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Research Article

Investigating the effects of *Sargassum glaucescens* extract on virulence gene expression of *Salmonella typhi* and it's anticancer potential in Caco$_2$ cancer cells

Hassannia M.[1], Aliasgari E.[1*], Kavousi M.[1]

1Department of Biology, ET.C., East Tehran Branch, Islamic Azad University, Tehran, Iran
*Correspondence: elahe.aliasgari@iau.ac.ir; E.asgari@gmail.com

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Abstract

The increasing prevalence of antibiotic resistance has led researchers to search for pharmaceutical products that can replace antibiotics or be used in combination with them. Among natural compounds, brown algae extract can have inhibitory effects on bacteria. In the present study, the effect of *Sargassum glaucescens* extract on virulence gene expression and its anticancer potential was investigated. Stool samples were collected from gastrointestinal infection individuals referred to Imam Khomeini and Milad Hospitals in Tehran from March 21, 2022, to September 21, 2022. *Salmonella typhi* isolates were identified using phenotypic and chemical tests. Next, *Vir* gene was confirmed through a polymerase chain reaction (PCR). In the next step, the minimum inhibitory concentration (MIC) of the methanolic extract of *S. glaucescens* was determined. Furthermore, the expression level of Vir, P53, and Bcl-2 was investigated using the Real-Time PCR method. Among 110 collected samples, 60 isolates of *S. typhi* were identified, all of which had the *Vir* gene. The MIC of the methanolic extract was *S. glaucescens* 16 µg/ml. The expression level of Vir and Bcl-2 in Caco$_2$ cells infected with S. typhi, and treated with *S. glaucescens* extract was significantly decreased (p<0.001). Besides, the expression level of P53 showed a significant increase (p<0.0001). The obtained results showed that the methanolic extract of *S. glaucescens* has strong anti-bacterial and anti-cancer effects.

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Introduction

Antibiotic resistance is one of the problems that threatens public health in the world. In 2013, the Centers for Disease Control and Prevention (CDC) published a report titled "Threats of Antibiotic Resistance in the United States, 2013" (Frieden, 2013). The report estimates that approximately two million people in the United States are infected by antibiotic-resistant pathogens each year and that at least 23,000 people die (Jernigan *et al.*, 2020).

Salmonella are gram-negative motile bacilli (except Gallinarum and Polurum) with capsules and pili, known as obligate intestinal parasites of animals. *Salmonella abortus* in sheep, *Salmonella gallinarum* in chickens, *Salmonella typhi* in humans, and *Salmonella typhisuis* in pigs are pathogenic (Mims *et al.*, 2004). The most important pathogenic factor of Salmonella is the ability to attack tissues and survive inside macrophages (Murray *et al.*, 2020). Various genes are involved in the pathogenicity of this bacterium, which is located on the main single chromosome of the bacterium or the virulence plasmid. Among these genes, we can mention Vir genes, which are located in an operon including *Vir* A, *Vir* B, *Vir* C, *Vir* D, *Vir* E, and *Vir* H genes. Two genes, *Vir* A and *Vir* B, which are located on the bacterial plasmid, have a great effect on the virulence of *Salmonella typhimurium* (Amprazi *et al.*, 2021).

On the other hand, cancer is one of the chronic diseases that occur in every individual and age group that affects the health of society (Mousavi *et al.*, 2007). Colon cancer is one of the most common cancers in men and women, especially in developing countries, and with about 1.4 million new cases in 2012, it was the third most common cancer in the world (Moussavou *et al.*, 2014). Statistics show that the prevalence of colon cancer in Iran is increasing (Azadeh *et al.*, 2008). Despite much cancer research, this disease is still considered one of the biggest health problems in human societies.

