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Biochemistry

Review on Amphora coffeaeformis: Chemical Composition, Bioactive Properties, and Potential Applications

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ABSTRACT

Amphora coffeaeformis (*A. coffeaeformis*) is a unicellular microalga existing in the majority of marine environments and can accommodate various levels of salinity. It could be harvested easily and has a very plentiful amount of macronutrients and micronutrients, essential amino acids, proteins, lipids, vitamins, minerals, and antioxidants. *A.coffeaeformis* has recently received a lot of interest from researchers as a promising source of nutraceuticals and for its potent biological capabilities, such as antioxidant, anti-inflammatory, therapeutic, and immunological attributes. Raising our knowledge of the metabolic composition of this alga is critical to recognising the future potential of its co-products. Consequently, the purpose of this review is to spotlight on *A. coffeaeformis*, its chemical composition, nutritional importance, and the bioactive components that are responsible for its beneficial properties, thus offering opportunities for using this alga as a feed additive or ameliorating agent against several poisons. Future studies should expand *A. coffeaeformis* screening attempts to cover its novel applications.

Keywords: *Amphora coffeaeformis*, Chemical composition, bioactive compounds, Therapeutic agent, Feed additive.

INTRODUCTION

There is a growing interest in the usage of natural medicinal plants as sources of new pharmacologically active compounds due to their accessibility and affordability (Shahrajabian and Sun, 2023). Algae are photosynthetic species with many different shapes and sizes. They do photosynthesis because they can obtain energy from sunlight. They range from unicellular microscopic creatures (microalgae) to multicellular organisms (macroalgae) (Sharma et al., 2016).

Microalgae are unicellular photosynthetic microorganisms. It might be eukaryotic or prokaryotic. They are classified as freshwater or marine according to their habitats (Aslam et al., 2020). Many of which are high in bioactive and pharmacological constituents, making them an important food source for marine species (Yarnold et al., 2019). Microalgae have a high level of biodiversity, photosynthetic products, growth, productivity, and adaptive metabolism. These features may be employed to provide a variety of applications (Dolganyuk et al., 2020).

Microalgae produce many of active phytochemicals known as "co-products" in their surroundings as a defence against predators and herbivores under environmental stress (Bhadury and Wright, 2004). Its metabolites include remarkable substances that can be used as nutritional supplements in animals, such as pigments, polyunsaturated fatty acids, polysaccharides, vitamins, and sterols (Mavrommatis et al., 2023). Furthermore, Coulombier et al. (2021) report that the majority of them are bioactive chemicals having anti-inflammatory, antibacterial, antifungal, anticancer, and/or antioxidant effects.

Diatoms are eukaryotic, unicellular microalgae. They are distinguished by their capacity to produce an exterior silica wall known as a frustule. The cell wall is mostly consisting of polymerised silica acid, which has no crystalline structure and is organised uniformly. It also has protein, polysaccharides, and lipids. Diatoms create insoluble mucilage, which interacts with glycoprotein to form a mucilaginous skin, limiting waves and tidal scour erosion (Sharma et al., 2021). *Amphora coffeaeformis* (*A. coffeaeformis*) is one of recognized benthic diatoms (Lachnit et al., 2019).

Amphora coffeaeformis Diatom

a-Source and chemical composition

A. Coffeaeformis is a widely distributed diatomic microalga with excellent nutritional value, the ability to tolerate various salinities, and existence in most regions (Sala et al., 2021). It's also called *Halamphora coffeaeformis* and belongs to the *Catenulaceae* family and *Bacillariophyta* division (Yousof et al., 2021). Its opaline silica cell wall (Buhmann et al., 2014) provides an essential food element for several kinds of fish species, invertebrates, and zooplankton (Kaparapu, 2018). *A. coffeaeformis* has a great metal absorption capacity and a rapid proliferation rate (Anantharaj et al., 2011), allowing it to withstand the harmful effects of environmental toxins (Gaur and Rai, 2001).

A. coffeaeformis powder contains many chemical components, macronutrients, and micronutrients. El-Sayed et al. (2018) concluded that carbs, ash, and protein were the most important chemical components discovered in *A. coffeaeformis*. Carbohydrate makes up the majority of *A. coffeaeformis'* chemical makeup (approximately 56% of total cellular carbons), followed by ash and proteins (Bhosle et al., 1993). The ash content of *A. coffeaeformis* dry powder is extremely valuable in the pharmaceutical sector (Stonik and Stonik, 2015).

