC [Rf: 0.85, hexane:ethyl acetate (9:1)], the reaction mixture was poured into cold water and extracted with ethyl acetate. Organic layer was washed with brine, dried over Sodium sulfate and concentrated. The crude mixture was subjected to column chromatography on silica gel using hexane/ethyl acetate mixtures as eluents, to give 6-decaprenyl-2, 3, 4, 5-tetramethoxy toluene (5.10, 2% of ethyl acetate in hexane, 540 mg, 79%).

1H NMR (CDCl₃, 400 MHz): δ 5.06 - 5.02 (10H, t, J = 6.40 Hz), 3.83 (3H, s), 3.82 (3H, s), 3.71 (3H, s), 3.70 (3H, s), 3.2 (2H, d, J = 6.4 Hz), 2.1 (3H, s), 2.03 - 1.97 (20H, m), 1.94 - 1.89 (18H, m), 1.69 (3H, s), 1.61 (6H, s), 1.53 (24H, s); 13C NMR (CDCl₃, 100 MHz): δ 147.9, 147.7, 144.6, 144.3, 135.1, 135.0, 134.9, 134.8, 131.2, 129.8, 124.9, 124.5, 124.3, 124.2, 124.1, 123.4, 123.2, 122.9, 61.0, 60.9, 60.6, 60.5, 40.1, 39.7, 27.0, 26.8, 26.7, 26.2, 25.8, 25.6, 23.3, 17.6, 16.2, 16.0, 11.7, 11.5; IR (CHCl₃, νmax): 2963, 2925, 2854, 1465, 1407, 1382, 1352, 1196, 1105, 1043, 979 cm⁻¹; LC-MS: m/z 915.9 (M+Na)⁺, +ve ion mode.

5.4.1.10. Coenzyme Q₁₀ (5.01): 6-Decaprenyl-2, 3, 4, 5-tetramethoxytoluene (5.10, 300 mg, 0.34 mmol) was dissolved in acetonitrile (1.25 mL) and dichloromethane (1.25 mL). Ceric ammonium nitrate (560 mg, 1.02 mmol) was slowly added to reaction mixture at 0°C and 50% aqueous acetonitrile (3 mL) was also added to the reaction mixture. Reaction mixture was stirred at 10°C for 1 h. After completion of the reaction which was monitored by TLC [Rf: 0.82, hexane:ethyl acetate (9:1)], the reaction mixture was poured into ice cold water and extracted with ethyl acetate. Organic layer was washed with brine, dried over sodium sulfate and concentrated. The residue was subjected to column chromatography on silica gel using hexane/ethyl acetate mixtures as eluents to yield coenzyme Q₁₀ (5.01, 2% of ethyl acetate in hexane, 210 mg, 72%).

1H NMR (CDCl₃, 400 MHz): δ 5.11 - 5.04 (9H, m), 4.95 - 4.92 (1H, m), 3.99 (3H, s), 3.97 (3H, s), 3.18 (2H, d, J = 6.4 Hz), 2.08 - 2.04 (20H, m), 2.01 (3H, s), 1.99 - 1.97 (18H, m), 1.73 (6H, s), 1.67 (3H, s), 1.59 (21H, s); 13C NMR (CDCl₃, 100 MHz): δ
187.7, 183.8, 144.5, 144.3, 141.7, 138.8, 137.8, 137.6, 135.2, 134.9, 134.8, 131.2, 124.4, 124.3, 124.2, 123.9, 118.9, 39.8, 39.7, 26.8, 26.7, 26.5, 25.6, 25.3, 17.6, 16.3, 16.0, 11.9; IR (CHCl₃, ν_max): 2923, 2853, 1743, 1653, 1611, 1146, 1265, 1201, 1150, 1098, 1023 cm⁻¹; LC-MS: m/z 863.8 (M+H)+, 885.7 (M+Na)+, 901.7 (M+K)+, +ve ion mode.

5.4.2. Synthesis of Nona prenylsulphates

5.4.2.1. Procedure for nona prenyl phenols 5.15 and 5.16: To a mixture of phenol (5.13 or 5.14; 0.01 mol) and solanesol (6.3 g, 0.01 mol) in 20 mL of carbon tetrachloride, boron trifluoride etherate (0.49 mL, 0.004 mol) was added and the reaction mixture stirred at room temperature for 1 h. After completion of the reaction which was monitored by TLC [Rf: 0.86, hexane:ethyl acetate (9:1)], the mixture was poured into cold water and the mixture extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulphate and concentrated under vacuum. The residue was subjected to column chromatography on silica gel, hexane/ethyl acetate mixtures used as eluents. The fractions eluted with 2-5% ethyl acetate/hexane mixtures yielded pure prenylated compounds 5.15 or 5.16.