Extensive efforts have been started to find appropriate and effective treatment methods, among which we can mention the investigation of antimicrobial effects and cell toxicity of compounds extracted from plants (Shamsoddini *et al.*, 2025; Khaledi and Meskini, 2020; Tavakoli *et al*., 2025). In addition to their significant therapeutic effects, these natural resources can also have lower production costs and side effects. Among these resources, we can mention marine resources (Meskini and Esmaeili, 2018; Meskini *et al.*, 2020), seaweeds are among the richest natural resources (Senthilkumar *et al.*, 2013). Various therapeutic effects such as anticancer, antioxidant, and antimicrobial properties have been reported for brown algae (Remya *et al.*, 2022). In several studies, the effect of the extract of these algae on inhibiting the growth of a wide range of bacteria, including *Enterococcus faecium*, *Streptococcus mutans*, *Shigella boydii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Vibrio Harveyi, Escherichia coli*, *Bacillus cereus*, etc. has been promising (Mahianeh *et al.*, 2014; Zobeidy Nezhad *et al.*, 2018; Amirsharifi *et al.*, 2016).

The effect of metabolites obtained from algae in the control and treatment of cancer has also been recently discussed and

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researched. The findings of these studies are promising for cancer prevention and treatment approaches (Sanjeewa *et al.*, 2017; Yao *et al.*, 2022). One of the most important characteristics of cancer cells is the dysregulation of the expression of tumor suppressor genes such as P53 and proto-oncogenes such as Bcl-2. These genes are mutated in many cancers and direct the carcinogenesis process (Falanga *et al.*, 2019; Kontomanolis *et al.*, 2020). Algae are rich in dietary fibers, proteins, minerals, vitamins, antioxidants, unsaturated fatty acids, and biologically active compounds such as phycocyanin, terpenes, fucosterols, and phenols (Zorofchian Moghadamtousi *et al.*, 2014). Alkaloids, terpenoids, and flavonoids are secondary metabolites that have anti-cancer effects (Kawashty *et al.*, 2000). Evidence shows that bioactive substances obtained from algae produce anticancer effects through multiple functional mechanisms, including inhibiting cancer cell growth, invasion and metastasis, and inducing cell death in cancer cells (Moussavou *et al*., 2014).

Also, it has been shown that the methanolic extract of *Padina pavoni*, in addition to having cytotoxic activity against cancer cells, has no cytotoxic activity against normal lung fibroblast cells (MRC-5) (Stanojković *et al.*, 2013). The rich extract of phlorotannin from the brown algae, *Himantothallus grandifolius*, also has selective cytotoxicity against non-tumor cells (Gambato *et al.*, 2014). Seaweed, *Samrgassum glaucescens*, is found on the coasts of southern cities of Iran and can be used in different seasons of the year. 118. Several studies have been conducted on different extracts of this algae, which have identified the methanolic extract as the most effective extract (the article itself). Hence, in the present study, we intend to investigate the effect of *S. glaucescens* methanolic extract on *Vir* gene expression in *S. typhi* cultured with $Caco_2$ cancer cell culture. Besides, we evaluated the anticancer potential of *S. glaucescens* methanolic extract by measuring the expression level of *P53* and *Bcl-2* genes in the $Caco_2$ cell line.

Materials and methods

Sample collection

This is a cross-sectional descriptive-analytical study. It was conducted over 6 months from the beginning of April 2022 to the end of September 2022. In this study, 110 stool samples were randomly collected from those referring to Imam Khomeini and Milad hospitals in Tehran. The inclusion criteria for the study were infliction by gastrointestinal infection diarrhea and abdominal pain, as well as a positive *S. typhi* culture. Cochran's statistical formula was used to calculate the sample size.

Identification of salmonella

To isolate *S. typhi* isolates from the collected stool samples, Rappaport-Vassiliadis (RVS) Broth, Sulfide, Indole, Motility (SIM), Tripol Sugar Iron Agar and selenite f (SF), blood agar, kligler's iron agar, Urea Agar differential, sugar and microbial diagnostic media of the Enterobacteriaceae family kit (https://assets.publishing.service.gov.uk) media were used. Then, by comparing the obtained biochemical characteristics with

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the guide table of the kit, the desired strain was identified.

Detection of Vir a genes by PCR method

After identifying the bacterial isolates using standard biochemical, bacteriological, and serological methods, they were also identified by molecular method. For this purpose, DNA extraction was done using a Cinagen kit. The PCR process was carried out at the annealing temperature of 60 ℃. The sequence of primers included *Vir* F-5′GGGGCGGAAATACCATCTACA-3′ and *Vir* R-5′ GCGCCCAGGCTAACACG-3′. The PCR process was confirmed by gel electrophoresis method.

