

Extraction of Bioactive Compounds from *Rosmarinus Officinalis* L. and its Anticancer Activity against HeLa Cell Line

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Abstract: In an extensive search for anticancer property from plant sources, the extract of rosemary leaves collected from the region of Bargur, Tamil nadu, India, was studied. Rosemary a woody, perennial herb contains number of bioactive compounds. These bioactive compounds were extracted from *Rosmarinus officinalis* L. and characterized by phytochemical analysis, Thin Layer Chromatography and UV spectrophotometry. The antioxidant study and anticancer study were carried out on the methanol extract of *R. officinalis* L. The anticancer study was performed against He La cell line using MTT assay.

Keywords: *Rosmarinus officinalis*, bioactive compounds, antioxidant activity, anticancer activity.

1. Introduction

Rosmarinus officinalis, commonly known as **rosemary**, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, native to the Mediterranean region. Rosemary is an aromatic evergreen shrub with leaves similar to that of hemlock needles.[1] It is native to the Mediterranean region and Asia, but is reasonably hardy in cool climates. It can withstand droughts, surviving a severe lack of water for lengthy periods.

Forms range from upright to trailing; the upright forms can reach 1.5 m (5 ft) tall, rarely 2 m (6 ft 7 in). The leaves are evergreen, 2–4 cm (0.8–1.6 in) long and 2–5 mm broad, green above, and white below, with dense, short, woolly hair.[2] The plant flowers in spring and summer in temperate climates, but the plants can be in constant bloom in warm climates; flowers are white, pink, purple or deep blue.

The leaves are used as a flavoring in foods such as stuffings and roast lamb, pork, chicken and turkey. Fresh or dried leaves are used in traditional Mediterranean cuisine.[3]

They have a bitter, astringent taste and a characteristic aroma which complements many cooked foods. Herbal tea can be made from the leaves. When roasted with meats or vegetables, the leaves impart a mustard-like aroma with an additional fragrance of charred wood compatible with barbecued foods.[4] In amounts typically used to flavor foods, such as one teaspoon (1 gram), rosemary provides no nutritional value. Since it is attractive and drought-tolerant, rosemary is used as an ornamental plant in gardens and for landscaping, especially in regions of Mediterranean climate. It is considered easy to grow and pest-resistant. Rosemary can grow quite large and retain attractiveness for many years, can be pruned into formal shapes and low hedges, and has been used for topiary.[5] It is easily grown in pots. The groundcover cultivars spread widely, with a dense

and durable texture. Rosemary grows on friable loam soil with good drainage in an open, sunny position. It will not withstand water logging and some varieties are susceptible to frost. It grows best in neutral to alkaline conditions (pH 7–7.8) with average fertility.[6] It can be propagated from an existing plant by clipping a shoot (from a soft new growth) 10–15 cm (4–6 in) long, stripping a few leaves from the bottom, and planting it directly into soil. Upon cultivation, the leaves, twigs, and flowering apices are extracted for use.[7]

The herb not only tastes good in culinary dishes, such as rosemary chicken and lamb, but it is also a good source of iron, calcium, and vitamin B-6. It is typically prepared as a dried whole herb or a dried powdered extract, while teas and liquid extracts are made from fresh or dried leaves. [8]

The herb has been hailed since ancient times for its medicinal properties. Rosemary was traditionally used to help alleviate muscle pain, improve memory, boost the immune and circulatory system, and promote hair growth.

Scientists have found that rosemary may also be good for your brain. Rosemary contains an ingredient called carnosic acid, which is able to fight off damage by free radicals in the brain. Some studies in rats have discovered that rosemary might be useful for people who have experienced a stroke.[9] Rosemary appears to be protective against brain damage and might improve recovery. Research published in Oncology Reports found that "crude ethanolic rosemary extract (RO)" slowed the spread of human leukemia and breast carcinoma cells." Another study, published in Bioscience, Biotechnology and Biochemistry, concluded that rosemary may be useful as an anti-inflammatory and anti-tumor agent.[10] In addition, a report published in the Journal of Food Science revealed that adding rosemary extract to ground beef reduces the formation of cancer-causing agents that can develop during cooking.[11]

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2. Materials and Methods

Collection of Sample

Rosmarinus officinalis L. leaves were collected from the region of Bargur, Tamil nadu, India. Mercuric cholride, copper sulphate, potassium hydroxide and sodium potassium tartarate were bought from Himedia Laboratories Pvt. Ltd., (Mumbai). Iodine solution, chloroform, sulphuric acid and acetic acid were bought from Central Drug House Pvt. Ltd., (New Delhi). Ferric chloride, hydrochloric acid, nitric acid and magnesium ribbon were bought from Hi-Pure Fine Chem Industries (Chennai). Agarose for electrophoresis was bought from S.D Fine Chem Limited (Mumbai), India.

