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# Assessment of the Potential Anti-diabetic, Anti-Alzheimer, and Anti-inflammatory activities of *Amphora coffeaeformis* Extracts, *in vitro* Study

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## ABSTRACT

Our study aimed to evaluate the *in vitro* biological efficacy of various extracts of *Amphora coffeaeformis* against disease-causing enzymes. The key active phytochemicals level (total polyphenolic compounds, total condensed tannins, and total flavonoid content) was quantified in all extracts. Antioxidant activity, as indicated by total antioxidant capacity (TAC) and iron reducing power (IRP), was assessed along with the ability to scavenge 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide (NO) radicals. The anti-diabetic potential was determined by measuring the inhibitory effects of each extract on α-amylase and α-glucosidase enzymes. Anti-Alzheimer's activity was evaluated by calculating the inhibition percentage of acetylcholinesterase (AChE). Anti-arthritic properties were assessed by measuring the inhibition percentage of protein denaturation and proteinase activity. Additionally, the *in vitro* anti-inflammatory effects were evaluated by measuring the inhibition percentage of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and 5-Lipoxygenase (5-LOX). Cytotoxicity was tested against human hepatocellular (HepG-2), colon carcinoma (Caco-2), and lung cancer (A549) cells, and the enzymatic activity of each extract on caspase-3 and Bcl-2 within these cancer cells was quantified. The results showed that the methanolic extract of *A. coffeaeformis* had the highest concentration of active phytochemicals, followed by acetone and ethyl acetate extracts. Consequently, it exhibited the highest antioxidant, scavenging, anti-diabetic, anti-Alzheimer's, anti-arthritic, anti-inflammatory, and cytotoxic activities, supported by increased caspase-3 activity and decreased Bcl-2 levels in the cancer cells studied. The ethyl acetate extract of *A. coffeaeformis* showed the lowest *in vitro* biological activities. In summary, the methanolic extract of *A. coffeaeformis* demonstrated the highest *in vitro* biological activities, followed by acetone and ethyl acetate extracts.

***Keywords:*** *Amphora coffeaeformis*, Polyphenolics, Tannins, Anti-diabetic Activity, Anti-Alzheimer Activity, Anti-inflammatory Activity.

## 1. Introduction

The development of diabetes, Alzheimer's, and arthritic disorders is due to degenerative processes that lead to inflammation, neuronal damage, and protein misfolding, triggered by oxidative stress and excessive lipid oxidation (Dash *et al*., 2025). Diabetes mellitus is a metabolic disorder characterized by high levels of glucose in the blood. The enzymes α-amylase and α-glucosidase play a role in regulating blood glucose levels. α-amylase breaks down carbohydrates into disaccharide molecules, and α-glucosidase converts these disaccharides into monosaccharide molecules (Hasaninezhad *et al*., 2020). As a result, the inhibitory effects of various natural compounds on both enzymes were assessed to measure their anti-diabetic properties, which were compared to those of acarbose, a standard reference drug (Kicel *et al*., 2022).

Alzheimer's disease is a neurodegenerative condition caused by oxidative stress, characterized by mood changes, disruptions in rational thinking, and a decline in memory and cognitive function. In

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cholinergic synapses associated with β-amyloid plaques, acetylcholinesterase (AChE) is a reliable enzyme that breaks down acetylcholine. Blocking AChE is one treatment strategy for managing Alzheimer's disease (Chen *et al*., 2022).

Protein denaturation and increased proteinase enzyme activity are the underlying factors of arthritis, a type of inflammatory disease. Therefore, inhibiting these processes is considered a promising treatment strategy for arthritis. Understanding the relationships between the diverse biological functions of natural substances is essential for a comprehensive evaluation of their potential therapeutic advantages (Tarjányi *et al*., 2025).

The primary approach to preventing and managing chronic diseases caused by excessive lipid oxidation and inflammation involves the utilization of exogenous antioxidants (Blagov *et al*., 2024). The researchers have been tasked with exploring nature to discover natural products that are both effective and economically feasible for treating a range of chronic diseases (Aware *et al*., 2022). Marine algae contain a wide range of beneficial phyto-constituents, leading to a diversity of biological activities. The primary and secondary metabolites produced by metabolic enzymes have been identified and developed as a viable antibiotic substitute, gaining significance in the fight against various diseases (Carpena *et al*., 2022).

The characteristics of the maritime environment, such as the varying levels of salt, temperature, and light, make compounds derived from marine species very intriguing (Gallego *et al*., 2019). To increase the availability and chemical diversity of marine functional components, more research is being conducted to identify and create marine chemicals using biotechnological techniques. Medical technology has greatly benefited from the use of organic compounds derived from marine algae and invertebrates, and there is potential for further diversification (Sigwart *et al*., 2021).

Marine bio-resources produce a wide range of specialized and powerful bioactive compounds, including natural organic molecules such as peptides, proteins, polyethers, fatty acids, polysaccharides, and enzymes (Karthikeyan *et al*., 2022). Because of their many important and unique characteristics, such as their ability to foam, form gels, and have antimicrobial properties, proteins from marine sources show promise as functional ingredients in food (Shahidi and Saeid, 2025).

Phenolic compounds are attractive for biotechnological applications because they are part of a class of secondary metabolites with recognized biological roles. One of the most promising resources for creating environmentally friendly, bioactive metabolites is microalgae (Del Mondo *et al*., 2021). Microalgae are photosynthetic organisms that live in watery settings and generate carotenoids and fatty acids. Their biological and functional variety is extensive. Additionally, they contain many other advantageous chemical groups, such as phenolic compounds (Cadar *et al*., 2025).

