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Comparative Study on Hydrogen Peroxide Scavenging Assay of Methanolic Extract of Thuja occidentalis And Araucaria heterophylla

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Abstract
In terms of antioxidant activity, the hydroalcohol extract of the leaves and stem came in second place after the methanol extract. The extract obtained from methanol provided the highest percentage of scavenging activity, followed by the extract obtained from hydroalcoholic. Methanol extract performed significantly better than hydroalcohol extract did in the hydrogen peroxide assay. The hydrogen peroxide scavenging experiment was used to investigate the in-vitro H_2O_2 scavenging potential of methanol extract of leaf and stem derived from T. occidentalis, and A. heterophylla (TOLMe, TOSMe, AHLMe, AHSMe). The suppression of free radicals was dose dependent and occurred at concentrations ranging from 20 to 100 g/ml in the methanol extract of the plants. % Inhibition of H_2O_2 radical by methanol extract of plants follows the order i.e., TOLMe>TOSMe>AHLMe>AHSMe. The hydrogen peroxide scavenging assay was used to determine the in-vitro H_2O_2 scavenging potential of hydro alcohol extract of leaves and stem derived from T. occidentalis, and A. heterophylla (TOLHA, TOSHA, AHLHA, AHSHA). The suppression of free radicals was dose dependent and occurred at concentrations ranging from 20 to 100 g/ml in the hydro alcohol extract of the plants. % Inhibition of H_2O_2 radical by hydro alcohol extract of plants follows the order i.e., TOLHA>TOSMe>AHSHA>AHLHA.
Kew Words-: *Anioxidant, Thuja occidentalis, Araucaria heterophylla, hydroalcohol extract, scavenging potential*

I. INTRODUCTION

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. Compounds that have been obtained via extraction from live organisms are known as natural products. They have been extracted from a limitless variety of different substances. 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. Medicinal properties can be extracted from plants. 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. Resin is the primary component of gymnosperm plants. Resins are plant exudates that can be harvested from conifers through tapping. Resins are soluble in organic solvents.

Araucaria is a member of the Araucariaceae family of plant families. ***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 gastro protective activities, were carried out. Compounds that have been obtained via extraction from live organisms are known as natural products. They have been extracted from a limitless variety of different substances. 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 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.

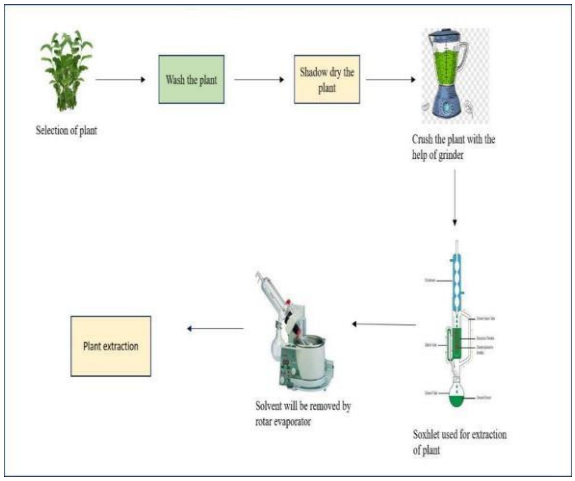
Metals are the group of materials that are used the most frequently, particularly in mechanical engineering, the transportation business, electronic manufacturing, and the construction industry. Metals can be mined from the earth in the form of ores, where they are found in the form of oxides, silicates, chlorides, and other compounds. Metals have a broad variety of applications in both commercial and domestic settings. They are extracted from the ores by a number of metallurgical processes that require a significant amount of energy.

As a result of having a higher energy state, pure metal has a greater propensity to transform back into its complex state. At the surface, metals undergo a transformation that results in the formation of metallic compounds that are known as corrosion products. 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.

II. MATERIALS AND METHODS

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.



Process of making the plant extract

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 .

Hydrogen peroxide scavenging assay

When an antioxidant molecule is incubated with hydrogen peroxide in this experiment, the amount of hydrogen peroxide that degrades or is lost can be detected at 230 nm using aspectrophotometer. A phosphate-buffered saline solution (0.05 mM, pH 7.4) containing 40 mM H2O2 was created. The H2O2 solution was then mixed with the extracts at various doses (0.6 ml, 40 mM). After 10 minutes, the reaction mixture was incubated, and the hydrogen peroxide absorbance was measured at 230 nm in comparison to a blank solution.

