

General introduction

The search for natural resources for production and development of bioactive compounds of plant origin for environmental health and safety reasons is the main scope of this study. To achieve this aim, the work would focus on two approaches. Firstly, to obtain bioactive products, safe to our environment, from carnation stems. Secondly, to recycle the wasted plants; specially the infected ones, and utilize them as potential antimicrobial products of plant origin.

Moreover, the resistance of microbes to synthetic compounds, promoted us to explore the synthesis of new potential antimicrobial compounds. Benzoxazinone compounds are an important class of heterocyclic compounds. They are used in many industrial, agricultural and clinical applications such as the benzoxazinone derivatives based which are compounds used as antiphlogistic drugs¹. 4*H*-3,1-benzoxazin-4-one compounds exhibit excellent inhibitory activities against the human leukocyte elastase². Other benzoxazinone derivatives have been reported as starting materials for synthesis of quinazolin-4-ones derivatives. Pharmacologically, quinazolin-4-ones possess versatile type of biological activities, some of these are well known for their anticancer, antitubercular, antibacterial, antifungal, anti-HIV, antihelmintic, anti-inflammatory and of antihypertensive activities.³

In plant-pathogenic systems, benzoxazinones compounds play also an important role. For example, 2,4-dihydroxy-1,4-benzoxazin-one (DIBOA) and its 7-methoxy analogue (DIMBOA) are found in several plants i.e. wheat and maize.⁴ They have been reported to be important in the resistance of these plants to insects and to some plant pathogens.⁴⁻⁵

Other bioactive aromatic compounds like the phytoalexin dianthalexine and its pathway has been well characterized⁶ in carnation, and this

pathway involves numerous branches leading to the accumulation of dianthalexine, 2s-Phenyl-7-hydroxy-3,1-benzoxazin-4-one compound, and others such as a large array of dianthramides⁷⁻⁹. These Phytoalexins are secondary metabolites and they have been reported to produce in carnation plants (*Dianthus caryophyllus*) after infection with *F oxysporum f.sp.dianthi*¹⁰ or *Phytophthora parasitica*¹¹⁻¹⁵, namely dianthalexine and a large array of dianthramides⁷⁻⁹].

They have been reported to exhibit antimicrobial properties against the fungal pathogen *F oxysporum f.sp.dianthi*^{6,16} which is the causal agent of Fusarium wilt disease in carnation¹⁷⁻¹⁹ besides another fungal pathogenic such as *Cladosporium herbarum*⁶, the causal agent of rot disease in cherry, pear and figs²⁰. Based on the importance of dianthalexine, this work, mainly focuses on synthesizing dianthalexine chemically and comparing it with the dianthalexine produced from the carnation plants that were naturally infected by *F oxysporum (FO)*. The synthesis of dianthalexine compound was a challenging trail because the raw compound for the synthesis of this compound, 4-hydroxy anthranilic acid, was not available in the international market. Moreover, the chemical synthetic methods for the synthesis of 4-hydroxyanthranilic acid, are proved very difficult. Furthermore, these methods are very dangerous and are not safe to the environment and human health. 4-hydroxyanthranilic acid could be obtained from 2-nitro-4-hydroxybenzoic acid, the latter also is unavailable in the market. A solution of 2-nitro-4-hydroxybenzoic acid in ethanol was shaken with hydrogen and platinum (from platinum oxide) at room temperature and under pressure, until hydrogen absorption was complete. Then, the product was recrystallized from water to afford 4-hydroxyanthranilic acid²¹. There are methods for obtaining 2-nitro-4-hydroxybenzoic acid. For example, a warm solution of 2-nitro-4-aminobenzoic acid in water and concentrated sulphuric acid was rapidly

chilled to 0°C. After the addition of crushed ice, a solution of sodium nitrite in water was added during 1 hour at 0-5°C. The mixture was slowly added to a boiling solution of sulphuric acid in water and the mixture heated under reflux for three hours. On cooling, 2-nitro-4-hydroxybenzoic acid was separated²¹. Another limitations, is that 2-nitro-4-aminobenzoic acid, 2-nitro-4-hydroxybenzoic acid and 4-hydroxyanthranilic acid are also unavailable in the market. Another method for obtaining 4-hydroxyanthranilic acid, is that it can be extracted from ten day old bean plants, which were incubated for five hours with anthranilic acid. Homogenated plants have been reported to exhibit all the hydroxy derivatives of added anthranilic acid that were formed²². But, extracted 4-hydroxyanthranilic acid can be formed in traces and insufficient for performing experiments. Fortunately, 300 mg of 4-hydroxyanthranilic acid, namely 4-hydroxy-2-aminobenzoic acid, was kindly provided by Prof. Michel Ponchet. But, this amount was not sufficient for this work. These reasons led to focus, instead, on chemically synthesis 2-Phenyl-3,1-benzoxazin-4-one compound which is the dianthalexine derived compound. In addition to synthesis other derivatives of this benzoxazinone. Secondly, to clarify the biosynthesis of the phytoalexin dianthalexine, artificially and naturally produced and accumulated in *Fusarium carnation* infected plants. Thirdly, comparing the potential antimicrobial chemically synthesized benzoxazinone compounds with the extracts of carnation stems (*Dianthus caryophyllus*), naturally infected by *F oxysporum f.sp. dianthi* (FOD), that contained the phytoalexin dianthalexine in relevance to their potential antimicrobial activities towards a number of selected bacteria and fungi which are pathogenic to human and plants. A long side of the strain of the bacterium *B subtilis* that acts as a biocontrol agent against different plant pathogenic microorganisms was included in this study.