TO STUDY THE ANTIMICROBIAL PROPERTIES OF PLANT EXTRACT RHODODENDRON PONTICUM

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ABSTRACT

Natural products continue to play an important role in the discovery and development of new pharmaceuticals. The present study is designed for extraction of bioactive compound by HPLC from *Rhododendron ponticum* and, also includes the antimicrobial activity of the bio active compound obtained by crude and the column extract.

Key Words: Antimicrobial activity, *Rhododendron ponticum, Natural products, Pharmaceuticals, bio active compound, mammalian toxicity, Antibacterial activity.*

INTRODUCTION

Rhododendron ponticum is a large evergreen shrub which grows up to 8 m tall and is tolerant of a wide range of conditions and soil types (Maguire et al., 2008). It has been widely distributed as an ornamental species due to its attractive flowers and can subsequently become naturalized through the large number of seeds produced as well as its ability to propagate through vegetative means (Maguire et al., 2008). The toxicity of R. ponticum gives it a competitive advantage over native species through herbivore avoidance and later helps suppress the regeneration of other species through the accumulation of toxic leaf litter (Maguire et al., 2008). This aids in the creation of dense impenetrable thickets which have been reported reduce the diversity of both plant and animal communities (Edwards, 2006; Maguire et al., 2008). In Northern Ireland, R. ponticum is also known to host the fungus-like pathogen, Phytophthora ramorum which has the potential to attack a variety of native woody plant species and is the causative agent of 'Sudden Oak Death' (Maguire et al., 2008). Control of R. ponticum is known to be expensive and very labour intensive due to its prolific seeding, rapid growth rate and ability to resprout vigorously from cut stems (Barron, undated) and as such it is essential to properly plan management programs considering the ecology and infestatio age of R. ponticum as well as the surrounding environment (Maguire et al., 2008).

Rhododendron ponticum is an annual herbaceous plant found in moist places. However, the widespread use of antibiotics in human medicine and agriculture has caused serious problem of bacterial resistance (Beovic et al.,2006). Therefore, plant derived antimicrobial agents with high potency and low mammalian toxicity, useful for food preservation and human health, have gained special interest in recent decades (Smid, E. J.& Gorris, L. G. M (1999), & Reische et al.,1998). Recent pharmacological interest has focused on its anti-allergy and antibacterial effects (Wu et al., 1991). the aerial parts of the plant are used to treat headaches, head colds, conjunctivitis, piles and malaria (Perry, 1980).phytochemical studies of its composition have led to the identification of a number of terpenes, including sesquiterpene lactones and triterpenes (Wu et al., 1991, Bohlmann, F. & Chen, Z.L. (1984)) .The former class contained the major active constituents contributing to the anti-allergy and anti-bacterial activities of the herb (Essawi, T & Srour, M (2000)). Despite it is used in the Chinese folk medicine to treat naso pharyngeal carcinoma (NPC) (Cheng, J.H & Li (1998) & Zhang., (2000)). Besides, both the anti-nasopharyngeal carcinoma potential and the potent constituents of *R ponticum* .

In the present investigation, we report the analysis of bioactive compounds available in the plant *Rhododendron ponticum* by HPLC, anti-bacterial activity of the crude and column extractions of the active component.

Materials and Methods

Rhododendron ponticum plants were collected from botanical garden, and plant's identity was confirmed by plant taxonomist.

Extraction and Analysis

Fresh flowers, stem, root & leaves, were collected, washed, and weighed (10 g each). The materials were then macerated in 10 ml of water, methanol, acetone & benzene separately and then kept for 6 h at room temperature. The mixtures were then filtered through sterile Whatmann filter paper No.1. The filtrates obtained were then centrifuged at 5000 rpm for 5 min. The supernatants were collected in a beaker and the solvents were allowed to evaporate. Then the dry extracts were stored at 4°C. These extracts were dissolved in 1-3 mL (w/v) of dimethyl sulfoxide (DMSO) (Priya and Ganjewala 2007). The samples were further extracted by passing through the column of cotton, silica gel, activated charcoal and again silica gel in ratio 1:2:1 to obtain the extracts. Thus, the collected extract was passed into the column, number of times, to obtain the pure compound.

HPLC Analysis

The column cleaned compounds, *Rhododendron ponticum* extracts (Root, Stem, Leaf & Flower), were tested for the compound conformity and purity in the HPLC mobile and stationary phases were used for testing. The gradient program was set up and the peak analysis was estimated by observing the graph and comparing the obtained chromatogram with that of the already available data (Plumb et al., 2004).

