Extraction of Humic Acid from Peat soil and Evaluation of its Anti-Inflammatory, Antioxidant, Antacid properties.

By

MD AJMAIN KHAN
21146051

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B.Pharm)

School of Pharmacy
BRAC University
2025

© 2025. BRAC University
All rights reserved.

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

MD AJMAIN KHAN
21146051

Approval

The thesis titled Extraction of Humic Acid from Peat soil and Evaluation of its Anti-Inflammatory, Antioxidant, Antacid properties." Submitted by MD AJMAIN KHAN (21146051), of Spring 2021, has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy on 20-11-2025.

Supervisor By:

Luluel Maknun Fariha
Senior Lecturer, School of Pharmacy
BRAC University

Approved By

Chairperson:

Raushanara Akter, PhD
Professor and Acting chairperson
School of Pharmacy
BRAC University.

Dean:

A.F.M Yusuf Haider, PhD
Acting Dean
School of Pharmacy
BRAC University

Ethics statement

I voluntarily donated my own blood for the purpose of this study. At the time of donation, I was in completely good health and followed all necessary precautions. Before proceeding, I also obtained approval from Brac University medical center. This procedure did not cause any harm.

Abstract

This study aimed to isolate and characterize humic acid from peat soil and assess its biological activity. The peat soil was validated with soil characterization tests, which assessed bulk density, organic matter content, and color. The degree of humification was assessed using the E4/E6 ratio (1.334) and log K value (0.058), showing a well-humified humic material. Humic acid was obtained by the alkaline-acid precipitation technique. The antioxidant activity, evaluated via the DPPH radical scavenging assay, demonstrated 58.69% inhibition at a dosage of 800 µg/mL concentration. The anti-inflammatory efficacy was assessed via the HRBC membrane stabilization method with human blood at three distinct concentrations, with E2 (200 µg/mL) demonstrating the greatest inhibition (35%) relative to diclofenac sodium as the reference standard. The Acid Neutralizing Capacity (ANC), obtained by the acid-base back titration method, validated modest buffering capability. Overall, the extracted Humic acid demonstrated promising antioxidant, anti-inflammatory, and neutralizing properties.

Keywords: Humic acid, Peat soil, E4/E6 ratio, log K value, DPPH assay, Anti-inflammatory activity, Acid Neutralizing Capacity.

Dedication

First and foremost, I am deeply grateful to Almighty Allah for granting me the strength, patience, and opportunity to complete this work successfully. I would like to express my heartfelt gratitude to my beloved parents for their endless love, support, and encouragement throughout my journey. I would like to express my heartfelt gratitude to Erena, who has supported me throughout my entire university life. Her constant encouragement and dedication have meant a great deal to me.

Table of content

Acknowledgement

Firstly, I would like to express my thankfulness to Almighty Allah for his unwavering blessings and assistance in completing the project. Also, my profound appreciation to all individuals who aided in the effective execution of this project.

I extend my profound gratitude to our beloved acting dean, A.F.M. Yusuf Haider, PhD, and also to Raushanara Akter, PhD, professor and chairperson of the School of Pharmacy at Brac University.

I wish to extend my heartfelt gratitude to my esteemed supervisor, Luluel Maknun Fariha, Senior lecturer, School of pharmacy, Brac University, for her exceptional guidance, steadfast support, and invaluable insights throughout the course of this project. Her profound expertise, patience, and constant encouragement have been pivotal in shaping my work and inspiring me to strive for excellence.

I express my gratitude to all faculty and personnel of the School of Pharmacy, Brac University, for furnishing the essential laboratory facilities and technical assistance necessary for the execution of this study.

I extend my sincere gratitude to the members of Dhaka University for their help for some Lab test.

I express my profound thanks to my family for their steadfast support, patience, and encouragement during this effort.

List of Figure

List of Table

List of Acronyms

WHO – World Health Organization

ROS - Reactive oxygen species

UV VIS- Ultra violet visible spectroscopy

DPPH- 2,2 Diphenyl-1picrylhydrazyl

FRAP- (Ferric Reducing Antioxidant Power)

FDA - U.S. Food and Drug Administration; drug discovery audits cited

IC_{50} — Half-maximal Inhibitory Concentration; potency metric

DNA- Deoxyribonucleic Acid.

DEA- Drug Enforcement Administration.

CHAPTER 1:

Introduction

People usually administer medication only in situations where it is considered essential, as their main function is to ease symptoms or completely remove the underlying illness. These medicines or drugs often come from natural sources like plants, animals, or soil. Later, they are processed or prepared through laboratories. Not only that, it's also created many synthetic medicines to treat diseases. Synthetic drugs are medicines that are chemically made in laboratories without using any natural sources. Since ancient times, people have been using natural ways to fight diseases. People often used whatever they had around them such as plants, herbs, soil and mixed them to create something that could heal. Over a period of time people learned how to use these natural elements more effectively. Even today, many of the medicines that are made in labs are based on things that originally come from nature. If we look a few hundred years back then, people relied fully on natural sources for treatment. By gradually making use of ancient resources, people are now able to develop modern medicines. For example, streptomycin is an antibiotic that was first discovered in a type of bacteria called *Actinobacteria*, which lives in the soil. It was the first antibiotic used to treat a serious disease like tuberculosis (Waksman & Schatz, 1944). Now, with the support of lab work, and using those same natural materials in a more precise way. This helps us make medicines that are more effective, purer, and faster to produce In Bangladesh, it can observe various types of medicine being practiced, such as folk medicine, Unani, Ayurveda, and homeopathy. Ayurveda is an ancient medical system that originated in India. In this method, diseases are diagnosed and treated based on an imbalance of the body's three supposed "doshas" or "tridoshas" Vata, Pitta, and Kapha.Since Bangladesh is part of the Indian.

In recent years, there has been a growing interest in alternative treatment methods and natural products that are derived from soil or vegetation. The primary reasons for this is misuse of synthetic drugs and that can result in severe complications, conventional medicines are occasionally ineffective or cause adverse effects, and a significant number of individuals worldwide still lack access to modern healthcare. Furthermore, it is widely believed that natural products are safer. (World Health Organization [WHO], 2002) Nevertheless, regulatory authorities do not always grant sanction to these herbal or soil-derived medicines. The World Health Organization has acknowledged the efficacy and potential of medicines

derived from soil and other natural sources. However, it has also emphasized the importance of ensuring the safety and purity of these medications through appropriate evaluation. Since peat soil is known to be a significant source of humic compounds, recent study has focused more on bioactives derived from soil. A significant portion of these compounds, humic acid, has a variety of functional groups, such as carboxyl and phenolic moieties, which support its membrane-stabilizing and antioxidant properties. These characteristics emphasize humic acid's potential therapeutic importance by allowing it to scavenge free radicals, anti-inflammatory activity, and demonstrate acid-neutralizing capability (ANC).

Humic acid's structural complexity, including molecule size and aromatic condensation, affects its biological action. Fractions with high solubility and aromatic content are obtained during extraction from peat, and these are frequently associated with increased bioactivity. Its cytoprotective benefits against oxidative and hypotonic stress can be assessed under controlled settings using in vitro assays, such as antioxidant testing and HRBC membrane stabilization.

This work is a systematic investigation of the antioxidant, Anti-inflammatory and acid-neutralizing qualities of humic acid generated from peat, correlating its structural features to observed biological effects in light of the increased interest in natural bioactives.

We hypothesize that peat-derived humic acid exhibits measurable antioxidant and membrane-stabilizing activities in vitro and demonstrates moderate ANC, supporting its potential as a natural therapeutic agent.