2-nonaprenyl-4-acetoxyphenol (5.15): Yield: 4.8 g (62%); ¹H NMR (CDCl₃, 400 MHz): δ 6.81 (1H, d, J = 2.4 Hz), 6.80 (1H, dd, J = 2.4, 6.8 Hz), 6.76 (1H, d, J = 7.2 Hz), 5.31 (1H, t, J = 6.4 Hz), 5.13 - 5.08 (8H, m), 3.33 (2H, d, J = 7.6 Hz), 2.25 (3H, s), 2.13 - 2.04 (18H, m), 1.99 - 1.96 (16H, m), 1.75 (3H, s), 1.67 (3H, s), 1.59 (24H, s); ¹³C NMR (CDCl₃, 100 MHz): δ 168.5, 151.0, 145.1, 137.9, 135.3, 134.9, 134.8, 132.4, 130.1, 128.3, 127.9, 124.5, 124.3, 124.2, 123.7, 122.5, 120.9, 120.0, 116.2, 39.7, 39.4, 29.7, 26.8, 26.7, 26.6, 25.6, 21.01, 17.7, 16.1, 16.0; IR(CHCl₃, ν_max): 3436, 2921, 1748, 1638, 1507, 1412, 1219, 772 cm⁻¹; LC-MS: m/z 763(M-H) -, -ve ion mode.

2-nonaprenylphenol (5.16): Yield: 3.9 g (59%); ¹H NMR (CDCl₃, 400 MHz): δ 7.11 - 7.08 (2H, m), 6.84 (1H, td, J = 1.2, 7.2 Hz), 6.79 (1H, dd, J = 1.2, 8.4 Hz), 5.34 - 5.31 (1H, m), 5.11 - 5.08 (8H, m), 3.36 (2H, d, J = 7.2 Hz), 2.13 - 2.06 (18H, m), 1.99 - 1.95 (16H, m), 1.77 (3H, s), 1.67 (3H, s), 1.59 (24H, s); ¹³C NMR (CDCl₃, 100 MHz): δ 149.7, 138.4, 137.3, 135.2, 135.0, 134.9, 134.9, 134.5, 131.2, 129.9, 129.4, 129.4.
5.4.2.2. Procedure for nonaprenylphenolic sulfates 5.17 or 5.18:
Nonaprenylphenol (5.15 or 5.16, 0.001 mol) was dissolved in dry tetrahydrofuran (25 mL) and treated with sulfurtrioxide-pyridine (0.24 g, 0.0015 mol), pyridine (1.04 mL, 0.013 mol) and DMAP (10 mg) and the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction which was monitored by TLC [Rf: 0.62, hexane:ethyl acetate (8:2)], the reaction mixture was poured into cold water and the mixture extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate and concentrated under vacuum at room temperature to yield nonaprenylphenolic sulfate 5.17 or 5.18.

2-nonaprenyl-4-acetoxy-phenolic sulfate (5.17): Yield: 759 mg (90%); 1H NMR (CDCl3, 400 MHz): δ 7.17 (1H, dd, J = 1.6, 7.6 Hz), 7.11 (1H, d, J = 3.0 Hz), 6.57 (1H, s), 6.44 (1H, d, J = 7.6Hz), 5.32 - 5.29 (1H, m), 5.12 - 5.07 (8H, m), 3.33 (2H, d, J = 7.2 Hz), 2.24 (3H, s), 2.13 - 2.04 (18H, m), 1.99 - 1.94 (16H, m), 1.67 (3H, s), 1.59 (27H, s); 13C NMR (CDCl3, 100 MHz): δ 169.9, 152.1, 144.1, 138.9, 135.6, 135.0, 134.9, 134.9, 134.8, 134.7, 131.2, 130.0, 128.3, 128.0, 124.5, 124.3, 124.2, 123.7, 122.5, 121.0, 120.0, 116.2, 39.8, 39.7, 29.7, 29.5, 26.8, 26.7, 26.6, 26.5, 25.6, 23.5, 22.7, 21.0, 17.6, 16.2, 16.1, 16.0; IR (CHCl3, νmax): 3444, 2918, 1746, 1662, 1506, 1440, 1376, 1231, 1146, 1098, 1017, 865 cm⁻¹; LC-MS: m/z 843.7 (M-H), -ve ion mode.