It is important to note that algae are capable of producing such useful and beneficial natural compounds. Because of this, the use of algae in therapeutic applications has garnered international interest in recent years (Penggalih *et al*., 2023).

*Amphora coffeaeformis* (Agardh) Kutzing is commonly found in alkaline freshwater and brackish water environments (Mansour *et al*., 2023). Heavy metals, particularly copper and cadmium, are present in the aqueous A. coffeaeformis waste, which affects the development of the organism and enhances the chelating effectiveness of the metals. These metals also significantly decrease the amounts of fat, protein, and carbohydrates (Anantharaj *et al*., 2011). *A. coffeaeformis* was found to contain numerous photosynthetic pigments, such as chlorophyll, β-carotene, and fucoxanthin, which exhibit various biological activities, including antibacterial, antiobesity, anticancer, and antioxidant properties. These pigments also have potential medical applications due to their abundance in the organism (Abdoul-Latif *et al*., 2024). Compared to the chlorophyll content (5.0%), the carotenoid content (1.083% of the dry matter) was more significant (Chtourou *et al*., 2015). Carotenoids and phenolics both have strong antioxidant properties that protect against peroxidation reactions and free radical attacks. Accordingly, the algal extract from *A. coffeaeformis* shown encouraging scavenging capabilities against hydrogen peroxide-induced DNA damages (Pérez-Gálvez *et al*., 2020). In view of the above, this study has been designed to evaluate the potential *in vitro* biological activities such as anti-diabetic, anti-Alzheimer, anti-inflammatory, and cytotoxic effects of various *A. coffeaeformis* extracts.

## 2. Materials and Methods

### 2.1. Alga mass production

*A. Coffeaeformis* was isolated from drainage water in Ismailia Governorate and identified by the Algal Biotechnology Unit at the National Research Centre in Dokki, Giza, Egypt. The F2 nutrient solution was used for laboratory growth, and outdoor mass production took place in a semi-closed photobioreactor with a final capacity of 1200L. The outdoor nutrient solution was created using commercial fertilizer compounds as recommended by El-Sayed *et al*. (2001). The biomass was obtained, cleaned through water washing and centrifugation to remove impurities, then freeze-dried and stored separately at -20°C until needed (Matloub *et al*., 2017).

### 2.2. Preparation of different algal extracts

After 72 hours of incubation at 50 °C, the new vegetative algal material was ground into a powder. Methanol, ethyl acetate, and acetone were used individually to extract the dried algal powder multiple times before it was left in the dark at 4°C overnight. After centrifuging for five minutes at 10,000 rpm, all the extracts were filtered. Following the method described by Sarkar *et al*. (2012), all filtrates were evaporated under pressure at 40°C until completely dry.

### 2.3. *In vitro* study on the algal extracts

#### 2.3.1. Quantitative determination of major phyto-constituents

The concentrations of total polyphenolic compounds, total condensed tannins, and total flavonoid content were measured in all extracts using the procedure recommended by Singleton and Rossi (1965), Broadhurst and Jones (1978), and Arvouet-Grand *et al*. (1994), respectively. The results were reported as mg gallic acid/100g, µg/mL, and mg quercetin/100g, respectively.

#### 2.3.2. *In vitro* biological activities

All biological activities were assessed in triplicate for all studied extracts.

##### 2.3.2.1. Antioxidant activity

The total antioxidant capacity (TAC) was measured following the procedure outlined by Prieto *et al*. (1999) and reported as mg gallic acid equivalent per gram weight. The iron reducing power (IRP) was determined as µg/mL using the method described by Oyaizu (1986) with ascorbic acid as the standard.

##### 2.3.2.2. Scavenging activity

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity was evaluated using the method described by Rahman *et al*. (2015). Ascorbic acid was used as a positive control at the same concentrations. The inhibition percentage of the DPPH free radical was calculated. The median inhibitory concentration ($IC_{50}$) of each tested extract was determined by plotting a curve using a series of sample concentrations against the percentage of DPPH inhibition.

The procedure for the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay followed the method suggested by Arnao *et al*. (2001). The ABTS scavenging capacities of the samples were compared with that of ascorbic acid. The percent inhibition of the ABTS radical was calculated. The $IC_{50}$ of each tested extract was determined by plotting a curve using a series of sample concentrations against the percent of ABTS inhibition.

The nitric oxide (NO) radical scavenging activity was estimated by the reaction with Griess Illosvory reagent based on the method proposed by Chakraborthy (2009). The NO scavenging capacities of the samples were compared with that of ascorbic acid. The percent inhibition of the NO radical was calculated. The $IC_{50}$ of each tested extract was determined by plotting a curve using a series of sample concentrations against the percent of NO inhibition.

##### 2.3.2.3. Anti-diabetic activity

The enzyme assay included determining the inhibition percentage (%) of α-amylase (Wickramaratne *et al*., 2016) and α-glucosidase enzymes (Pistia-Brueggeman and Hollingsworth, 2001) using acarbose as the standard drug. The $IC_{50}$ for each sample was calculated by creating a curve with various sample concentrations plotted against the enzyme inhibition percentage.

**2.3.2.4. Anti-Alzheimer activity**

The assay required determining the level of inhibition of the acetylcholinesterase (AChE) enzyme using Ellman's method, with donepezil serving as the reference drug (Ellman *et al*., 1961).

**2.3.2.5. Anti-arthritic activity**

The assay included measuring the percentage of inhibition of protein denaturation and proteinase inhibition using diclofenac sodium as a standard drug, following the methods described by Das and Sureshkumar (2016) and Oyedapo and Famurewa (1995) respectively. The $IC_{50}$ values for each tested extract were determined by constructing a curve with different sample concentrations against the percentage of proteinase inhibition, as outlined by Meera *et al*. (2011).

