Gelidiella acerosa Compounds Target NFκB Cascade in Lung Adenocarcinoma

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Abstract
In carcinogenesis, increased metabolism, abnormal functioning of mitochondria, peroxisomes, aberrant cell signaling, and prolonged inflammation can result in the overproduction of reactive oxygen species (ROS). In turn, excess ROS can upregulate the expression of various signaling pathways including the MAP kinase, PI3K/Akt, and NFκB cascades in cancer. The constitutive expression of NFκB causes drug resistance in lung cancer. Hence, drugs that can enhance the antioxidant activity of enzymes and regulate the NFκB activity are of prime target to manage the drug resistance and inflammation in cancer. This study evaluated the effect of compounds present in ethyl acetate extract of Gelidiella acerosa on inflammation and on antioxidant enzymes in lung cancer. The anti-inflammatory activity was determined under in silico and in vitro conditions. The in silico analysis showed that the phyto-constituents of G. acerosa inhibit the IKBα-NFκB-p65-p50 complex in a similar way as that of doxorubicin and dexamethasone. Similarly, G. acerosa treatment enhanced the efficiency of antioxidant enzymes peroxidases and superoxide dismutase in A549 lung cancer cells. Furthermore, the results of in vitro analysis showed that G. acerosa can decrease the activation of NFκB and production of pro-inflammatory cytokines and upregulate the expression of IL 10. As inflammation causes cancer progression, the inhibition of inflammation inhibits tumorigenesis. Hence, based on the results of the study, it can be concluded that G. acerosa exerts anti-inflammatory activity by decreasing the expression of NFκB cascade and moreover, the phyto-constituents of G. acerosa may have the potential to regulate the inflammatory response.

Keywords Gelidiella acerosa · NFκB · Pro-inflammatory cytokines · Anti-inflammatory marker · IKBα-NFκB-p65-p50 complex · ROS

Abbreviations
GAE Gelidiella acerosa Ethyl acetate extract
NFκB Nuclear factor kappa B
TNF α Tumor necrosis factor α

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**Introduction**

Reactive oxygen species (ROS) are group of highly reactive molecules generated as a normal by-product of cellular metabolism. ROS are removed by the cellular antioxidant enzymes including superoxide dismutase, peroxidase, and catalase families and by non-enzymes, such as the flavonoids, vitamins, and glutathione to maintain homeostasis. Superoxide dismutase (SOD) is a metallo enzyme, ubiquitously expressed in cells. They convert the superoxide generated to oxygen and hydrogen peroxide. The hydrogen peroxide is removed by peroxidases, thus protecting the cells from oxidative stress [1]. It is well established that increased metabolism, abnormal functioning of mitochondria, peroxisomes, cyclooxygenase, lipoxygenase, and aberrant cell signaling, and prolonged inflammation can result in the overproduction of ROS in carcinogenesis [2]. Furthermore, the interleukins and growth factors upregulate the production of hydrogen peroxide and nitric oxide in tumor cells [3]. The production of TNFα and IL-1β by macrophages also induces ROS generation in tumor cells. ROS upregulates the expression of various signaling pathways including the MAP kinase, PI3K/Akt, and NFκB cascades in cancer [4]. In breast cancer, exposure to TNFα– and IL-1β-induced hydrogen peroxide production, which in turn activated NFκB resulting in prolonged cell proliferation [5]. In the case of oral squamous cancer, an inhibition of SOD resulted in an upregulation of ROS levels which in turn activated NFκB pathway [6]. In lung cells, ROS are generated in response to environmental factors. Impaired clearance of ROS causes damage to the lung cells. The major antioxidant enzyme, glutathione peroxidase, plays a vital role in detoxifying the ROS [7] and regulates the cytokine production [8]. Either the lack or loss of GPX activity results in chronic inflammation of the lungs. Earlier studies have shown a constitutive activation of NFκB-p65 in lung cancer [9]. Moreover, the conventional therapies employed in the management of the disease also activate NFκB signaling resulting in drug resistance [10]. The activation of Rel A (p65) form of NFκB is considered a predictive marker for drug resistance in cancer [11].