2-nonaprenylphenolic sulfate (5.18): Yield: 700 mg (84%); 1H NMR (CDCl3, 400 MHz): δ 7.98 - 7.96 (1H, m), 7.07 - 7.02 (2H, m), 6.79 - 6.77 (1H, m), 5.38 - 5.36 (1H, m), 5.14 - 5.10 (8H, m), 4.09 - 4.07 (2H, m), 2.10 - 2.07 (18H, m), 1.99 - 1.96 (16H, m), 1.66 (3H, s), 1.60 (27H, s); 13C NMR (CDCl3, 100 MHz): δ 148.2, 136.3, 134.5, 134.2, 134.0, 133.9, 133.8, 133.5, 130.2, 128.9, 128.4, 127.8, 126.4, 125.9, 125.7, 124.5, 123.5, 123.3, 123.1, 122.8, 120.9, 120.8, 120.7, 119.6, 114.7, 38.8, 38.7, 28.7, 28.5, 26.7, 25.9, 25.8, 25.7, 25.5, 24.6, 16.6, 15.2, 15.1, 15.0; IR (CHCl3, νmax):
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3434, 2926, 1661, 1456, 1388, 1256, 1165, 1102, 1040, 771 cm$^{-1}$; LC-MS: m/z 785.5 (M-H)$^-$, -ve ion mode.

5.4.2.3. Sodium salt of 2-(nonaprenyl)-4-acetoxyphenylsulphonic acid (5.11): To a solution of 2-(nonaprenyl)-4-acetoxyphenylsulphonic acid (5.17, 0.5 g, 0.59 mmol) in methanol (3 mL) was added sodium hydroxide (47 mg, 1.17 mmol) and the reaction mixture was stirred overnight at room temperature. After completion of the reaction which was monitored by TLC [Rf: 0.42, hexane:ethyl acetate (8:2)], the reaction mixture was poured into cold water, acidified with 2N HCl and mixture extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude was dissolved in methanol (4 mL), then treated with 23 mg of sodium hydroxide, stirred over night and concentrated to obtain the sodium salt of 2-(nonaprenyl)-4-hydroxyphenylsulphonic acid (5.11, 426 mg, 90%).

$^1$H NMR (CDCl$_3$, 400 MHz): δ 7.17 (1H, dd, J = 1.2, 7.6 Hz), 7.11 (1H, d, J = 3.0 Hz), 6.57 (1H, s), 6.44 (1H, d, J = 7.6Hz), 5.21 - 5.18 (1H, m), 5.13 - 5.09 (8H, m), 3.36 - 3.34 (2H, m), 2.09 - 2.03 (18H, m), 1.99 - 1.92 (16H, m), 1.67 (3H, s), 1.59 (24H, s), 1.55 (3H, s); $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 152.7, 144.6, 137.3, 135.3, 135.1, 135.0, 134.9, 134.8, 132.4, 131.2, 130.1, 128.3, 124.5, 124.3, 124.2, 124.1, 121.5, 121.4, 40.0, 39.8, 39.7, 29.7, 29.3, 27.1, 27.0, 26.9, 26.8, 26.7, 25.7, 17.7, 16.3, 16.1, 16.0; IR (CHCl$_3$, $\nu_{\text{max}}$): 3444, 2917, 1662, 1506, 1440, 1376, 1231, 1146, 1097, 1017, 865 cm$^{-1}$; LC-MS: m/z 801.6 (M-Na)$^-$, -ve ion mode.

5.4.2.4. Sodium salt of 2-(nonaprenyl)-phenylsulphonic acid (5.12): To a solution 2-(nonaprenyl)-phenylsulphonic acid (5.18, 0.5 g, 0.63 mmol) in methanol (3 mL) was added sodium hydroxide (32.4 mg, 0.81 mmol) and the reaction mixture was stirred overnight at room temperature. After completion of the reaction which was monitored by TLC [Rf: 0.4, hexane:ethyl acetate (9:1)], the reaction mixture was poured into cold water and the contents extracted with ether. The organic layer was washed with brine, dried over sodium sulfate and concentrated to obtain the sodium salt of 2-(nonaprenyl)-phenylsulphonic acid (5.12, 452 mg, 88%).
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1H NMR (CDCl₃, 400 MHz): δ 6.84 (1H, d, J = 7.6 Hz), 6.78 (1H, t, J = 7.6 Hz), 5.19 - 5.16 (1H, m), 4.96 - 4.93 (8H, m), 3.34 (2H, d, J = 7.6 Hz), 1.97 - 1.90 (18H, m), 1.84 - 1.80 (16H, m), 1.55 (3H, s), 1.54 (3H, s), 1.43 (24H, s); 13C NMR (CDCl₃, 100 MHz): δ 154.5, 138.5, 135.6, 135.0, 134.9, 134.8, 131.2, 129.9, 127.5, 126.9, 124.5, 124.4, 124.3, 123.8, 121.7, 120.7, 115.8, 39.8, 39.7, 29.7, 26.8, 26.7, 26.6, 26.5, 25.7, 17.7, 16.3, 16.1, 16.0; IR (CHCl₃, νmax): 3439, 2917, 1590, 1449, 1380, 1213, 1152, 1095, 1043, 855, 754 cm⁻¹; LC-MS: m/z 785.5 (M-Na⁻), -ve ion mode.