**2.3.2.6. Anti-inflammatory activity**

The *in vitro* anti-inflammatory activities were assessed by measuring the inhibition of two isoenzymes, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (ovine/human), as well as the 5-LOX enzyme (human recombinant). The inhibition percentages of COX-1 and COX-2 were determined using the COX-1 and COX-2 kit described by Alaa *et al*. (2016), while the inhibition percentages of 5-Lipoxygenase (5-LOX) were evaluated using the 5-LOX kit as outlined by Huang *et al*. (2019). The $IC_{50}$ values were calculated using linear regression analysis.

**2.3.2.7. Cytotoxic activity**

Cytotoxic activities were assessed against human hepatocellular (HepG-2), colon carcinoma (Caco-2), and lung cancer (A549) cells using the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay following the method described by Vichai and Kirtikara (2006). The percentage of cell growth inhibition and the $IC_{50}$ were determined using $IC_{50}$ calculation software.

**2.3.2.8. The enzymatic activity**

The enzymatic activities of the various extracts were assessed against caspase-3 and Bcl-2 within the cancer cells (HepG-2, Caco-2, and A549 cells) using established techniques from the literature (Pandey *et al*., 2021).

## 3. Results and Discussion

### 3.1. The major phyto-constituents

The findings in **Table 1** indicate that the methanolic extract of *A. coffeaeformis* had higher levels of flavonoids (42.91 ± 0.32 mg quercetin/100 g), polyphenols (179.74 ± 1.52 mg/100 g), and tannins (75.17 ± 0.91 µg/mL) compared to the other extracts. This aligns with the theory proposed by Abubakar and Haque (2020), suggesting that the choice of solvent used in the extraction process can impact the extraction efficiency of specific chemicals, leading to variations in the composition of the extracts. Methanol is typically the preferred solvent for extracting flavonoids and polyphenols from medicinal plants due to its higher extraction yield. The distinct chemical profiles of the methanolic extracts contribute to the significantly greater antioxidant activity observed in the methanol extract, as plant extracts rich in polyphenols and flavonoids are known to exhibit antioxidant properties (Gonzalez-Pastor *et al*., 2023).

**Table 1:** Concentrations of the major active phyto-constituents in different *Amphora coffeaeformis* algal extracts.

| | Total Polyphenols (mg gallic acid/100 g) | Total Tannins (µg/mL) | Total Flavonoid (mg quercetin/100 g) |
|---|---|---|---|
| **Methanolic Ext.** | **179.74 ± 1.52** | **75.17 ± 0.91** | **42.91 ± 0.32** |
| **E. Acetate Ext.** | 27.89 ± 0.25 | 14.17 ± 0.19 | 10.18 ± 0.07 |
| **Acetone Ext.** | 81.72 ± 0.59 | 37.70 ± 0.71 | 22.11 ± 0.17 |

The values were calculated from n=3/extract and given as mean ± SE. The green cell indicates the extract with the highest concentrations of the phyto-constituents.

In contrast, the E. acetate extract of *A. coffeaeformis* exhibited the lowest levels of flavonoids (10.18 ± 0.07 mg quercetin/100 g), polyphenols (27.89 ± 0.25 mg/100 g), and tannins (14.17 ± 0.19 µg/mL)

compared to the other extracts. This finding aligns with Gopčević *et al*. (2019), who demonstrated that the choice of extracting agent and the original plant material significantly influence the composition of the extracts. The extraction process was notably affected by the reduced solubility and extractability of metabolites in ethyl acetate.

### 3.2. *In vitro* biological activities

#### 3.2.1. Antioxidant activity

The biological properties of these extracts, such as their anti-inflammatory, anti-diabetic, anti-Alzheimer's, antioxidant, and radical scavenging properties, were evaluated and compared with the reference drug for each test. Therefore, the methanolic extract of *A. coffeaeformis* had higher TAC and IRP (242.01 ± 1.27 mg gallic acid/g and 197.26 ± 1.27 µg/mL, respectively) compared to the other extracts (Table 2).

The presence of high levels of active phenolic compounds with multiple hydroxyl groups and the substitution of electron-donating alkyl or methoxy groups may explain this phenomenon, as it enhances the antioxidant properties of the compounds (Charlton *et al*., 2023). The structure of phenolic compounds is closely related to their antioxidant activity, with increased activity observed in compounds with multiple hydroxyl groups and electron-donating substituents like alkyl or methoxy groups. This is attributed to the improved ability of these structural features to donate electrons or hydrogen, thereby neutralizing free radicals and reducing oxidative stress, as noted by Nagarajan *et al*. (2020).

#### 3.2.2. Scavenging activity

At the same concentration of 100 µg/mL, the methanolic extract of *A. coffeaeformis* exhibited the highest inhibitory percentages (57.14 ± 0.75, 50.93 ± 0.35, and 41.43 ± 0.35%, respectively) against DPPH, ABTS, and NO radicals, as shown in Table 2. The extracts with higher antioxidant activity had lower $IC_{50}$ values (Hussien *et al*., 2024a), with the lowest $IC_{50}$ values of 7.71 ± 0.09, 5.61 ± 0.04, and 7.31 ± 0.06 µg/mL compared to the other extracts studied. The E. acetate extract of *A. coffeaeformis* showed the lowest scavenging activity (12.58 ± 0.09, 16.33 ± 0.09, and 6.83 ± 0.09%, respectively), resulting in the highest IC50 values (21.74 ± 0.26, 14.51 ± 0.05, and 44.35 ± 0.60 µg/mL, respectively). In comparison, the ascorbic acid standard had the lowest $IC_{50}$ values (4.38 ± 0.02, 3.58 ± 0.02, and 5.34 ± 0.02 µg/mL, respectively) and the highest Inhib. % (62.50 ± 0.11, 66.25 ± 0.11, and 56.75 ± 0.11%, respectively) at the same concentration. This finding aligns with Lahlou *et al*. (2022), who suggested that the antioxidant activity of plant extracts is influenced by the structure of bioactive compounds like flavonoids and phenolic acids, which can neutralize reactive oxygen and nitrogen species. The methanolic extract, rich in phenolic compounds, was identified as a potent free radical inhibitor with effective scavenging of free radicals.