NFκB is a major transcription factor that occurs as homo or heterodimers. Among the major forms of NFκB, the Rel A—a heterodimer of p65 and p50 subunits—is the predominant one. In resting cells, the NFκB dimer is bound with inhibitor (IKBα) and remains inactive. Upon activation, the NFκB translocates from cytosol to the nucleus and regulates the expression of nearly 200 genes involved in cell cycle, proliferation, survival, differentiation, migration, adhesion, and inflammation. Activation of NFκB confers activation of these genes in cancers including lung cancer [12]. Alternatively, NFκB also suppresses the expression of tumor suppressor genes such as p53 and PTEN, thus promoting carcinogenesis. In addition, activation of NFκB-p65 induces anti-inflammation and immunosuppression in tumor-induced macrophages. NFκB is a well-established transcription factor that regulates inflammation through the production of pro-inflammatory cytokines [13]. Hence, the regulation of NFκB directly affects tumor growth and tumor microenvironment. Thus, the inhibition of NFκB is reported to enhance the efficacy of anti-cancer drugs both under in vitro and in vivo conditions [14]. IL-10 is an anti-inflammatory cytokine that antagonizes the expression of TNFα by inhibiting the NFκB activity. It is a cytokine synthesis
inhibitory factor reported to inhibit the production of inflammatory cytokines [15]. As the inhibition of NFκB is the primary mechanism behind the regulation of inflammatory process, the expression level of IL-10 was considered an anti-inflammatory marker. In addition, the regulation of NFκB is a key factor in the management of inflammation and cancer [16].

Based on these reports, the drugs that can enhance the antioxidant activity of enzymes and regulate the NFκB activity are of prime target to manage the drug resistance and inflammation in cancer. Marine natural products (MNPs) are employed in the management of various diseases including cancer [17]. MNPs, especially terpenoids, alkaloids (Hymenialdisine and its derivatives), pigments (Prodigiosins) and steroids, macrolides, peptides, depsipeptides, and polysaccharides are reported for their anti-inflammatory activities [18, 19]. MNPs are reported to exhibit anti-inflammatory activity through the inhibition of NFκB. Cycloprodigiosin is a red pigment from marine bacteria which can exhibit immunosuppressive and apoptotic activities through the inhibition of NFκB [20]. The marine red algae are rich in phytocompounds with varied bioactivities. In an earlier study, [21, 22] we analyzed the anti-cancer and antimetastatic activities of marine red algae Gelidiella acerosa under both in vitro and in vivo conditions. The current study now analyzed the interaction of isolated algal compounds with IkBα-NFκB-p65-p50 complex under in silico, to determine the efficacy of the algal extract on SOD and POX activities in adenocarcinoma cell line A549, to analyze the expression of NFκB, pro-inflammatory cytokines (TNF α and IL-1β), and anti-inflammatory cytokine IL 10 under in vitro conditions.

Materials and Methods

Chemicals and Reagents

DMEM, FBS, and antibiotic cocktail were purchased from HiMedia. Nitroblue tetrazolium blue, riboflavin, methionine, and pyrogallol were purchased from Merck, Germany.

Seaweed Collection, Extraction, and Characterization

The seaweed was procured from the Mandapam coast of Tamil Nadu, India. It was authenticated and a voucher specimen was deposited at CMFRI, Mandapam, Tamil Nadu, India (Accession No: MMR- CMFRI17002). The seaweed was extracted as previously described [22] and the ethyl acetate extract (GAE) was investigated for anti-inflammatory activity. The structures of the algal compounds analyzed by GC–MS were retrieved from PubChem and subjected to in silico analysis.

Cell Culture and Treatment

The human adenocarcinoma cell line A549 was obtained from NCCS, Pune, India. The cells were cultured in DMEM, supplemented with 10% FBS and 1% antibiotic cocktail, incubated at 37 °C, 5% CO2 in CO2 incubator. Actively dividing cells were seeded in a six-well plate at a density of 1.5 × 10^5 cells/ml and incubated till they became 100% confluent. The cells were treated with the algal extract (1.5 mg/ml) for 24 h and untreated cells represented the control. The cells were treated with lysis buffer (10 mM Tris pH7.5, 150 mM
NaCl, 0.1 mM EDTA) and the cell lysate was collected, sonicated, and centrifuged to isolate the total protein. The protein samples from both the control and treated cells were analyzed for their protein content by Lowry’s method, and used for further analysis. For all the studies carried out in the study, the protein lysate obtained by treating the cells with and without algal extract (1.5 mg/ml) for 24 h was used.