Preparation of *Rosmarinus officinalis* L. Extract

The leaves of *R. officinalis* L. were dried, ground and made into a powder. The powder was made into two samples of methanol and water. A little quantity of rosemary powder was added to methanol and water in two conical flasks and incubated overnight under required conditions. After incubation, the samples were filtered into a clear solution for further study.

Phytochemical Analysis

The phytochemical analysis for both water sample and methanol sample was performed separately and the sample which contained more number of bioactive compounds was taken for further procedure. There are number of tests involved in Phytochemical analysis of the plant extracts as follows:

2.1 Test for Alkaloids

A small amount of sample was taken in a test tube and one ml of Mayer's reagent was added along with a drop of Iodine solution.

2.2 Test for Terpinoids

To a small amount of sample, two drops of chloroform, followed by one ml of concentrated hydrochloric acid was added and heated for two minutes.

2.3 Test for Phenol and Tannin

To a small amount of sample two ml of ferric chloride was added and kept for incubation at room temperature for five minutes.

2.4 Test for Sugars

A small amount of sample was taken in a test tube, felling's solution A and B were added and kept at water bath for five minutes.

2.5 Test for Saponin

A small amount of sample was taken, two to three drops of distilled water was added and mixed well.

2.6 Test for Flavanoids

A small piece of magnesium ribbon and one ml of concentrated hydrochloric acid were added to a small amount of sample.

2.7 Test for Quinine

One ml of sodium hydroxide solution was added to a small amount of sample and kept for 2 minutes.

2.8 Test for Protein

A small amount of sample was taken in a test tube, a drop of concentrated nitric acid was added to it.

2.9 Test for Steroids

One ml of chloroform, one ml of concentrated sulphuric acid were added to a small amount of sample and incubated for five minutes.

Thin Layer Chromatography

The sample was subjected to TLC. Solutes contained sample and standard as methanol sample plant extract and ascorbic acid respectively. The solvent contained five ml of methanol, two ml of formic acid, two ml of butanol and one ml of distilled water.

UV Spectrophotometry

The sample that turned into yellow in the thin layer was scrapped by a sharp blade. The powder was dissolved in one ml of methanol and was scanned under UV spectrometer at about 300-800 nm wavelength.

Antioxidant Study

Antioxidant activity of the methanol sample was determined by the following two tests.

- 1) DPPH (Di Phenyl Picryl Hydroxide) activity test in which to a small amount of sample 0.1 ml of DPPH solution was added and mixed well. To that, 400 µl of 50mM Tris hydrochloric acid was added and incubated for about 30 minutes at room temperature and the results were determined at 517 nm.
- 2) Test for total phenol content in the sample was performed, in which 0.5 ml of Folin Ciocateau reagent was added to a small amount of sample followed by the addition of 2 ml of sodium carbonate. Then the sample was subjected to incubation at 45°C for about 30 minutes.

DNA damage Study

DNA damage study was performed using DNA nicking assay. In this study, 2 µl of sample was added to 2 µl of plasmid DNA (PBR322) along with 2 µl of hydrogen peroxide. This mixture was kept for incubation (1 hour). Later the sample was run in agarose gel electrophoresis with three lanes loaded with DNA sample, marker and RNA sample in Lane 1, Lane 2 and Lane 3 respectively. The results were recorded by using a UV transilluminator.

Anticancer Study in He La Cell line (MTT ASSAY)

The anticancer study was performed by MTT assay by using DMSO (Dimethyl Sulfoxide) and DMEM (Dulbecco's

Modified Eagles Medium). The He La cells were cultured in a tissue culture flask and placed in a CO₂ incubator for 72 hours. Various concentrations of methanol sample were taken and added in the wells of the microtiter plate. To that, 100µl of cell line (along with growth medium) was added. Here, 50µl of DMSO and 100µl of cell line served as blank and control respectively. The plate is placed for overnight incubation under required conditions.