Furthermore, *A. coffeaeformis* is a good source of almost all necessary vitamins (A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, and pantothenic acid) as well as a balanced mineral

composition (e.g., Na, K, Ca, Mg, Fe, Zn, and trace minerals) (Ragheb and Alahmadi, 2020). It is also a major source of PUFA, and they may produce both omega-3 and omega-6 FA. (Rajaram et al., 2018; Riccio et al., 2020).

PUFAs are the essential components for the biosynthesis of prostaglandins and leukotrienes, which regulate T cell activity and cytokine production, and therefore affect immunity (Nicolaou et al., 2014). Amino acid analysis of *A. coffeaeformis* identified all non-essential amino acids. All essential amino acids were identified, with the exception of phenylalanine (Lachnit et al., 2019). Amphora sp. also contains bioactive components such as hexadecanoic acid (antioxidant), phytol (anti-inflammatory), 2,6-dimethyl-4[3H]-quinazolinone (anticancer), and neophytadiene (antiviral) (Saleh et al., 2020).

Among the numerous constituents present in *A. coffeaeformis* algae, antioxidants are most likely the ones that have captured the health and pharmaceutical industries' interest. *A. coffeaeformis* contains a variety of bioactive carotenoids and polyphenolic chemicals, including methyl gallatenaringenin, kaempferol, taxifolin, gallic acid, cinnamic acid, and syringic acid, supporting its antioxidant effect (Hassan et al., 2021). β-coumaric acid is the highest phenolic content (Yousof et al., 2021). They demonstrated a potential scavenging mechanism of action against hydrogen peroxide and reduced DNA damage (Karawita et al., 2007). The n-hexane fraction of *A. coffeaeformis* revealed a significant free radical capture effect. As a result, it was obvious that the biochemical compounds demonstrating activity were concentrated in the n-hexane fraction, implying that the bioactive constituents are fat-soluble and may be composed of pigments and steroids (Lee *et al*., 2009).

b. Amphora Coffeaeformis uses and biological properties

The use of *A. coffeaeformis* algae in therapeutic applications has received widespread interest in recent years (Enwereuzoh and Onyeagoro, 2014). It's regarded to be a particularly beneficial and adaptive organism in a range of industries, including medicine, food, nutritional supplements, and treatment of wastewater (Mansour et al., 2023). The high level of bioactive substances such as polyphenols, vitamins, and fatty acids provided it with antioxidant, antiviral, antifungal, and anti-inflammatory effects, as previously stated (El-Sayed et al., 2018; Mekkawy et al., 2020). Amphora found to be safe, with no deleterious effects on physiological or nutritional condition.

b.1 Antioxidant Activity

A. coffeaeformis has lately gained popularity as a natural antioxidant, because of its safety and efficacy in substituting existing and commercial synthetic antioxidants. *A. coffeaeformis* has stronger antioxidant activity than α-tocopherol (Lee et al., 2009). It is high in hydrophilic and hydrophobic anti-oxidative chemicals with varying anti-oxidative characteristics that can activate a particular set of biochemical and physiological responses to counteract the damaging effects of environmental contaminants (Mekkawy et al., 2020). *A. coffeaeformis* analysis indicated the presence of fatty acid methyl ester (FAME), polyphenols, flavonoids, and

vitamins. These chemicals operate synergistically to provide antioxidant and radical scavenging action in vitro owing to their significant hydrogen-donating capacity (Hassan et al., 2021).

Moneeb et al. (2020) hypothesised that *A. coffeaeformis* extracts may replenish antioxidant enzymes in arsenic-intoxicated fish. *A. coffeaeformis* appears to have a role in restoring antioxidant levels in rat liver to near-normal levels in response to paracetamol's deleterious effects (El-Sayed et al., 2018). Furthermore, El-Sonbaty et al. (2021) stated that administering algal extract increased antioxidant levels while decreasing lipid peroxidation in d-galactosamine (D-GalN) nephrotoxicity in rats. Karawita et al. (2007) reported that algal extracts might reduce oxidative stress via regulating intracellular elevated Ca2+ and caspase-3 generation.