% Inhibition was calculated using following formula:

$$\% \text{ Inhibition} = \frac{(AC\ 230nm - AS\ 230nm\ ) * 100}{AC\ 230nm}$$

III. RESULTS AND DISCUSSIONS

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of methanol extract of different plants

The hydrogen peroxide scavenging experiment was used to investigate the in-vitro H_2O_2 scavenging potential of methanol extract of leaf and stem derived from T. occidentalis, and A. heterophylla (TOLMe, TOSMe, AHLMe, AHSMe). The suppression of free radicals was dose dependent and occurred at concentrations ranging from 20 to 100 g/ml in the methanol extract of the plants.[

In the methanol extract of the plants, TOLMe exhibited the greatest percentage of inhibition of the H_2O_2 radical at concentrations ranging from 20 to 100 g/ml, with an IC50 value of 77.259 g/ml. On the other hand, TOSMe showed the least percentage of inhibition of the H_2O_2 radical atconcentrations ranging from 20 to 100 g/ml, with an IC50 value of 91.427 g/ml. The IC50 value for the percentage of H_2O_2 radical inhibition caused by AHLMe was found to be 107.036 g/ml, whereas the IC50 value for the percentage of H_2O_2 radical inhibition caused by AHSMe was found to be 156.098 g/ml. Ascorbic acid showed a percentage suppression of the H_2O_2 radical at concentrations ranging from 20 to 100 ng/ml, with an IC50 value of 65.179 ng/ml, as shown in Figure . % Inhibition of H2O2 radical by methanol extract of plants follows the order i.e., TOLMe>TOSMe>AHLMe>AHSMe.

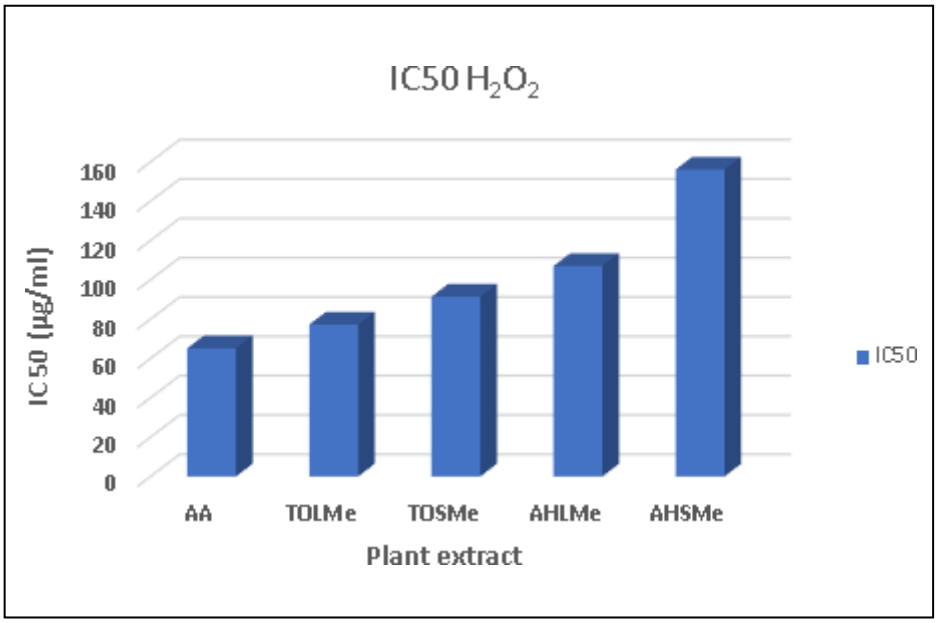


Figure : H2O2 antioxidant potential of leaf and stem extracts in methanol of *Thujaoccidentalis* and *Araucaria heterophylla*

Hydrogen peroxide scavenging potential of hydro alcohol extract of different plants

The hydrogen peroxide scavenging assay was used to determine the in-vitro H_2O_2 scavenging potential of hydro alcohol extract of leaves and stem derived from T. occidentalis, and A. heterophylla (TOLHA, TOSHA, AHLHA, AHSHA). The suppression of free radicals was dose dependent and occurred at concentrations ranging from 20 to 100 g/ml in the hydro alcohol extract of the plants.

TOLHA showed the most percentage inhibition of H_2O_2 radical at (20-100 g/ml) with the lowest IC50 value of 132.223 g/ml, whereas TOSHA showed the least percentage inhibition of H_2O_2 radical at (20-100 g/ml) with the highest IC50 value 133.643 g/ml. Both of these results were found in the hydro alcohol extracts of the plants. The IC50 value for the percentage of H_2O_2 radical inhibition caused by AHLHA was found to be 159.199 g/ml, whereas the IC50 value for the percentage of H_2O_2 radical inhibition caused by AHSHA was found to be 147.195 g/ml. As shown in Figure 16, ascorbic acid displayed a percent suppression of the H_2O_2 radical at concentrations ranging from 20 to 100 g/ml, with an IC50 value of 65.179 g/ml.

% Inhibition of H2O2 radical by hydro alcohol extract of plants follows the order i.e., TOLHA>TOSMe>AHSHA>AHLHA.