3. Results and Discussion

Characterization of bioactive compounds

The extract was subjected to phytochemical analysis. This resulted in the determination of bioactive compounds that were present in the methanol sample of plant extract. The compounds identified in the sample were found to be alkaloids, terpenoids, phenol, sugar, saponin, flavanoids and proteins. The results are shown in table and Fig.1

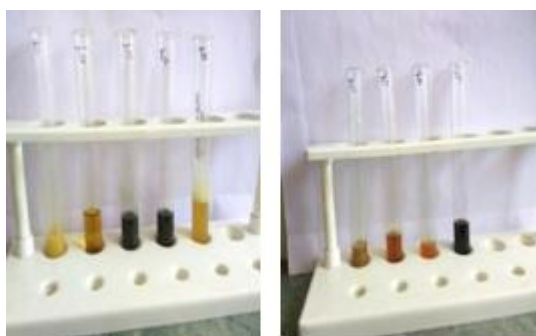


Figure 1: Results of phytochemical analysis

Table 1: Results of phytochemical analysis

S.No	Test	Reaction	Result
1.	Test for alkaloids	Formation of yellow colour	Alkaloids are present
2.	Test for terpenoids	Formation of grey colour	Terpenoids are present
3.	Test for phenol and tannin	Formation of bluish green colour	Phenol and tannin are present
4.	Test for sugar	Formation of red colour	Sugars are present
5.	Test for saponin	Formation of foam	Saponins are present
6.	Test for flavanoids	No formation of pink colour	Flavanoids are present
7.	Test for quinine	No formation of bluish green colour	Quinines are present
8.	Test for proteins	Formation of yellow colour	Proteins are present
9.	Test for steroids	No formation of red coloured ring	Steroids are present

Thin Layer Chromatography

The compound present in the sample was characterized by thin layer chromatography. The yellow coloured spots were identified as **alkaloids** since the retention factor value of the compound was found to be **0.9687**. The result of thin layer chromatography has been shown in Fig.2



Figure 2: Results of thin layer chromatography

UV Spectrophotometer

The compounds were analysed using UV spectrophotometer. The graph obtained is shown in Fig.3

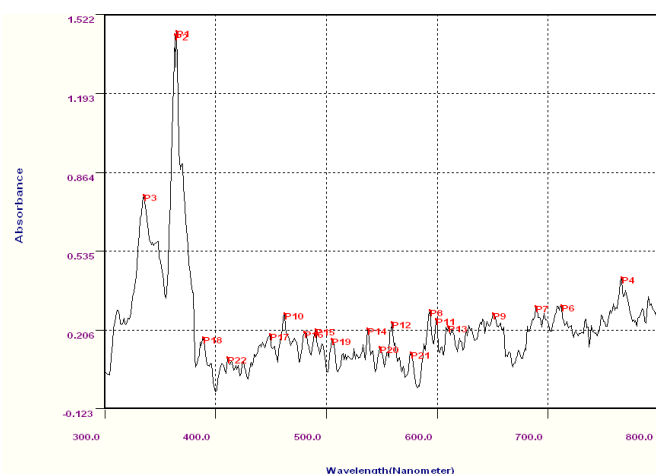


Figure 3: Results plotted from spectrophotometer

Antioxidant Study

The results obtained from DPPH activity and total phenol content showed an absorbance of **1.556** at 517 nm and **3.000** at 765 nm.

DNA damage Study

The DNA sample, marker and RNA sample were made to run in Lane 1, Lane 2 and Lane 3 respectively. The results observed in UV transilluminator is shown below in Fig.3

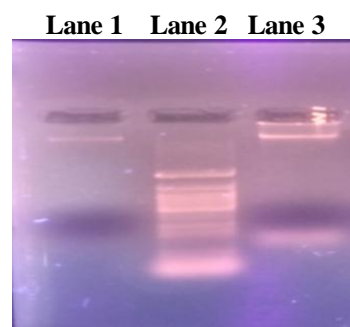


Figure 4: Results of DNA damage study

Anticancer Activity

The results in ELISA plate after an incubation of 24 hours were recorded at 570 nm. As the concentration of the sample

was increased, the viability of cancer cells decreased gradually. The readings are shown in the table below.

Table 2: Results in anticancer study

S.No	Percentage of cell viability
1	100
2	92.56
3	83.33
4	76.98
5	68.73

4. Conclusion

Thus in the present study, phytochemical analysis was performed for the extract of *Rosmarinus officinalis* L. and was characterized by thin layer chromatography and UV spectrophotometer. DNA nicking assay was used to study the DNA damage caused by the extract. The anticancer activity against He La cell line was done. The results indicated that the plant species had good anticancer activity.

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