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Investigation of Total Tannin, Phenolic & Flavonoid Content of *Araucaria Heterophylla* for Antioxidant Source

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Abstract

Secondary metabolites, a type of bioactive molecule with many functions, are abundant in Araucaria Heterophylla. The secondary metabolites flavonoids, phanolic acid, and tannin have been found to have anti-inflammatory, antiviral, antibacterial, antioxidant, and anticancer properties. The present study was conducted to analyze the phytochemical and determine the total flavonoid, tannin, phenolic content of Araucaria Heterophylla leaves extract using two different solvents (methanol and dichloromethane) using UV-Vis spectrophotometric. The plant was identified by NHB (ID No: DACB 48435) and prepared the methanolic extract to estimate the total content of tannin, phenolic & flavonoid in Araucaria Heterophylla. Folin-ciocaltu method was used for the investigation of total phenolic and tannin content. The A. heterophylla leaf methanolic and dichloromethane extract had remarkable antioxidant effects due to its high flavonoid and phenolic content. This standardised bioactive ingredient could be used in many phytopharmaceutical preparations

Keywords: Tannin, Phenolic Acid, Flavonoids, Araucaria Heterophylla, Antioxidants

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1 Introduction

Many pharmacological effects can be elicited by the bioactive chemical substances found in plants. These bioactive substances, which are agents of plant medicines, are commonly known as secondary metabolites. Scientific reports have indicated that bioactive components generated from plant extracts have a range of biological actions. Chemicals such as phenols, alkaloids, flavonoids, steroids, glycosides, tannins, and so on are the most crucial components with biological activity. The anticipated beneficial characteristics are mostly caused by these chemical components. Astringents, diuretics, antioxidants, antiseptics, anti-inflammatory, and hemostatic medications are all made from plant extracts that include tannin. [1] These extracts are also used to combat stomach and duodenal tumors. Tannins, when applied topically, draw out all skin irritants. Particularly, treatments that are rich in tannins are utilized as antimicrobials, antioxidants, antivirals, and anthelmintics [2]. Plants rely on alkaloids for protection and survival since these compounds have the ability to ward off insects, herbivores, and microbes (thanks to their antibacterial and antifungal properties).[3-5]

Phenolic acid has a wide range of biological effects, including the ability to alter gene expression, anti-inflammatory, antibacterial, antihypertensive, anti-carcinogenic, and anti-mutagenic properties [6,7,8]. Because of their wide range of pharmacological effects on human health, flavonoids are considered nutraceuticals. Many beneficial qualities are attributed to flavonoids, including their antioxidant, anti-inflammatory, anti-allergic, estrogenic, antibacterial, vascular, and cytotoxic anticancer activities. the eleventh the antioxidant and free radical scavenging functions of plant phenolics and flavonoids are supported by their widespread distribution in plant tissues. Antioxidants found in nature boost plasma antioxidant capacity and lessen illness risk.[9-12]

Araucaria heterophylla leaf aqueous extract was found to include alkaloids, carbohydrates, flavanoids, tannins, and phenols according to the results of a phytochemical screening. Secondary metabolites, which include alkaloids, carbs, flavanoids, saponins, tannins, and terpenoids, are responsible for medicinal plants' pharmacological actions; these compounds may also have antioxidant properties. Due of their antioxidant activity, economic viability, and side effects, the therapeutic qualities of plants have been studied worldwide [13-16]. To avoid kidney disorders, cholesterol, and carcinomas, phytochemicals are vital. Therefore, the objective of this work was to estimate the total tannin, alkaloid, phenolic, and flavonoid content of *Araucaria Heterophylla.*

2 Methods

2.1 Plant Identification & Collection

Botanists at the National Herbarium Bangladesh determined that the plant was Araucaria heterophylla; the DACB 48435 identification code corresponds to this. Plants belonging to the Araucariaceae family include Araucaria heterophylla Franco. The Ranada Prasad Shaha University campus in Shitalakhya, Narayanganj, was scoured for *Araucaria heterophylla* tree leaves on August 20, 2019. After picking them up, lightly dust the leaves. The leaves were subsequently allowed to dry in a clean, shaded area from August 23, 2019, until September 30, 2019. Following a thorough drying process, the leaves were ground to a powder using a domestic grinder.

