ORIGINAL ARTICLE

Evaluation of *in vitro* anti-oxidant and anti-inflammatory activity of ethanolic extract of *Hevea brasiliensis* roots

Moushumi Baidya[1], Plabani Shil[2], Soubhik De[2], Lipika Das[2], Subrata Debnath[2]

[1]*Department of Pharmacy, Milestones Institute of Pharmaceutical Sciences, Udaipur, Tripura, India,*
[2]*Department of Pharmacy, Bharat Pharmaceutical Technology, Agartala, Tripura, India*

Abstract

This study examines the *in vitro* antioxidant and anti-inflammatory activities of the ethanolic extract of *Hevea brasiliensis* roots. The potential medical benefits of *H. brasiliensis*, a plant farmed largely for latex production, are being investigated. To create the crude extract, the roots were collected, dried, and then extracted using ethanol. After being dried, the resulting extracts underwent a preliminary phytochemical screening procedure 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging tests was used to measure antioxidant activity. DPPH studies showed that the extract had a substantial ability to neutralize free radicals, indicating significant antioxidant activity. The protein denaturation inhibition assay was used to assess the anti-inflammatory properties. The extract showed a significant reduction in the denaturation of proteins. The ethanolic extract of *H. brasiliensis* roots has significant antioxidant and anti-inflammatory properties, indicating its potential as a natural source of therapeutic agents for oxidative stress and inflammation-related conditions. More *in vivo* studies and compound isolation are needed to fully explore the medicinal potential of *H. brasiliensis* roots.

Key words: Anti-inflammatory, antioxidants, *Hevea brasiliensis* roots, phytochemicals

INTRODUCTION

The majority of people on the planet, over 80% of them, rely on herbal or traditional medicine for their primary medical needs. The history of human interactions with the environment in Asian countries is significantly influenced by the use of herbal treatments.[1] Many different compounds found in plants can be used to treat both acute and chronic ailments. Plants are valuable medical resources because they contain chemical substances that affect human physiology in particular ways. Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant bioactive substances present in plants. In many developing countries' rural areas, the traditional practice of using medicinal plants as remedies is well-known.

Traditional healers assert that compared to current therapy, traditional medicine is more affordable and efficient. Traditional medicine is used to treat common illnesses by low-income individuals in developing nations, including farmers, those living in small, remote communities, and indigenous tribes.[2] In general, phytochemical research that is grounded in ethnopharmacological data is thought to be a successful method of finding novel higher plant anti-infective compounds.[3]

The most popular source of natural rubber is *Hevea brasiliensis*. *H. brasiliensis* is a member of the *Euphorbiaceae* family of trees, usually known as the Sharinga tree, the Para rubber tree, or just the rubber tree. It is the most significant member of the genus *Hevea* in terms of economy. Because the main source of natural rubber is the milky latex that is removed from the tree, it is extremely important economically.[4]

Oxidation can contribute to the synthesis of active chemicals in medicinal plants as well as their possible therapeutic effects.[5] The history of life's emergence on Earth is accompanied by antioxidants. Antioxidants played a crucial

Address for correspondence:
Moushumi Baidya, Milestones Institute of Pharmaceutical Sciences, Udaipur, Tripura, India. Mobile: +91-7829461721.
E-mail: baidyamoushumi@gmail.com

Received: 01-08-2024
Revised: 23-09-2024
Accepted: 30-09-2024

role in the structure of complex molecules because of the unfavorable and harsh environment that led to the production of free radicals.[6]

Traditional medicine reports that extracts from medicinal plants work very well as anti-inflammatory medicines. They provide an excellent foundation for the development of novel anti-inflammatory medications and are abundant sources of phytochemicals used in herbal remedies. There have also been reports of the potent anti-inflammatory properties of isolated substances derived from medicinal plant extracts. To determine the possible anti-inflammatory properties of natural plant compounds, more ethnopharmacological research is needed. As a result, numerous studies have demonstrated that the type of living cells involved and the distribution method employed both affect the anti-inflammatory properties of phytochemicals and their derivatives.[7] Although there have been no research on the antioxidant capacity of *H. brasiliensis* roots, the plant includes phytochemicals such as flavonoids, phenolic compounds, tannins, and others that have anti-inflammatory and antioxidant properties. A review of the literature showed that the anti-inflammatory and antioxidant properties of *H. brasiliensis* root extract had not been investigated. Thus, the study's objective was to investigate the ethanolic extract of *H. brasiliensis* root's anti-inflammatory and antioxidant properties. This work has revealed which phenolic and flavonoid chemicals give each extract its antioxidant and anti-inflammatory properties, which may pave the way for further research on the characterization of extracts with greater potential.

