International Journal of Education and Science Research Review
Volume-11, Issue-2 March-April-2024 E-ISSN 2348-6457
(Peer-Reviewed, Open Access, Fully Refereed International Journal) P-ISSN 2349-1817
www.ijesrr.org Impact Factor - 7.02 Email- editor@ijesrr.org

High-Performance Thin-Layer Chromatography (HPTLC) for Comparative Quantification of Quercetin in Medicinal Plants

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ABSTRACT

The *T. occidentalis* extract was applied in volumes of 0.2 µL, 0.5 µL, and 0.10 µL, with corresponding sample application positions at 68.9 mm, 76.6 mm, and 84.3 mm. The solvent front extended to 80 mm, and the spacing between tracks was 7.7 mm. Scanning was conducted at a speed of 20 mm/s, with a data resolution of 100 µm per step. HPTLC plates were analyzed at a wavelength of 254 nm under a D_2 lamp using an automatic detection mode. Similarly, the *A. heterophylla* extract was applied in volumes of 0.2 µL, 0.5 µL, and 0.10 µL, with respective sample application positions at 67.2 mm, 75.9 mm, and 84.6 mm. The track spacing measured 8.7 mm, while scanning was performed at 20 mm/s with a data resolution of 100 µm per step. HPTLC analysis was carried out at 254 nm under D_2 lamp illumination using an automatic detection mode.

Kew Words-: HPTLC, *Thuja occidentalis*, , *Araucaria heterophylla*, quercetin

INTRODUCTION

Araucaria is a member of the Araucariaceae family of plant families. The plant known as *Thuja occidentalis*, which is a member of the *Cupressaceae* family, is yet another option. Rheumatism, migraines, and uterine carcinomas are some of the conditions that can be helped by using this. Anticancer, antiviral, anti-inflammatory, insecticidal, and diabetes-fighting properties have all been uncovered by it . *Araucaria* and *Agathis* are the only two genera within this small family, which has a total of 38 different species of tree. Christmas trees are typically made out of Araucaria heterophylla, which is a type of generic columnar tree.

Investigations on the chemical and biological properties of the resin of this genus, which was rumored to exhibit gastroprotective activities, were carried out. Medical plants serve as a key source of organic chemicals, which are used for physiological effects as well as medical purposes. Some species used primarily for decoration have tremendous value in the field of medicine, such as the separated components of Catharanthus roseus that are employed in cancer treatment. The use of plants in the production of medicine is a practice that has been passed down through generations. Plants have served as a fertile breeding ground for medicinal compounds, which are now an essential component of the healthcare system . Because India is the largest producer of medicinal herbs, the country is sometimes referred to as the botanical garden of the globe, which is an apt description. Plants are capable of producing secondary metabolites, which have a wide variety of biological and pharmacological properties, such as anti-allergic, antibacterial, hypoglycemic, and anti-carcinogenic. Plants that are utilized as remedies include a wide array of elements that have the potential to treat a number of ailments, including those that are chronic and infectious. Over the course of the past few years, gas chromatography–mass spectrometry has established itself as the primary technological program for characterizing secondary metabolites across all plant groups. Natural materials have, in addition to their use in the treatment of human ailments, also been put to use in the treatment of a variety of diseases. Unani and Ayurveda are two of the most well-known medical practices in the world that make use of ingredients derived from natural sources. These methods, along with other forms of folklore from a variety of countries, continue to rescue a significant section of the world's population by making use of items derived from the natural world. The majority of well-known inhibitors are organic inhibitors, but in addition to having strong corrosion inhibition efficacy, these inhibitors are expensive, non-biodegradable, and hazardous for both life and the environment. As a result of the drawbacks of existing inhibitors, the researchers were motivated to look for an eco-friendly green inhibitor. The use of extracts derived from plants as corrosion inhibitors is becoming increasingly common in modern times.

MATERIALS AND METHODS

Collection of plants

Plants of ***Thuja occidentalis*** and ***Araucaria heterophylla*** of procured from Saharanpur, Uttar Pradesh.

