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Evaluation of Antioxidant and Anti-inflammatory Activity of Ethanolic Extract of Ficus heterophylla var. assamica Fruits

Md. Khalequeuzzaman, Ananta Kumar Das, Mohammad Rezwanur Alam, Sadia Afroz, Kazi Sumaiya, and Sadia Siraj

ABSTRACT

The objective of the current study was to investigate the antioxidant, anti-inflammatory, and analgesic activities of the ethanolic extract from the fruits of Ficus heteorphylla var. assamica (FHVAEE). Several in-vitro tests were performed on the plant's fruit ethanol extract. The antioxidant capability of diverse compounds was assessed using tests for total phenol content, total flavonoid content, DPPH free radical scavenging activity, and total antioxidant capacity. Protein denaturation was tested against anti-inflammatory properties. On a test for total phenol content, the fruit extract had a modest antioxidant capability; TPC as GAE was 120 ±3.14 mg/gm. Total flavonoid content assay results indicated exceptional antioxidant activity; TFC as QE was 428.33 ±7.07 mg/gm. The IC_{50} value for the DPPH free radical scavenging experiment was 61.17 µg/ml, while standard ascorbic acid was found to have an IC50 value of 16.97 µg/ml, indicating modest antioxidant activity. According to the anti-inflammatory assay, the plant fruit extract has excellent anti-inflammatory potential. The presence of several phytoconstituents in the extract could be the cause of these effects. To assess these effects and the results, which call for further advanced research, more studies must be done.

Keywords: Anti-inflammatory activity, antioxidant, DPPH, ethanolic extract, Ficus heterophylla var. assamica.

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I. INTRODUCTION

Since the beginning of human history, a wide variety of medicinal preparations have been made using plants. It was established that plants possessed the potential to be used as a source of medication after a series of investigations and experiments were carried out. As a direct consequence of this, continuing treatments utilising a wide variety of plants were carried out during the early phases of human civilization. Despite the dearth of evidence on the majority of medicinal plants, medicines derived from plants are typically safer than those made from synthetic ingredients [1], [2]. It is a well-established fact that free radical reactions can play a role in the pathophysiology of several diseases. In addition, it is well established that the reactions in question have a role in a number of acute and chronic illnesses that are found in humans, such as diabetes, atherosclerosis, ageing, immunosuppression, and neurodegenerative disorders [3]. In recent years, there has been a discernible rise in individuals' interest in naturally occurring antioxidants for use in various goods, including those utilised in the food, beauty, and medical industries. Because naturally occurring antioxidants have polyphase in their plurality and amount of activity, they offer a wide range of possibilities for redressing imbalances [4], [5]. The amount of foods high in antioxidants that are included in a person's diet has been shown to have a negative correlation with the number of human diseases that are recorded in research that was just recently made public. It is common knowledge that the denaturation of proteins is a factor in the development of inflammatory disorders such as rheumatoid arthritis and other types of arthritis. It has been proven that anti-inflammatory drugs such as phenylbutazone, salicylic acid, and flufenamic acid, amongst others, have the capability to inhibit thermally induced protein denaturation, and this ability is dose-dependent. Other anti-inflammatory treatments include salicylic acid and flufenamic acid. As part of the investigation into the mechanism that underlies the anti-inflammatory activity of the substance, the possibility of the extract being able to prevent the denaturation of proteins was investigated. Locally known as "Bhui dumur," the creeping or erect scan dent fig species Ficus heterophylla var. assamica (Family: Moraceae) belongs to the family Moraceae and can be found in rural and hamlet areas. Additionally, it can be found in South and Southeast Asian countries. As a

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form of traditional medicine on the Indian subcontinent, the juice extracted from the leaves and roots is employed [6].

II. Materials and Method

A. Collection and Identification of Plant

The regions of Amdala, Shibalaya, and Manikganj in Bangladesh were searched thoroughly in order to find fruits of the Ficus heterophylla variety assamica. After some time, the Department of Botany at Jahangirnagar University in Savar, which is located in Dhaka, Bangladesh, recognised that it existed.