MATERIALS AND METHODS

Plant Collection and Identification

H. brasiliensis roots were obtained in February 2024 from Baralutma, Dhalai, Tripura, and were identified by Prof. Badal Kumar Dutta, a professor and botanist taxonomist in the department of botany at Tripura University.

Chemicals and Drugs

99.9% ethanol, α-naphthol, concentrated H_2SO_4, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), methanol, ascorbic acid, hydrochloric acid (HCL), Mayer's reagent (potassium mercuric iodide), NaOH, ferric chloride, aspirin, egg albumin, distilled water.

Preparation of *H. brasiliensis* Extract

Once the roots have been collected, they are all carefully cleansed with distilled water multiple times before being chopped into little pieces and allowed to dry for 2 weeks. Once this time has passed, the roots are coarsely ground using an appropriate grinder. A thimble of Soxhlet is filled with coarsely ground root powders, and ethanol is used as a solvent for 48 h to begin the extraction process. Then, to achieve the final extract, the solvent containing the extract is filtered and boiled in a water bath. The yield value is then calculated and tests for phytochemical screening are then conducted.

Yield value is calculated using the mentioned equation:

% yield value = Weight of the extract/Weight of the plant material ×100

Phytochemical Screening

H. brasiliensis ethanolic root extract underwent a number of significant first phytochemical tests, including those for alkaloids, glycosides, phenolic compounds and tannins, flavonoids, and saponins. All of the extracts underwent phytochemical screening using established techniques.[8]

- Detection of alkaloids: Mayer's test – a few drops of diluted HCL are added to the extract solution, which is then filtered. Now add a few drops of Mayer's reagent (potassium mercuric iodide) to the filtrate. The presence of alkaloids is now indicated by the formation of a cream-colored precipitate.
- Detection of flavonoids: Alkaline reagent test-one milliliter of the test solution is mixed with a few drops of sodium hydroxide solution. The presence of flavonoids is indicated by the formation of a strong yellow color that eventually vanishes upon the addition of diluted HCL.
- Detection of phenolic compound and tannins: Ferric chloride test – sample solution is prepared by diluting the extract with water. After adding 5% ferric chloride solution to the test solution, the presence of tannins and phenolic compounds is indicated by the creation of a blue-black color.
- Detection of saponins: Foam test – extracts are shaken for 15 min after being diluted to 20 mL with distilled water. The presence of saponins is indicated by the creation of a 1 cm foam layer.

In vitro Anti-Oxidant Activity

With minor adjustments, the technique described by Chang *et al*. (2002) for DPPH scavenging was employed to evaluate the plant extract's antioxidant activity.[9] Ascorbic acid solution (1 mg/mL sample and standard) is first made with methanol acting as the solvent. The DPPH solution of 0.004% (4 mg in 100 mL) is now made with methanol. One milliliter of DPPH solution is added to each test tube containing varying concentrations of sample and standard solution (100, 200, 300, 400, and 500) and the tubes are left to react in a dark environment for half an hour. Following that, an ultraviolet (UV) spectrometer set at 517 nm is used to measure all test, standard, and control samples. The

anti-oxidant activity percentage is then computed using the equation that follows:

% Scavenging capacity of test drug = Abc-Abt/Abc × 100

% Scavenging capacity of standard drug = Abc-Abs/Abc × 100

In vitro Anti-Inflammatory Activity

The technique that was used to measure the inhibition of protein denaturation is as follows: 500 microliters of 1% egg albumin were first mixed with varying concentrations of root extract (100, 200, 300, 400, and 500 µL), and the mixture was allowed to cool at room temperature before being heated for 20 min at 51°C. After bringing the resultant solution down to room temperature, a UV spectrometer set to measure wavelengths of 660 nm is used to measure each sample. A positive control was aspirin.[10] Three times of this process were conducted, and the following formula was used to get the % inhibition:

% of inhibition of test drug= (At-Ac)/Ac × 100

% of inhibition of standard drug = (At-Ac)/Ac × 100.

RESULTS AND DISCUSSION

The plant has notable therapeutic properties that demonstrate its ability to treat a wide range of illnesses. This is corroborated by phytochemical study, which has identified a variety of secondary metabolites with therapeutic significance, including as phenol, alkaloids, flavonoids, saponins, and tannins. By assisting in the selection of particular extracts for additional research, the qualitative evaluation of phytoconstituents provides insightful information on the composition of the extracts and assistance for future researchers aiming to isolate the active principles.

Notable medicinal qualities of the plant show that it can be used to cure a variety of ailments. A range of secondary metabolites having medicinal relevance, including as phenol, alkaloids, flavonoids, saponins, and tannins, has been identified through phytochemical research, which supports this. In addition to offering valuable information on the composition of the extracts and support for future researchers seeking to isolate the active principles, the qualitative evaluation of phytoconstituents aids in the selection of certain extracts for further investigation.