- **Thuja occidentalis**

This species is a member of the ***Cupressaceae*** plant family. Rheumatism, migraines, and uterine carcinomas are some of the conditions that can be helped by using ***Thuja occidentalis***. Anticancer, antiviral, anti-inflammatory, insecticidal, and diabetes-fighting properties have all been discovered in it.

- ***Araucaria heterophylla***

Araucaria is a member of the Araucariaceae family of plants. There are just 38 different species of trees that belong to this tiny family, which include the genera agathis and araucaria. It is a common columnar tree that is decorated to seem like a Christmas tree . Investigations on the chemical and biological properties of the resin of this genus, which was rumored to exhibit gastro protective activities, were carried out.

Preparation of plant extract

The components made from the fresh plant parts were allowed to dry out and were then pulverized roughly. After defatting the plant parts powder, a soxhlet extractor was utilized in order to perform a sequence of consecutive methanolic extractions. A rotary vacuum evaporator was used to remove moisture from the methanolic extract as the pressure was lowered. Following extraction using a Soxhlet apparatus maintained at a temperature lower than 60 degrees Celsius, the extract was obtained. The Soxhlet extraction method was chosen for the process of plant extraction because it is simple to implement, requires a short amount of time, is economical due to the fact that only one sample is required for the entirety of the extraction, makes it simple to determine when the extraction process is complete, and presents a lower risk of contamination due to the fact that it is a closed system.

Materials

During the course of the experiment, many types of analysis, including weight loss, EIS, potentiodynamic polarization, and surface analysis, were performed using the metal sheet. To create a surface that was perfectly smooth, the metal sheet was first cut into squares measuring 2.5 cm2 by 2.5 cm2 before being cleaned using abrasive papers or Emery sheets of grades 320, 600, 1000, 1500, and 2000 respectively. They were cleaned with acetone to remove any oil, washed with distilled water, and then allowed to air dry before being submerged in the corrosive media.

High-performance thin layer chromatographic (HPTLC) analysis

After being filtered, the extract was put through a rota evaporator so that it could be evaporated (Heidolph, Germany). A methanolic solution was used to bring the volume of the 100 ml volumetric flask, which was used to collect the filtrates, up to the appropriate level. In order to conduct an HPTLC analysis, plant extracts and standards were suitably diluted at a range of concentrations. MERCK KGaA's 60-F254 silica gel plates

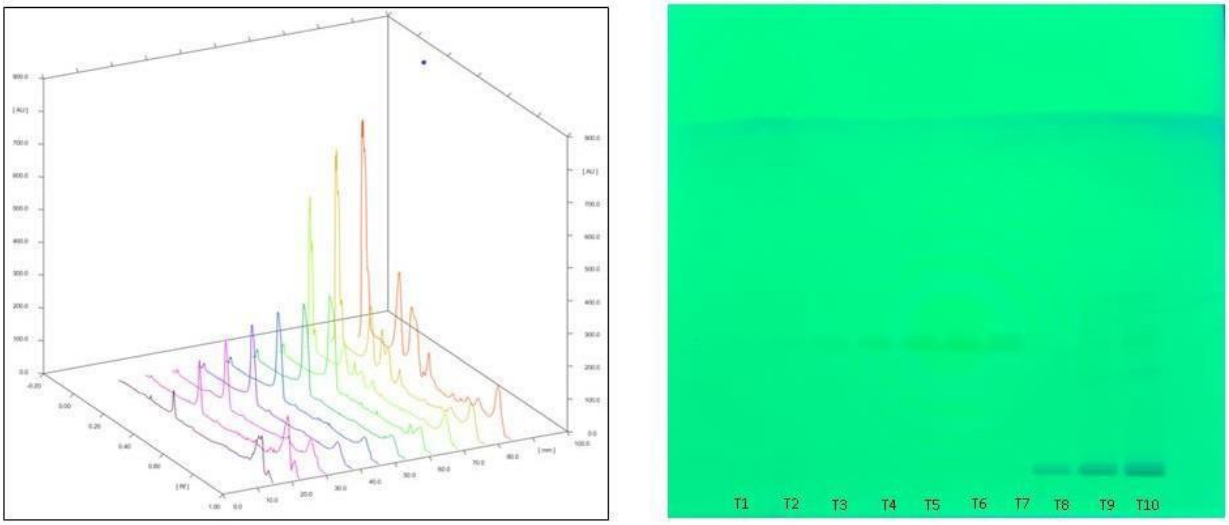
with a size of 10.0 × 10.0 cm were used for the analysis. A syringe containing 100 uL was used to apply each of the samples. In bands with a width of 10 millimeters, 15 l of the standard and 30 l of the plant extract samples were applied. After a period of saturation lasting for thirty minutes, the chromatograms were produced. Toluene, ethyl acetate, methanol, and formic acid were the components of the mobile phase that were utilized in the analysis. Following saturation, plates were analyzed using an ultraviolet spectrophotometer at wavelengths of 254 and 365 nanometers.