In accordance with this, treatment with *A. coffeaeformis* algae reduced monosodium glutamate-induced nitrosative stress in rats (Yousof et al., 2021). *A. coffeaeformis'* antioxidant activity is attributed to the presence of carotenoids such as β-carotene and fucoxanthin, which include effective groups such as epoxy, hydroxyl, carbonyl, and carboxyl moieties that participate in the antioxidant action (Boukhris et al., 2017). Furthermore, adding *A. coffeaeformis* to semen freezing extender mends buffalo sperm cryopreservation efficiency and lessens cryo-damage by enhancing sperm cells' antioxidant defence and reducing lipid peroxidation (Badr et al., 2017).

In addition, *A. coffeaeformis* exhibited iron-chelating activity. Ferrous may induce lipid peroxidation through the Fenton reaction and increase it by degrading lipid hydro-peroxides into peroxyl and alkoxyl radicals (Abu Affan et al., 2007). Different organic solvent fractions of the benthic diatom revealed a noteworthy capability for iron binding, implying their potential to defend against peroxidation, which is related to their ferrous binding capacity (Mal et al., 2022).

b.2. Feed additive

A. coffeaeformis can be utilised to improve the nutritional content of animal feed due to its well-balanced chemical structure. When Nile tilapia was fed an *A. coffeaeformis*-enriched diet, they showed improved growth parameters and physiological markers (serum protein profile and serum lysozyme) (Ayoub et al., 2019). Furthermore, Cui et al. (2023) concluded that *A. coffeaeformis* may be employed as a feed supplement to improve the lipid content of crucian carp flesh. The addition of *A. coffeaeformis* to fish diets altered the fish's primary elements, increasing ash and moisture content. The increased fish ash content might be attributed to the high ash content of *A. coffeaeformis* (Saleh et al., 2020).

Supplementing rabbit drinking water with *A. coffeaeformis* algae improved growth rate and dietary intake (Salim et al., 2019). Diets containing *A. coffeaeformis* significantly enhanced the quantity of essential and non-essential amino acids in broiler muscles (El-Bahr et al., 2020). *A. coffeaeformis* also increases broiler chicken growth performance, serum biochemical markers, and intestine microbial population (Alwaleed et al., 2021). Furthermore, *A. coffeaeformis* nanoparticles in the diet enhance heights

of intestinal villi and crypt depth, which improves nutrient digestion and absorption in Nile tilapia (Raslan et al., 2024).

A. coffeaeformis promotes development through the presence of physiologically active components such as polyphenolic compounds, omega-3 FA. and PUFA, gallic acid, catechin, and β-coumaric acid, all of which are antioxidants (Lee et al., 2009). These components improved feed intake, intestine integrity, and overall beneficial microbiota, counteracting the impacts of detrimental dietary elements (El-Sayed et al., 2018), and improving nutrient absorbability and digestibility (Alwaleed et al., 2021), providing an evidence for biotechnology applications of this highly nutritional value microalgae.

b.3. Hepato-protective effect

A. coffeaeformis extract can be applied as a therapeutic agent on arsenic-induced hepatotoxicity in fish because of its biologically active elements as it improved the alterations in GSH and SOD levels as well as the ultrastructure changes in the hepatic tissue of arsenic-induced fish (Moneeb et al., 2020). Also, Elsaman et al. 2023 provide compelling molecular evidence in favour of *A. coffeaeformis's* hepto-ameliorative action against D-Galactosamine-induced hepatotoxicity. This ameolirating effect induced by altering signalling molecular targets linked with antioxidant, anti-inflammatory, and anti-proliferative properties. Furthermore, El-Sayed et al. (2018) hypothesised that this algal extract has a contribution in restoring antioxidant levels to almost normal levels in the rat liver in response to the detrimental effects of paracetamol.

b.4. Prebiotic

A. coffeaeformis algae is a potential bioactive source that has been widely used as a source of prebiotic compounds. Implementing *A. coffeaeformis* as a prebiotic element in fermented milk can help to increase the starter bacterial strains (Badr et al., 2023). The combination of prebiotics and probiotics has been exhibited a reduction in the gastrointestinal tract (GIT) infections (Śliżewska et al., 2020). *A. coffeaeformis* has been shown to boost the ileocecal Lactobacillus intestinal bacteria population in broiler chickens (Alwaleed et al., 2021). *A. coffeaeformis* can serve as a prebiotic due to its high polysaccharide content, which is known to act as fermentable targets for beneficial gut microbes. Additionally, its bioactive compounds contribute to digestive health by promoting gut barrier integrity and minimising inflammation (Stirk and van Staden, 2022).