Algae sampling and extraction

In May and April 2022, brown algae, *S. glaucescense*, were collected from the protected marine area of the Oman Sea around Chabahar City, Sistan and Baluchistan province, Iran. Upon transfer to the laboratory, all samples were washed in distilled water to separate sand and epiphytes. After air-drying at 25°C in the shade, the algae powder was ground using a mortar and pestle. A total of 150 grams of sample were extracted using methanol, in batches of 800 mL at room temperature. Filtered extracts were re-extracted from residues. Filtrates were collected, dried, and evaporated under vacuum (Amirsharifi *et al*., 2016).

Evaluation of minimum inhibitory concentrations (MICs) and toxicity of Sargassum algae

Sargassum algae extract was dissolved in four solvents, ethyl acetate, diethyl ether, methanol, and chloroform, and was used for MIC testing on 60 clinical isolates of *S. typhi* by broth dilution method. In the MTT test, Caco-2 cells were treated with dilutions of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 mg/mL of brown algae extract in different wells. After incubating this mixture and treating it with MTT and DMSO solution, the optical absorbance of each well was measured at a wavelength of 590 nm.

Infection of $Caco_2$ cells and treatment with algae extract

The standard pathogen strain used in this process was *S. typhi* (PTCC: 1609), confirmed after culturing the strain on differential and selective media. This bacterium was cultured in LB agar and LB broth until it reached the logarithmic phase. Also, the $Caco_2$ clone cancer cell line was purchased from a cell bank (Institute of Pasture, Tehran, Iran). The $Caco_2$ cells were infected in triplicate with a dilution of 107 cfu/ml of standard *S. typhi* and fecal isolates. The samples were taken from the cultures each time (zero, two, and four hours after infection). The infected $Caco_2$ cells were treated with *Sargassum algae* extract, and some cell cultures were not treated and were used as controls. The expression level of *Vir* gene before and after treatment with *Sargassum algae* .

The level of *Vir* gene expression in *S. typhi* isolates propagated in $Caco_2$ cells was evaluated in the two groups receiving treatment and the group without treatment. For this purpose, RNA extraction was done using cold chloroform and isopropanol and RNX solution produced by Cinagen Company (2). cDNA synthesis was performed using the Revert AidTM First

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Strand cDNA Synthesis Kit (Fermentas) after determining the amount and concentration of the resulting RNA by nanodrop device. The real-time PCR process was performed by SYBER green method and using primers 5'ACTCTGTTATTAGGGAAGAA3' and 5'AACGCTTGCCACCTACGTAT3' for the *16SrRNA* reference gene and 5'GGGGCGGAAATACCATCTACA3' and 5'-GCGCCCAGGCTAACACG-3' genes for the *Vir* gene. All reactions were repeated twice.

Bcl-2 and P53 expression evaluation

The effect of brown algae extracts on the expression of *Bcl-2* and *P53* genes was evaluated in the control and treatment groups including $Caco_2$ cells not infected and infected with *S. typhi* respectively. Both groups were treated with brown algae extract and expression was measured by Real-time PCR method. The primers for *bcl-2* gene included F- 5′ GTGGATGACTGAGTACCT -3′ and R- 5′- CCAGGAGAAATCAAACAGAG -3′, and for *p53* gene included F-5′- GTATTTCACCCTCAAGATCC- 3′ and R-5′- TGGGCATCCTTTAACTCTA -3′.

Statistical analysis

Statistical calculations were performed using REST 2009 SPSS 16 software and the results were analyzed using one-way ANOVA. The difference between the control and treatment samples in the expression of the target genes was calculated by Tukey's HSD post-hoc test. $p<0.05$ is considered as significant. Real-time PCR data analysis was done by the ΔΔCt method.

Results

The mean age of the 110 patients participating in the study was 57±4.6 years. Among them, 63 cases were male (57.27%) and 47 (42.73%) were female. Identification of bacteria by phenotypic and molecular methods Gram staining and standard differential biochemical tests such as oxidase, lactose and glucose fermentation, motility, indole, H2S, citrate and urea test, MR/VP, lysine iron agar, arginine, and orentine decarboxylase, confirmed the identity of 60 *S. typhi* isolates. Also, the PCR results confirmed that all these 60 isolates had the *Vir* gene (Fig. 1).