B. Preparation of Plant Samples

The entire fruit was taken for examination, and its selection was random. They were exposed to the sun for a period of time in order to dry out before being handled, crushed, and dried once again. After that, the fruit pieces that were previously crushed were put through a mechanical grinder in order to produce a coarse powder. The fruit powders were stored in a location that was cool, dark, and dry before any analysis was performed on them. They were also kept in an airtight container.

C. Extraction of Powdered Samples

After being dried and ground, 500 grammes of dried fruit powder were placed in an amber glass jar with 1000 millilitres of ethanol and allowed to soak for five days while being stirred on a regular basis. After that, the extract was filtered not once but twice, the first time through a sterile cotton plug and the second time through Whatman filter paper. After the extraction process was complete, the solvent was eliminated by passing the sample through a rotating vacuum evaporator at a temperature of 40 degrees Celsius and a pressure of 2 pascals. The oily crude extract that was left over after the evaporation procedure was finished was collected on a fresh Petri plate. After that, the crude extract was kept in an area with a temperature of four degrees Celsius [7].

D. Chemicals

At each stage of the investigation, reagent-grade chemicals and several other compounds were utilised alongside their more general counterparts.

E. In-vitro Antioxidant Potential Evaluation

The antioxidant activity of the selected oily crude extract of Ficus heterophylla var. assamica was evaluated using a variety of in-vitro models, which are discussed further down in this piece of writing.

F. Determination of the Total Phenolic Content

With the help of the Folin-Ciocalteu Reagent (FCR), the total amount of phenolic compounds that were found in the sample could be determined [8]. The FCR and 1.5mL of sodium carbonate at a concentration of 20% were added to an extract solution that had a concentration of 200µg/mL. Before adding the distilled water to get the total amount up to 10 ml, the mixture was given a thorough shake. After allowing the mixture to settle for two hours, it was then ready for use. After that, the absorbance at 765 nm was determined to be measured. The total phenol content, expressed in terms of mg of gallic acid equivalent, was calculated with the help of an equation derived from a typical gallic acid graph.

G. Total Flavonoid Content Determination

The flavonoid content of the extract was determined with the use of the method developed by Kumaran and Karunakaran [9]. 3mL of ethanol was added to a test tube that already contained 1 mL of plant extract at a concentration of two 200 µg/mL. The contents of the test tube were then mixed with an additional 200 µl of a solution containing 10% aluminium chloride and 200 microliters of a solution containing 1M potassium acetate. Following that, 5.6 ml of distilled water was added to the test tube, and it was allowed to react at room temperature for a period of thirty minutes. The Standard (Quercetin) solutions were made in the same manner but with varying amounts of the active ingredient. In comparison to a blank, the absorbance of the solutions was determined using a UV-VIS spectrophotometer at a wavelength of 415 nm. After that, the amount of flavonoid content was calculated as mg of equivalent quercetin using an equation that was derived from a typical quercetin graph.

H. DPPH Radical Scavenging Assay

Braca *et al.* [10] described the method to evaluate the fruit extract's capacity to scavenge DPPH free radicals. The stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used to estimate the plant extract's ability to neutralise free radicals. A 0.004% ethanol DPPH solution and 0.1 ml of plant extract were combined. A UV-VIS spectrophotometer was used to measure the absorbance at 517 nm after 30 minutes. The percentage inhibitory activity was computed using the formula:

$$[(A0-A1)/A0] \times 100$$

where A0 represents the absorbance of the control, and A1 represents the absorbance of the extract/standard.
The IC_{50} values were calculated after the inhibition curves were produced.