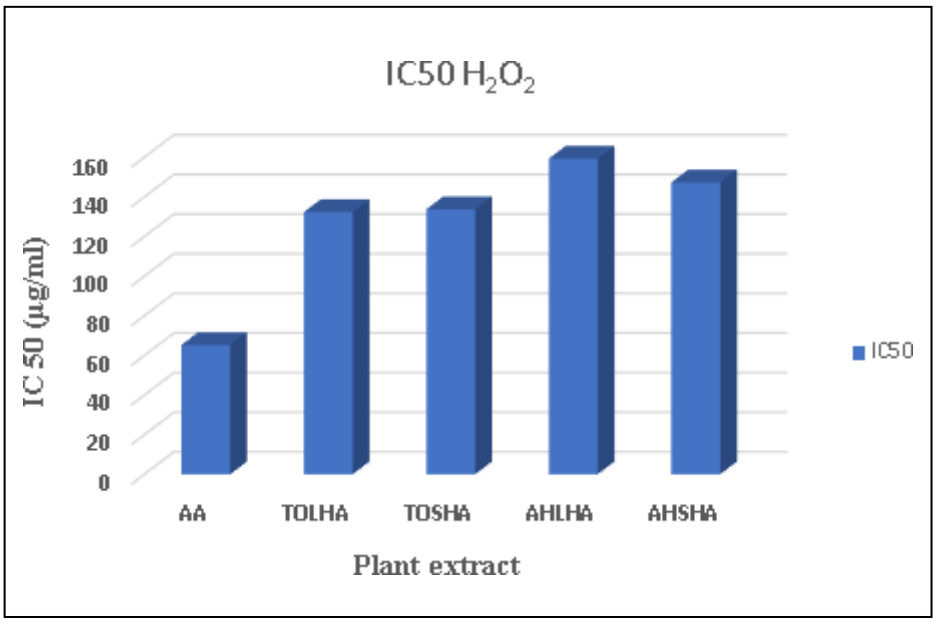


Figure : H2O2 antioxidant potential of leaf and stem extracts in hydro alcohol of *Thujaoccidentalis* and *Araucaria heterophylla*

Table - Antioxidant capacity of leaf and stem extracts of *Thuja occidentalis* and *Araucaria heterophylla*

EXTRACTS	H2O2 (IC50 µg/ml)
AA	65.179±0.0003
TOLMe	77.529±0.0006
TOLHA	132.223±0.0006
AHLMe	107.036±0.0006
AHLHA	159.199±0.0009
TOSMe	91.427±0.0009
TOSHA	133.643±0.0012
AHSMe	156.098±0.0003
AHSHA	147.195±0.0018

The data represent mean± SEM of three determinants

*p< 0.05, significantly different from standard

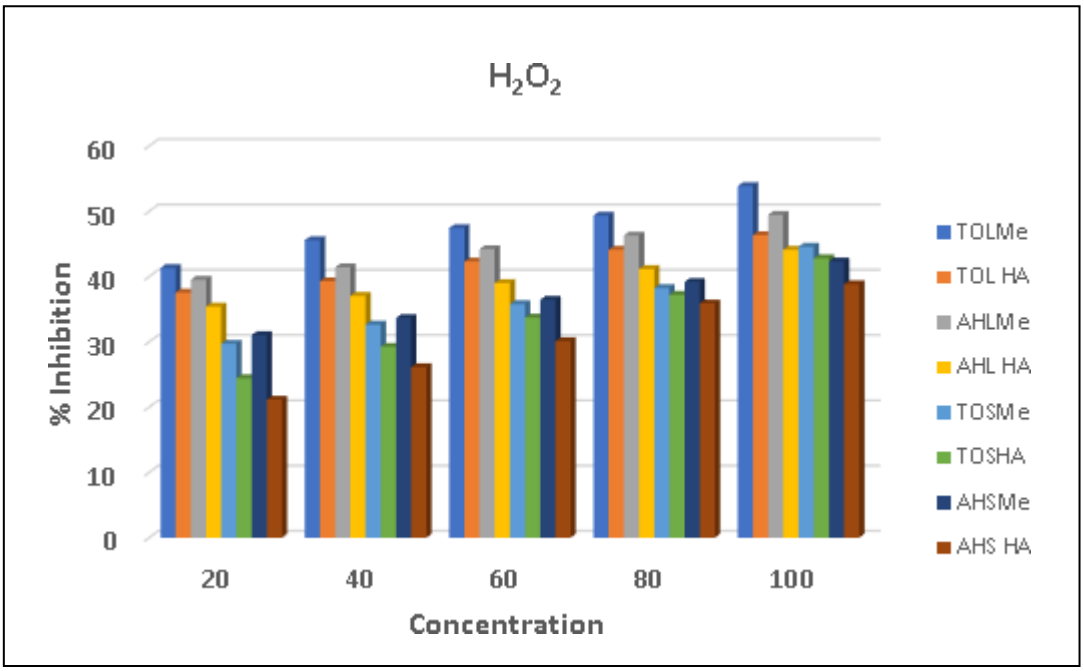


Figure : Comparison of % inhibition of *T. occidentalis* and *A. heterophylla* leaves andstem extracts against concentrations

IV. CONCLUSION

In terms of antioxidant activity, the hydroalcohol extract of the leaves and stem came in second place after the methanol extract. In the hydrogen peroxide assay procedure, the extract obtained from methanol provided the highest percentage of scavenging activity, followed by the extract obtained from hydroalcoholic. Methanol extract performed significantly better than hydroalcohol extract did in the hydrogen peroxide assay.

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