2.2 Extraction Process

An electrical balance and a vacuum pump are crucial tools for this procedure. Using methanol as a solvent, the plant material, weighing approximately 300g, was thoroughly mixed in a plastic container for several days. After immersing the 300g of dried plant powder in the solvent for an extended period, it required approximately 1400 ml of methanol to fully dissolve it. Later, the water component was separated by passing it through a vacuum pump. Following that, the mixture was allowed to naturally dry at room temperature for approximately ten to twelve days. A substance with a thick, sticky, dark green, coarse texture was produced through this procedure. Using an extraction efficiency of approximately 15.6%, we determined the quantity of extract obtained from 300g of plant material.

2.3 Investigation of total phenolic content

The total phenolic content of the extract was ascertained using the modified Folin-ciocalteu technique. Sodium carbonate (7.5%) and Folin-ciocaltu reagent (2.5 mL, diluted 10 fold) were combined with 1.0 mL of the extract (200µg/mL) in a brief manner. To bring out the colours, the mixture was vortexed for 15 seconds before being left to stand at 20°C for 40 minutes. A UV-1800 spectrophotometer from Shimadzu, Japan, was used to measure the absorbance at 765 nm. Using the equation derived from a standard gallic acid calibration curve, [14] the total phenolic content was calculated as milligrammes of gallic acid equivalent per gramme (Figure 1).

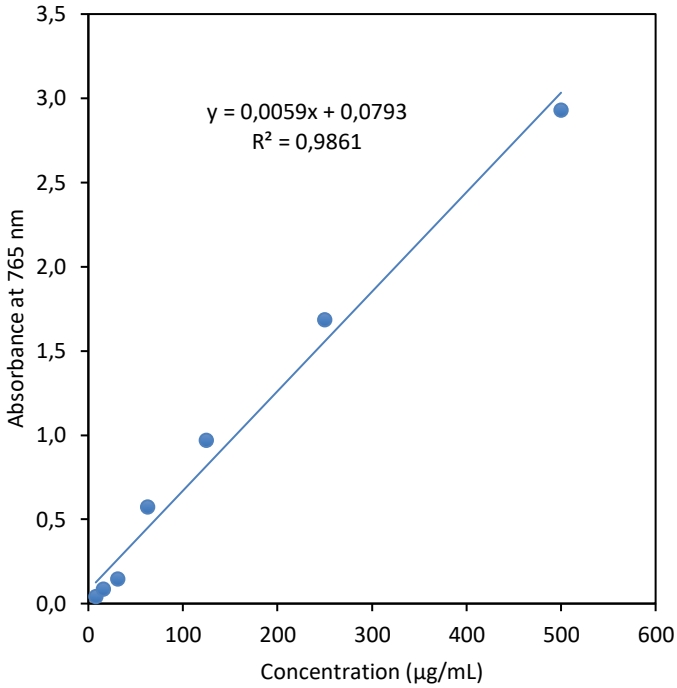

Figure 1: Total Phenolic content of gallic acid (standard).

2.4 Investigation of total flavonoids content

Each sample of the leaves crude extract (0.025 g) was suspended in methanol (5 ml). 3 mL of each suspension was transferred to a tube followed by addition of 200 µL of 1M potassium acetate solution and 200 µL of 10% aluminum chloride solution. Finally, 5.6 mL of distilled water was mixed with the reaction mixture. After that the reaction mixture then Incubated for 30 minutes at room temperature to complete the reaction [15]. Then, measurement of absorbance was taken by UV–visible spectroscopy at 415 nm wavelength against blank (Figure 2).