**Table 2:** The *in vitro* antioxidant and radicals scavenging activities of different *Amphora coffeaeformis* algal extracts.

| | **Antioxidant Activity** | | **Scavenging Activity** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **DPPH** | | **ABTS** | | **NO** | |
| | **TAC (mg gallic acid/g)** | **IRP (µg/mL)** | **Inhib. (%)** | **$IC_{50}$ (µg/mL)** | **Inhib. (%)** | **$IC_{50}$ (µg/mL)** | **Inhib. (%)** | **$IC_{50}$ (µg/mL)** |
| **Methanolic Ext.** | **242.01 ± 1.27** | **197.26 ± 1.27** | **57.14 ± 0.75** | **7.71 ± 0.09** | **50.93 ± 0.35** | **5.61 ± 0.04** | **41.43 ± 0.35** | **7.31 ± 0.06** |
| **E. Acetate Ext.** | 61.34 ± 0.45 | 33.59 ± 0.45 | 12.58 ± 0.09 | 21.74 ± 0.26 | 16.33 ± 0.09 | 14.51 ± 0.05 | 6.83 ± 0.09 | 44.35 ± 0.60 |
| **Acetone Ext.** | 106.98 ± 0.79 | 79.23 ± 0.79 | 21.94 ± 0.16 | 12.47 ± 0.15 | 25.69 ± 0.16 | 9.22 ± 0.04 | 16.19 ± 0.16 | 18.70 ± 0.19 |
| **Ascorbic Acid** | - | | **62.50 ± 0.11** | **4.38 ± 0.02** | **66.25 ± 0.11** | **3.58 ± 0.02** | **56.75 ± 0.11** | **5.34 ± 0.02** |

### 3.3. Anti-diabetic activity

Elevated glucose levels are a key feature of diabetes mellitus (DM), a chronic metabolic disorder (Borai *et al*., 2016; Hussien *et al*., 2024b). α-amylase breaks down carbohydrates into disaccharides,

while α-glucosidase breaks them down into monosaccharides. Inhibiting the activity of these enzymes is considered the most effective approach to managing hyperglycemia (Aboulthana *et al*., 2025). The anti-diabetic efficacy of the extract is assessed by measuring its inhibition of α-amylase and α-glucosidase and comparing it to the effectiveness of acarbose, a commonly prescribed medication (Hussien and Aboulthana, 2025).

The current study evaluated the effects of α-amylase and α-glucosidase enzymes. The methanolic extract of *A. coffeaeformis* showed the lowest $IC_{50}$ values (4.26 ± 0.03 and 3.40 ± 0.01 µg/mL, respectively) and had a higher inhibitory effect on both enzymes (48.51 ± 0.33 and 38.26 ± 0.33%, respectively) at a concentration of 100 µg/mL. Following this extract, the acetone extract of *A. coffeaeformis* exhibited similar trends. The ethyl acetate extract of *A. coffeaeformis* had the highest $IC_{50}$ values (13.29 ± 0.09 and 24.52 ± 0.24 µg/mL, respectively) and the lowest inhibitory effect on both enzymes (15.55 ± 0.09 and 5.30 ± 0.09%, respectively) at the same concentration. In comparison, acarbose, used as a standard, showed the strongest anti-diabetic activity at the same concentration (63.10 ± 0.10 and 52.85 ± 0.10%, respectively). Among all the extracts tested, acarbose had the lowest $IC_{50}$ values (3.28 ± 0.02 and 2.46 ± 0.02 µg/mL, respectively) (Table 3).

The extract containing higher levels of active phenolic phyto-constituents exhibited a more potent inhibitory impact on the enzymes α-amylase and α-glucosidase compared to the other extracts being studied, despite having lower concentrations of phyto-constituents. This finding aligns with the research by Quan *et al*. (2019), which suggested that the inhibition of these enzymes could be influenced by the quantity and arrangement of functional groups in the phenolic compounds, as well as the relationship between structure and activity. Additionally, the potential refolding of enzymes, leading to an increase in the α-helix shape and a decrease in random coils and β-sheets, demonstrates that protein-protein interaction could partially or completely counteract the impact of mineral-protein contact (Sheng *et al*., 2022).

### 3.4. Anti-Alzheimer's activity

The typical approach to managing chronic diseases caused by high levels of lipid oxidation and inflammation, like atherosclerosis, cancer, and rheumatoid arthritis, is to intake external antioxidants (Hassan and Aboulthana, 2023). The enzyme AChE, a serine hydrolase, is responsible for halting nerve impulse transmission at cholinergic synapses by breaking down the neurotransmitter acetylcholine into two inactive molecules (choline and acetic acid) (Tuzimski and Petruczynik, 2022). AChE inhibitors are utilized to regulate the levels of acetylcholine, a neurotransmitter present in cholinergic synapses, to prevent its breakdown. This approach is crucial in the treatment of Alzheimer's disease. Therefore, the development of potent AChE inhibitors could be vital for the advancement of effective therapies for neurological disorders (Stanciu *et al*., 2020).