1.1 Soil as drug source

Bacteria and fungi naturally generate potent antibiotics and immunosuppressive medications in soil, which is a significant source of drug discovery. In the soil, a variety of microorganisms, including bacteria and fungi, generate critical chemicals that are beneficial to living. The utilization of drugs derived from soil has been prevalent for an extended period. However in recent years, researchers have devoted significantly more attention to this question. An examination of approved pharmaceuticals from 1981 to 2019 indicates that approximately 50% of the medications were derived from natural sources. Naturally these, 20–25% were sourced from sediment or microorganisms, while approximately 25% were derived from plants. The remaining 30% of medications were entirely manufactured using synthetic processes (Newman & Cragg, 2020). The reason for this is that these medications are not only effective in treating diseases, but they are also natural. Consequently, they have

fewer adverse effects and other complications than synthetically produced medications. Many of these compounds are employed as medications to aid in the prevention and treatment of a variety of human diseases. Over the past few decades, numerous significant pharmaceuticals have been identified in soil by scientists. The antibiotic streptomycin, which is derived from the soil bacterium Streptomyces, is a well-known example. This medication represented a significant advancement in the treatment of infectious diseases. Tetracycline, rifamycin, and cyclosporine are additional medications that are derived from soil. (Demain & Sanchez, 2009) Special chemicals known as "secondary metabolites" are generated by the microorganisms in the soil. These chemicals have the capacity to eliminate cancer cells, fungi, and hazardous germs. Consequently, scientists isolate these microorganisms from soil and investigate their capacity to generate these beneficial chemicals. Researchers are capable of analyzing the DNA of soil microorganisms with the assistance of contemporary technology. This facilitates the identification of previously unknown medications. Despite the presence of obstacles, such as the difficulty of cultivating certain microorganisms in the laboratory or the re-discovery of previously discovered medications, soil continues to possess significant potential for the discovery of novel pharmaceuticals. Different microorganisms are present in various varieties of soil in the country, including peat soil, forest soil, and desert soil. This diversity enhances the likelihood of discovering novel medications. In summary, soil is a critical ingredient in the production of pharmaceuticals. By effectively managing and safeguarding our soil, we can continue to develop vaccines and medications for a variety of diseases in the future.

1.2 Benefits and source of Peat Soil

Peat soil is a highly organic soil that is a specialized type that is produced by a combination of plant components that have endured partial decomposition over extended periods of time in anaerobic conditions that are saturated with water. This substance is characterized by a velvety texture, a high concentration of carbon-based organic materials, particularly Humic compounds, and a dark brown to black color.

Figure 1: Peat soil

According to the peat soil we collected, a substantial quantity of fibrous material and charcoal is frequently present in peat soil. The structural characteristics and the quantity of organic matter in the peat can be determined from the presence of these components. The quality of the peat soil has a positive relationship with the quantity of ash and fiber, which suggests a high concentration of organic residues (Joosten, H., & Clarke, D. 2002). This can be beneficial for the extraction of valuable compounds or for further chemical analysis.

The majority of peat soils in Bangladesh are situated in low-lying wetland regions, particularly in districts such as Gopalganj, Khulna, Sunamganj, and specific sections of Sylhet (Soil Resource Guide of Bangladesh 2005). The conditions in these regions are ideal for the development of peat, as they are characterized by dense vegetation, seasonal flooding, and inadequate drainage. The local community frequently employs the terms "marshy fields" or "peat basins" to describe these regions.

It is crucial to recognize that peat soil is essential for both ecological and economic reasons. In terms of the environment, it serves as a carbon sink, which aids in the regulation of the climate and the reduction of greenhouse gases on a global scale. Furthermore, it contributes to the preservation of the distinctive ecosystems of wetland areas, which provide habitats for a diverse array of animal and plant species.

Peat has been employed as a raw material for the production of organic fertilizer, a source of energy, and to improve the character of soil from a cost and value perspective. In recent years, there has been an increasing interest in the extraction and utilization of humic acid from peat soil due to its antibacterial, anti-inflammatory, and plant growth-promoting properties (Senesi, Plaza, Brunetti, & Miano, 2009).

1.3 Importance of Humic Acid extracted from Peat Soil

The microbial decomposition of plant and animal residues in terrestrial and aquatic environments results in the production of humic acid, a high molecular weight organic substance that is naturally formed. We can rapidly extract a substantial quantity of humic acid from peat soil, which is advantageous for us (Zhou, Xu, & He, 2016). Its chemical composition is intricate, encompassing a variety of functional groups, including carboxyl, hydroxyl, and phenolic groups, as well as aromatic rings. Humic acid is effective in a variety of biological activities as a result of these characteristics. A rich and efficient source for extracting humic acid is peat soil, which contains a high quantity of partially decomposed organic matter. The biological activities of humic compounds have garnered increasing scientific attention in recent years. Humic acid's antibacterial activity is one of its most critical attributes. It is beneficial in the prevention of infections because it has been demonstrated to inhibit the growth of specific hazardous bacteria and fungi in numerous studies. Humic acid is now recognized as a promising natural alternative in light of the global concern on antibiotic resistance. Furthermore, humic acid possesses anti-inflammatory properties that may be beneficial in the treatment of inflammation-related conditions, including cutaneous inflammation and arthritis. It is generally regarded as secure for use due to its tendency to produce fewer side effects. Additionally, humic acid possesses gastroprotective (anti-ulcer) properties. It is a potential natural remedy for gastric issues because it can help prevent the formation of stomach ulcers and supports their restoration. Furthermore, by eliminating toxins from the body and supplying nutrients to cells, humic acid enhances the immune system. It also contributes to the restoration of vitality and overall physical health. (Senesi, Plaza, Brunetti, & Miano, 2009). Humic acid is regarded as a promising natural compound in the pharmaceutical industry for all of the aforementioned reasons. It may be feasible to identify novel classes of safe, effective, and cost-effective medications that are derived from this compound through additional research and development in the future. Humic acid exerts its effects on microorganisms through a variety of mechanisms. Some of them are explicated in the subsequent points.

1.4 Biological Activity of Humic Acid

1) Targeting and damaging the bacterial cell membrane

The bacterial cell membrane can be attacked and disrupted by specific active compounds in humic acid, such as phenolic compounds and quinone structures. Consequently, the membrane degrades, resulting in the leakage of intracellular components and the bacterial cell's eventual demise.

2) Reactive oxygen species (ROS)

Reactive oxygen species (ROS) can be generated by humic acid. including molecules such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2). Ultimately, these reactive molecules cause damage to the cells by disrupting critical bacterial functions, including DNA synthesis and protein production.

Oxidative stress is the term used to describe this condition. The bacteria, as oxidative stress increases, become progressively weaker over time and ultimately perish.

3) Interaction with the bacterial cell wall

Humic acid has the ability to bind to critical structural components of the bacterial cell wall, such as lipopolysaccharides and peptidoglycan. This binding has the potential to compromise the cell wall's stability, resulting in its eventual vulnerability and reduced viability.

In conclusion, humic acid exhibits potent antibacterial properties through a variety of mechanisms. It induces the production of reactive oxygen species (ROS) and damages the bacterial cell membrane, resulting in oxidative stress. It deprives microbes of essential nutrients necessary for survival by binding essential metal ions, including iron and zinc. Furthermore, it leads to the death of bacterial cells by disrupting critical enzymatic and genetic processes and compromising the cell wall structure (OlK et al., 2005).

1.5 Rational of the project

The significance of extracting bioactive compounds from peat soil is steadily increasing, as it is a prospective field for the acquisition of medicinal and other valuable substances from a natural and sustainable source. Humic acid, fulvic acid, and other organic compounds are among the numerous natural molecules that compose peat soil, which is exceedingly abundant in organic matter. These substances are instrumental in the prevention and treatment of numerous diseases, as they possess natural antioxidant, antimicrobial, anti-inflammatory, and cellular protective properties.