**Analysis of Antioxidant Enzyme Activity**

**Determination of SOD Activity**

SOD activity was determined photometrically as previously described [23]. Briefly, 300 µl of the reaction mixture contained phosphate buffer (0.5 M, pH7.5), EDTA (0.1 mM), methionine (13 mM), nitro blue tetrazolium (63 mM), riboflavin (1.3 mM), and 20 µg/ml of total cell protein. The contents were incubated for 15 min and the reaction was initiated by exposure to fluorescent lamp (15 W) for 10 min. The reaction was terminated by switching off the lamp and the contents were covered with a black cloth. The absorbance was read at 560 nm in Multimode plate reader. The experiment was performed in triplicate and repeated thrice and the values presented as mean ± SD (standard deviation).

**Determination of Peroxidase Activity**

The peroxidase activity was determined based on the standard protocols [24]. Briefly, the reaction mixture contained 200 µl of phosphate buffer (0.1 M, pH7), 100 µl of H₂O₂ (0.005 M), 100 µl of pyrogallol (0.01 M), and 20 µg/ml of total cell protein. The contents were incubated for 5 min at 25 °C and 100 µl of 2.5 N sulfuric acid was added to terminate the reaction. The purpurogallin formed was measured at 420 nm using a spectrophotometer. The enzyme activity was determined as the amount of protein sample that brought changes in absorbance by 0.1 min/mg of protein. The experiment was carried out in triplicate and repeated thrice and the values represent mean ± SD (standard deviation).

**Analysis of Anti-inflammatory Activity**

**Determination of Anti-inflammatory Activity In Silico**

The X-ray crystallographic structure of the receptor protein IKBα-NFκB-p65-p50 complex Homo sapiens was retrieved from the Protein data Bank (PDBID: 1NFI). The protein was prepared as per the standard protocol of SYBL X 1.3 which is an automated docking tool, designed to evaluate the interactions of small molecule inhibitors and drug molecules with various target proteins in three dimensions. The interacting residues, atoms involved, the number of polar bonds, bond lengths, and energy of interaction were predicted by the docking tool. The ligands (compounds identified in GAE) were prepared as described by the ligand preparation program. The prepared ligands were docked with the receptor protein and the interaction was visualized through Pymol. The interaction with anti-cancer drug, doxorubicin, and anti-inflammatory drug, dexamethasone, with NFκB was evaluated and the results of the interaction were taken as reference standards.
Determination of Anti-inflammatory Activity Under In Vitro Condition

The efficacy of GAE on inflammation was determined by analyzing the expression levels of NFκB-p65, pro-inflammatory cytokines, and IL1β by real-time PCR. Total RNA was isolated from the GAE-treated A549 cells by TRizol method, followed by synthesis of cDNA using the high capacity cDNA Reverse Transcription kit (Applied Biosystem). The cDNA was utilized for analysis of gene expression using BioRad SYBR Green PCR Master mix in CFX96 TOUCH BioRad. The program (95 °C for 3 min, 40 cycles of 95 °C for 15 s and 55 °C for 30 s) was followed. Melt curve analysis was also carried out. The primers used are NFκB-p65 (F) 5′-ATCCCATCTTTGGACAATCGTG-3′, (R) 3′-CTGGTGCCCG TGAAATACACCTC-5′, TNF α (F) 5′-CCCAGGGACCTCTCTCTATAATCA-3′, (R) 3′-GCT TGAGGTTTGTACAACTAG-5′, IL 1β (F) 5′-AAATACCTGTCGGCTTGGGC-3′, (R) 3′-TTGGGATCTACACTTCCGCT-5′, IL 10 (F) 5′-CATCGATTCTCTCCCTGT GAA-3′, (R) 3′-CTTTGGAGCCTATAAAGGCCATC-5′, β Actin (F) 5′-TAGAACCTTTCATGGGACAAC-3′, (R) 3′-GTATCAGGCGATGAAAACAAG-5′. The analysis was carried out in triplicate and the fold change in gene expression was determined based on $2^{-ΔΔct}$.

Statistical Analysis of Data

All the control and test data were analyzed by using Student’s $t$-test and ANOVA. All values are presented as mean ± standard deviation (SD). Test and control data were compared statistically and a value of $p < 0.05$ was taken as significant.

Results

Algal Compounds Enhanced SOD and POX Activity

The SOD and POX activities of the A549 cells treated with or without GAE were determined. The results showed that sample from treated cells exhibited strong SOD activity and removed the superoxide anions generated more efficiently than the untreated sample. Similarly, the level of peroxidase activity was increased in the GAE-treated cells when compared to the control cells. The results are shown in Fig. 1. The antioxidant efficacy of GAE was previously determined by DPPH assay [21] and the outcomes of the current study strongly correlated with our previous observation. This is due to the increased activity of superoxide dismutase and peroxidases which are generally downregulated or inhibited in cancer. The results strongly confirmed the antioxidant efficacy of the algal compounds to detoxify the ROS.