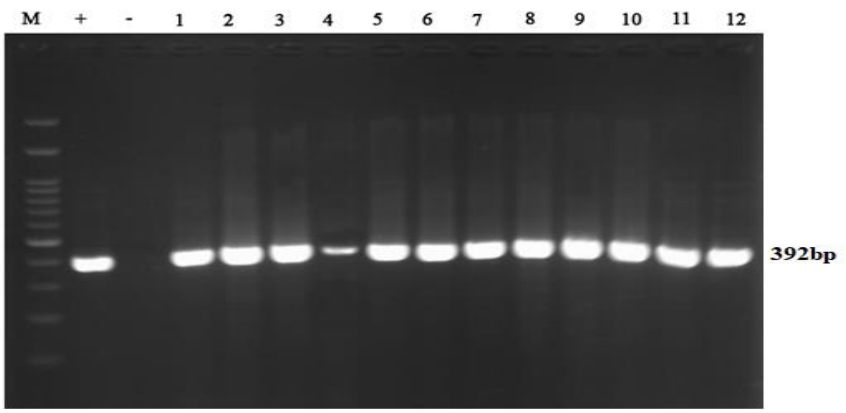

Figure 1: The PCR product for *Vir* gene is 392 bp long. M, 50 bp DNA marker, -: negative control, +: positive control, 1-12 Clinical isolates of *S. typhi.*

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MICs and toxicity results

The identity of the collected *Sargassum algae* was confirmed based on the expected systematic characteristics (Fig. 2A) (Duc Thinh *et al.*, 2013, Kantachumpoo *et al.*, 2015). In the MIC test, the methanolic extract of Sargassum showed the greatest inhibitory effect on *S. typhi* isolates. The range of MIC obtained was 1-16 µg/mL. The methanolic extract (2 µg/mL) showed the lowest MIC50. The survival of $Caco_2$ cells treated with different concentrations of algal supernatant after 24, 48, and 72 hours in the MTT test is shown in Figure 2, B. Also, the IC50 was estimated as 37.14 mg/mL (Fig. 2C).