As a result of the increasing global concern regarding the adverse effects of synthetic and chemical-based pharmaceuticals, as well as environmental pollution, there is a growing demand for safe and effective medicines that are derived from natural sources. Peat soil is regarded as a sustainable and environmentally benign source that has the potential to create new opportunities for pharmaceutical innovation from this perspective. The scientific investigation and evaluation of the bioactive components of peat soil, which include chemical analysis, biological assays, and pharmacological assessments, not only establish a scientific foundation for the validation of traditional uses but also facilitate the discovery of new medicinal compounds. Furthermore, these bioactive substances may be employed in agricultural and environmental conservation applications, including the enhancement of soil quality and the stimulation of plant growth. Therefore, the potential to bring about revolutionary changes in healthcare, agriculture, and environmental management is present in the extraction of bioactive compounds from peat soil and the assessment of their efficacy. This endeavor will not only substantially contribute to the sustainable utilization of the country's natural resources and socio-economic development, but it will also add new dimensions to scientific research.

1.5.1 Aim of the Study

The objective of this investigation is to assess the antioxidant and antibacterial properties of the extract extracted from peat soil through in vitro testing. Specifically, the potential and efficacy of humic acid as a bioactive agent will be examined after it is extracted from peat soil. The objective of this investigation is to investigate the potential of humic acid extract as a natural antioxidant and antibacterial agent, with potential applications in the pharmaceutical and environmental sectors.

1.5.2 Objective of the study

- Humic acid is extracted from peat soil by following an appropriate procedure.
- The antioxidant property is evaluated using the humic acid obtained from peat soil through in vitro methods.
- The anti-inflammatory and antacid properties are checked using in vitro methods.
- To evaluate and contrast the extract's antioxidant and antibacterial properties in anticipation of its potential application in pharmaceutical formulations.

1.6 Strength and limitation of the study

Number of studies have recently revealed numerous noteworthy biological activities of humic acid. It has been observed to possess antibacterial, antioxidant, and anti-inflammatory properties. Humic acid has been demonstrated to be effective in suppressing the proliferation of a variety of hazardous microorganisms, according to research. Furthermore, it aids in the regulation of the immune system and the mitigation of inflammation that is the result of various biological processes. Consequently, humic acid is being regarded as a potential natural therapeutic agent, which may provide new opportunities for its application in both medical and agricultural fields in the near future. Additionally, the utilization of modern instruments, including UV–Vis, FTIR, and NMR spectroscopy, has facilitated a more comprehensive and in-depth comprehension of the structure and functionality of humic compounds. Despite the fact that numerous studies have been conducted on humic acid, there are still some substantial limitations. The geographical diversity of the research has been

restricted by the use of leonardite, lignite, or commercially prepared humic products in the majority of these investigations. Particularly, there is a dearth of research on humic acid that is extracted from peat soil in South Asia.

The opportunity to analyze multiple effects simultaneously is diminished by the fact that many studies have concentrated on only one or two biological activities, such as antibacterial or anti-inflammatory effects. Additionally, it is challenging to replicate previous studies or conduct comparative analyses as a result of variations in extraction methods, dosage applications, and experimental protocols. (Muscolo et al., 2013).

CHAPTER 2:

Methodology:

2.1 Collection and Authentication of peat soil:

As mentioned earlier, this specific type of peat soil is found in very limited areas and was collected from near an enclosed wetland in the Koyra upazila of Khulna. Its precise geographical location has been documented (Haque et al., 2015). A verification was done from the Soil and Land Use Research Division, Khulna for authentication purposes. Peat soil has a High water retention capacity, allowing it to hold large amounts of moisture. It is usually acidic, with a pH ranging from about 3.5 to 5.5. Due to its high organic matter content, peat soil has a low bulk density, making it lightweight compared to mineral soils. However, it is generally poor in mineral nutrients when compared to mineral-based soils. Further confirmation, I conducted tests at the Soil and Water Environment Department of Dhaka University, where I obtained results for organic matter content, Color, pH, Bulk density as well. This is important to identify peat soil.

মৃত্তিকা, পানি ও পরিবেশ বিভাগ
ঢাকা বিশ্ববিদ্যালয়
ঢাকা ১০০০
বাংলাদেশ

Department of Soil, Water and Environment
University of Dhaka
Dhaka 1000
Bangladesh

04 September, 2025

Md. Ajmain Khan
School of Pharmacy
ID 21146051
BRAC University
Kha 224, Pragati Sarani
Merul Badda, Dhaka 1212.
Cell: 01647409817

Subject: Analytical Results on Supplied Soil Sample

Dear Sir,
As per your request, the supplied soil sample has been analyzed and the analytical results are given below;

Soil sample tested	Properties/Compositions	Characteristics/Contents
Soil sample for Peat Identification	Color	5YR 2.5/1 (Black)
	pH	3.14
	Organic matter contents (%)	21.34
	Bulk Density (g/cm³)	0.24

Thanks and regards,

(Professor Dr. ASM Mohiuddin)
Chairman

Telephone : 9661920-73/7470, Fax: (880-2) 9667222, e-mail: swed@du.ac.bd

Figure 2: Soil test result

From above we can observe. The organic matter content of the soil sample was found to be 21.84% (Dhaka University Lab, 2025).

In Bangladesh, soils with organic matter content exceeding 20% are classified as peat soils. These soils are characterized by high organic matter levels, often ranging from 18.2% to 45.5% in specific soil series like Satla and Harta(Bhuiyan, Haque, & Islam, 1999)

2.2 Extraction process:

Initially, 20 grams of peat soil were placed in an oven for drying, as some moisture was observed. The soil was kept in the oven for approximately 6–7 hours, and then passed through a mesh to obtain a particle size of 0.25 mm. In total, 15.6 grams of dried peat soil were obtained. On the other hand, 4 grams of sodium bicarbonate ($NaHCO_3$) were mixed with 80 mL of distilled water to prepare 80 mL of a 5% $NaHCO_3$ solution. Next, 15 grams of the dried peat soil were placed in a beaker and mixed with the 80 mL of 5% sodium bicarbonate solution. The mixture was then kept in an isolated condition for 24 hours to ensure it remained protected.

Subsequently, distilled water was added to the mixture based on a solid-to-liquid ratio of 1:20. Then the pH of the solution was maintained within the range of 10 to 12 by the addition of 5% sodium hydroxide (NaOH) solution because Humic acid is typically insoluble in water or other solvents under neutral conditions. However, when the solution's pH is elevated to an alkaline range of 10 to 12, the molecular structure of humic acid is altered, enhancing its solubility in aqueous media. The addition of sodium hydroxide (NaOH) raises the pH, facilitating the dissolution of humic acid into the liquid phase, thereby enabling its effective separation from the soil matrix. Without this increase in pH, humic acid remains strongly bound to the soil particles, making its extraction difficult. The mixture was then stirred continuously at 80°C for 2 hours. After the stirring process, the solution was subjected to centrifugation to separate the solid residues from the liquid extract.

The separated supernatant is then subjected to drying over a period of 24 to 48 hours, gradually maintaining the temperature between 40°C and 60°C. This controlled drying process ensures complete evaporation of moisture, ultimately yielding the final product in powdered form. Around 2.17 g humic acid powder obtained by this process. Gradual drying helps preserve the structural and functional integrity of humic acid, which may degrade at high temperatures.

Figure 3: Supernatant (Fulvic acid) and Humic acid powder

Schematic Representation of Humic Acid Extraction:

Peat Soil

↓

Drying

↓

Acid Treatment (HCl)

↓

Vaccume Filtration / Separation

↓

Humic Acid Dissolution (NaOH)

↓

Precipitation (pH adjust)

↓

Washing & Drying

↓

Powdered Humic Acid

2.2.1 Chemical, apparatus and Instruments

Table 1: Chemical and Apparatus

CHEMICAL	APPARATUS
NaHCO3	Falcon tube
NaOH	Oven
H2SO4	Beaker
	Volumetric flask
	Test tube
	Pipette
	Measuring Cylinder

Instruments

1. Centrifuge machine
2. Oven
3. Sonicate
4. PH meter
5. UV VIS Spectrometry
6. Weight machine
7. Water bath

2.2.2 Determination of E4/E6 Ratio for Humic Acid Characterization

0.03 grams of the dried humic acid powder were dissolved in 10 mL of 5% NaOH solution for absorbance measurement. A 5% NaOH solution was used as the blank. The absorbance values were recorded as 0.994 at 465.0 nm and 0.877 at 665.0 nm. We also performed 1:2, 1:4, 1:8 and 1:16 dilution and Obtained absorbance values at 465.0 nm were 0.382, 0.237, 0. 124, 0.070, respectively on the other side for 665.0 nm the observed absorbance is 0.375, 0.154, 0.092, 0.055 which follow Beer's Lambert law.