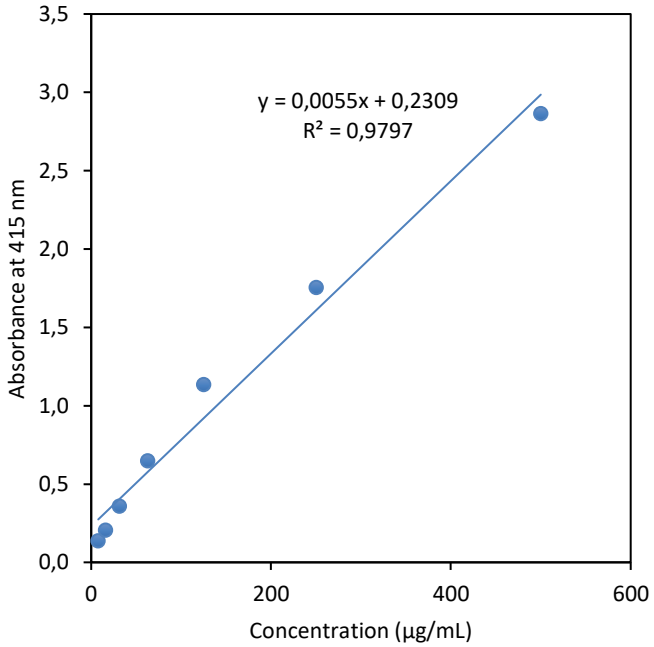

Figure 2: Total Phenolic content of quercetin (standard).

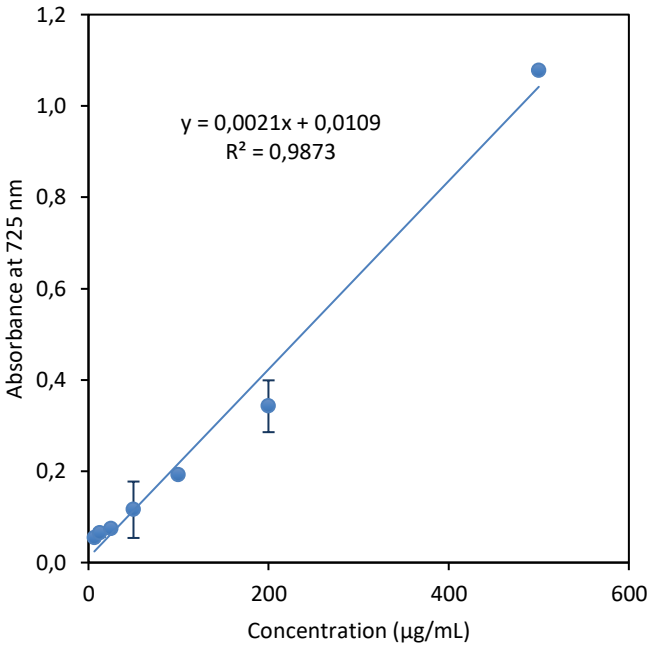

Figure 3: Total tannin content of gallic acid (standard).

2.5 Investigation of total tannins content

Total tannin in plant extract was determined by Folin-ciocaltu method. The total tannin content was measured by adding 1 mL of plant extract (200 µg/mL) or a standard with different concentrations to individual test tubes. Then, 7.5 mL of distilled water was added to each tube. Next, 0.5 mL of Folin-Ciocalteu phenol reagent (diluted 10 times) was added to each tube, along with 1 mL of 35% Na_2CO_3 solution. The mixtures were shaken vigorously and allowed to sit at room temperature for 30 minutes. The absorbance readings for both the test and standard solutions were measured against the blank at 725 nm using a UV/Visible spectrophotometer. The solution contained a combination of 7.5 mL water and 2.5 mL methanol [16]. Using this approach, the absorbance readings were used to quantify the total tannin content, offering valuable information about the concentration of tannins in the analysed samples (Figure 3).

3 Results and Discussion

The amount of total phenolic content was quite high in the methanolic crude extract of *Araucaria heterophylla* (202.57± 11.44 mg/g of gallic acid equivalent). Then, the amount of total flavonoids content was moderately high in the methanolic crude extract of *Araucaria heterophylla* (132.26±78.24 mg/g of quercetin equivalent). And, the amount of total tannin content was quite comparatively in the methanolic crude extract of *Araucaria heterophylla* (145.48±2.38 mg/g of gallic acid equivalent).

Table 1: Total content of secondary metabolites (phenolics, flavonoids and tannins) of Araucaria heterophylla in dichloromethane & methanol extract.