The methanolic extract of *A. coffeaeformis* showed a higher inhibitory effect on the AChE enzyme (43.99 ± 0.38 %) compared to the other extracts at the same concentration (100 µg/mL), as indicated in Table 3. The ethyl acetate extract of *A. coffeaeformis* had the lowest $IC_{50}$ value (43.23 ± 0.62 µg/mL). In contrast to the other extracts tested, donepezil, used as a standard at the same concentration, exhibited the most potent inhibitory effect on the AChE enzyme (60.77 ± 0.12 %) and the lowest $IC_{50}$ value (4.34 ± 0.02 µg/mL).

The AChE enzyme was more effectively inhibited by the methanolic extract compared to the other extracts tested at the same concentration. This observation aligns with the findings of Magalhães *et al*. (2021), who established a significant correlation between antioxidant and scavenging properties and anti-Alzheimer activity. Therefore, an extract with antioxidant properties is likely to possess anti-Alzheimer properties as well. Phenolic compounds interact with the amino acid residues that make up the AChE active site in two ways: through hydrogen bonds and hydrophobic interactions. Phenolic substances containing multiple hydroxyl groups enhance the inhibitory effect on AChE due to their greater binding capacity (Ahmad *et al*., 2024).

**Table 3:** The *in vitro* anti-diabetic and anti-Alzheimer activities of different *Amphora coffeaeformis* algal extracts.

| | Anti-diabetic Activity | | | | Anti-Alzheimer Activity | |
|---|---|---|---|---|---|---|
| | α-Amylase | | α-Glucosidase | | AChE | |
| | Inhib. (%) | $IC_{50}$ (µg/mL) | Inhib. (%) | $IC_{50}$ (µg/mL) | Inhib. (%) | $IC_{50}$ (µg/mL) |
| **Methanolic Ext.** | **48.51 ± 0.33** | **4.26 ± 0.03** | **38.26 ± 0.33** | **3.40 ± 0.01** | **43.99 ± 0.38** | **5.99 ± 0.04** |
| **E. Acetate Ext.** | 15.55 ± 0.09 | 13.29 ± 0.09 | 5.30 ± 0.09 | 24.52 ± 0.24 | 6.10 ± 0.10 | 43.23 ± 0.62 |
| **Acetone Ext.** | 24.47 ± 0.15 | 8.45 ± 0.06 | 14.22 ± 0.15 | 9.14 ± 0.04 | 16.35 ± 0.18 | 16.12 ± 0.14 |
| | Acarbose | | | | Donepezil | |
| **STD** | **63.10 ± 0.10** | **3.28 ± 0.02** | **52.85 ± 0.10** | **2.46 ± 0.02** | **60.77 ± 0.12** | **4.34 ± 0.02** |

The values were calculated from n=3/extract and given as mean ± SE. The green cell indicates the most effective extract.

### 3.5. Anti-arthritic activity

Arthritis, an autoimmune condition, is marked by inflammation. Inflammatory conditions like arthritis are caused by both protein denaturation and the proteinase enzyme. Therefore, inhibiting these factors is considered a potential treatment strategy for arthritis (Heo *et al*., 2024). In comparison to the other extracts tested at the same concentration (100 µg/mL), the methanolic extract of *A. coffeaeformis* exhibited a more potent inhibitory effect on protein denaturation (38.80 ± 0.26 %) and the activity of the proteinase enzyme (Inhib. 35.65 ± 0.26 %; $IC_{50}$ 8.70 ± 0.07 µg/mL). The ethyl acetate extract of *A. coffeaeformis* showed lower inhibitory activity on protein denaturation (12.44 ± 0.07 %) and proteinase enzyme activity (Inhib. 9.29 ± 0.07 %; $IC_{50}$ 33.36 ± 0.28 µg/mL) among all tested extracts. Diclofenac sodium, the standard used at the same concentration, exhibited the most potent inhibitory effect on protein denaturation (50.48 ± 0.08 %) and proteinase enzyme activity (Inhib. 47.33 ± 0.08 %; $IC_{50}$ 6.55 ± 0.02 µg/mL) when compared to all tested extracts (Table 4).

The presence of high concentrations of phenolic substances may enhance the scavenging ability against free radicals, which are significant contributors to inflammation and arthritis (Platzer *et al*., 2022). Hydrogen bonds formed between the hydroxyl groups of phenolics and oxygen or nitrogen, particularly the hydroxyl and amino groups of proteins, may inhibit the proteinase enzyme (Makarewicz *et al*., 2021). Additionally, the hydroxyl groups of phenolics and charged groups on proteins may interact electrostatically to inhibit the proteinase enzyme, or phenolic compounds with non-polar aromatic rings may associate with hydrophobic regions of the protein molecules, which are the main sources of hydrophobic interactions (Yilmaz *et al*., 2022).

**Table 4:** The *in vitro* anti-arthritic and anti-inflammatory activities of different *Amphora coffeaeformis* algal extracts.

| | Anti-arthritic Activity | | | Anti-inflammatory activity | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Protein Denaturation | Proteinase | | COX-1 | | COX-2 | | 5-LOX | |
| | Inhib. (%) | Inhib. (%) | $IC_{50}$ (µg/mL) | Inhib. (%) | $IC_{50}$ (µg/mL) | Inhib. (%) | $IC_{50}$ (µg/mL) | Inhib. (%) | $IC_{50}$ (µg/mL) |
| **Methanolic Ext.** | **38.80 ± 0.26** | **35.65 ± 0.26** | **8.70 ± 0.07** | **48.13 ± 0.36** | **7.77 ± 0.06** | **50.38 ± 0.36** | **5.73 ± 0.03** | **43.13 ± 0.36** | **8.64 ± 0.10** |
| **E. Acetate Ext.** | 12.44 ± 0.07 | 9.29 ± 0.07 | 33.36 ± 0.28 | 12.55 ± 0.10 | 29.79 ± 0.25 | 14.80 ± 0.10 | 19.51 ± 0.10 | 7.55 ± 0.10 | 49.39 ± 0.77 |
| **Acetone Ext.** | 19.58 ± 0.12 | 16.43 ± 0.12 | 18.87 ± 0.16 | 22.18 ± 0.17 | 16.86 ± 0.14 | 24.43 ± 0.17 | 11.82 ± 0.07 | 17.18 ± 0.17 | 21.69 ± 0.28 |
| | Diclofenac Sodium | | | Indomethacin | | | | Zileuton | |
| **STD** | **50.48 ± 0.08** | **47.33 ± 0.08** | **6.55 ± 0.02** | **63.89 ± 0.11** | **5.85 ± 0.02** | **66.14 ± 0.11** | **4.36 ± 0.02** | **50.89 ± 0.11** | **7.32 ± 0.02** |