2.2.3 Ratio interpretation chart

Before dilution, (E4)/(E6) ratio of concentrated solution was calculated. The absorbance at 465 nm (E4) was 0.994, and at 665 nm (E6) it was 0.877.

The E4/E6 ratio, (E4)/(E6) = 0.994 ÷ 0.877 = 1.334

Approximately 1.35. Since this ratio is below 5, it indicates a high degree of humification and good quality of the humic acid.

According to Chen et al. (1977), the E4/E6 ratio can be used to assess the degree of humification and molecular characteristics of humic substances. The following chart summarizes the interpretation:

Table 2: Interpretation Chart

E4/E6	Interpretation	Quality
<5	Humic acid is characterized by a substantial molecular mass, compact structural configuration, and a high level of maturity in the humification process.	Good / Ideal
5-8	Moderate molecular weight, medium condensation	Moderate
>8	Low molecular weight, less humified, more fulvic in nature	poor

2.2.4 Calculation of LogK

Recorded absorbance at 456 nm is 0.997

Recorded absorbance at 665 nm is 0.877

According to Stevenson, F. J the formula is,
logK= log10 (ABS 456) - log10(ABS 665) = (log10 x 0.997) - (log10 x 0.877) = 0.058

According to Stevenson, F. J the interpretation is given below:

Table 3: Interpretation Chart

logK range	Characteristics
0.0-0.3	Highly condensed, high molecular weight, more aromatic, less soluble
0.3 - 0.5	Moderately condensed, medium molecular weight, moderately aromatic
0.6-0.9	Well humified, high aromaticity

The measured logK value of 0.058 (E4/E6 = 1.334) signifies that the humic acid derived from peat soil is substantially condensed, possesses a substantial molecular weight, and exhibits a stronger aromatic configuration. These humic acids demonstrate reduced solubility while enhanced stability, showcasing superior complexing and adsorptive characteristics. This observation aligns with prior research on humic chemicals (Stevenson, 1982).

2.2.5 Characterization of peat-derived humic acid (Yield, E4/E6 ratio, log K and pH)

Yield = (2.17 g ÷ 20 g) × 100 = 10.85

E4/E6 = 0.465 nm absorbance ÷ 0.665 nm absorbance = 1.33

log K = log10 (0.465 absorbance ÷ concentration) = 0.058

pH = 3.14

Parameter	Yield (%)	E_4/E_6	log K	pH
Peat-derived Humic Acid	10.85	1.334	0.058	pH 3.14

2.3 In-Vitro Antioxidant Activities:

The in vitro antioxidant test is conducted in a laboratory, and the main purpose of antioxidants is to neutralize free radicals. This allows us to observe the scavenging capacity of a sample. There are several methods for conducting antioxidant tests, among which notable ones are:

1. DPPH radical scavenging assay,
2. ABTS radical cation decolorization assay,
3. FRAP (Ferric Reducing Antioxidant Power), etc.

The results are usually expressed in terms of % inhibition. The higher the % inhibition, the greater the antioxidant property.

2.3.1 DPPH scavenging activity:

Humic substances contain phenolic and many other types of redox-active groups, which help scavenge free radicals. According to Braga et al., 2015, humic acid extracted from peat soil can be evaluated for free radical scavenging activity using the DPPH assay.

2.3.2 Reagents/chemicals

Name of reagent/chemical supplies

1. DPPH SIGMA ALDRICH USA
2. Methanol Active Fine Chemical LTD, Bangladesh
3. L-Ascorbic acid, Merck, German

Figure 4: DPPH SIGMA ALDHRIC USA

2.3.3 Reagent preparation

2mg DPPH Dissolved in 50 ml methanol and 0.004%(w/v). DPPH solution was established and stored in -4 degree celsius before use.

2.3.4 Sample and standard

Initially, 120 mg of humic powder is dissolved in 10 mL of methanol to prepare a 12 mg/mL stock solution.

The primary purpose of this stock solution is to carry out serial dilutions to obtain six different concentrations: 1200, 800, 400, 200, 100, and 50 µg/mL.

Subsequently, L-ascorbic acid is prepared in the same manner as a standard, following the same concentration range from 1200 to 50 µg/mL.

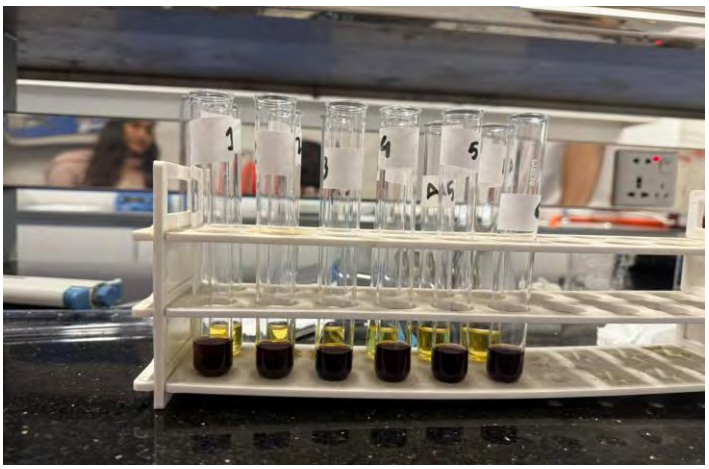


Figure 5: Different concentration of sample & standard

2.3.5 Preparation of blank solution

3 ml methanol was used to prepare the blank solution.

2.3.6 Experimental procedure

1. 1 mL of each sample and standard (L-ascorbic acid) is taken separately in individual test tubes.
2. 2 mL of 0.004% DPPH solution is added to each test tube.
3. The test tubes are incubated in the dark at room temperature for 30 minutes to allow the reaction to take place.

4. After incubation, the absorbance of each sample and standard is measured at 517 nm using a UV-Vis spectrophotometer (six absorbance readings for the standards and six for the samples).

5. The percentage of inhibition is then calculated using the appropriate equation given bellow:

   According to Brand-Williams (1995), %inhibition of free radical scavengers = (A0-A1) x 100/A0
   Where,
   A0 is the absorbance of the control
   A1 is the absorbance of the sample/ standard.

2.4 In vitro Anti Inflammatory Test

2.4.1 HRBC Membrane Stabilization Assay.

Preparation of Phosphate Buffer Saline (PBS):

- A total of 180 mL distilled water was taken.
- The following salts were added sequentially and dissolved completely: NaCl, KCl, Na_2HPO_4, KH_2PO_4.
- The pH of the solution was adjusted to 7.4 using dilute HCl.
- The final volume was made up to 250 mL with distilled water and labeled as PBS.

Table 4: Required amount of chemicals needed to make 250 ml PBS.

Component	Formula	Amount for 250 mL
Sodium chloride	NaCl	2.25 g
Potassium chloride	KCl	0.050 g
Dibasic phosphate	K_2HPO_4 (anhydrous)	0.060 g
Monobasic phosphate	NaH_2PO_4 (anhydrous)	0.360 g
Distilled water	H_2O	Up to 250ml

2.4.1 Collection of Blood Sample

• 5 mL of venous blood was collected from a healthy male donor in a tube where sodium citrate was added to the tube beforehand to prevent blood clotting.
• The sample was gently inverted 8–10 times to ensure proper mixing.