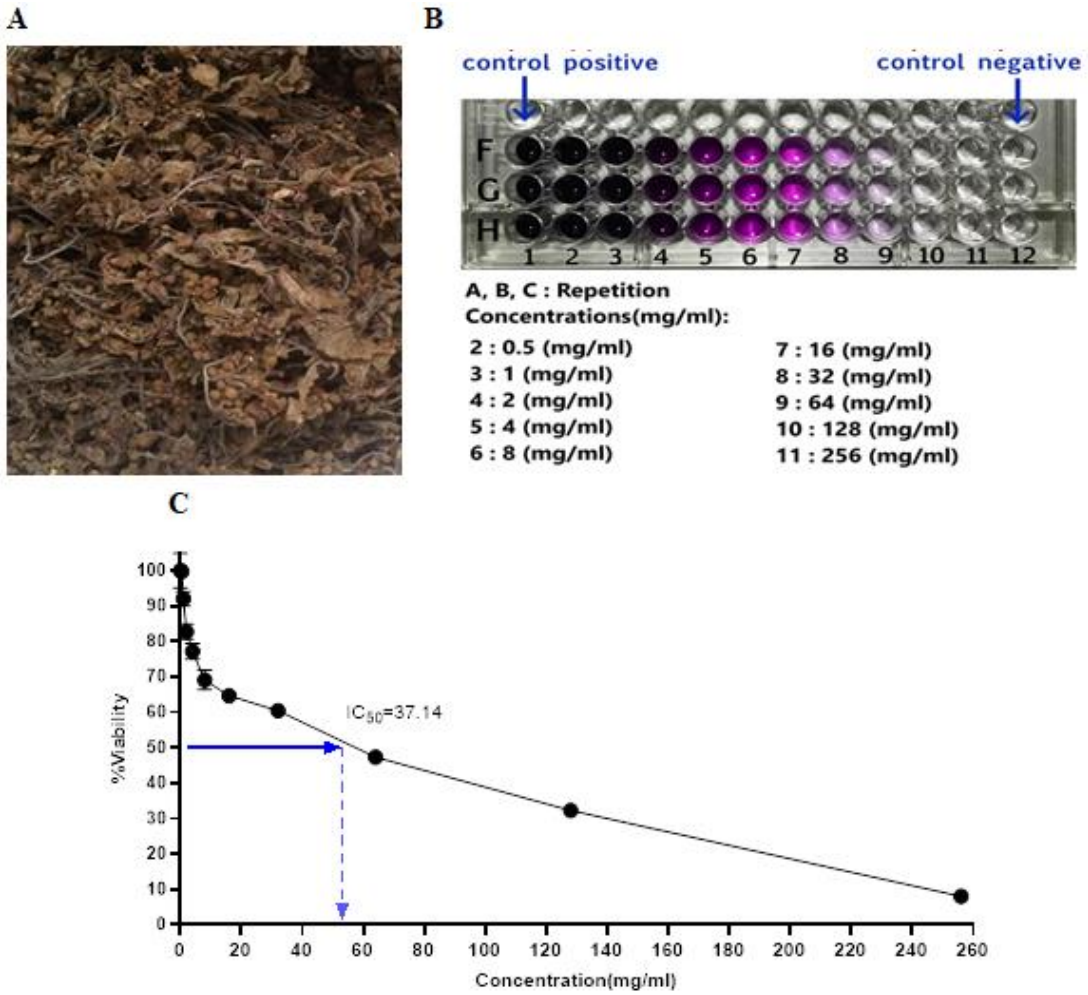

Figure 2: *S. glaucescens* (A), results of MTT (B) and IC50 (C) in the treatment of Caco2 cell line by *S. glaucescens* extract.

Evaluation of the vir expression

The results obtained in the Real-time PCR method indicated that the expression level of the *Vir* gene in *S. typhi* strains propagated in the $Caco_2$ cells receiving treatment with the methanolic extract of *Sargassum algae* reduced compared to the reference gene (*16SrRNA*) ($p<0.05$). Additionally, there was a decrease in the expression of this gene in the treated cells compared to the untreated cells, although the difference was not statistically significant ($p>0.05$) (Fig. 3).

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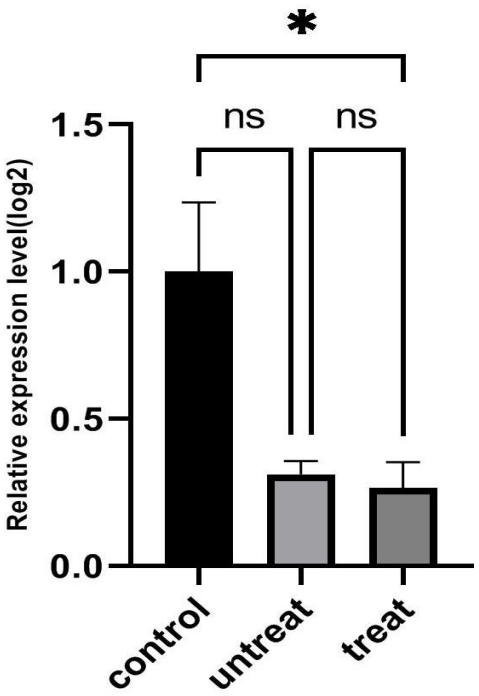

Figure 3: Comparison of *vir* gene expression in *S. typhi* cultured in Caco2 cells with and without treatment using methanolic extract of Sargassum algae. *: $p<0.05$. ns: not significant.

Evaluation of the Bcl-2 and P53 gene expression

The results of ΔΔCT evaluation showed that the expression of the *Bcl-2* gene in $Caco_2$ cells treated with brown algae extract before being infected with *S. typhi* decreased by 2.07 times compared to the control ($p<0.001$). After *the S. typhi* infection, the expression of the *Bcl-2* gene in the group treated with brown algae extract showed a decrease of 1.78 times compared to the control ($p<0.001$). These findings indicate that brown algae extract can reduce *Bcl-2* gene expression in $Caco_2$ cells, especially when the cells are infected with *S. typhi* bacteria (Figure 4). Also, the expression of *the P53* gene in $Caco_2$ cells treated with brown algae extract before being infected with *S. typhi* increased by 0.594 times compared to the control ($p<0.0001$). After infection with *S. typhi*, the expression of *the P53* gene in the group treated with brown algae extract increased by 0.65 times compared to the control (*P*-value= 0.038). Therefore, brown algae extract can increase the expression of the *P53* gene in $Caco_2$ cells, especially when the cells are infected with *S. typhi* bacteria (Fig. 4).