ANTI BACTERIAL ACTIVITY

Pour Plate Technique

Nutrients required for the growth of micro-organisms were taken into a 250ml conical flask and 100 ml of distilled water was added. pH was adjusted to 7.2 and 2 gm of agar was added. Then the nutrient agar medium was sterilized in an autoclave at 121°C under 15 lbs pressure for 15-20 minutes.

The bacterial strains were collected from microbial type culture collection consisting of Escherichia *coli*, *Candida albicans*, *Bacillus subtilis* and *Streptococcus aureus.*

Pure cultures nutrient agar plates were prepared by taking a loopful of culture from stock cultures and it was streaked on Petri plates in streak plate method to obtain fine isolated colonies. The petriplates were incubated at 37°C for 24 hrs.

Pour plates allow for the growth of isolated colonies on the surface of the agar. The standard procedure, agar well diffusion method, was followed to test the antibacterial activity by pour plate method

(Deena and Thoppil 2000). A loopful of inoculums containing the microorganism from the broth was poured on a sterile agar medium plate. Again, the loop was sterilized on the flame and continued to pour the bacteria again. The plate was rotated for about 90C and the bacteria were spread. The process was repeated as per the requirement. Later the plates were incubated. The crude and column cleaned up HPLC compound were used to test the antibacterial activity by disc method. The plates were observed for inhibition of culture growth by the tested compounds.

HPLC Analysis

The column cleaned up compound was analyzed in the HPLC, and the compound was plenolin (Figure 1), of peak height 7.5871. This plenolin was used for testing antibacterial activity.

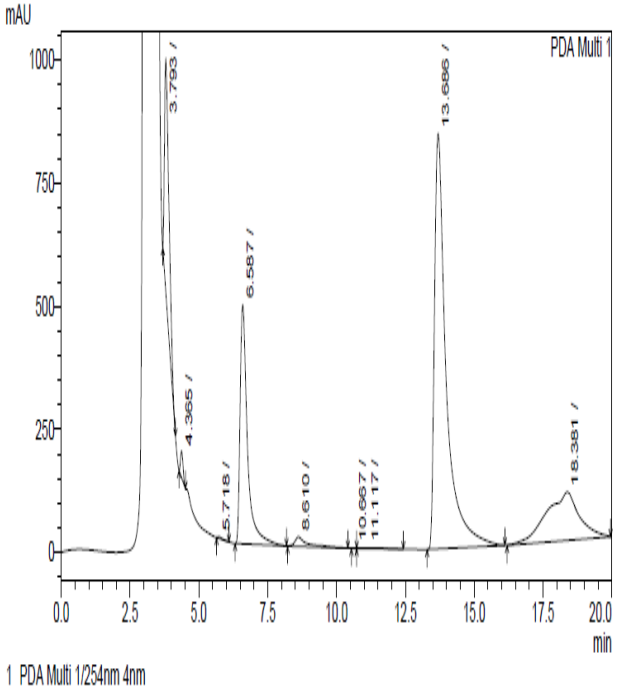

Figure 1: HPLC Analysis of *Rhododendron ponticum* extracts showing the peaks of active component**.**

Anti-Bacterial Activity

R ponticum flower extracts possess strong antibacterial activities more than the corresponding leaf extracts. Extracts were prepared in acetone, methanol, and water. *C. minima crude* extracts of flower and leaf showed the highest inhibitory effects against *B. subtillis* with a measured value of zone of inhibition area ranging from 6 -9.2 mm. Column extracts compared to the crude extracts, displayed less inhibitory effects against all the bacteria tested with a relatively smaller zone of inhibition area ranging from 3.3-6.6mm. *E. coli* was found to be the most sensitive bacteria to all *R ponticum* column and crude extracts. *P. aeruginosa* and *E. fecalis* was also found to be highly susceptible to all *R ponticum* crude and column extracts.

Table 1: Anti-Bacterial activity of crude and column extracts *Rhododendron ponticum*

Microbes	**Zone of Inhibition in mm Mean ± SD**	
	Crude	**Column**
E. coli	7.6±0.6	4.4±0.6
S. aureus	7.7±0.8	4.5±0.2
B. subtilis	7.2±1.2	5.8±0.8
Candida albicans	8.4±0.4	5.6±0.8

The order of susceptibility of plenolin isolated from *Rhododendron ponticum* on microorganisms is as follows: P.aeruginosa > B.subtilis > S.aureus> E .coli(Table 1).

Previous phytochemical studies on *C. minima reported* that they *have* more than ten sesquiterpene lactones which are all pseudo-guanianolide or guaianolide types (Taylor et al.,1998). Sesquiterpenoids were also found to have antagonistic activities for platelet activating factor and antibacterial activities (Iwamaki et al.,1992). The plenolin and helanalin have same activity against the Bacillus and Sreptococcus species (lee et al.,1977). Since micro-organism is becoming resistant against the drugs in use, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

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