RESULTS AND DISCUSSIONS

Assessment of quercetin in methanolic extract of *T. occidentalis*

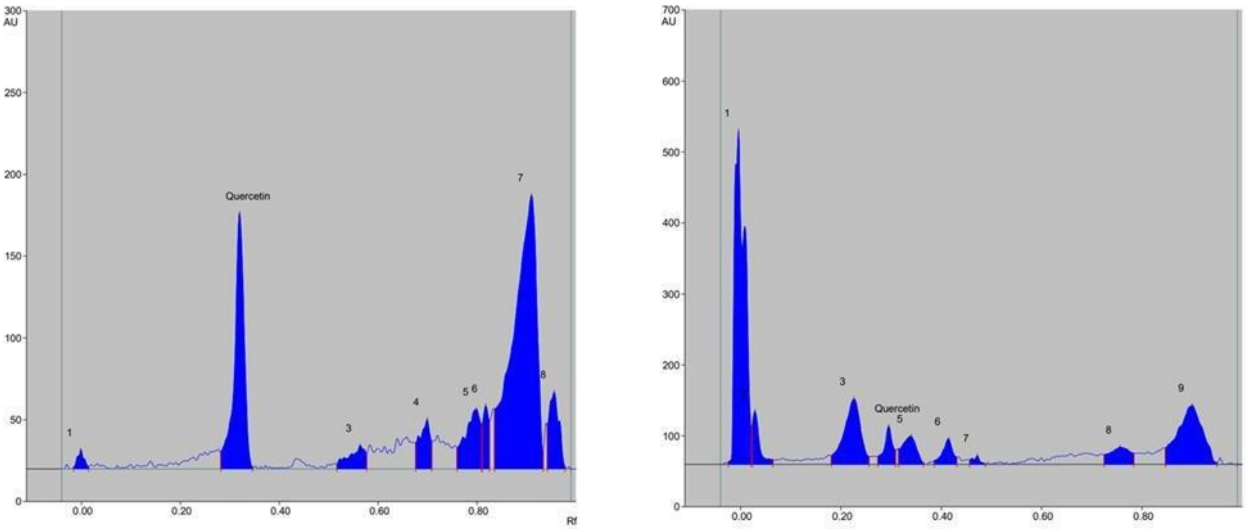
The HPTLC (CAMAG Linomat 5) instrument was used to evaluate the amount of quercetin present in the methanolic extract of the T. occidentalis leaves. 10.0 cm × 10.0 cm plates of silica gel 60 F 254 (E. MERCK KGaA) were used in the preparation of HPTLC plates. Toluene, ethyl acetate, methanol, and formic acid were the components of the mobile phase that were employed to generate the chromatograms. Methanol was used as the solvent, while inert gas was utilized for the spraying process. A syringe with a capacity of one hundred microliters was used to inject the sample. The length of the band was 6.0 mm, and the position of the sample in the application was 8.0 mm. The volume of the predosage was changed to 0.2 microliters, and the rate of the dosage was changed to 150 nanoliters per second. As may be seen in Figure 18, there were a total of ten different tracks. Quercetin was applied at various volumes, such as 0.1 l, 0.2 l, 0.3 l, 0.4 l, 0.5 l, 0.6 l, and 0.7 l, and at various sample application positions, such as 15.0 mm, 22.7 mm, 30.4 mm, 38.1 mm, 45.8 mm, 53.5 mm, and 61.2 mm correspondingly. Each volume was applied at a separate location. The T. occidentalis extract was administered at quantities of 0.2 l, 0.5 l, and 0.10 l, with the sample application position being, respectively, 68.9 mm, 76.6 mm, and 84.3 mm. The front position of the solvent was 80 millimeters. 7.7 millimeters was the distance between each track. The scanning speed was 20 millimeters per second, and the data resolution was 100 micrometers per step. At a wavelength of 254nm and under the illumination of a D2 lamp, HPTLC plates were analyzed using an automatic detection mode.