5.5. Biological Activities:

5-Lipoxygenase enzyme inhibitory activity was measured by Schewe et al.⁷⁴ method and modified by Reddanna et al.⁷⁵ The procedure was described in 2.5.1 and the percentage inhibitions of compounds 5.01, 5.11 and 5.12 are summarized in Table 5.1. Brine shrimp (Artemia salina) nauplii were hatched using brine shrimp eggs⁷⁶ in a conical shaped vessel (1 L), the procedure was described in 2.5.2 and the results of the compounds 5.01, 5.11 and 5.12 are summarized in Table 5.1.

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<th>S.No</th>
<th>Test Substance</th>
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<td>Brine shrimp</td>
</tr>
<tr>
<td>1</td>
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<tr>
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<tr>
<td>5</td>
<td>Podophyllotoxin</td>
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</table>

Table 5.1: 5-lipoxygenase inhibitory activity and Brine shrimp lethality bioassay of compounds 5.01, 5.11 and 5.12

5.5.1. Antioxidant activity:

Superoxide radical scavenging activity: Superoxide radical scavenging activity of compounds 5.01, 5.11 and 5.12 were determined spectrophotometrically (560 nm) by following the nitro blue tetrazolium (NBT) photo reduction method of McCord and
Fridovich. The assay mixture contained EDTA (6.6 µM), NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), test substance and phosphate buffer (58 mmol, pH 7.8) in a final volume of 3 mL. Each mixture in a tube was shaken well, and the optical density was measured at 560 nm. Each tube was then uniformly illuminated with an incandescent lamp for 15 min, and the optical density was measured again at 560 nm. The percentage inhibition of superoxide radical-generation was measured by comparing the absorbance values of the control and that of the test substance. The IC₅₀ values were obtained from a plot drawn between concentrations (µM) versus the percentage inhibition. The super-oxide radical’s inhibitions exhibited by compounds 5.01, 5.11 and 5.12 have been summarized in Table 5.2.

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Table 5.2: Superoxide radical scavenging activity of compounds 5.01, 5.11 and 5.12

In vivo anti-inflammatory activity of nonaprenylsulphate1 in Freunds Complete Adjuvant (FCA) induced model of paw edema: The anti-inflammatory efficacy of 5.11 was evaluated in an in vivo study in Freund’s Complete Adjuvant induced arthritis model of Sprague Dawley rats. The rats of either sex were randomly selected and divided into three groups containing six animals in each group. The treatment groups of rats were supplemented with 50 mg/kg body weight of 5.11. The positive control group was supplemented with Prednisolone at 10 mg/kg body weight. All supplements were diluted in 10 mL of 1% CMC for administration. The animals of control group received same volume of 1% CMC. At 14th day, Freund’s Complete Adjuvant (FCA) was injected subcutaneously in the sub-plantar region of the left hind paw of each animal. At the end of experiment, the animals were sacrificed and liver tissue samples were excised and stored in aliquot at -80°C. Blood samples were collected from each animal at a regular interval and paw volumes were measured by Plethysmography equipment on the day of FCA injection and after 13 days of FCA
inoculation. The difference in volume of edema at the day of FCA injection and at 13\textsuperscript{th} day after induction is considered as the inflammatory response. The data is summarized in figure 5.2. The \textit{in vivo} anti-inflammatory responses of 5.11 and Prednisolone were estimated by calculating the percentage of inhibition of paw edema when compared to the CMC supplemented control.

The nonaprenylsulphate 5.11 showed 26.42\% inhibition of paw edema when compared to control group of animals. The positive control prednisolone in comparison showed 37.99\% improvement as summarized in figure 5.2.

![Figure 5.2](image)

\textbf{Figure 5.2}: Bar diagrammatic representation of mean paw edema, each bar represents mean ± SD, n=6, *p<0.05.