I. Evaluation of In Vitro Anti-inflammatory Activity

To evaluate the extract's effectiveness as an anti-inflammatory agent, different quantities of the extract were submitted to an incubation process with egg albumin under carefully monitored experimental circumstances, after which the absorbance of the mixture was measured. The medicine diclofenac sodium was chosen to serve as the standard [11]. 1 mL of plant extract and 1 ml of standard medication, each at a different concentration (500, 400, 300, 200, and 100 µg/ml), were combined with 1 ml of egg albumin at a concentration of five percent. After adjusting the pH of the reaction mixtures to 5.6±0.2 with a solution of 1 N HCl, they were allowed to incubate at 27 ºC for 15 minutes. The mixture was heated to 70 ºC to induce denaturation and maintained in a water bath for ten minutes. After lowering the temperature, the turbidity was evaluated using spectrophotometry at 660 nm. The percentage of inhibited denaturation was calculated using the control group, in which no medication was introduced.

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III. RESULTS

A. In-vitro Antioxidant Potential Evaluation

1) Total Phenolic Content

The gallic acid standard curve (y = 0.0098x + 0.0458) was used to compute the total phenolic contents (Fig. 1). Gallic acid equivalents (GAE) milligram per gram of the fruit extract was calculated at 120 ± 3.14.

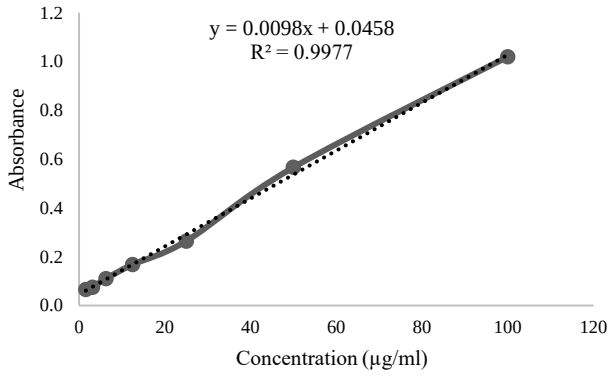

Fig. 1. Calibration curve of gallic acid.

2) Total Flavonoid Content

Flavonoid contents of the test sample were calculated using the Quercetin (y = 0.0043x - 0.0182) standard curve (Fig. 2). Quercetin equivalents (QE) milligram per gram of fruit extract were found to be 428.33 ± 7.07.

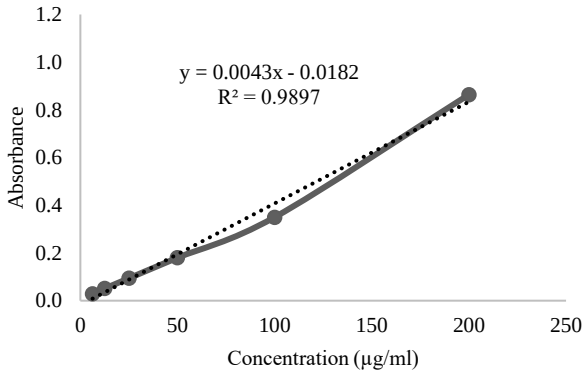

Fig. 2. Calibration curve of quercetin.

3) Free Radical Scavenging Activity

The plant extract's capacity to scavenge free radicals was dose-dependent. Fig. 3 shows the percentage of inhibition. The extract had a higher IC_{50} value for DPPH free radical scavenging than standard ascorbic acid (16.97 vs. 61.17).

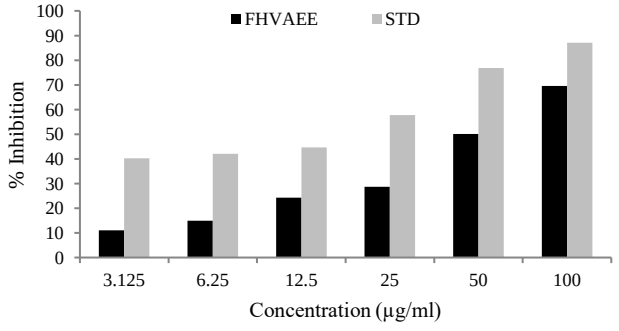

Fig. 3. Percentages of the inhibition of Ascorbic Acid (STD) and FHVAEE.