Within the scope of the investigation, the quercetin calibration plot demonstrated strong linearity in terms of the test range. Under the settings of the chromatograph that was utilized, the limit of detection was determined to be 1.77 ng, and the limit of quantification was determined to be 5.90 ng. These values were obtained in a clear and distinct manner at signal-to-noise ratios (S/N) of 3, and 10, respectively.



a b

Figure a) 3D spectra showing track details of quercetin in *T. occidentalis* and b) HPTLC photograph of methanolic extract of *T. occidentalis*



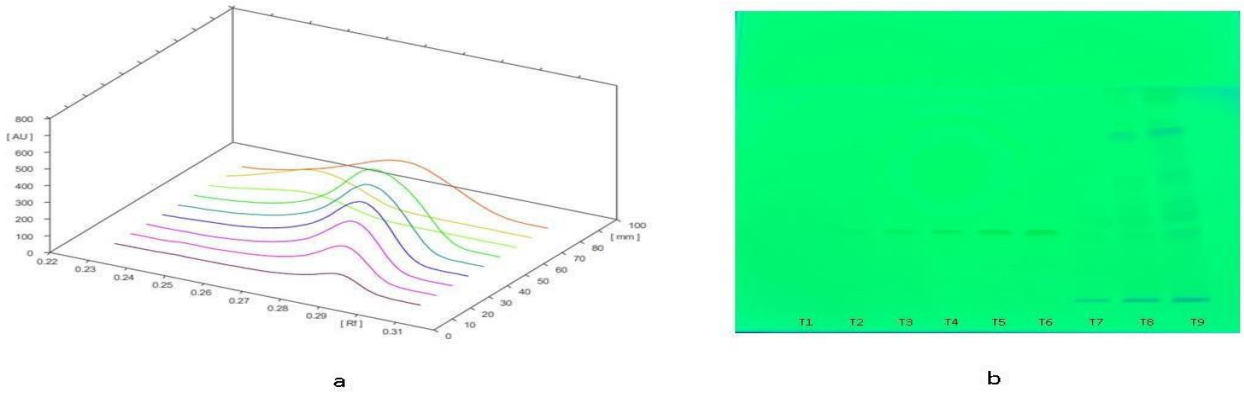
c d

Figure : (C) Standard peak of quercetin and (d) peak of quercetin in *T. occidentalis*

Assessment of quercetin in methanolic extract of *A. heterophylla*

The HPTLC (CAMAG Linomat 5) instrument was used to evaluate the amount of quercetin present in the methanolic extract of the leaves of A. heterophylla. 10.0 cm × 10.0 cm plates of silica gel 60 F 254 (E. MERCK KGaA) were used in the preparation of HPTLC plates. Toluene, ethyl acetate, methanol, and formic acid were the components of the mobile phase that were employed to generate the chromatograms. Methanol was used as the solvent, while inert gas was utilized for the spraying process. A syringe with a capacity of one hundred microliters was used to inject the sample. The length of the band was 6.0 mm, and the position of the sample

in the application was 8.0 mm. The volume of the predosage was changed to 0.2 microliters, and the rate of the dosage was changed to 150 nanoliters per second. As may be seen in Figure 20, there were a total of nine different tracks. It was administered at various volumes of quercetin, such as 0.1 l, 0.2 l, 0.3 l, 0.4 l, 0.5 l, and 0.6 l, with the sample being positioned at 15.0 mm, 23.7 mm, 32.4 mm, 41.1 mm, 49.8 mm, and 58.5 mm, respectively. The A. heterophylla extract was administered at quantities of 0.2 l, 0.5 l, and 0.10 l, with the sample application position being, respectively, 67.2 mm, 75.9 mm, and 84.6 mm. The space between each track measured 8.7 millimeters. The scanning speed was 20 millimeters per second, and the data resolution was 100 micrometers per step. At 254 nm and using automatic detection mode, HPTLC plates were analyzed under D2 lamp illumination. Within the scope of the analyzed test range, the linearity of the calibration plot for quercetin was quite satisfactory. Under the conditions of this study, the limit of detection is set at 3.044 ng, and the limit of quantification is set at 10.147 ng. At signal-to-noise ratios (S/N) of 3 and 10, respectively, distinct chromatographic conditions were determined and applied.