Figure 6: Human Blood

2.4.2 Separation of Red Blood Cells (RBC)

•The collected blood sample was centrifuged at 3000 rpm for 10 minutes.
• The upper plasma layer was discarded, and the packed RBC layer was retained.

Figure 7: Centrifuged Blood

2.4.3 Washing of RBC

• The packed RBCs were washed three times with 5 mL PBS each time.

• After each washing, the sample was centrifuged again at 3000 rpm for 10 minutes.

• The supernatant was discarded after each tube

2.4.4 Preparation of 10% HRBC Suspension

• The final packed cell volume (approximately 0.75 mL) was measured.

• PBS was added to make the final volume 5.0 mL.

• The resulting suspension represented 10% v/v HRBC suspension, stored on ice and used within 1 hour.

2.4.5 Preparation of Humic Acid Extract Solution

•First 1000 mg of dried humic acid extract was dissolved in 50 mL PBS and put into the sonicator.

Figure 8: Sonicator machine

•Then another 50 ml PBS was added to obtain a stock solution (10 mg/mL).

• From this stock, three concentrations were prepared using $C_1V_1 = C_2V_2$:

Table 5: Required amount of chemical

Target Conc. (µg/mL)	Stock Used (10mg/mL)	PBS Added (mL)	Final Volume (mL)
400	0.20 mL	4.80	5.0
200	0.10 mL	4.90	5.0
100	0.05 mL	4.95	5.0

2.4.6 Preparation of Standard Drug (Diclofenac Sodium)

- 10 mg diclofenac sodium was dissolved in 80 mL PBS.
- The final volume was adjusted to 100 mL with PBS.
- The solution was mixed thoroughly.

2.4.7 Preparation of Hypotonic PBS (0.25% NaCl)

The following chemicals were used to prepare 250 mL of hypotonic PBS:

Table 6: Chemical name and amount

Chemical	Amount (g)
NaCl	0.625
KCl	0.050
Na_2HPO_4	0.360
KH_2PO_4	0.060

- 250 ml Distilled water needed

2.4.8 Preparation of Assay Mixture

Table 7: Calculation of amount that needed to add

Tube	Hypotonic PBS (mL)	Test Solution (mL)	10% HRBC (mL)
B	—	0.5 mL PBS + 2.5 mL extract	—
N	2.0	0.5 mL PBS	0.5
S	2.0	0.5 mL Diclofenac	0.5
E1	2.0	0.5 mL Extract (100 µg/mL)	0.5
E2	2.0	0.5 mL Extract (200 µg/mL)	0.5
E3	2.0	0.5 mL Extract (400 µg/mL)	0.5

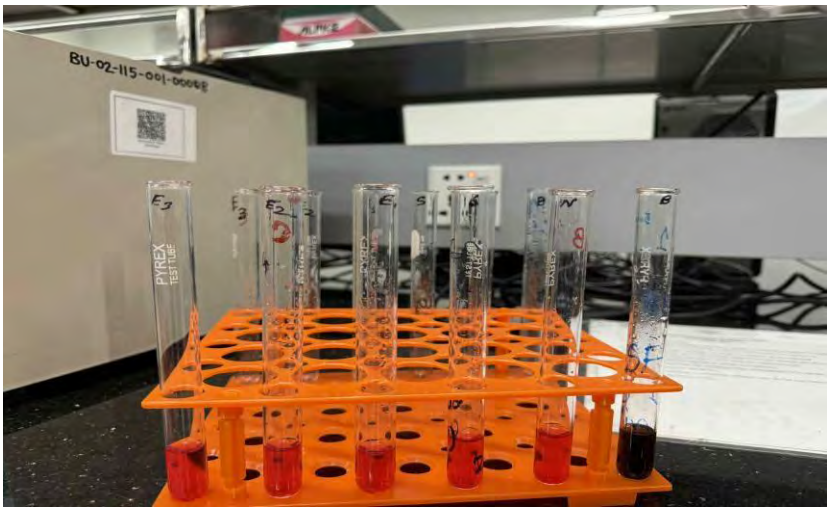


Figure 9: Different concentration of sample

2.4.9 Incubation and Measurement of Absorbance

• All tubes were incubated at room temperature for 30 minutes.
• Tubes were gently inverted after 15 minutes.
• After incubation, centrifugation was performed at 3000 rpm for 10 minutes.
• Supernatant was collected carefully.
• Absorbance was measured at 540 nm using a UV–Visible spectrophotometer.
• The blank was used to set the zero absorbance.

2.5 Back-titration ANC method

2.5.1 Materials

- Peat extract humic acid powder
- 0.1 N Hydrochloric acid (HCl)
- 0.1 N Sodium hydroxide (NaOH)
- Distilled water
- pH meter (or high-precision pH indicator)
- Beakers (100 mL or 250 mL)
- Burette, pipette
- Magnetic stirrer or glass rod

2.5.2 Sample Preparation

1. Analytical balance was used to weigh 0.10 g of humic acid powder.
2. In a 100 mL beaker, 50 mL distilled water was taken.
3. The weighed humic acid powder was added to the water and stirred using a magnetic stirrer for 5–10 minutes to form a uniform suspension.
4. Used sonicator for 10 minutes.

2.5.3 Acid Addition (Acidification)

1. The pH meter was calibrated using pH 4 and pH 7 buffers.
2. 1.3 mL of 0.1 N HCl was slowly added to the suspension while stirring.

3. Acid was added until the pH reached approximately 3.0 ± 0.1
4. The pH was allowed to stabilize for 10 minutes.

2.5.4 Back Titration

1. 0.1 N NaOH was taken in a burette.
2. 3.5 ml NaOH was added slowly to the suspension with continuous stirring.
3. The pH was monitored with a pH meter and when pH reached 7(+_ 1) Addition of NaOH stopped. The total volume of NaOH used was recorded.

CHAPTER 3:

Result

3.1 DPPH scavenging activity results

The sample and standard tests were repeated three times to ensure accuracy, and the results were averaged over the three trials. The percentage of inhibition was calculated according to DEA, 2003 is % Inhibition = ((A_control − A_sample) / A_control) × 100

3.1.1 Percentage Inhibition

The DPPH assay was performed to evaluate the antioxidant activity of the peat extract (humic acid) and the standard L-ascorbic acid. According to Brand-Williams (1995), the percentage of inhibition was calculated using the following equation:

% Inhibition = ((A0 − A1) / A0) × 100

Where, A0 is the absorbance of the control (DPPH solution without sample or standard), and A1 is the absorbance of the sample or standard. In this experiment, the control (A0) consisted of 2 mL of 0.004% DPPH solution and 1 mL of methanol, without any extract or ascorbic acid added.

Table 8: Results of 3 sample, mean & ± SD are given bellow:

Concentration ug/ml	N1%	N2%	N3%
1200	58.08	58.65%	58.43%
800	58.79	58.35	58.39
400	48.70	49.52	49.24
200	40.11	39.31	38.28
100	38.93	38.31	38.28
50	33.27	33.39	33.61

Mean, ± SD are given bellow:

- 1200 µg/mL: 58.38 ± 0.30
- 800 µg/mL: 58.50 ± 0.25
- 400 µg/mL: 49.15 ± 0.42
- 200 µg/mL: 39.97 ± 0.49
- 100 µg/mL: 38.51 ± 0.34
- 50 µg/mL: 33.42 ± 0.17

Table 9: Different samples % of inhibition

Concentration ug/ml	**N1%**	**N2%**	**N3%**
1200	63.16	63.02	63.34
800	59.27	59.42	59.50
400	55.77	56.10	55.51
200	48.26	48.47	47.80
100	42.91	42.47	42.37
50	39.05	39.07	38.91

Positive Control (% Inhibition, Mean ± SD)

- 1200 µg/mL: 63.18 ± 0.16
- 800 µg/mL: 59.40 ± 0.12
- 400 µg/mL: 55.79 ± 0.30
- 200 µg/mL: 48.13 ± 0.30
- 100 µg/mL: 42.58 ± 0.29
- 50 µg/mL: 39.01 ± 0.09

