



Preliminary Phytochemical analysis and Estimation of Total Phenolic Content in Rosemary Extract.

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ABSTRACT

Natural products have been the single most productive source leads for development of drugs. Herbs and spices have many phytochemicals which are source of natural antioxidants, such as flavonoids, phenolic diterpens, tannins and phenolic acids. *Rosmarinus officinalis*, is a woody herb with a needle like leaves. . It has been considered as the most effective herb for treating headache, poor circulation, inflammatory diseases and physical and mental fatigue. The potent antioxidant properties of rosemary have been mainly attributed to its major diterpenes, carnosol and carnosic acid, as well as to the essential oil components. The total phenolic content was determined using Folién-ciocalteu reagent as described by Salär.

Keywords: Natural products, *Rosmarinus officinalis*, phenolic content.

INTRODUCTION

In this world most of the population still rely on traditional medicine practitioners and local medicinal plants for primary health care¹. Natural products have been the single most productive source leads for development of drugs². Herbs and spices have many phytochemicals which are source of natural antioxidants, such as flavonoids, phenolic diterpens, tannins and phenolic acids³. Phytochemical analysis was established in 1991. It was published by John Wiley and Sons. It deals with research on the utilization of analytical methodology in botany. Phytochemistry or plant chemistry deals with the analysis and characterization of organic compounds present in plant materials. Phenols, terpenoids, organic acids, lipids, sulphur compounds, amino acids, alkaloids, cytokines, purines, pyridines, sugar and their derivatives and many more are common compounds that are used in Phytochemical analysis. Phytochemical analysis helps in determining the medicinal properties in different kinds of herbs. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables, roots that have defense mechanism and protect various diseases⁴.

Rosemary often called as *Rosmarinus officinalis*, is a woody herb with a needle like leaves. It is native to the Mediterranean region. It is a good source of iron, calcium and vitamin B6. This herb has been used from ancient times for its medical properties. It was traditionally used to help alleviate muscle pain, improve memory, boost the immune system and promote hair growth. It has been considered as the most effective herb for treating headache, poor circulation, inflammatory diseases and physical and mental fatigue. Rosemary has also been used empirically as a choleric and hepatoprotective agent in folk medicine. Most pharmacological effects of rosemary are the consequence of high antioxidant activity of its main chemical constituents, which include carnosol,

carnosic acid, ursolic acid, rosmarinic acid, and caffeic acid. The potent antioxidant properties of rosemary have been mainly attributed to its major diterpenes, carnosol and carnosic acid, as well as to the essential oil components⁵. The total phenolic content was determined using Folién-ciocalteu reagent as described by Salär. The amount of total phenolic content was calculated as Gallic acid equivalent from the standard calibration curve of Gallic acid and expressed as mg Gallic acid equivalents /g dry weight basis⁶.

MATERIALS AND METHODS

The present study involves preliminary Phytochemical analysis and estimation of total phenolic content of plant *Rosmarinus officinalis*. The medicinal plants were collected locally and were used for the purpose of their Phytochemical analysis. The chemicals which were used in this study were Fehling solution A and Fehling solution B, ethanol, distilled water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid, ammonium solution, picric acid and hexane. The leaves of the selected plant were removed from the plant and then washed under running tap water to remove any dust and dirt. The plant samples were then air dried for a few days and the leaves were crushed into powder and stored in polythene bag for use. The powder of the plant was then taken in a test tube and distilled water was added to it and was shaken well. The solution was then filtered and the filtrate was then used for further Phytochemical analysis. This filtrate was later used to test phlobatannins, reducing sugar, terpenoids, flavonoids and alkaloids. The total phenolic content was determined using Folin-ciocalteu reagent with analytical grade Gallic acid as standard⁷. Samples containing polyphenols are reduced by Folin-ciocalteu reagent thereby producing blue colored complex⁸.