Algal Phytocompounds Interact with NFκB

IKBα-NFκB-p65-p50 complex is made up of 6 chains such as chains A&C-p65 subunit, chains B&D-p50 subunits, and chains E&F-IKBα. Chain A constitutes 301 residues, chain B has 107 residues, and chain E has 213 residues. They are represented by 3 sequence-unique entities (Fig. 2). In this study, the interaction of IKBα-NFκB-p65-p50 complex with
the anti-cancer drug doxorubicin and the anti-inflammatory drug dexamethasone (Fig. 3) was performed. The results showed that doxorubicin interacted with residues GLN 162, ARG 73, ASN 138, ASN 139, and ARG 174 of NFκB complex. The interaction was stabilized by 8 polar bonds with bond length varying between 1 and 3 Å. Similarly, dexamethasone was found to interact with GLU 92, ARG 174, 95, and 73. The interaction was stabilized by 5 polar bonds with bond lengths between 1 and 3 Å. The results of in silico analysis are shown in Fig. 3 and Table 1.

The structures of the algal compounds identified by GC–MS were downloaded from PubChem and docked with IKBα-NFκβ-p65-p50 complex using the SYBL X 1.3 docking suite. The results showed that, among the 15 algal compounds identified by GC–MS, only 6 compounds, namely n-heneicosylformate, n-hexadecanoic acid methyl ester, 1, 2 benzenedicarboxylic acid mono ester, 6,4,10 trimethylpentadecanone, and carbamic acid phenyl (2 nitro phenyl) methyl ester, interacted with the NFκB complex (Fig. 4). The total score and C scores of these interactions were calculated based on the energy required for binding and number of bonds involved. The results are shown in Table 2.
The outcomes of the in silico analysis showed that both the standard drugs doxorubicin and dexamethasone interacted with the target protein at some common residues (ARG 174, ARG 95, and ARG 73). Hence, these residues are essential for regulation.

![Molecular interaction of IKBα-NFκBp65/p50 with standard drugs.](image)

**Fig. 3** Molecular interaction of IKBα-NFκBp65/p50 with standard drugs. a Structure of doxorubicin. b Interaction of doxorubicin with IKBα-NFκBp65/p50. c Structure of dexamethasone. d Interaction of IKBα-NFκBp65/p50 with dexamethasone

| Compound      | Interacting residues | Bond length Å | Total score | C score |
|---------------|----------------------|---------------|-------------|---------|
| Doxorubicin   | GLN 162              | 1.3           | 7           | 4       |
|               | ARG 73               | 1.8           |             |         |
|               | ASN 138              | 1.4           |             |         |
|               | ASN 139              | 2.6           |             |         |
|               | ARG 174              | 2.0           |             |         |
| Dexamethasone | GLN 922              | 1.6           | 7           | 5       |
|               | ARG 95               | 1.5           |             |         |
|               | ARG 73               | 1.4           |             |         |
|               | ARG 174              | 2.3           |             |         |

Table 1 Molecular docking analysis of standard drugs with NFκB-p65
of NFκB activity in both cancer and inflammation. Similarly, the algal compounds also interacted with residues ARG 174 and ARG 95 of NFκB complex. The results further showed that the algal compounds interacted and regulated the inhibition of NFκB complex in a way similar to the standard drugs.

**Table 2** Molecular docking of GAE compounds with NFκB-p65

| Compound                                      | Interacting residues | Bond length Å | Total score | C score |
|-----------------------------------------------|----------------------|---------------|-------------|---------|
| Heneicosyl formate                            | ARG 174              | 2.2           | 7           | 5       |
| n-hexadecanoic acid                          | THR 164, ARG 95      | 1.9, 2.3      | 7, 3        |         |
| Hexadecanoic acid methyl ester                | ARG 174              | 2.0           | 6           | 4       |
| Mono(2 ethyl-6-(tetrahydropyranoxy)hexyl) Phthalate | ARG 96              | 1.6           | 5           | 3       |
| 6,10,14-trimethyl Pentadecan-2-one            | ARG 96, 95           | 1.4, 1.7      | 5, 3        |         |

**Fig. 4** Molecular interaction of IκBα-NFκB-p65/p50 with GAE compounds. a Heneicosyl formate. b n-hexadecanoic acid. c hexadecanoic acid methyl ester. d Mono (2 ethyl-6-(tetrahydropyranoxy) hexyl) Phthalate. e 6,10,14-trimethyl Pentadecan-2-one. f Methyl ((2-nitro-4-(phenylmethoxy) phenyl) amino) thioxomethyl) carbamate
**Algal Compounds Decreased Expression of NFκB**