Table 10: Average of triplicates

Concentration (µg/mL)	**Sample (% Inhibition)**	**Positive Control (% Inhibition)**	**Sample Absorbance (A1)**	**Standard Absorbance (A1)**
1200	58.20%	63.23%	0.380	0.335
800	58.69%	59.63%	0.376	0.367
400	49.14%	55.81%	0.463	0.402
200	39.90%	48.25%	0.547	0.471
100	38.60%	42.80%	0.559	0.521
50	33.59%	38.60%	0.604	0.559

It shows that the antioxidant property of a sample increases with its concentration. This is because dilution reduces the amount of antioxidant present, whereas in a concentrated

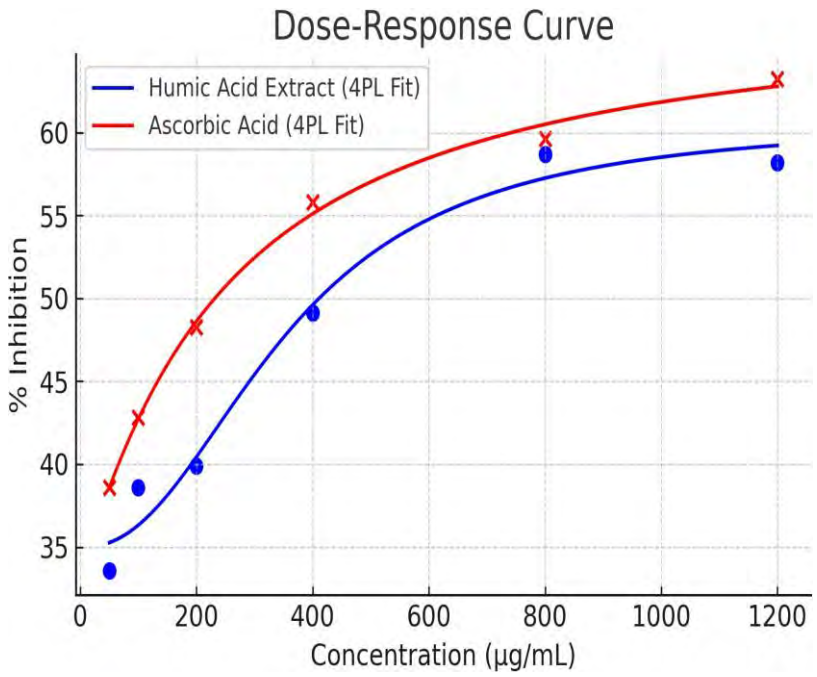
solution, a higher antioxidant content results in greater free radical scavenging activity. These results indicate moderate antioxidant activity for humic substances (Braca et al., 2002).

3.1.2 IC50 Value:

The % inhibition data at six concentrations were used to fit a 4-parameter logistic (4PL) dose-response curve. The 4PL equation used is:

Y = Bottom + (Top - Bottom) / (1 + (IC50 / X)^HillSlope)

where Y is % inhibition, X is concentration (µg/mL), Bottom and Top are minimum and maximum % inhibition, IC50 is the concentration at which 50% inhibition occurs, and HillSlope determines the curve steepness.



The percentage of inhibition in the sample increased with concentration in a dose-dependent manner. The maximum inhibition, which was somewhat lower than that of the positive control (63.23%), was recorded at 1200 µg/mL (58.20%). At 800–400 µg/mL (58.69–49.14%), moderate inhibition was seen, coming close to but falling short of IC_{50}. Significantly less inhibition was observed at lower values (200–50 µg/mL). Overall, the sample's IC_{50} fell short of the measured range, but the positive control showed more activity.

Sample	IC50 (µg/mL)
Humic Acid Extract	354.60
Ascorbic Acid	253.06

3.2 HRBC Membrane Stabilization Assay results

Table 11: Results of 3 sample are given bellow:

Sample	Replicate 1	Replicate 2	Replicate 3
Negative control	0.910	-	-
Standard	0.578	0.581	0.576
E1(100ug/ml)	0.685	0.688	0.687
E2(200 ug/ml)	0.613	0.616	0.615
E3(400ug/ml)	0.721	0.724	0.723

Table 12: Average result of triplicates.

Tube	Absorbance (540 nm)	% Protection
Standard (S)	0.58	36.26%
E1 (100 µg/mL)	0.687	25.27%
E2 (200 µg/mL)	0.615	32.42%
E3 (400 µg/mL)	0.723	20.55%
Negative control (hypotonic PBS)	0.910	_____

Calculation

% Inhibition: (Absorbance of negative control−Absorbance of sample)×100 is a standard calculation in HRBC membrane stabilization or antioxidant assays to determine the percent inhibition or protection of a sample compared to the control (Sultana et al., 2009).

Negative control (Hypotonic PBS) is 0.910.

Standard (S = 0.580)

% inhibition = (0.910 − 0.580) / 0.910 × 100 ≈ 36.26%

E1 (100 µg/mL, A = 0.680)

% inhibition = (0.910 − 0.687) / 0.910 × 100 ≈ 25.27%

E2 (200 µg/mL, A = 0.615)

% inhibition= (0.910 − 0.615) / 0.910 × 100 ≈ 32.42%

E3 (400 µg/mL, A = 0.723)

% inhibition = (0.910 − 0.723) / 0.910 × 100 ≈ 20.55%

Brief Analysis of % inhibition Results: The % Protection results indicate the relative efficacy of each sample in inhibiting the reaction compared to the control.

Table 13: Absorbance and %inhibition of Samples and Standard.

Tube	Absorbance (540)	% inhibition
S (standard)	0.580	36.26
E1	0.678	25.27
E2	0.615	32.42
E3	0.723	20.55

Interpretation:

- Standard (S) showed the highest % Protection (36.26%), indicating it is the most effective in inhibiting the reaction among all samples (Rahman et al. 23)
- E2 (200 µg/mL) showed moderate protection (32.42%), suggesting some efficacy.

- E1 (100 µg/mL) and E3 (400 µg/mL) exhibited lower % Protection (25.27% and 20.55%, respectively), indicating comparatively lower activity.

- Thus, the trend of potency is:
  Standard (S) > E2 > E1 > E3, where a higher % Protection corresponds to greater potency (Singleton et al., 1999).

3.3 Back titration results

All samples were analyzed in triplicate. ANC was calculated for each sample using the following formula:

ANC (meq/g) = (Volume of NaOH (mL) × Normality of NaOH (N)) / Weight of sample (g)

Table 14: Required amount of chemical

Sample	**pH Before HCl**	**HCl Added (mL)**	**NaOH Added (mL)**	**pH After NaOH**	**Weight of sample (g)**	**ANC (meq/g)**
1	5.2	1.4	3.6	7.4	0.1	3.6
2	4.7	1.7	3.8	7.4	0.1	3.8
3	4.7	1.6	3.2	7.4	0.1	3.2

According to (according to Wilde, 2008) ,

ANC (meq/g) = Volume of titrant (mL) × Normality of titrant (N) / Weight of sample (g)

Where:

V = Volume of titrant used (in mL)

N = Normality of the titrant (in meq/L)

W = Weight of the sample (in grams)

ANC = (3.6 mL × 0.1 N) / 0.1 g = 3.6 meq/g

ANC = (3.8 mL × 0.1 N) / 0.1 g = 3.8 meq/g

ANC = (3.2 mL × 0.1 N) / 0.1 g = 3.2 meq/g

The Acid Neutralizing Capacity (ANC) of the peat humic acid samples was determined using back titration, where NaOH was used to titrate the residual HCl (ANC = (Volume of NaOH × Normality of NaOH) / Weight of sample). The ANC values obtained for the three samples were 3.6, 3.8, and 3.2 meq/g, respectively, indicating moderate buffering capacity. These results suggest that humic acid extracted from peat soil possesses potential antacid activity (Rahman et al., 2020). Also its suggest that the humic acid can effectively neutralize acid, which is consistent with previous studies on soil organic matter and antacid formulations (Schulten & Schnitzer, 1997; Ayensu et al., 2020). The slight variation between samples may be due to differences in pH and HCl consumption during titration, reflecting the natural heterogeneity of peat extracts. Overall, the measured ANC values demonstrate that the samples possess significant acid-neutralizing potential, suitable for studies involving soil buffering or pharmaceutical applications.