### 3.6. Anti-inflammatory activity

Cyclooxygenase (COX) is a well-known pro-inflammatory enzyme commonly used to evaluate the effectiveness of anti-inflammatory drugs. The two forms of COX, COX-1 and COX-2, are essential in converting arachidonic acid into prostaglandins (Pannunzio and Coluccia, 2018). Lipoxygenase (5-LOX) is the main enzyme responsible for producing leukotrienes, particularly hydroperoxides, which are important in the development of various inflammatory conditions. One approach to demonstrate antioxidant properties involves inhibiting the enzymatic peroxidation process that leads to the creation of lipid hydroperoxides. This method could potentially decrease the amount of lipid substrate available for the catalytic activity of 5-LOX (Rådmark *et al*., 2015).

The methanolic extract of *A. coffeaeformis* displayed a higher inhibitory effect on the activities of COX-1, COX-2, and 5-LOX enzymes (48.13 ± 0.36, 50.38 ± 0.36, and 43.13 ± 0.36 %, respectively) compared to the other extracts studied at the same concentration (100 µg/mL). Consequently, this extract exhibited lower $IC_{50}$ values ($IC_{50}$ 7.77 ± 0.06, 5.73 ± 0.03, and 8.64 ± 0.10 µg/mL, respectively). On the other hand, the ethyl acetate extract of *A. coffeaeformis* demonstrated the weakest inhibitory effect on the activities of COX-1, COX-2, and 5-LOX enzymes (12.55 ± 0.10, 14.80 ± 0.10, and 7.55 ± 0.10 %, respectively) compared to the other extracts at the same concentration (100 µg/mL), resulting in higher $IC_{50}$ values ($IC_{50}$ 29.79 ± 0.25, 19.51 ± 0.10, and 49.39 ± 0.77 µg/mL, respectively). The concentrated extracts with higher polyphenolic content may have anti-inflammatory properties. It was also observed that the radical scavenging activity is closely associated with the inhibition of COX-1, COX-2, and 5-LOX. Moreover, the anti-inflammatory effect may be attributed to the characteristics of flavonoids, influenced by the number of OH groups, conjugations, and resonances (Metkin *et al*., 2025).

### 3.7. Cytotoxic activity and enzymatic assays

Cancer, one of the most severe illnesses, is caused by uncontrolled cell growth that invades and damages surrounding tissue, ultimately leading to death (Nurmik *et al*., 2020). The methanolic extract of *A. coffeaeformis* showed the most potent cytotoxic activity against HepG-2, Caco-2, and A549 cells, with the lowest $IC_{50}$ values (IC50 15.30 ± 1.26, 29.06 ± 4.76, and 33.28 ± 2.72 µg/mL, respectively). The ethyl acetate extract of *A. coffeaeformis* had higher $IC_{50}$ values of 134.98 ± 5.36, 105.87 ± 4.63, and 98.52 ± 2.19 µg/mL, respectively. Doxorubicin, used as a standard, exhibited the highest cytotoxic activity ($IC_{50}$ 12.85 ± 0.58, 15.39 ± 1.16, and 11.07 ± 1.03 µg/mL, respectively) (Table 5).

**Table 5:** The *in vitro* cytotoxic activities of different *Amphora coffeaeformis* algal extracts against human hepatocellular carcinoma (HepG-2), colon (Caco-2) and lung (A549) cancer cell lines.

| | **Median Inhibitory Concentration ($IC_{50}$) (µg/mL)** | | |
|---|---|---|---|
| | **HepG-2** | **Caco-2** | **A549** |
| **Methanolic Ext.** | **15.30 ± 1.26** | **29.06 ± 4.76** | **33.28 ± 2.72** |
| **E. Acetate Ext.** | 134.98 ± 5.36 | 105.87 ± 4.63 | 98.52 ± 2.19 |
| **Acetone Ext.** | 75.93 ± 2.58 | 82.72 ± 3.97 | 64.86 ± 5.08 |
| **DOX** | **12.85 ± 058** | **15.39 ± 1.16** | **11.07 ± 1.03** |

The values were calculated from n=3/extract and given as mean ± SE. The green cell indicates the most effective extract.

The methanolic extract showed the lowest $IC_{50}$ values and the highest cytotoxic activity against the three cell lines (HepG-2, Caco-2, and A549 cells), followed by the acetone extract, then the ethyl acetate extract (Tables 6, 7, and 8). This finding aligns with the study by Zahra *et al*. (2024), which demonstrated that the cytotoxic effects of extracts are influenced by their chemical composition. It has been shown that certain bioactive compounds, particularly flavonoids and tannins, possess cytotoxic and antiproliferative properties by inducing apoptosis, inhibiting cell division, and disrupting the progression of cancer cell cycles. Phenolic and flavonoidal compounds impede proliferation either through DNA intercalation and inhibition of DNA topoisomerases or by modulating cell cycle regulatory mechanisms (Huynh *et al*., 2025).