b.5. Immune modulation activity

Microalgae are classified as immunostimulants because of their capacity to elicit a broad immunological response (Riccio and Lauritano, 2019). A rise in both IgG and IgM levels, as well as an improvement in spleen tissue histological examination and lymphocyte count in immunosuppressive rats fed on an *A. coffeaeformis*-enriched diet (Ragheb and Alahmadi, 2020). According to Shawky et al. (2020), *A. coffeaeformis* activates the immune system and makes hens resistant to harmful microorganisms via increasing splenic mRNA production of interferon-gamma (IFN-γ). IFN-γ controls acquired immunity by triggering cells and boosting expression of major histocompatibility complex (MHC) class

II antigens. High levels of IFN-γ, a measure of cellular immunity, have been linked to protective immune responses against parasite infections (Lee et al., 2008; Priyanka and Muralidharan, 2014).

In the same line of raising immunity, Mansour et al. (2023) confirmed that *A. coffeaeformis* algae may be employed as an immuno-stimulant for control of *Schistosoma mansoni* in *Biomphlaria alexandrina* snails. Also, *A.coffeaefformis* nanoparticles have the ability to replace antibiotics in getting rid of harmful bacteria *Aeromonas veronii* and preventing infectious diseases in Nile tilapia by provoking an innate immune system response (Raslan et al., 2024). *A. coffeaeformis* contains a lot of pigments and polyphenolic substances such as catechin, gallic acid, and β-coumaric acid, which may activate the immune system (El-Sayed et al., 2018). Polysaccharides of marine origin enhanced immunity in mice by increasing bone marrow activities, thymus and spleen growth, and also WBC production (Hirahashi et al., 2002).

Khan et al. (2012) found that plant-based feed additives have immunomodulatory properties by increasing macrophage phagocytosis, producing interleukins, IFN-γ, tumour necrosis factor, and enhancing antigen-presenting cells and antioxidant function. These findings were consistent with those of (Pelvan et al., 2022), who suggested that herbal supplements might boost immune response since globulin levels were utilised as an index of immunological responses and a source of antibody formation.

b.6. Modulation on hematological parameters

Feeding rats *A. coffeaeformis* increased the blood picture with all of its constituents, mainly RBCs, Hb, and all types of WBCs, including lymphocytes and neutrophils (Ragheb and Alahmadi, 2020). Administration of *A. coffeaeformis* extract before or after deltamethrin resulted in substantial improvements in all haematologic parameters (Hassan et al., 2021). *A.coffeaeformis* feed supplementation has been demonstrated to preserve the haematological parameters of Nile tilapia poisoned with microplastic (Ismail et al., 2021). Furthermore, Mekkawy et al. (2020) found that *A. coffeaeformis* in the diet ameliorates arsenic-induced haematological alterations in African catfish (Clarias gariepinus). Ayoub et al. (2019) discovered that *A. coffeaeformis* in fish feeds improved haematological and biochemical markers in Nile tilapia. This improvement in hematological parameters might be attributed to *A. coffeaeformis'* high level of polyphenol antioxidants and PUFA (particularly omega-3 FA.) anti-inflammatory (El-Moghazy et al., 2014).

b.7. Amelioration of Reproductive Toxicity

A. coffeaeformis (7.5%) supplementation alleviated testicular damage induced by microplastic in Nile tilapia fish through restoring testis histological changes and sustaining LH and testosterone levels (Ismail et al., 2021). Furthermore, Saleh et al. (2020) discovered that *A.coffeaeformis* supplementation improves male gonadal development and increases female fish fertility. Also, combining treatment of *A. coffeaeformis*

extract with deltamethrin-intoxicated rats resulted in a considerable increase in testosterone hormone levels (Hassan et al., 2021).

b.8. Anti-inflammatory

A. coffeaeformis extract reduces TNF-α and NF-κB levels in D-GalN-induced kidney injury in rats, demonstrating its anti-inflammatory action (El-sonbaty et al., 2021). Also, Shaban et al. (2023) found that *A. coffeaeformis* had anti-arthritic capabilities by decreasing oxidative stress and inflammatory mediators of arthritis in rats induced by monosodium iodoacetate (MIA). Elsaman et al. (2023) found that *A. coffeaefomis* effectively reduced serum TNF-α and IL-6 levels in a rat model of hepatitis. *A. coffeaefomis* extract decreased inflammatory cytokine levels (TNF-α, AFP, and IL6) in rats treated with deltamethrin (Hassan et al., 2021). The impact of A. coffeaeformis may be associated with the substantial levels of anti-inflammatory linolenic acid (omega-3), linoleic acid (omega-6), and hespertin molecule (Riccio et al, 2020; El Moghazy et al., 2014). *A. coffeaeformis* bioactive substances may lower pro-inflammatory indicators and cyclooxygenase-2 enzyme expression, as well as lower secretion of pro-inflammatory cytokines by suppressing pro-fibrotic mediator expression (Eltahir et al., 2020; Salahuddin et al., 2017).