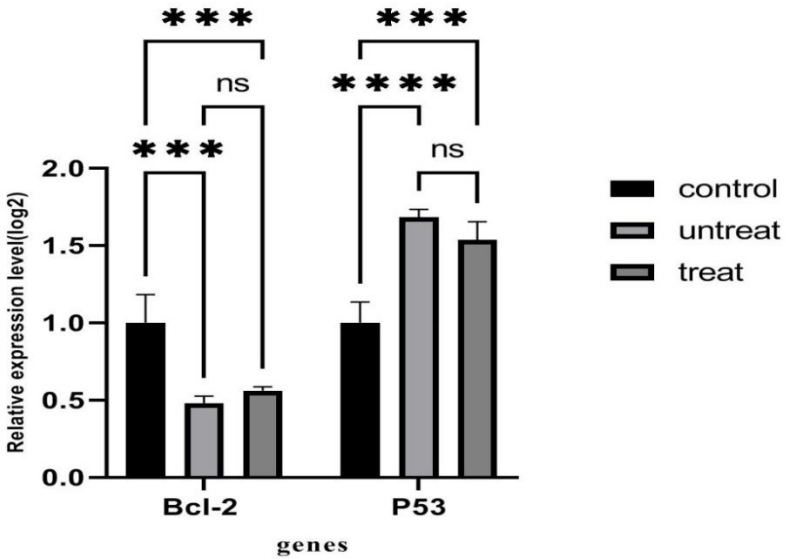

Figure 4: Relative expression of *Bcl-2* and *P53* genes in Caco2 cells treated with brown algae extract compared to untreated cells before and after infection with *S. typhi*. ***: $p<0.001$ and ****: $p<0.0001$.

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Discussion

Salmonella is one of the most common bacteria that can be transmitted from animals to humans, and due to the diversity of animal reservoirs, it is considered one of the most important causes of foodborne diseases and one of the health problems around the world (Rowe *et al.*, 1997). Due to the spread of antibiotic resistance, especially foodborne bacteria and nosocomial infections, the discovery and development of antibacterial drugs have attracted the attention of many researchers (Peymani *et al.*, 2013). Research in recent years has shown that resistance-virulence plasmids, which carry factors effective in resistance and virulence, can also be formed (Kidgell *et al.*, 2002). Salmonella carries chromosomal and plasmid genes that play a major role in the virulence and invasion of this bacterium. Among the most important virulence factors of Salmonella are the Vir, Inv, and Int genes (Guiney and Fierer, 2011). Due to the importance mentioned about the *Vir* gene, this study was designed to investigate the effect of brown seaweed extract on the expression of this gene in *S. typhi*.

Several studies have shown that different marine sources have very good anti-viral, anti-fungal, and anti-bacterial effects (Ghaednia *et al.*, 2011). Algae have valuable polysaccharides such as agar, carrageenan, and alginate that can have anti-microbial and anti-cancer properties (Ajdari *et al.*, 2016). In Iran, on the tidal beaches of Chabahar, seaweed from three families of green, brown, and red algae can be found, and all these algae can be used in different seasons of the year. Unfortunately, in Iran, few studies have been conducted on the biological and medical properties of these valuable resources, and it is necessary to conduct several studies in this field. Sargassum, brown algae, is one of the most famous algae on the southern coast of Iran and is known as seaweed or rockweed (Peymani *et al*., 2013). On the other hand, according to the conducted research, some macroscopic algae have considerable antibacterial and antifungal properties, the antibacterial activity of algae depends on factors such as the season of their collection, the environment and different stages of algae growth. In the chemical studies conducted on algae, the presence of compounds such as phenol, tannin, saponin, flavin, and steroid has been proven.