Extract Name	Total Phenolic Content (TPC) (mg GAE/g)	Total Flavonoids Content (TFC) (mg QE/g)	Total Tannin Content (TTC) (mg GAE/g)
Dichloromethane extract	160.68±9.06	158.01± 52.9	120.08±1.37
Methanolic Extract	202.57± 11.44	132.26±78.24	145.48±2.38

Due to its high flavonoid and phenolic content, the methanolic and dichloromethane extract of *A. heterophylla* leaves exhibited strong antioxidant properties; this standardized bioactive constituent has the potential to be included into numerous significant phytopharmaceutical preparations. Research on the stability and bioavailability of plant extracts, as well as standards for their preparation, are being developed [17,21]. The use of plant extracts as a key ingredient in the production of certain biopharmaceuticals has the potential to aid in the global fight against microbial diseases. However, this won't happen until toxicity studies and pharmaceutical pharmacy standards are applied to the use of natural products in biopharmaceutical preparations. Further research and the development of novel medications can be aided by conducting clinical trials. The methodologies employed in this study involved using UV-Vis spectrophotometry to measure the absorbance of the extracts at specific wavelengths, indicating the presence and concentration of tannins, phenolics, and flavonoids [18, 21]. The Folin-Ciocalteu method was utilized for phenolic and tannin content estimation, while the aluminum chloride method was used for flavonoids. The methanolic extract exhibited a TPC of 202.57±11.44 mg GAE/g, significantly higher than the dichloromethane extract, which had a TPC of 160.68±9.06 mg GAE/g. This aligns with findings from the other study, where the highest TPC was observed in the n-BuOH extract, followed by methanol and other solvents. The dichloromethane extracts surprisingly had a higher TFC (158.01±52.9 mg QE/g) compared to the methanolic extract (132.26±78.24 mg QE/g). This discrepancy suggests that flavonoids in *A. heterophylla* may be more soluble in dichloromethane than methanol [19, 21]. The methanolic extract again showed a higher TTC (145.48±2.38 mg GAE/g) compared to the dichloromethane extract (120.08±1.37 mg GAE/g). This higher content is indicative of methanol's superior extraction efficiency for tannins, consistent with the general understanding that polar solvents like methanol are more effective for extracting phenolic compounds. The high levels of phenolics and tannins in the methanolic extract correlated with its stronger antioxidant activity. Polyphenols, including tannins and flavonoids, contribute to the antioxidant potential by acting as reducing agents, hydrogen donors, and metal chelators. The study's results showed that the methanolic extract's antioxidant capacity was notably high, which is in agreement with the findings from a study where polyphenolic content directly correlated with antioxidant activities measured by different assays such as the phosphomolybdenum antioxidant assay and the reducing power assay. The methanolic extract of *A. heterophylla* leaves demonstrated superior levels of phenolics, tannins, and antioxidant activity compared to the dichloromethane extract. These findings suggest that methanol is a more effective solvent for extracting antioxidant compounds from *A. heterophylla*, making it a potential candidate for use in phytopharmaceutical preparations. Further research on the stability, bioavailability, and clinical applications of these extracts could pave the way for their incorporation into therapeutic formulations [20, 21].

4 Conclusions

Due to its high flavonoid and phenolic content, the methanolic and dichloromethane extract of *A. heterophylla* leaves exhibited strong antioxidant properties; this standardized bioactive constituent has the potential to be included into numerous significant phytopharmaceutical preparations. Research on the stability and bioavailability of plant extracts, as well as standards for their preparation, are being developed. The use of plant extracts as a key ingredient in the production of certain biopharmaceuticals has the potential to aid in the global fight against microbial diseases. However, this won't happen until toxicity studies and pharmaceutical pharmacy standards are applied to the use of natural products in biopharmaceutical preparations. Further research and the development of novel medications can be aided by conducting clinical trials.

5 Declarations

5.1 Author Contributions

The names of the authors listed in this journal contributed to this research.

5.2 Funding Statement

This research was not supported by any funding sources.

5.3 Conflicts of Interest

The authors declare no conflict of interest.

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