The activation of NFκB is essential for mediating the secretion of cytokines and hence inflammation. Therefore, to evaluate the involvement of NFκB in the inflammatory response, this study analyzed the expression levels of NFκB in GAE-treated and control A549 cells. The experimental data are presented in Fig. 5. The outcomes of the real-time PCR analysis showed that the expression of NFκB was downregulated in GAE-treated A549 cells when compared to untreated control cells. The treatment with GAE significantly ($p<0.05$) suppressed the expression levels of NFκBp65 in A549 cells. These findings suggest that GAE can suppress inflammation. As the activation of NFκB is essential for the production of pro-inflammatory cytokines, the expression levels of TNFα and IL 1β in the GAE-treated and control cells were also investigated for comparison. The results showed that GAE treatment significantly ($p<0.05$) decreased the expression levels of both TNFα and IL 1β cytokines. These outcomes demonstrated that GAE can downregulate the secretion of pro-inflammatory cytokines through the regulation of NFκB phosphorylation.

**Algal Compounds Upregulated IL-10**

As GAE treatment decreased the expression of NFκB-p65 and pro-inflammatory cytokines, the study further investigated the efficacy of GAE on the expression of the anti-inflammatory marker IL-10. Also earlier studies have shown that the deficiency of IL 10–induced inflammation and that the overexpression of IL 10 caused tumor rejection in vitro. The outcomes of the current study showed that the expression of IL-10, the major anti-inflammatory cytokine transcribed by NFκB, was decreased in the control cells whereas the expression levels of IL 10 were upregulated in the GAE-treated cells (Fig. 5). These results revealed that IL 10 is probably exerting anti-inflammatory activity by decreasing NFκB activation. These findings strongly support the results of previous reports where IL 10 is shown to suppress the secretion of inflammatory cytokines especially IL 1β, IL 6, IL 8, and TNF α by preventing gene transcription of NFκB to the nucleus [25].

![Fig. 5 Analysis of anti-inflammatory activity of GAE in vitro.](image-url)
Discussion

In our body, the lung encounters a plethora of substances ranging from the inhaled microbes, environmental pollutants like vehicle exhaust, smoke, fine particulate matter from industries, toxic gases, pollen, cigarette smoke and tobacco smoke, and high oxygen pressure. All these induce the generation of free radicals resulting in oxidative stress in the lung tissue. However, the presence of antioxidants such as antioxidant enzymes (SOD, POX, and Catalase) and vitamins A, D, E, C, and β-carotene offer protection to the lungs from the ROS-induced cellular damage. Antioxidant enzymes are the primary line of defense to the lungs from ROS. The levels of these enzymes are reported to be associated with the initiation of carcinogenesis in the lung [26]. It is well established that smoking causes lung cancer. \( \text{H}_2\text{O}_2 \) and superoxide radicals in cigarette smoke are shown to induce DNA damage in lung cell lines. Furthermore, studies have shown that cigarette smoke exhausts the intracellular antioxidant enzymes and upsurges the neutrophils in the lungs which are a source of ROS. Accumulation of ROS accompanied by decreased antioxidant enzymes is directly related to increased activation of signaling pathways including the NFκB in cancer. NFκB is the most frequently activated pathway in chronic inflammation and also in lung cancer [27].

Inflammation is a protective response of the body against any foreign stimulus. However, uncontrolled inflammation leads to disorders including arthritis, neurodegeneration, diabetes, cardiovascular disease, cancer, and autoimmune diseases thus, causing huge economic burden on the human society [28]. Various therapeutic agents including steroids, nonsteroid anti-inflammatory drugs, and immunosuppressant are employed in controlling and suppressing inflammatory response but these medications are associated with adverse side effects. Hence, there is a surge to identify and utilize anti-inflammatory factors from natural origin with increased pharmacological response and devoid of unwanted side effects [28]. Plants of both terrestrial and marine origin are used as medicine since ancient times. They are utilized in the forms of teas, dry powders, formulations, and tinctures. Plant products and secondary metabolites serve as new chemical entities or lead molecule for novel drug development. Medicinal plants are a rich source of natural products with varied therapeutic potential. Hence, plants are widely used in the development of new and potent drugs.