In the study conducted by Khan *et al*. (2022), it was observed that the cytotoxic extract reduced the levels of Bcl-2 in the cancer cells that were treated, while simultaneously increasing the activity of the caspase-3 enzyme compared to the untreated cells. The stimulation of proapoptotic molecules and the inhibition of anti-apoptotic molecules are common strategies in anti-cancer treatments (Karimi Dermani *et al*., 2021). Following treatment with various extracts of *A. coffeaeformis*, the activities of caspase-3

and Bcl-2 enzymes were evaluated in the treated cells at IC50 values, specifically in HepG-2, Caco-2, and A549 cells.

**Table 6:** The *in vitro* cytotoxic activity of different *Amphora coffeaeformis* algal extracts against human hepatocellular carcinoma cell lines (HepG-2) and compared to Doxorubicin used as standard.

| **Methanolic Ext.** | | | | | | |
|---|---|---|---|---|---|---|
| **Conc.** | **0.00** | **6.25** | **12.50** | **25.00** | **50.00** | **100.00** |
| **Mean OD** | 0.39 | 0.33 | 0.28 | 0.19 | 0.14 | 0.07 |
| **Viability %** | 100.00 | 76.27 | 64.37 | 46.11 | 35.14 | 17.38 |
| **Cytotoxicity %** | 0.00 | 23.73 | 35.63 | 53.87 | 64.87 | 82.62 |
| **$IC_{50}$** | | | **15.30 ± 1.26 µg/mL** | | | |
| **Ethyl Acetate Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.34 | 0.26 | 0.21 | 0.16 | 0.09 | 0.05 |
| **Viability %** | 100.00 | 81.19 | 75.92 | 56.29 | 42.68 | 17.88 |
| **Cytotoxicity %** | 0.00 | 18.81 | 24.08 | 43.71 | 57.33 | 82.12 |
| **$IC_{50}$** | | | **134.98 ± 5.36 µg/mL** | | | |
| **Acetone Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.32 | 0.27 | 0.22 | 0.15 | 0.09 | 0.05 |
| **Viability %** | 100.00 | 78.27 | 68.92 | 54.26 | 41.14 | 17.24 |
| **Cytotoxicity %** | 0.00 | 21.73 | 31.08 | 45.74 | 58.86 | 82.77 |
| **$IC_{50}$** | | | **75.93 ± 2.58 µg/mL** | | | |
| **Doxorubicin** | | | | | | |
| **Conc.** | **0.00** | **6.25** | **12.50** | **25.000** | **50.000** | **100.00** |
| **Mean OD** | 0.35 | 0.25 | 0.22 | 0.17 | 0.09 | 0.06 |
| **Viability %** | 100.00 | 73.54 | 63.03 | 33.57 | 17.21 | 10.19 |
| **Cytotoxicity %** | 0.00 | 26.46 | 36.97 | 66.43 | 82.79 | 89.81 |
| **$IC_{50}$** | | | **12.85 ± 0.58 µg/mL** | | | |

**Table 7:** The *in vitro* cytotoxic activity of different *Amphora coffeaeformis* algal extracts against human colon cancer cell lines (Caco-2) and compared to Doxorubicin used as standard.

| **Methanolic Ext.** | | | | | | |
|---|---|---|---|---|---|---|
| **Conc.** | **0.00** | **6.25** | **12.50** | **25.00** | **50.00** | **100.00** |
| **Mean OD** | 0.31 | 0.25 | 0.21 | 0.12 | 0.08 | 0.05 |
| **Viability %** | 100.00 | 82.61 | 60.65 | 43.99 | 33.60 | 16.79 |
| **Cytotoxicity %** | 0.00 | 17.39 | 39.35 | 56.00 | 66.39 | 83.21 |
| **$IC_{50}$** | | | **29.06 ± 4.76 µg/mL** | | | |
| **Ethyl Acetate Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.32 | 0.27 | 0.24 | 0.17 | 0.10 | 0.07 |
| **Viability %** | 100.00 | 82.37 | 69.62 | 57.46 | 43.85 | 19.05 |
| **Cytotoxicity %** | 0.00 | 17.63 | 30.37 | 42.53 | 56.14 | 80.94 |
| **$IC_{50}$** | | | **105.87 ± 4.63 µg/mL** | | | |
| **Acetone Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.32 | 0.26 | 0.22 | 0.16 | 0.09 | 0.06 |
| **Viability %** | 100.00 | 79.40 | 62.49 | 55.39 | 42.27 | 18.37 |
| **Cytotoxicity %** | 0.00 | 20.59 | 37.51 | 44.60 | 57.72 | 81.62 |
| **$IC_{50}$** | | | **82.72 ± 3.97 µg/mL** | | | |
| **Doxorubicin** | | | | | | |
| **Conc.** | **0.00** | **6.25** | **12.50** | **25.00** | **50.00** | **100.000** |
| **Mean OD** | 0.33 | 0.28 | 0.19 | 0.11 | 0.09 | 0.04 |
| **Viability %** | 100.00 | 70.01 | 59.18 | 48.84 | 37.27 | 16.20 |
| **Cytotoxicity %** | 0.00 | 29.98 | 40.82 | 51.15 | 62.72 | 83.80 |
| **$IC_{50}$** | | | **15.39 ± 1.16 µg/mL** | | | |

**Table 8:** The *in vitro* cytotoxic activity of different *Amphora coffeaeformis* algal extracts against human lung colon cancer cell lines (A549) and compared to Doxorubicin used as standard.