a b

Figure : a) 3D spectra showing track details of quercetin in *A. heterophylla* and b) HPTLC photograph of methanolic extract of *A. heterophylla*

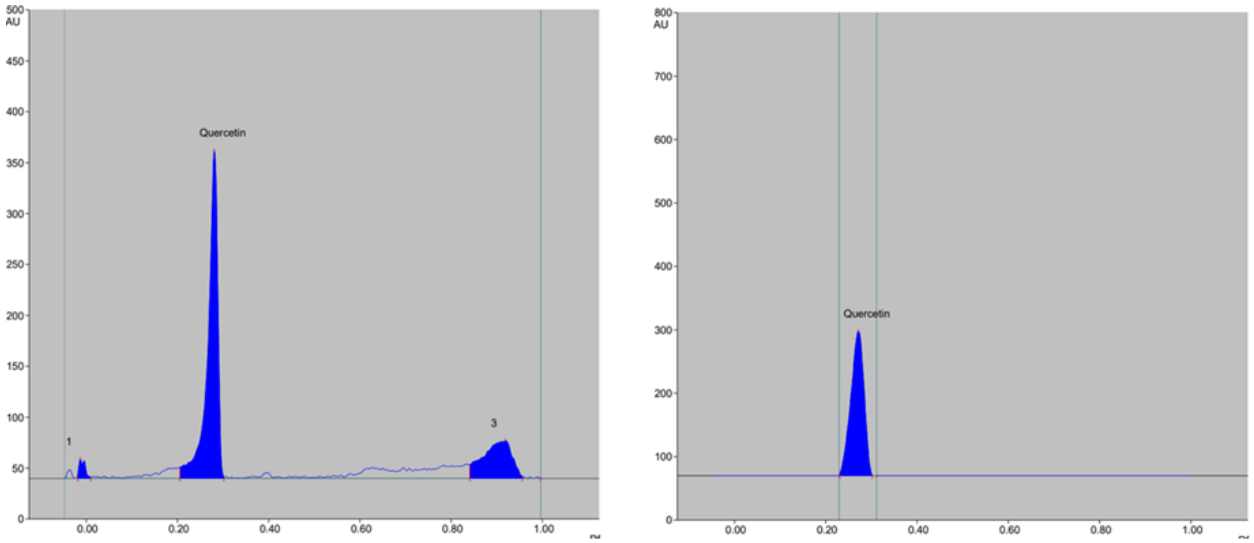


Figure: (c) Standard peak of quercetin and (d) peak of quercetin in *A. heterophylla*

CONCLUSION

Within the scope of the investigation, the quercetin calibration plot demonstrated strong linearity in terms of the test range. The findings of a investigation of extract of Thuja occidentalis and Araucaria heterophylla leaves and stems have been given. The investigation was conducted on both of these plants. This study made use of gas chromatography-mass spectrometry in order to determine the nature of the bioactive chemicals that were found in the plant extract. In order to evaluate the levels of quercetin in both plants, HPTLC was utilized.

REFERENCES

[1]. Singh, I. P., Ahmad, F., Chatterjee, D., Bajpai, R., Sengar, N. (2021). Natural products: Drug discovery and development. In: Drug Discovery and Development.Springer, 11-65.

[2]. Mukherjee, P. K. (2019), Traditional Systems of Medicine and Harmonization, Quality Control and Evaluation of Herbal Drugs, 1- 28.

[3]. Ahmad, R. S., Hussain, M. B., Saeed, F., Waheed, M., Tufail, T. (2017). Phytochemistry, metabolism, and ethnomedical scenario of honey: A concurrent review. International journal of food properties, 20, S254-S269.