Test for phylobatannins

Plant powder sample was mixed with distilled water in a test tube, then shaken well and filtered to take the plant extract. Then to the plant extract, 1% aqueous hydrochloric acid was added and the sample was then boiled with the help of hot plate stirrer. Formations of red colored precipitate confirmed a positive result.

Test for reducing sugar

An amount of 0.50g of Rosemary was added in 5 ml of distilled water. Then 1 ml of ethanol mixed in plant extract. After that we took 1 ml of Fehling's solution A and 1 ml of Fehling's solution B in a test tube, heated it to boiling and then poured it in the aqueous ethanol extract. When color reaction was observed, it shows a positive result.

Test for terpenoids

An amount of 0.8 g of selected plant sample was taken in a test tube then poured 10 ml of methanol in it. Shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of sample and 3 ml of sulphuric acid were added in the sample extract. Formation of reddish brown color indicates the presence of terpenoids in the sample.

Test for flavonoids

Table 1: Total amount of phytochemicals present

<i>Rosmarinus officinalis</i>	Phytochemical
+++	Phylobatannins
++++	Reducing sugar
+++	Flavonoids
+++	Alkaloids
++++	Terpenoids

Table 2: Total phenolic content

Total Phenolic Content	Extracts
86 mg/gm	<i>Rosmarinus officinalis</i>

Plant such as herbs have long been used in traditional/folk medicine in various cultures throughout the world¹⁰. Medicinal values of plants have assumed a great importance in the past decade. Plants produce secondary metabolite and antioxidant property¹¹. Secondary metabolites are reported to have many biological and therapeutic properties, so this specie is expected to have many medicinal uses¹². Rosemary (*Rosmarinus officinalis*) belongs to the lamiaceae family, is a pleasant smelling perennial herb that grows in several

For the confirmation of flavonoids in Rosemary extract, 0.5g of Rosemary extract were added in the test tube and 10ml of distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of Rosemary extract followed by addition of 1 ml concentrated H₂SO₄. Indication of yellow color shows the presence of flavonoids in Rosemary.

Test for alkaloids

For the purpose of Phytochemical analysis of carrot, 0.2 g of the sample were added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then 5 ml of 2% HCL was taken and poured in a test tube having the mixture, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicated the presence of alkaloids.

For determining the test for total phenolic content, folin ciocalteu reagent was used. The total phenolic content was measured 86 mg/gm of dry weight of extracts expressed as Gallic acid equivalent

RESULTS AND DISCUSSION

Phytochemicals are increasing acceptor as health promoting, maintains and repairing agents in cells, tissues, or the whole human body. The phytochemicals that are frequently associated with human health are polyphenols, carotenoids and tocopherols⁹.

regions all over the world. It is well known valuable medicinal herb that is widely used in pharmaceutical products and traditional medicine as digestive, tonic, astringent, diuretic, diaphoretic and useful for urinary ailments³. Phenolic compounds are very important plant constituents because their hydroxyl groups confer scavenging ability. Phenolic present in leaves have received considerable attention because of their potential antioxidant activities. Plant materials. The quantitative phytochemical analysis of this species exhibited the presence of from the above study we can see that the herb *Rosmarinus officinalis* (Rosemary) contained phylobatannins, reducing sugar, flavonoids, alkaloids and terpenoids. Among that phylobatannins, flavonoids and alkaloids showed moderate concentration. Whereas reducing sugar and terpenoids showed high concentration. The total phenolic content was measured to be about 86 mg/gm of dry weight of extracts expressed as Gallic acid equivalent. Estimating the total phenolic content and phytochemical contents can pave way for the application of herbal extract in various medicinal use.

CONCLUSION

Phytochemical screening revealed the presence phylobatannins, reducing sugar, flavonoids, alkaloids and terpenoids in Rosemary extract. It was observed that the leaf extracts contained high level of phenolic content. These findings suggest that they could have great importance as medicinal plants in treating various health problems. Further research may be needed to identify the active principle in rosemary extract and to elucidate its

mechanism of action in metabolic pathways and its role in health and disease.

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