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TO STUDY THE PHYTOCHEMICAL CHEMICAL ANALYSIS *RHODODENDRON PONTICUM* (ROHITAKA)– A REVIEW ARTICLE

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ABSTRACT

Natural products continue to play an important role in the discovery and development of new pharmaceuticals. Several chemical compounds have been extracted and identified from its species known as Rhododendron ponticum (R.ponticum). The phytochemical screening of all the extracts showed the presence of various phytochemicals that are biologically important. The total phenolic and flavonoid content of the plant are comparable to other medicinal plants. Even though the enormous progress on the phytochemistry and pharmacology of R. arboreum have been made, there still require more conclusive studies on the safety, efficacy, and in vivo toxicity of extracts and pure compounds to gain a better understanding

INTRODUCTION

Rhododendron ponticum is an annual herbaceous plant found in most 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), Essawi, T.& Srour, M (2000) & Reische et al .,1998). Recent pharmacological interest has focused on its anti-allergy and antibacterial effects (Wu et al., 1991). 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. 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 remain elusive. Sesquiterpene lactones, most widely distributed within the Compositae, have received considerable attention for their anticancer properties (Zhang et al., 2005). 6-O-angeloylenolin, a sesquiterpene lactone containing an, β-unsaturated cyclopentenone, isolated from R.ponticum was reported to induce apoptosis in HL-60 cells in vitro and inhibit the solid cancer growth in Lewis lung cancer xenograft model (Li et al., 2008). In the present investigation, we report the Phytochemical analysis of available in the plant Rhododendron ponticum. Phytochemicals give flavor, color, fragrance, and create a natural defense system for host plants. Till date, more than 4000 of these compounds have been discovered. The phytochemicals give protection to plants and also possess curative potential such as immunomodulatory activity, anti-diabetic, anticancer, anti-oxidant, adaptogenic property, enhancing memory, and cholesterol reducing effects. Discovery of thousands of phytochemicals was grouped based on source and function. Potential of natural compounds with immunostimulating activity will be classified as low molecular immunomodulatory compounds such as alkaloids, phenolic, and terpenoids whereas high molecular weight compounds are polysaccharides .

Rhododendron species where arboreum means tree-like. Rhododendron is the state flower of Nepal and the state tree of Uttarakhand. It is enormously variable in stature, leaf characteristics, hardness, and flower color. It grows at elevations of 4500–10,500 ft. The tree attains a height of 40–50 ft tall occasionally attaining above 100 ft. This is an evergreen much-branched tree up to 2.4 m in girth and 14 m in height . Flowering period is from March to April/June to September bearing crimson to pale pink

or deep red flowers. R. arboreum methanolic extract of different parts (roots, stem, leaves, bark, and flowers) shows good antimicrobial and antifungal activity . Chauhan et al. discovered that the antibacterial activity of R. arboreum leaf extract was found effective as compared to flower extract against Staphylococcus aureus. Methanolic extract of R. arboreum leaves through gas chromatography-mass spectrometry (GC-MS) analysis confirms the presence of main phytoconstituents, i.e., 22-stigmasten-3-one (14.59%) with highest area percentage, followed by 1, 1, 6-trimethyl-3-methylene-2-(3, 6, 10, 13,14-pentamethyl-3-ethenyl-pentadec-4-enye) cyclohexane (12.26%), beta-amyrin (7.62%), and linoleyl alcohol (6.50%) having antimicrobial, anticancer, antiarthritic, anti-inflammatory, and antiviral properties.This plant is reported to possess several medicinal and pharmacological properties such as hepatoprotective, antioxidant, immunomodulatory, anti-inflammatory, anti-diabetic, anticancer, antinociceptive, adaptogenic, and antidiarrheal, oxytocic, estrogenic, prostragl, synthetase inhibitory activity, and central nervous system depressant activity.

METHODOLOGY

Phytochemical Screening Specific qualitative tests were performed for detection of metabolites in leaf and flower extracts. Alkaloids were estimated from previously published procedures (Clarke and Williams 1955). The presence of sterols was confirmed by the addition of 2 ml of acetic anhydride to 0.5 g of dried ethyl acetate extract with 2 ml of concentrated sulphuric acid. For the identification of phenolics, one ml of neutral ferric chloride was added to one ml of the extract. For the identification of terpenes, the extracts were treated with tin and thionyl chloride. For the identification of Flavones, 10 % sodium hydroxide was added. To reveal the presence of tannins, 0.5 g of the dried powder of the leaves and flowers were boiled with 5 ml of water in a test tube and then filtered. To the filtrate, ferric chloride was added and kept undisturbed for the observation. Phospholipids and glycolipids were estimated based on previously published procedures (Roughan and Batt 1969, Lowry and Tinsley 1976). To reveal the presence of Fixed oils, small quantity of petroleum ether and benzene extract was pressed separately between two filter papers

Half (0.5) mg of the extract was treated with 3 ml concentrated sulfuric acid followed by 2 ml of chloroform. Allow the mixture to stand for a minute. Formation of reddish-brown color in chloroform layer confirmed the presence of phytosterol .Liebermann Burchard's testFour mg of extract was treated with 0.5 ml of chloroform and 0.5 ml of acetic anhydride and then filtered. To the filtrate, few drops of concentrated sulfuric acid were added carefully along the side's wall of the test tubes. Formation of greenish-blue color indicates the existence of steroids .