[4]. Waseem, R., Low, K. H. (2015). Advanced analytical techniques for the extraction and characterization of plant-derived essential oils by gas chromatography with mass spectrometry. Journal of separation science, 38(3), 483-501.

[5]. Caines, S., Khan, F., Shirokoff, J. (2013). Analysis of pitting corrosion on steel under insulation in marine environments. Journal of Loss Prevention in the process Industries, 26(6), 1466-1483.

[6]. Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. -M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H. (2023). Discovery andresupply of pharmacologically active plant-derived natural products: A review. Biotechnology advances, 33(8), 1582-1614.

[7]. Shukla, S., Mehta, A. (2015). Anticancer potential of medicinal plants and their phytochemicals: a review. Brazilian Journal of Botany, 38(2), 199-210.

[8]. Verma, R. S., Padalia, R. C., Goswami, P., Verma, S. K., Chauhan, A., Darokar, M. P. (2014) .Chemical composition and antibacterial activity of foliage and resin essential oils of Araucaria cunninghamii Aiton ex D. Don and Araucaria heterophylla (Salisb.) Franco from India. Industrial Crops and Products, 61, 410-416.

[9]. Akkol, E. K., İlhan, M., Demirel, M. A., Keleş, H., Tümen, I., Süntar, İ. (2022). Thuja occidentalis L. and its active compound, α- thujone: Promising effects in the treatment of polycystic ovary syndrome without inducing osteoporosis. Journal of ethnopharmacology, 168, 25-30.

[10]. Singh, P., Ebenso, E. E., Olasunkanmi, L. O., Obot, I., Quraishi, M. (2016). Electrochemical, theoretical, and surface morphological studies of corrosion inhibition effect of green naphthyridine derivatives on mild steel in hydrochloric acid. The Journal of Physical Chemistry C, 120(6), 3408-3419.

[11]. Aljamali, N. M., Mohsin, N. M. B., Ali, H. N. (2019). Review on corrosion and rust inhibition of machines in chemical engineering field. International Journal of Thermodynamics and Chemical Kinetics, 5(1), 1-9.

[12]. Cragnolino, G. A. (2021). Corrosion fundamentals and characterization techniques. In: Techniques for corrosion monitoring. Elsevier, 7-42.

[13]. Sastri, V. S. (2015). Challenges in corrosion: costs, causes, consequences, and control. John Wiley & Sons, 95-402.

[14]. Yang ,Y., Khan, F., Thodi, P., Abbassi, R. (2017). Corrosion induced failure analysis of subsea pipelines. Reliability Engineering & System Safety, 159, 214-222.

[15]. Dobkowska, A., Castillo, M. D. H., Turnbull, J. P., Ramamurthy, S., Zagidulin, D., Moser, D. E., Behazin, M., Keech, P. G., Shoesmith, D. W., Noël, J. J. (2021). A comparison of the corrosion behavior of copper materials in dilute nitric acid. Corrosion Science, 192, 109778-109789.

[16]. Esmaily, M., Svensson, J., Fajardo, S., Birbilis, N., Frankel, G., Virtanen, S., Arrabal, R., Thomas, S., Johansson, L. (2017). Fundamentals and advances in magnesium alloy corrosion. Progress in Materials Science, 89, 92-193.

[17]. Srinivasan, S. (1992). Electrode Kinetic and Electrocatalytic Aspects of Electrochemical Energy Conversion. Electrochemistry in Transition, 577-602.

[18]. Ujjain, S., Ahuja, P., Kanojia, R. (2023). Electrochemical studies of green corrosion inhibitors. Theory and Applications of Green Corrosion Inhibitors, 86, 91-126.

[19]. Khan, A., Qurashi, A., Badeghaish, W., Noui-Mehidi, M. N., Aziz, M. A. (2020). Frontiers and challenges in electrochemical corrosion monitoring; surface and downhole applications. Sensors, 20(22), 6583-6617.

[20]. Quraishi, M., Nayak, D., Kumar, R., Kumar, V. (2017). Corrosion of reinforced steel in concrete and its control. An overview. Journal of Steel Structures & Construction, 3(1), 1-6.