| **Methanolic Ext.** | | | | | | |
|---|---|---|---|---|---|---|
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.42 | 0.34 | 0.28 | 0.21 | 0.13 | 0.07 |
| **Viability %** | 100.00 | 59.68 | 43.82 | 31.78 | 24.27 | 12.13 |
| **Cytotoxicity %** | 0.00 | 40.32 | 56.17 | 68.21 | 75.72 | 87.86 |
| **$IC_{50}$** | | | **33.28 ± 2.72 µg/mL** | | | |
| **Ethyl Acetate Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.45 | 0.31 | 0.23 | 0.16 | 0.09 | 0.05 |
| **Viability %** | 100.00 | 83.54 | 56.23 | 27.01 | 20.63 | 10.31 |
| **Cytotoxicity %** | 0.00 | 16.45 | 43.76 | 72.98 | 79.36 | 89.68 |
| **$IC_{50}$** | | | **98.52 ± 2.19 µg/mL** | | | |
| **Acetone Ext.** | | | | | | |
| **Conc.** | **0.00** | **31.13** | **62.50** | **125.00** | **250.00** | **500.00** |
| **Mean OD** | 0.42 | 0.29 | 0.21 | 0.16 | 0.10 | 0.07 |
| **Viability %** | 100.00 | 70.21 | 51.55 | 37.39 | 28.56 | 14.27 |
| **Cytotoxicity %** | 0.00 | 29.78 | 48.44 | 62.60 | 71.43 | 85.72 |
| **$IC_{50}$** | | | **64.86 ± 5.08 µg/mL** | | | |
| **Doxorubicin** | | | | | | |
| **Conc.** | **0.00** | **6.25** | **12.50** | **25.00** | **50.00** | **100.000** |
| **Mean OD** | 0.43 | 0.35 | 0.21 | 0.17 | 0.11 | 0.0 |
| **Viability %** | 100.00 | 61.01 | 47.79 | 22.96 | 17.54 | 8.76 |
| **Cytotoxicity %** | 0.00 | 38.98 | 52.20 | 77.03 | 82.46 | 91.23 |
| **$IC_{50}$** | | | **11.07 ± 1.03 µg/mL** | | | |

The caspase-3 enzyme showed the highest activity in HepG-2 (179.70 ± 1.20 pg/mL), Caco-2 (134.77 ± 0.90 pg/mL), and A549 (154.99 ± 1.04 pg/mL) cells treated with the methanolic extract of *A. coffeaeformis*, while the lowest activity of the Bcl-2 enzyme (4.83 ± 0.02, 6.44 ± 0.02, and 5.60 ± 0.02 ng/mL, respectively) was observed in these treated cells (Table 9). Aboulthana *et al*. (2024) reported that the bioactive components in the methanolic extract activate the apoptotic pathway by up-regulating caspase-3 and down-regulating the anti-apoptotic protein Bcl-2, leading to poly (ADP-ribose) polymerase disintegration, DNA fragmentation, and nuclear chromatin condensation. Additionally, Saddam *et al*. (2024) found that the phytochemicals' anti-Bcl-2 activity exhibited potent lethality by disrupting interaction of Bcl-2 with pro-apoptotic proteins, ultimately inducing cancer cell death.

Treatment with doxorubicin resulted in the lowest activity of the Bcl-2 enzyme (4.15 ± 0.01, 5.54 ± 0.02, and 4.82 ± 0.02 pg/mL in HepG-2, CACO-2, and A549 cells, respectively) and the highest activity of the caspase-3 enzyme (208.84 ± 1.40, 156.63 ± 1.05, and 180.12 ± 1.20 pg/mL, respectively).

**Table 9:** The enzymatic assay values after the treatment of human hepatocellular carcinoma (HepG-2), colon (Caco-2) and lung (A549) cancer cells with different *Amphora coffeaeformis* algal extracts.

| | **HepG-2** | | **Caco-2** | | **A549** | |
|---|---|---|---|---|---|---|
| | **Caspase-3 (Pg/mL)** | **Bcl-2 (ng/mL)** | **Caspase-3 (Pg/mL)** | **Bcl-2 (ng/mL)** | **Caspase-3 (Pg/mL)** | **Bcl-2 (ng/mL)** |
| **DMSO** | 97.13 ± 0.65 | 8.93 ± 0.03 | 72.85 ± 0.49 | 11.91 ± 0.04 | 83.78 ± 0.56 | 10.35 ± 0.04 |
| **Methanolic Ext.** | **179.70 ± 1.20** | **4.83 ± 0.02** | **134.77 ± 0.90** | **6.44 ± 0.02** | **154.99 ± 1.04** | **5.60 ± 0.02** |
| **E. Acetate Ext.** | 111.70 ± 0.75 | 8.12 ± 0.03 | 83.78 ± 0.56 | 10.82 ± 0.04 | 96.34 ± 0.64 | 9.41 ± 0.03 |
| **Acetone Ext.** | 145.70 ± 0.97 | 7.14 ± 0.03 | 109.27 ± 0.73 | 9.53 ± 0.03 | 125.67 ± 0.84 | 8.28 ± 0.03 |
| **DOX** | **208.84 ± 1.40** | **4.15 ± 0.01** | **156.63 ± 1.05** | **5.54 ± 0.02** | **180.12 ± 1.20** | **4.82 ± 0.02** |

The values were calculated from n=3/extract and given as mean ± SE. The green cell indicates the most effective extract.

## 4. Conclusion

The methanolic extract of *A. coffeaeformis* showed the highest levels of active phyto-constituents. As a result, this extract exhibited the most potent antioxidant, scavenging, anti-diabetic, anti-Alzheimer's, anti-arthritic, anti-inflammatory, and cytotoxic properties, with acetone and ethyl acetate extracts following closely behind.

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