\textbf{5.6. Conclusion}:

As part of our search for potent anti-inflammatory and anticancer agents, the author has selected nonaprenyl aromatic sulfates for further investigation. Limited availability of sponge material, coupled with tedious extraction and isolation process for nonaprenylsulfates from natural sources prompted us to develop a cost effective synthetic methodology for nonaprenyl hydroquinone sulfates to study their \textit{in vitro} and \textit{in vivo} efficacy. Due to the health benefits associated with of CoQ\textsubscript{10} and nonaprenylsulfates, the author has embarked on the synthesis of CoQ\textsubscript{10} and
nonaprenylsulfates and evaluation of their biological activities. The compounds 5.11 and 5.12 exhibited potent 5-LOX inhibitory activity with IC50 of 1.08, 3.4 µg/mL respectively and also showed strong cytotoxicity against brine shrimp lethality with IC50 of 2.26, 3.98 µg/mL respectively.
5.7. Spectra of Synthesized compounds:

**Figure 5.3:** $^1$H NMR spectrum of coenzyme qunone 10 (5.01)

**Figure 5.4:** $^{13}$C NMR spectrum of coenzyme qunone 10 (5.01)
Figure 5.5: $^1$H NMR spectrum of 2, 3, 4, 5-tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04)

Figure 5.6: $^{13}$C NMR spectrum of 2, 3, 4, 5-tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04)
Figure 5.7: Mass spectrum of 2, 3, 4, 5- tetramethoxy-6-(3-methyl-2-butene)-toluene (5.04)
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Figure 5.8: $^1$H NMR spectrum of 2-(2-methyl-2-en-1-al-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.05)

![Figure 5.8: $^1$H NMR spectrum of 2-(2-methyl-2-en-1-al-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.05)](image)

Figure 5.9: $^{13}$C NMR spectrum of 2-(2-methyl-2-en-1-al-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.05)

![Figure 5.9: $^{13}$C NMR spectrum of 2-(2-methyl-2-en-1-al-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.05)](image)
Figure 5.10: Mass spectrum of 2-(2-methyl-2-en-1-yl)-4, 5, 6-tetramethoxytoluene (5.05)
Figure 5.11: $^1$H NMR spectrum of 2-(2-methyl-2-en-1-ol-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.06)

Figure 5.12: $^{13}$C NMR spectrum of 2-(2-methyl-2-en-1-ol-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.06)
Figure 5.13: Mass spectrum of $2-(2$-methyl-2-en-1-ol-4-yl)-3, 4, 5, 6-tetramethoxytoluene (5.06)
Figure 5.14: $^1$H NMR spectrum of 2-(2-methyl-2-en-1-$p$-tolenesulphinate-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.08)

Figure 5.15: $^{13}$C NMR spectrum of 2-(2-methyl-2-en-1-$p$-tolenesulphinate-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.08)
Figure 5.16: Mass spectrum of 2-(2-methyl-2-en-1-\textit{p}-tolenesulphinate-4-yl)-3, 4, 5, 6-tetramethoxy toluene (5.08)
Figure 5.17: $^1$H NMR spectrum of 2-decaprenyl-3, 4, 5, 6-tetramethoxy toluene (5.10)

Figure 5.18: $^{13}$C NMR spectrum of 2-decaprenyl-3, 4, 5, 6-tetramethoxy toluene (5.10)
Figure 5.19: Mass spectrum of 2-decaprenyl-3, 4, 5, 6-tetramethoxy toluene (5.10)
Figure 5.20: $^1$H NMR spectrum of sodium salt of 2-(nonaprenyl)-phenylsulphonic acid (5.12)

Figure 5.21: $^{13}$C NMR spectrum of sodium salt of 2-(nonaprenyl)-phenylsulphonic acid (5.12)
Figure 5.22: Mass spectrum of sodium salt of 2-(nonaprenyl)-phenylsulphonic acid (5.12)
Figure 5.23: $^1$H NMR spectrum of o-nonaprenylphenol (5.16)

Figure 5.24: $^{13}$C NMR spectrum of o-nonaprenylphenol (5.16)
Figure 5.25: Mass spectrum of o-nonaprenylphenol (5.16)
5.8. References:


59. Naruta Y and Maruyama K; Regio and stereo controlled polyprenylation of quinines. A new synthetic method of Coenzyme Q\textsubscript{2}, Q\textsubscript{3}, Q\textsubscript{9} and Q\textsubscript{10}. *Chem. Lett.*, 1979, 885 - 888.