CHAPTER 4:

Discussion

4.1 Acid Neutralization Capacity (ANC)

The acid-neutralizing capacity (ANC) test for the humic acid extracted from peat soil showed a moderate buffering potential, with values between 3.2 and 3.8 meq/g and an average of 3.6 meq/g. This intermediate ANC indicates that the humic acid contains a balanced proportion of ionizable functional groups—primarily carboxylic (−COOH) and phenolic (−OH) groups—which are essential for proton exchange and acid–base interactions.

The moderate ANC further suggests that the material is neither highly inert nor overly reactive. Instead, it points to a partially humified structure made up of both condensed, stable regions and more reactive domains. From our tests, the logK value (0.058) reflects moderate aromatic condensation, while the low E4/E6 ratio (1.334) indicates a well-condensed aromatic structure with higher molecular weight. These parameters suggest that the humic acid demonstrates a moderate aromaticity effect and a certain level of maturation. Which is in accordance with its observed buffering capacity.

The humic acid obtained from peat soil normally shows an effect in the range of 2.5 meq/g to 5.0 meq/g. We ovserved this from several previous study reports also the humic acid extracted from peat exhibits chemical characteristics that are comparable to those of naturally occurring humic substances, It shows a moderate effect but demonstrates functional capacity, which is essential for medical use and can also be applied in various fields of agriculture and environmental applications.

4.2 In vitro Anti-inflammatory Test (HRBC membrane Stabilization assay)

The HRBC membrane stabilization method has been used to evaluate the anti-inflammatory potential of the extracted humic acid, with diclofenac (S) being used as the standard. The results indicated a concentration-dependent effect, as the percentage of protection increased with concentration up to E2 (200 µg/mL). This was demonstrated by a 32.42% protection, which was higher than the 25.27% protection at E1 (100 µg/mL) and the 20.55% protection at E3 (400 µg/mL). This trend (S > E2 > E1 > E3) suggests that humic acid demonstrates optimal membrane stabilization at an intermediate concentration, indicating a balance between the potential aggregation or saturation effects at higher dosages and the active

molecular availability. Diclofenac's optimized solubility, polarity, and short molecular size, which facilitate interaction with erythrocyte membranes, resulted in a consistently higher percentage of protection (36.26%) than humic acid. In contrast, the maximal stabilization efficiency of humic acid may be restricted by the moderate aromaticity, partial polarity, and complex macromolecular structure, as demonstrated by the previously obtained E_4/E_6 ratio (1.334) and log K (0.058). This is due to the restriction of membrane penetration and the accessibility of active functional groups.

These results are consistent with previous research on humic substances and other natural extracts, which has shown that moderate membrane stabilization activity increases with concentration but remains below pharmacological standards. In general, the pattern observed underscores the anti-inflammatory potential of peat-derived humic acid, with E2 serving as the optimal concentration for the most effective protective effect.

4.3 in Vitro Antioxidant test (DPPH scavenging activity)

The antioxidant effect was evaluated using the DPPH radical assay with humic acid obtained from peat soil extract. The test showed a dose-dependent effect, where higher concentrations of humic acid exhibited greater activity. This occurs because more functional groups remain active at higher concentrations, enabling them to donate electrons or hydrogen atoms to neutralize free radicals. The moderate free radical scavenging activity of humic acid was compared with a standard potent antioxidant, and the results showed that the IC50 value of peat-derived humic acid (approximately 354.6 µg/mL) was relatively higher than that of ascorbic acid (253.1 µg/mL).

Humic acid typically neutralizes DPPH radicals by donating electrons through its carboxylic and phenolic functional groups, thereby exhibiting free radical scavenging activity. Its moderate antioxidant capacity can be attributed to several factors, one of the most important being its condensed aromatic ring structure, which is evident from the E4/E6 ratio (1.334) and log K value (0.058). This structural feature helps stabilize unpaired electrons within the aromatic system.

Various factors influence the interaction between free radicals and humic acid, such as slower reaction kinetics, limited solubility, and minor experimental errors, all of which may cause variations in the percentage of inhibition.

We also found similarities between our results and several previous reports, which indicates that the humic acid obtained from peat soil exhibits properties consistent with a natural antioxidant. This confirms that humic substances can function as natural antioxidants,

despite their lower potency than small-molecule standards such as ascorbic acid. In general, these results underscore the antioxidant potential of humic acid, which exhibits an increase in efficacy as the concentration is increased.

Conclusion

In this study, peat soil is thoroughly examined from which humic acid was extracted having acid-neutralizing, antioxidant, and anti-inflammatory properties. Humic acid has a good capacity to neutralize acidic conditions and this was proved by ANC values that ranged from 3.2 to 3.8 meq/g showing moderate buffering potential which can be used in pharmaceutical applications as well as for soil improvement. In the anti-inflammatory test, the standard sample showed the highest protection at 36.26%, followed by E2 at 32.42%, E1 at 25.27%, and E3 at 20.55% which indicate that humic acid helps to stabilize protein structures and reduces denaturation giving anti-inflammatory effect. Differences in the samples activity may change from variations in pH and the chemical composition of the Humic Structure. As the concentration increased, the antioxidant effect also rise in a clear dose-dependent manner. The highest inhibition (58.20% at 1200 μg/mL), close to the standard of ascorbic acid, likely comes from the phenolic and carboxylic groups present in the humic Acid.

Overall, the results show that peat-derived humic acid has notable anti-inflammatory, antioxidant, and buffering properties, making it a promising natural bioactive compound. These findings also open the door for future research to explore its therapeutic, industrial, and environmental uses as a sustainable and effective material.

Reference

Ahmad, I. (2014). *Unani medicine: History and current status*. Journal of Ethnopharmacology, 152(2), 457–461.

Ayensu, I., et al. (2020). *Evaluation of acid neutralizing and buffering capacities of selected antacids.*

Bloom, P. R., Peranginangin, N., Wagner, S. W., & D.C. (2005). *Environmental and agricultural relevance of humic fractions extracted by different methods*. Geoderma, 126(3–4), 177–192. https://doi.org/10.1016/j.geoderma.2004.09.0

Brown, P. A., Gill, S. A., & Allen, S. J. (2000). *Metal adsorption by peat and peat-derived materials*. Journal of Chemical Technology & Biotechnology, 75(5), 394–402.

Demain, A. L., & Sanchez, S. (2009). *Microbial drug discovery: 80 years of progress*. The Journal of Antibiotics, 62(1), 5–16. https://doi.org/10.1038/ja.2008.16

Dragunova, T., Kalinina, O., & Tolpeshta, I. (2017). *Humic substances isolated from peat soils: Extraction efficiency and chemical characteristics*. Eurasian Soil Science, 50(8), 907–915.

Hsu, J. H., & Lo, S. L. (2000). *Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure*. Environmental Pollution, 109(2), 201–208.

Joosten, H., & Clarke, D. (2002). *Wise use of mires and peatlands: Background and principles including a framework for decision-making*. International Mire Conservation Group and International Peat Society.

Kujawinski, E. B., & Behn, M. D. (2006). *Automated analysis of complex natural organic matter using Fourier transform ion cyclotron resonance mass spectrometry*. Analytical Chemistry, 78(13), 4363–4373.

MacCarthy, P. (2001). *The principles of humic substances chemistry*. Soil Science, 166(11), 738–751.

Muscolo, A., Sidari, M., & Nardi, S. (2013). *Humic substances: Relationship between structure and activity. Deeper insights into the effects on soil and plants*. Geoderma, 209–210, 65–73.