Amirsharifi *et al*. (2016) evaluated the effect of brown algae extract on inhibiting *Enterococcus faecium*, *Streptococcus mutans*, *Shigella boydii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella enteritidis*, by microdilution method. They reported methanolic extract of algae (*S. glaucescens*) had the best inhibitory effect on all pathogens, followed by hexane extract. In accordance with our study, the methanolic extract of *Sargassum* had the best effect against *S. typhi* isolates. The range of MIC was 1-16 µg/mL and the lowest MIC_{50} was related to the methanolic extract (2 µg/mL). Mahianeh *et al*. (Mahianeh *et al*., 2014) investigated the antibacterial effect of hot water brown algae, extract *S. glaucescens*as, on *Staphylococcus aureus*, *Vibrio harveyi*, *Escherichia coli*, and *Bacillus cereus* by disc diffusion agar method. Ethanol extract showed better antibacterial activity compared to other extracts (p<0.05).

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Zobeidy *et al*. (Zobeidy Nezhad *et al*., 2018) also investigated the effect of methanolic extract of *Padina gymnospora* from10 to 80 mg/mL on *Vibrio cholerae*, *Morganella morganii*, *Proteus mirabilis*, and *Proteus vulgaris* using disc diffusion method. The results showed that *P. vulgaris* is most sensitive to algae extract at a concentration of 80 mg/mL (Mahianeh *et al*., 2014). According to the results obtained in the present study, the methanolic extract had the most antibacterial properties, which shows the agreement of the results with the mentioned studies.

In another study, the antibacterial activity of *Sargassum tenerrimum Yersinia ruckeri*, *Streptococcus iniae*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhi*murium, and *Escherichia coli* was investigated by disk diffusion method. The results indicated that 50 mg/mL of extract has a higher inhibitory effect against *Salmonella typhi*murium compared to 25 mg/mL, there was no significant inhibitory effect against the other bacteria (Shahhosseini *et al.*, 2020). The findings of the present study also indicate that the methanolic extract of brown algae has significant antibacterial effects on *S. typhi*. However, the differences in the results of this study with other studies are attributable to the difference in the bacterial strains, algae, and the geographical area of sampling.

In the present study, the level of expression of the Vir in the strains treated with the methanolic extract of *Sargassum* algae decreased compared to the reference gene (16SrRNA). Mohkami and Habibi-Pirkoohi (2019) examined the inhibitory effect of *Sargassum angustifolium* extract on the growth and pathogenicity of *Staphylococcus aureus* and investigated the expression level of staphylococcal enterotoxins and bacterial capsules (cap8). The results revealed that the extract of *S. angustifolium* inhibits the growth of bacteria in a dose-dependent manner, in such a way that the inhibitory effect of the extract increases with increasing concentration. The results of the Real-time PCR test showed that seaweed extract was able to significantly reduce the expression of virulence genes, again in a dose-dependent manner.

The findings of previous studies have shown that algae bioactive substances have anti-cancer effects by inhibiting cancer cell growth, invasion, metastasis, and cell death (Aziz *et al.*, 2021, Minhas *et al.*, 2024). In the present study, the effect of brown algae extract on cancer inhibition has also been observed. Some studies have shown the dose-dependent role of red, brown, and green algae on breast cancer cell lines (Pangestuti and Kim, 2011) and the most important human cancer cell lines (MCF-7, MDA-MB-231, HeLa HepG2, and HT-29) (Namvar *et al.*, 2014). *The P53* gene is a tumor suppressor gene and has a very important role in regulating the cell cycle. Mutations in this gene lead to the development and progression of cancer by inhibiting apoptosis (Borrero and El-Deiry, 2021). Also, the important role of *P53* gene mutations, which lead to the inactivation of the caspase cascade in $Caco_2$ cells and subsequently create resistance to drugs such as 5FU, has been proven in various studies (Liu and Bodmer, 2006; Thant *et al.*, 2008). *The P53* gene inhibits the growth

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and proliferation of cancer cells by inducing apoptosis through endoplasmic reticulum stress. Studies have shown that apoptosis can be induced in $Caco_2$ cells even in the presence of some *P53* gene mutations (Hiraishi *et al.*, 2019).