The current experiment's findings demonstrated that the extracts from the roots of *H. brasiliensis* provided a respectable yield of extracts (15.56%). Small quantities of alkaloids, particularly plant alkaloids, are presented in every edible plant element and support a variety of pharmacological actions in the bodies of both humans and animals.[11] Alkaloids can have detrimental side effects over time, including headaches, liver damage, nausea, and infections of the skin and pores.[12]

Any plant element has a broad spectrum of pharmacological effects, as evidenced by the existence of flavonoids, phenol, and tannin.[13] The current study discovered that extracts from the roots of *H. brasiliensis* included presence of phenol, flavonoids, carbohydrates, and alkaloids. These extracts may have active pharmacological effects.

Free radicals are associated with pain, inflammation, diabetes, cancer, liver damage, and several degenerative disorders. Antioxidants fight free radicals and offer protection against several degenerative disease types. With the correct reducing agents, DPPH, a strong unbound radical that targets nitrogen, can easily summarize an electron or hydrogen radical to build a powerful molecule. Consequently, the single electron in DPPH is coupled off to generate the corresponding hydrazine. Ethanolic extract of the roots of *H. brasiliensis* specifically inhibited the DPPH radical. This result showed that the extracts from *H. brasiliensis* roots might donate hydrogen or an electron to the DPPH radical in order for it to react. The findings of the study, which examined the anti-oxidants properties of natural sources and examined an extract from the roots of *H. brasilliensis*, are shown in Figure 1.

Inflammation is primarily caused by denaturation of proteins. The extract's capacity to reduce protein denaturation was tested as part of the inquiry into the mechanism of the anti-inflammatory effect.[14,15] The selected extract prevented denaturation of albumin. The findings of the study, which examined the anti-inflammatory properties of natural sources and examined an extract from the roots of *Hevea brasilliensis*, are shown in Figure 2. The study's findings indicate that the extract has the ability to prevent protein denaturation, indicating a potential major reduction in inflammation.[16] The study's findings suggested that the plant extract may inhibit the synthesis of two different kinds of inflammatory

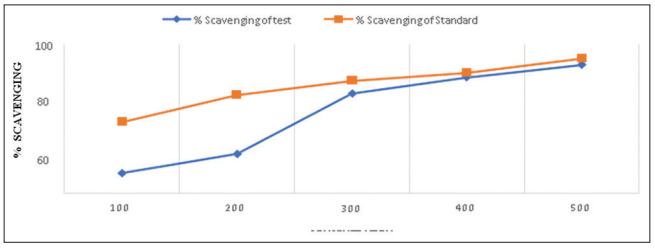

Figure 1: *In vitro* anti-oxidant activity of *Hevea brasiliensis* root extract

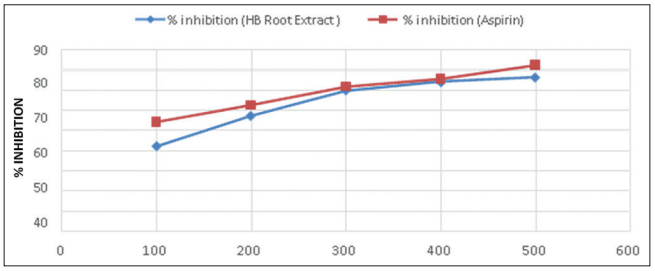

Figure 2: *In vitro* anti-inflammatory activity of *Hevea brasiliensis* root extract

Table 1: Results of phytochemical screening of ethanolic root extract of *Hevea brasiliensis*

Phytochemicals	Ethanolic root extract of *Hevea brasiliensis*
Carbohydrates	+ve
Alkaloids	+ve
Flavonoids	+ve
Phenolic compound and tannins	+ve
Saponins	-ve

"+ve" indicate the presence and "-ve" indicate absence

mediators, prostaglandins and leukotrienes. The presence of polyphenols and alkaloids in *H. brasilliensis* root extract may indicate its possible anti-inflammatory capabilities.

% Yield of the *H. brasiliensis* root extract:

% yield value = Weight of the extract/weight of the plant material ×100

= 4.675/30.028 × 100

= 15.56%

The qualitative analysis of plant secondary metabolites of roots of *H. brasiliensis* in ethanolic extract is represented in Table 1.