Newman, D. J., & Cragg, G. M. (2020). *Natural products as sources of new drugs from 1981 to 2019*. Journal of Natural Products, 83(3), 770–803.

Schulten, H.-R., & Schnitzer, M. (1997). *The chemistry of soil organic matter* (pp. 225– 230). John Wiley & Sons.

Senesi, N., Plaza, C., Brunetti, G., & Miano, T. M. (2009). *Chemical characterization of humic substances as tools for a sustainable agriculture*. Advances in Agronomy, 97, 267–348.

Stevenson, F. J. (1982). *Humus chemistry: Genesis, composition, reactions*. Wiley-Interscience.

Stevenson, F. J. (1994). *Humus chemistry: Genesis, composition, reactions* (2nd ed.). Wiley-Interscience.

Sultana, B., Anwar, F., & Przybylski, R. (2009). *Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolana Lam. trees*. Food Chemistry, 104(3), 1106–1114. https://doi.org/10.1016/j.foodchem.2007.11.021

Tan, K. H. (2014). *Humic matter in soil and the environment: Principles and controversies* (2nd ed.). CRC Press.

Waksman, S. A., & Schatz, A. (1944). *Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria*. Proceedings of the Society for Experimental Biology and Medicine, 55(1), 66–69.

World Health Organization. (2002). *Traditional medicine strategy 2002–2005*. Geneva: WHO.

Ye, R., Wright, A. L., & Orem, W. (2017). *Characteristics of peat soils and implications for extraction and environmental applications*. Soil Science Society of America Journal, 81(4), 937–948.

Zhou, Y., Xu, W., & He, H. (2016). *Extraction and characterization of humic acid from peat soil: An efficient approach*. International Journal of Environmental Science and Technology, 13(5), 1281–1288.

Kujawinski, E. B., & Behn, M. D. (2006). *Automated analysis of complex natural organic matter using Fourier transform ion cyclotron resonance mass spectrometry*. Analytical Chemistry, 78(13), 4363–4373.

Brown, P. A., Gill, S. A., & Allen, S. J. (2000). *Metal adsorption by peat and peat-derived materials*. Journal of Chemical Technology & Biotechnology, 75(5), 394–402.

Project

ORIGINALITY REPORT

10%	8%	4%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to BRAC University Student Paper	1%
2	dspace.bracu.ac.bd Internet Source	1%
3	pdffox.com Internet Source	<1%
4	clinphytoscience.springeropen.com Internet Source	<1%
5	pmc.ncbi.nlm.nih.gov Internet Source	<1%
6	Submitted to Shaheed Benazir Bhutto Women University, Peshawar Student Paper	<1%
7	Submitted to University of Wolverhampton Student Paper	<1%
8	www.actascientific.com Internet Source	<1%
9	ebin.pub Internet Source	<1%
10	Eskıer, Nazli Eda. "Anti-Kanser Tek Domainli Antikor Takımlarının Geliştirilmesi.", Dokuz Eylul Universitesi (Turkey) Publication	<1%
11	www.bindingdb.org Internet Source	<1%

12	biomedpharmajournal.org Internet Source	< 1 %
13	Submitted to Vels University Student Paper	< 1 %
14	www.doublehelixresearch.com Internet Source	< 1 %
15	www.freepatentsonline.com Internet Source	< 1 %
16	"Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems", Wiley, 2009 Publication	< 1 %
17	Submitted to De Montfort University Student Paper	< 1 %
18	Santwana Padhi, Anindita Behera. "Targeted Nanotechnology - Enhancing the Efficacy of Natural Anticancer Drug Therapies", CRC Press, 2025 Publication	< 1 %
19	Uche-Okereafor, Nkemdinma Chinezurum. "Studies on Solanum Mauritianum Scopoli Bacterial Endophytes and the Effects of their Crude Secondary Metabolites on Common Pathogenic Bacteria and Resistant Human Cancer Cells", University of Johannesburg (South Africa), 2023 Publication	< 1 %
20	bmccomplementmedtherapies.biomedcentral.com Internet Source	< 1 %
21	nmbu.brage.unit.no Internet Source	< 1 %

22	Submitted to Florida International Baccalaureate Consortium Student Paper	< 1 %
23	Submitted to Maharishi University Of Information Technology, Lucknow Student Paper	< 1 %
24	Romero, E.. "Humic acid-like fractions in raw and vermicomposted winery and distillery wastes", Geoderma, 20070515 Publication	< 1 %
25	jopr.mpob.gov.my Internet Source	< 1 %
26	www.plantsjournal.com Internet Source	< 1 %
27	docshare.tips Internet Source	< 1 %
28	dspace.bracu.ac.bd:8080 Internet Source	< 1 %
29	patents.glgoo.top Internet Source	< 1 %
30	www.researchgate.net Internet Source	< 1 %
31	depot.umc.edu.dz Internet Source	< 1 %
32	file.scirp.org Internet Source	< 1 %
33	Submitted to Yeditepe University Student Paper	< 1 %
34	chembioagro.springeropen.com Internet Source	< 1 %
35	hdl.handle.net	

	Internet Source	<1%
36	icfre.gov.in Internet Source	<1%
37	mail.rmib.mx Internet Source	<1%
38	patentimages.storage.googleapis.com Internet Source	<1%
39	va.mite.gov.it Internet Source	<1%
40	Malise, Tshifhiwa. "Vegetation Classification, Soil Organic Carbon and Water Levels of the Waterkloof Spruit Peatland System, Kgaswane Mountain Reserve, South Africa", University of South Africa (South Africa) Publication	<1%
41	Romeo M. Flores, Tim A. Moore. "Peats, peatlands, peat gases, and depositional systems", Elsevier BV, 2024 Publication	<1%
42	Tatsuya Ohkawara, Jun Nishihira, Hiroshi Takeda, Kencho Miyashita, Kanji Kato, Mototsugu Kato, Toshiro Sugiyama, Masahiro Asaka. "Geranylgeranylacetone protects mice from dextran sulfate sodium-induced colitis", Scandinavian Journal of Gastroenterology, 2009 Publication	<1%
43	Ton Duc Thang University Publication	<1%
44	Submitted to UCL Student Paper	<1%
	baptcb.org	

45	Internet Source	<1%
46	bbrc.in Internet Source	<1%
47	ijpsr.com Internet Source	<1%
48	ijrpr.com Internet Source	<1%
49	ris.utwente.nl Internet Source	<1%
50	www.omicsonline.org Internet Source	<1%
51	Mahmuda Akter, Sadia Afrin, Sk. Nazmus Sakib, Rana Biswas, Md. Morsaline Billah, Mohammad Shahedur Rahman, Umme Salma Zohora. "Investigation of Antibacterial, Cytotoxic and Antioxidant Properties of the Mangrove Plant Xylocarpus mekongensis", Advances in Bioscience and Biotechnology, 2016 Publication	<1%
52	Palanisamy Prakash, Ekambaram Gayathiri, Mostafizur Rahaman, Govindasami Periyasami et al. "Exploring the potential of targeting insulin-like growth factor-1 through network pharmacology, molecular docking, molecular dynamics, and experimental validation of antioxidant and anti-inflammatory activities", South African Journal of Botany, 2023 Publication	<1%
53	Biswakanth Kar, RB Suresh Kumar, Indrajit Karmakar, Narayan Dola, Asis Bala, Upal K	<1%

Mazumder, Pallab K Hadar. "Antioxidant and in vitro anti-inflammatory activities of Mimusops elengi leaves", Asian Pacific Journal of Tropical Biomedicine, 2012
Publication

54 Romina Torres-Carro, María Inés Isla, Samanta Thomas-Valdes, Felipe Jiménez-Aspee et al. "Inhibition of pro-inflammatory enzymes by medicinal plants from the Argentinean highlands (Puna)", Journal of Ethnopharmacology, 2017 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		