4) In Vitro Anti-inflammatory Activity

It is possible that the ability of the fruit extract of Ficus heterophylla var. assamica to prevent heat-induced protein denaturation contributes to the anti-inflammatory effects of this extract. When compared to standard Diclofenac Sodium, the extract demonstrated a dose-dependent reduction in protein denaturation, showing that it had powerful anti-inflammatory activities.

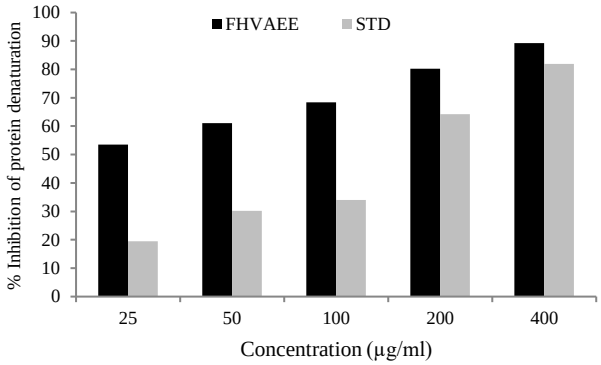

Fig. 4. Anti-inflammatory activity of diclofenac sodium and FHVAEE.

IV. DISCUSSION

In this study, the antioxidant properties of an ethanolic extract of Ficus heterophylla var. assamica fruits were analysed. Total phenolic content, flavonoids, and the DPPH scavenging assay were measured to determine the level of antioxidant activity.

The oxidation-reduction capabilities of phenolic compounds are the key mechanism that underlies the antioxidant action of these compounds. These properties can help absorb and neutralise free radicals by quenching single and triplet oxygen or degrading peroxides [12]. It is possible that the antioxidative effect of the extract is caused by the presence of phenolic components in the extract. The aluminium chloride colorimetric method was utilised in order to determine the total flavonoid concentration of the extract. Flavonoids, which are classified as a subclass of polyphenols, are the polyphenolic chemicals that are found in nature the most frequently. Flavonoids can be further subdivided into flavones, flavonols, isoflavones, anthocyanins, and proanthocyanidins. Flavones are the most common type of flavonoid. Flavonoids exert their antioxidative actions in a number of different ways, including by scavenging and chelating free radicals, blocking the enzymes that create free radicals, and chelating metal ions such as iron and copper [13]. The decolourisation caused by DPPH accepting an electron given to it by an antioxidant chemical can be calculated from changes in absorbance. Due to the harmful impact that free radicals have on dietary and biological systems, antioxidant characteristics, particularly radical scavenging activities, are crucial. Excessive free radical generation increases lipids' oxidation in food, reducing food quality and consumer acceptance. Since free radicals are well-known to play a major part in the cellular damage caused by oxidative stress, the DPPH assay is often used to estimate an antioxidant's capacity to suppress them. According to reports, phenolic compounds and flavonoids have an

antioxidative effect on biological systems by scavenging singlet oxygen and free radicals [14]. Ascorbic acid, carotenoids, and phenolic compounds are among the several naturally occurring antioxidants that are more efficient. In addition to preventing lipid peroxidation, they are known for their ability to bind heavy metal ions. Inflammation has been linked to the denaturation of proteins, which is widely known. Studies have shown that the power of anti-inflammatory drugs to inhibit thermally induced protein denaturation varies with the dose.

V. Conclusion

The antioxidant capacity of the fruit extract of Ficus heterophylla var. assamica was found to be moderate, but its anti-inflammatory capacity was found to be remarkable. It is possible that the extract contains pharmacologically active phytochemicals, and these effects are a direct result of their presence. Because the experiments in this study were all conducted with crude extract, the results should be regarded as preliminary at the very least, and additional, more sophisticated research is required before any firm conclusions can be drawn.

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Conflict of Interest

All the authors declare no competing interests.

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