Antioxidant qualities of naturally occurring chemicals in higher plants have long been recognized. It is known that antioxidants affect the biochemical system in a number of ways, such as by scavenging free radicals, promoting reductive capacity, and preventing chain initiation. Because phytochemicals are complex compounds found in plant materials, evaluating their antioxidant capacity requires the application of multiple techniques.[17] Consequently, the current study assessed the lowering capability and DPPH free radical scavenging activity. In comparison to the standard ascorbic acid, the roots of *H. brasiliensis* demonstrated a notable scavenging activity in the DPPH scavenging assays. Figure 1 shows the extract's % scavenging values in relation to ascorbic acid for the DPPH scavenging techniques.

The albumin denaturation assay was utilized to assess the *in vitro* anti-inflammatory activity, and aspirin was the study's reference medication. Figure 2 shows the prevention of protein denaturation (mean %) in the *in vitro* anti-inflammatory activity of an ethanol extract derived from the roots of *H. brasiliensis*.

Chemicals known as antioxidants interact with free radicals and neutralize them to protect the cells of the organic system.[18] The body creates a large amount of antioxidants to reduce free radicals. These protecting substances are known as "endogenous antioxidants." However, the body receives the remaining antioxidants from exogenous (external) sources, mostly food.[19] These exogenous antioxidants are generally referred to as "nutritional antioxidants." Fruits, vegetables, and grains are rich sources of antioxidants that are high in nutrients.[20,21]

CONCLUSION

In the current investigation, DPPH, a free radical scavenger, was used to investigate the antioxidant capabilities of the plant extracts. Root extracts from *H. brasiliensis* are useful in treating some diseases because of their ability to scavenge free radicals. Antioxidant activities found in root extracts *of H. brasiliensis*.

Major anti-inflammatory properties are observed in the ethanolic extract of the roots of *H. brasiliensis*. It is therefore logical to conclude that the phytoconstituents present in the root extracts are what give them their anti-inflammatory benefits. The study findings support the traditional application of ethanolic extract from the roots of *H. brasiliensis* for the management of inflammatory diseases.

REFERENCES

1. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech 2005;4:685-8.
2. Baidya M, Anbu J, Akhtar MS. Antimicrobial evaluation of ethanolic extract of selected seed shells. Int J Curr Pharma Res 2020;12:74-6.
3. Deshmukh CD, Choudhari SP. Phytochemical and pharmacological profile of *Emblica officinalis* Linn. J Med P'ceutical Allied Sci 2021;10:2698-703.
4. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 2006;99:191-203.
5. Ndhlala A, Moyo M, Van Staden J. Natural antioxidants: Fascinating or mythical biomolecules? Molecules 2010;15:6905-30.
6. Ebo DG, Bridts CH, Rihs HP. *Hevea*latex-associated allergies: Piecing together the puzzle of the latex IgE reactivity profile. Exp Rev Mol Diagn 2020;20:367-73.
7. Justo OR, Simioni PU, Gabriel DL, Tamashiro WM, Rosa Pde T, Moraes ÂM. Evaluation of *in vitro* anti- inflammatory effects of crude ginger and rosemary extracts obtained through supercritical CO_2 extraction on macrophage and tumor cell line: The influence of vehicle type. BMC Complement Altern Med 2015;15:390.
8. Habib MA, Ismail MN. *Hevea brasiliensis* latex proteomics: A review of analytical methods and the way forward. J Plant Res 2020;134:43-53.
9. Chang LW, Yen WJ, Huang SC, Duh PD. Antioxidant activity of sesame coat. Food Chem 2002;78:347-54.
10. Zhang Y, Leclercq J, Montoro P. Reactive oxygen species

in *Hevea brasiliensis* latex and relevance to tapping panel dryness. Tree Physiol 2016;37:261-9.
11. Tripathi KD. Essentials of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers Pvt Ltd.; 2019.
12. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, *et al*. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci 2002;163:1161-8.
13. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20:69-73.
14. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
15. Bouayed J, Bohn T. Exogenous antioxidants-double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Long 2010;3:228-37.
16. Rahman MS, Islam MB, Rouf MA, Jalil MA, Haque MZ. Extraction of alkaloids and oil from Karanja (*Pongamia pinnata*) seed. J Sci Res 2011;3:669-75.
17. Soni P, Siddiqui AA, Dwivedi J, Soni V. Pharmacological properties of *Datura stramonium* L. as a potential medicinal tree: An overview. Asian Pac J Trop Biomed 2012;2:1002-8.
18. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res 2003;523-524:9-20.
19. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
20. Kotha RR, Luthria DL. Curcumin: Biological, Pharmaceutical, nutraceutical, and analytical aspects. Molecules 2019;24:2930.
21. Menon DB, Latha K. Phytochemical screening and *in vitro* anti- inflammatory activity of the stem of *Coleus forskohlii*. Pharmacogn J 2011;3:75-9.

Source of Support: Nil. **Conflicts of Interest:** None declared.