Test for phenols
Two ml of distilled water was added to 1 ml of extracts (1 mg/ml) followed by addition of some drops of 10% $FeCl_3$

(ferric chloride) and waited for the development of blue or green color.

Alkaloids

The extract was treated with 2% diluted HCL in boiling water bath for 2 min. Allow to cool the mixture was filtered and treated with some drops of 5% NaOH solution. The samples were observed for the presence of yellow precipitate or turbidity.

Glycosides

The extracts were neutralized by NaOH and hydrolyzed by HCl solution. Little drops of Fehling solution A and B were added and observed for red precipitate.

Terpenoids

Half ml of acetic anhydride was added to 4 g of extract. Then add 0.5 ml of chloroform and concentrated H2SO4 to the mixture and observed for red violet color.

Flavonoids

Lead acetate testThe extract was treated with some drops of lead acetate solution. Development of yellow color precipitate indicates the presence of flavonoids.

Alkaline reagent testSome drops of NaOH solution were added to the extract. Development of deep yellow color which disappears after adding diluted HCL shows the presence of flavonoids.

Tannins test

Mixed little amount of extract with water and heated on the water bath and filtered. Add few drops of ferric chloride to the filtrate. A dark green solution shows the existence of tannins [33].Saponins testHalf ml of extract was dissolved in 5 ml of distilled water and shaken for 15 min. Persistence of frothing indicates that leaves extract contains Saponins.

Anthocyanin and Betacyanin

testOne ml of leaves extract was dissolved in 1 ml of 2N NaOH and was heated at 100°C in a water bath for 5 min. The appearance of bluish-green color shows the presence of anthocyanin while the development of yellow color indicated the presence of Betacyanin.

Carbohydrate test

Molisch test: Add 2 ml of leaves extract to 1 ml of Molisch reagent, and little drops of concentrated H2SO4 were added. Development of purple or reddish color shows the presence of carbohydrates .

Fehling's testAdd 2 ml of leaves extract, 5 ml of Fehling's solution A and B was kept in hot water bath for 5 min. The development of yellow or red color shows the presence of carbohydrates.

Quantitative phytochemical screening

Determination of total phenolic content (TPC)Folin–Ciocalteu reagent (FCR) assay was performed for the evaluation of TPC in the isolated plant extract. Take 150 µl of the extract and mixed in 240 µl of water and 150 µl of 0.25 N FCR. It was incubated in the dark at room temperature for 3 min. After incubation, add 300 µl of 1N Na2CO3,then the mixture was incubated further for 2 h inside dark at room temperature. Different dilutions of gallic acid (0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.08 mg/ml, and 0.1 mg/ml) were used as a standard for drawing the calibration curve. The absorbance of each sample was measured at 765 nm, and results were expressed in terms of mg of gallic acid equivalent g−1 of

extract* and were calculated using a formula:GAE* = X×V÷MWhere X: Concentration of extract/standard (mg/ml), M: Weight of extract (g), V: Volume of extract (ml).

Determination of total flavonoid content

Total flavonoid content (TFC) assay was performed as suggested by Dae et al. [47], Dae through minor modifications. To 10 ml extract, add 2 ml of water and 150 µl of 5% NaNO2 solution. Allow the mixture to react for 6 min and then add 150 µl of 10%, AlCl3 solution and absorbance were measured after 15 min at the wavelength of 510 nm. Rutin at different concentrations (0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.08 mg/ml, and 0.1 mg/ml) were used as a standard for the quantification of total flavonoid. Triplicate measurements were carried out, and total flavonoid content was expressed in milligram of rutin equivalents (RE) mg/g of extract (mg RE g−1)* and was calculated using formula.RE* = X×V÷MWhere X: Concentration of extract/standard (mg/ml), M: Weight of extract (g), V: Volume of extract (ml).

DISCUSSION

The pink color appearance when crude extract is treated with tin and thionyl chloride indicates the presence of terpenes. The change in color from yellow to orange when the crude extract is treated with 10% NaoH shows the presence of Flavones. Oil stained on paper when benzene extract treated with petroleum ether indicates the presence of fixed oil. Appearance of cream color when treated with Mayer's reagent indicates the presence of an alkaloid Bioactive potential of Flavonoids has been reported. (Illic et al., 2004, Cushner and Lamb 2005).The present results are compared with Samy and Ignace Muthus (1999) & Sanchez et al (2005). Based on the fact that micro organism are 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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