b.9. Nephro-protective effect

A previous study by El-sonbaty et al. (2021) verified that the extract of *A. coffeaeformis* significantly improved kidney function tests and hindered renal damage caused by D-GalN in *A. coffeaeformis*-treated rats. Also, *A. coffeaeformis* bioactive activities improved histological abnormalities in the kidneys of arsenic-exposed fish, indicating its nephron-protective impact (Mahmoud et al., 2020).

b.10. Neuro-protective effect

The neuroprotective capabilities of *A.coffeaeformis* algae against monosodium glutamate neurotoxicity were shown by the reversal of histopathological damages caused by monosodium glutamate when coupled with the *A.*coffeaeformis extract (Yousof et al., 2021). *A.coffeaeformis* contains essential fatty acids (linoleic acid and α-linolenic acid "ALA") that may impact receptor interactions and should be included in the diet as their lack can cause neuropathies
affecting synaptogenesis, neurogenesis, and synaptic function (Narita et al., 2003).

b. 11. Antibacterial effect

A. coffeaeformis algae were shown to have an antibiotic effect on harmful microbes in the chicken digestive tract through lowering bacterial count of *E. coli* in the ileocecal content of chickens and raised the ileocecal count of healthy bacteria (*Lactobacillus*). It also improved physiology and biochemical markers compared to untreated chickens. (Alwaleed et al., 2021). In addition, Rosland et al. (2021) used this alga in the aquaculture to combat the pathogenic *Vibro bacterium* rather than using antibiotics. Ayoub et al. (2019) revealed that *A. coffeaeformis* has the ability to substitute antibiotics in *Oreochromis niloticus* aquaculture. These findings highlight *A. coffeaeformis'* effectiveness as a natural alternative to pharmaceutical antibiotics. When tested against Gram-negative bacteria (*Salmonella enterica*

and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Micrococcus luteus* and *Staphylococcus aureus*), *Amphora* species showed high antibacterial activity (Boukhris et al., 2017).

b.12. Biodiesel production

Microalgae require only a few things: light, water, CO2, and minerals for development and photosynthesis. They can rapidly synthesise enormous quantities of lipids, proteins, and carbs (Brennan and Owende, 2010). Under certain cultivation conditions, these algae store triglycerides. Lipids of microalgae are the green oil for environmentally friendly biodiesel synthesis (Ramasamy Sakthivel et al., 2011). *Bacillariophyceae* is a major source of microalgae biodiesel production (Mourya et al., 2022). Previous research indicates that a new diatom of A. coffeaeformis may be a viable contender for bio-crude manufacturing. It has the potential to satisfy future energy demands because of its high biomass content (Rajaram et al., 2018).

Conclusion

A.coffeaeformis is recognized as a multifunctional microalga and might be employed in a variety of biotechnological uses. It appears to be an effective choice for bioactive chemical production as it possesses anti-inflammatory, antibacterial, immune-stimulant, and antioxidant properties that diminish the detrimental impact of free radicals. As a result, this alga exhibits potential as a natural medicinal product and antioxidant agent. Additionally, it can be used as a dietary component in a variety of nutraceutical applications and used in fuel production.

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No funds have been donated.

CONFLICT OF INTEREST

The authors have declared that they have no conflicts of interest.

LIST OF ABBREVIATION

A. coffeaeformis	*Amphora coffeaeformis*
AFP	Alpha-fetoprotein
ALA	Alpha linolenic acid
CO2	Carbon dioxide
D-GalN	D-Galactosamine
E. coli	Escherichia coli
F.A	Fatty acid
GIT	Gastrointestinal tract
GSH	Glutathione
Hb	Hemoglobin
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin G
IL6	Interleukin 6
LH	Luteinizing hormone
MHC	Major Histocompatibility Complex
mRNA	Messenger ribonucleic acid
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells

PUFA	Poly unsaturated fatty acid
RBCs	Red Blood Cell
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor-alpha
WBC	White Blood Count

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