Hiraishi *et al*. (2019) reported the growth-limiting potential of *Lactobacillus plantarum* 06CC2 extract on $Caco_2$ colon cancer cells. However, the present study is the first study to investigate the effect of methanol extract of brown algae on the expression of anti-apoptotic *Bcl-2* and apoptotic *P53* genes in colorectal cancer $Caco_2$ cell line before and after infected with *S. typhi*. The results of this study showed that the treatment of $Caco_2$ cells with algae extract has a significantly decreasing effect on the expression of the anti-apoptotic gene *Bcl-2* and a significantly increasing effect on the expression of the apoptotic gene *P53*, and these effects are greater after infecting the cells with *S. typhi*. The fold change results of *P53* and *Bcl-2* gene expression showed that the preventive effect of brown algae extract is much higher than its therapeutic effect.

The results of the study by Yavari and Ahmadizadeh (2020) showed that the treatment of $Caco_2$ cells with *Lactobacillus casei* extract increases the expression of the P53 and is capable of inducing apoptosis. Also, in the study of Al-Aadily *et al*. (2022), the anticancer effect of the hydroalcoholic extract of *Sargassum oligocystom* was investigated on SW742, HT-29, WiDr, and CT-26 cancer cell lines, and the expression of P53 was investigated. The results showed that the extract used at a concentration of 2 mg/mL increases the expression of the P53 by 0.5-0.68 times. In the study conducted by Fahmy *et al*. (2023), the expression of P53 increased after the treatment of several cancer cell lines, including $Caco_2$, with the methanol extract of the algae *Sirophysalis trinodis*, *Polycladia myrica*, and *Turbinaria triquetra*. In our study, the evidence indicates that the compounds in brown algae extract increased the expression of the P53 in the $Caco_2$ cell line. This means that this extract probably leads to the death of cancer cells by activating apoptosis.

The *Bcl-2* gene family participates in the regulation of apoptosis in both anti-apoptotic and pro-apoptotic roles. The ratio of anti-apoptotic and pro-apoptotic members present in cells is regulated by different signaling pathways and influenced by cell stress such as available nutrients, DNA damage, and protein processing, and determines the initiation or inhibition of apoptosis (Warren *et al.*, 2019). In the present study, changes in the expression level of the *Bcl-2* family anti-apoptotic protein under the influence of brown algae extract were investigated. The results showed that the expression of this gene decreases under the influence of brown algae extract, which can confirm the significant effect of the extract in inducing apoptosis of cancer cells. In the study conducted by Kavousi *et al*., it was reported that the treatment of glioblastoma cancer U87 cell line with Spirulina Microalgae extract leads to a 3-fold decrease in the expression of *Bcl-2*, the anti-apoptotic gene (Kavousi and Fatemi, 2022). Also, in the study conducted by Begum *et al*., Gelidiella acerosa extract was able to induce apoptosis in the A549 cell line by reducing Bcl-2

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expression (Begum and Hemalatha, 2020). Based on the findings of the present study, it can be assumed that the metabolites in algae extract may control or treat cancer through two mechanisms: first by directly inhibiting the growth of pathogenic bacteria by inflammatory compounds, and second by indirectly inhibiting *Bcl-2* production and increasing the expression of apoptotic protein P53. This claim has been made earlier in other studies as well (Rengaraj *et al.*, 2018).

Conclusion

The results of this study showed that the methanolic extract of brown algae *S. glaucescens* has antibacterial effects by reducing the expression of *Vir* pathogenic gene in *S. typhi*. Also, this extract is capable of inhibiting cancer by reducing the expression of the anti-apoptotic gene *Bcl-2* and increasing the expression of the apoptotic gene *P53* in the $Caco_2$ cell line when the cells are exposed to *S. typhi* when they are not exposed. The preventive effect of brown algae extract was much higher than the therapeutic effect. It seems that the algae have valuable biological compounds that are effective in reducing the pathogenicity of *S. typhi* and the risk of cancer. By completing the studies and identifying the effective ingredients in the algae extract, it may be possible to develop new and safe anti-cancer drugs. It is also necessary to evaluate the stability and immunogenicity of these substances and design appropriate drug delivery systems.

Confilicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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