Recent studies have shown that approximately 80% of the drugs with anti-inflammatory, anti-cancer, anti-diabetic, and cardiovascular activities are derived from natural products. Most of the prescribed drugs are either a derivative of natural products or their synthetic analogue. Almost 50% of the prescribed drugs contain at least any one ingredient from natural origin [29]. Phytocompounds and secondary metabolites including polyphenols, resins, polysaccharides, carotenoids, fatty acids, flavonoids, and plant extracts are shown to modify the expression of genes involved in inflammatory pathway under both in vitro and in vivo conditions [30].

Since it is well established that the inhibition of NFκB can enhance the sensitivity to anti-cancer drugs, research is focused to eventually discover potent NFκB inhibitor(s) for lung cancer chemoprevention. Chemoprevention requires the continued usage of preventative drugs which may result in the intolerable side effects [28]. Natural products, dietary components of vegetable origin, and crude extracts of medicinal plants and fruits are more preferred than their synthetic counterparts in lung cancer prevention. Hence, the current study was designed to analyze the anti-inflammatory efficacy of GAE both under in silico and in vitro conditions.
The outcomes of the study revealed the efficacy of GAE to enhance the activity of the antioxidant enzymes superoxide dismutase and peroxidase, thereby protecting against ROS-induced cellular damages and ROS-induced activation of signaling pathways including the NFκB in A549 cells. The outcomes of the current study are in line with earlier studies where *G. acerosa* was shown to regulate SOD activity in Alzheimer’s disease [31] and to protect human peripheral mononuclear cells from TDCC-induced toxicity [32]. As excess ROS are associated with cellular damage, inflammation, and cancer progression, the enhanced activity of antioxidant enzymes may confer protection against free radical damage to cells.

Furthermore, the results of in silico analysis in this study revealed that the compounds in GAE targeted specific amino acids as doxorubicin and dexamethasone. In addition, the compounds in GAE interacted with the regulatory residues of NFκB which can modify the activation of NFκB. These data corroborate with earlier in silico studies where standard anti-cancer and anti-inflammatory drugs were reported to interact with NFκB [33].

The findings of the in vitro analysis showed that GAE treatment decreased the activation of NFκB and thereby suppressed the production of pro-inflammatory cytokines (IL 1β, TNF α) which mediate the inflammatory response in cancer. These data correlated with the earlier reports where algal extracts from *D. salina* were shown to decrease the production of inflammatory cytokines through the inhibition of NFκB cascade [34, 35]. Similarly, plant extracts are also shown to inhibit cytokine secretion through the inhibition of NFκB [36]. Furthermore, GAE treatment also increased the expression of anti-inflammatory cytokine (IL 10), thereby revealing the mechanism by which GAE can regulate inflammation in cancer. The current data correlated closely with previous findings [35] which revealed similar regulation of inflammation by IL 10. The results of the current study strongly support the anti-inflammatory activity of GAE since it can regulate the levels of both NFκB and pro-inflammatory cytokines.

Together, the in silico and in vitro results show the capacity of the algal compounds to regulate NFκB-p65, thereby offering protection from inflammatory cytokines. Furthermore, the upregulation of anti-inflammatory marker confirms the anti-inflammatory potential of the algal compounds. Our previous studies [21, 22] have revealed the anti-cancer and antimetastatic properties of the algal extract and this current investigation has revealed the anti-inflammatory property of the extract both under in silico and in vitro conditions. As inflammation can cause cancer progression, then the inhibition of inflammation must in turn inhibit tumorigenesis.

**Conclusion**

Based on the outcomes of the study, it can be concluded that the compounds in GAE seem to exert its anti-inflammatory property through the downregulation of NFκB activation and subsequently the production of inflammatory cytokines and upregulation of IL 10 expression in lung cancer. GAE compounds are effective as the standard drugs such as doxorubicin and dexamethasone and target the same amino acids to regulate NFκB and in vitro analysis also supported the same. As six phyto-constituents of GAE interacted with NFκB, the study can be extended to investigate the interaction of each of these constituents individually. Overall, our results suggest that GAE compounds have the moderate antioxidant and anti-inflammatory activities and thus can control various diseases mediated...
by oxidative stress. These findings suggest new applications of the alga. However, further studies are warranted.

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Author Contribution SMF performed the experiment, collected and analyzed the data, SH designed and conceived the study. Both the authors wrote and edited the manuscript. All authors read and approved the final manuscript.

Data availability The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate All the authors agreed to participate in the scientific work.

Consent for Publication All the authors agreed to submit the manuscript.

Conflict of Interest The authors declare no competing interests.

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